MEETING ABSTRACTS

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The 5th Congress of the Brazilian Biotechnology Society (SBBIOTEC): Meeting abstracts

Florianópolis, Brazil. 10-14 November 2013

Edited by Luiz Antônio Barreto de Castro and Dario Grattapaglia

Published: 1 October 2014

These abstracts are available online at http://www.biomedcentral.com/bmcproc/supplements/8/S4

INTRODUCTION

I1
5th Congress of the Brazilian Biotechnology Society: Frontiers in Biology

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BMC Proceedings 2014, 8(Suppl 4):I1

Despite Brazil’s increasing production of scientific articles in the last three decades, this growth has yet to translate into an effective contribution to the economic development of this large and diverse country and to the improvement of the life standards of its population. The advances in biotechnology, whose full development relies on the elucidation of the main tenets of biology, plays an increasingly crucial role in food security, environmental remediation, sustainable bio-based industry and public health.

Recognizing the key relevance of biotechnology to the overall development of our country, the Brazilian Biotechnology Society was founded in 1988. The SBBIOTEC was established to promote the overall progress of biotechnology by integrating biosciences, technology development and capacity building, aiming at contributing with positive impacts on the economic development and well-being of the Brazilian society. Scientific in its nature, from 1998 on SBBIOTEC has held Congresses in São Paulo, Salvador, Fortaleza and Santos. For the 5th edition of the SBBIOTEC Congress, held in the beautiful city of Florianópolis, the organizing committee thought it would generate a permanent memory of the content of the event by publishing a BMC Proceedings supplement. By capturing the current status of research and applications of biotechnology in Brazil, SBBIOTEC hopes to provide better visibility and value of the scientific contributions presented by the participants and make such an initiative an everlasting one for future Congress editions.

During five days, in eight scientific sessions, an outstanding team of scientists both from public and private organizations shared their results and visions on the present and future of this fast moving area of science and technology with an audience of over 600 attendees. Some of the current advances of biotechnology research and applications in Brazil were presented together with keynote contributions from international scientists. In total, 47 oral lectures and 264 poster presentations, these last ones mostly by undergraduate and graduate students, were delivered, totaling 311 papers now available online as extended abstracts into this BMC Proceedings supplement. Presentations covered four different areas, namely: agricultural, human health, animal and industrial biotechnology. Besides the Brazilian delegates, scientists from biotech companies in Canada, USA, and Europe also came to the Congress to discuss important issues such as industrial scaling up, animal and insect gene expressions and markers for the early detection of epithelial cancer, to name a few.

The program included a specific section on biofuels biotechnology, a challenging area that can be significantly boosted by metabolic and genomic engineering strategies applied to industrial yeast strains to improve bioethanol production. In human health, stem cells, tuberculosis and cancer were thoroughly discussed with emphasis on monoclonal antibodies in the therapeutic scenario. Pre-clinical and clinical trials were dealt as a critical issue to this process, particularly in light of a recent activist attack that destroyed one of the very few skilled institutions performing pre clinical trials in Brazil. In agricultural biotechnology, an area where Brazil is currently a worldwide key player, a number of important advances were presented in the area of nitrogen fixation in grasses, host pathogen interactions, and vaccine production in plants. Biological control of plant pests and human diseases such as dengue fever, and advances in RNA interference and intragenics were also hot topics of discussion. In animal husbandry the potential production of heparin in Brazil and several advances in animal gene expression were thoroughly discussed. Finally, specific sessions were devoted to the discussion of the legal framework for bioscience development, graduate training and initiatives to enhance biotechnology collaborative networks in Brazil.

In closing this introductory statement, a major acknowledgement is due to the outstanding financial support provided by the Federal District Research Foundation (Fundação de Amparo à Pesquisa do Distrito Federal FAP-DF) that made the Congress and this BMC Supplement possible. Besides the financial sponsorship of FAP-DF and an active Scientific Committee involved in abstract review, a number of people were involved in the organization and logistics. The conference would not have been possible without the valuable contributions of all these players. Given the rewarding feedback received after the Congress, we believe that the goal of providing a good mix of science and social interactions was truly accomplished, which we trust should be well illustrated by the abstracts that now follow these brief opening words.

ORAL PRESENTATION

O1
Recombinant proteins in plants

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Background: Over the last few decades, several studies have shown that plants can be a viable option for producing functional recombinant proteins with a wide range of structural characteristics [1]. In addition, the potential benefit for developing countries is a prominent feature that we have recently addressed [2]. Plant-produced recombinant proteins can already be considered a novel component in sustainable food production [3]. A major reason for this optimism relates to cost. Indeed, it is widely recognized that plants used as bioreactors to produce recombinant proteins would enable a significant reduction in overall manufacturing costs [2]. Although recombinant proteins can be functionally expressed in different plant systems, it is imperative to determine the platform that offers the most advantageous conditions for the expression and recovery of a particular protein [1]. In addition, because plant pathogenic organisms cannot cause human disease, this opens the possibility of exploiting plants as potential candidates for the production of orally administered antigens [1]. Basically, there are three strategies for recombinant protein production in plant-based systems: (1) use of cell-culture-based systems that are equivalent to mammalian, microbial and insect cell systems; (2) transient expression of foreign genes in plant tissues that are transformed by either agrobacterium or by viral infection and (3) development of transgenic plants carrying stably integrated transgenes [4,5]. Here, I will focus on some of our recent results on transient expression and soybean seed as bioreactor-based systems. Transient expression systems are very useful for research and are now being routinely used for the rapid production of valuable proteins. These systems allow high throughput production and straightforward manipulation, permitting the rapid validation of expression constructs and the production of large amounts of recombinant protein within a few weeks. As a direct consequence, the protein yields from transient expression in plants are normally higher than yields observed in other recombinant plant systems. Transient technology is based on the insertion of transgenes into plant cells using plant viruses, commonly the tobacco mosaic virus (TMV) and the potato virus x (PVX) as well as transgenic Agrobacterium tumefaciens. Transgene insertion occurs without stable chromosomal insertion, resulting in non-permanent and non-inheritable gene expression. Because the transfer rates of Agrobacterium T-DNA and viral-carried genes can reach a very high number of plant cells after infection. Tobacco leaves are the dominant choice for the development of commercial platforms using transient expression [1]. Seeds as bioreactors also provide a potential economical platform for the large-scale production and storage of recombinant proteins [1], soybean seed storage proteins are of great interest for the development of regulated large-scale production and storage of recombinant proteins [1,5]. Soybean developing seeds.

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Clinical trials in drug development: the Brazilian experience

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BMC Proceedings 2014, 8(Suppl 4):C02

Clinical research in Brazil today can be seen in three distinct areas. In the first, still acting shy and with few resources, the research is funded through government agencies that seek the understanding of some diseases or peculiar conditions in the country. These include neglected diseases still endemic in Brazil that do not interest large international industries. In the second area, funded with abundant resources coming from international pharmaceutical industries, research studies are carried out to evaluate the efficacy and safety of drugs in multicenter trials. The third area, new in the country, is the clinical research of bioavailability and bioequivalence of drugs that emerged due to the need for assessing the quality of generic drugs, which was funded in part with public funds and in part with funds from national pharmaceutical industries. Although there are criticisms to all three, they have contributed to boosting clinical research in the country. At the moment, it is necessary to use a policy to guide their future directions and coordinate the actions of groups that have academic competences and are already working traditionally in the sector, avoiding the opportunism that often takes place. It is always important to keep in mind that clinical research is not done only by dilettantism or on behalf of scientific knowledge. This research involves humans and therefore it should be conducted in compliance with ethical principles of autonomy, beneficence, non-maleficence, justice and equity, concerning the participation of the research subject and the social relevance of their results. It should also be considered that the research carried out in Brazil by major international pharmaceutical conglomerates had the merit of introducing the country to the international circuit of multicenter clinical research, to emphasize the quality of our scientists in academia and to introduce new research protocols. However, neither the design of these experiments nor the elaboration of protocols and even the management of the experiments were in charge of our research groups. When they are not the responsibility of the industry itself, they are outsourced to international CROs (Contract Research Organizations), leaving the Brazilian researcher only in an operational role. In another scenario we find domestic industries that are still groping in drug research and development. However, some have begun to realize the impossibility of surviving in the market doing just similar and generic drugs. Therefore, it is necessary that our clinical research is prepared to support the national pharmaceutical industry that invests in the development of new drugs, and do so with the same competence displayed in the quality control of generic medications. Furthermore, it is of critical importance that clinical research is also prepared to assist public and private institutions in obtaining information regarding the diseases prevalent in the population.

Contract Development and Manufacturing Organizations (CDMO): are they needed in Brazil?

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BMC Proceedings 2014, 8(Suppl 4):C03

Life-altering biologic therapeutics are complex large molecules which include hormones, growth factors, interferons, vaccines, monoclonal antibodies, recombinant proteins and others. The time, cost and risk for advancing a biologic through the pipeline comes at a steep price. It is estimated that the development of a biologic therapeutic is as high as US$1.2 billion [1] versus the cost of developing a small molecule Innovator estimated at US$350 million [2]. Consequently, it is necessary to coordinate the clinical research is also prepared to assist public and private institutions in obtaining information regarding the diseases prevalent in the population.

The growing demand of biologics is an added financial stress on Brazil's National Public Healthcare System SUS (Sistema Unico De Saude) which includes coverage of drug cost. With patent expiry approaching for several blockbuster biologics, Brazil is assessing multi-faceted programs and partnerships that facilitate the introduction of biosimilar therapeutics. The government initiative for the development of local biosimilars and improvement of national biologics capabilities, attempts to address cost savings measures to the healthcare system while reducing the risk of supply associated with imported branded biologics.

Through the coordination of the Ministry of Health and private and public companies, the Partnership for Productive Development (PPD) initiative was developed for the local manufacturing of 14 biological drugs seven of which are monoclonal antibodies (mAb) [7] that involve facilities with greater complexity and unique manufacturing processes. The manufacturing process is important for all drugs, but especially for biological medicines, which are more complex structurally requiring a high degree of technology and scientific expertise to stabilize the complex molecular structures. These differences are important to take into account when understanding how the manufacturing process for a biological medicine can have an impact on product efficacy, quality and safety. Establishing "biosimilarity" to a reference product for a biosimilar drugs requires more preclinical and clinical testing than is usually necessary for generic small-molecule drugs because of the complexities of the molecular structure and manufacturing process of biologics. Biosimilar development is a high-risk activity demanding significant resources with cost around US$75 - $250 million [8] versus the cost to develop a generic small molecule (US$1 million [9] - $3.5 million [10]). Additionally, building a biologics manufacturing facility is expected to be in the range of up to US$400 million [11]. One option to mitigate the high cost and risk of developing and manufacturing biosimilars is to partner with Contract Development and Manufacturing Organizations (CDMO). CDMOs specializing in the manufacturing of complex biologics can provide access to capacity with lower risk and investment and apply their scientific and technical expertise to serve as a strategic partner in the development and manufacturing of biosimilars.

References

The development of genetically modified (transgenic) animals for the production of human recombinant therapeutic proteins has been ongoing for more than 25+ years. Only recently in the last few years have products finally been approved that can now be found commercially on the market to the benefit of the medical community and patients that receive these lifesaving therapies. rEVO Biologics has a long history in transgenic technology for this purpose and has been recognized as the leader in this field. With the approval of ATryn® (antithrombin alpha) by both the EMA in 2007 and the FDA in 2009, rEVO Biologics set the standard and established significant milestones and “firsts” and is revolutionizing the application of this technology to recombinant therapeutic production. rEVO Biologics is now expanding upon this success by not only furthering the development of ATryn® into subsequent larger clinical indications but also by moving additional recombinant therapeutic proteins through the pipeline from development and into early and late stage clinical trials. The following presentation will highlight the company history and evolution for rEVO Biologics over the years and the developmental path for ATryn®. Specifically, areas to be reviewed will include the technology, the molecular biology involved, protein expression in the mammary gland, the purification scheme, antithrombin as a molecule and its clinical development, and the regulatory path for approval of ATryn® over the years. Additionally, the further clinical development of ATryn® into additional clinical indications and new proteins being taken through this development, clinical trial evaluation, and regulatory approval path will be discussed.

**OS**

**Development of Mycobacterium tuberculosis attenuated strains as live vaccine candidates for tuberculosis**

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BMC Proceedings 2014, 8(Suppl 4):OS

Tuberculosis (TB) is an infectious disease mainly caused by the bacillus *Mycobacterium tuberculosis*. The World Health Organization estimates that 8.6 million new TB cases occurred in 2012, resulting in 1.3 million deaths [1]. TB is the second leading cause of death caused by an infectious disease worldwide after immunodeficiency virus (HIV). Despite the availability of an effective chemotherapy and a moderately protective Bacille-Calmette-Guérin (BCG) vaccine, TB remains a major global health problem [1]. BCG provides efficient protection against TB in newborns, but its efficacy against the establishment of latent or pulmonary TB in adults is highly variable. The variability of BCG protection in adults might be related to the absence of more than a hundred genes when compared with the *Mycobacterium bovis* pathogenic strain. Among the missing genes in BCG is the RD1 region which encodes potent antigens and virulence factors [2]. Thus, there is an urgent need for the emergence of new prophylactic strategies to decrease TB incidence worldwide. The development of genetic tools to manipulate mycobacteria and the completion of *M. tuberculosis* genome sequencing have been contributing to a better understanding of genes involved in TB virulence and pathogenesis, and consequently to the emergence of novel vaccine candidates.

Two major strategies have been used to develop new vaccine candidates against TB: (i) substitution of BCG in which an improved version of BCG or a new attenuated live *M. tuberculosis* vaccine would have a higher efficiency than BCG and replace it as a prime vaccine; and (ii) a prime-boost strategy in which BCG continues to be given to neonates, and a new vaccine is given as a booster dose to extend the protection and efficacy [1]. Following these approaches, numerous vaccine candidates against TB are currently in preclinical and clinical trials, including recombinant BCGs, attenuated *M. tuberculosis* strains, recombinant viral-vector platforms, protein/eductants combinations and mycobacterial extracts [1]. The strategy to develop novel vaccines possessing the effects of both a traditional live-attenuated *M. tuberculosis* strain while not producing immunogenicity has to be reached, since over attenuated bacteria may not produce in vivo some key antigens for the induction of a protective immunity [4].

An efficient strategy to rationally attenuate *M. tuberculosis* consists on the construction of double-deletion mutant of *M. tuberculosis* in the *panC* and *panD* genes, both involved in the *de novo* biosynthesis of pantothenate. Pantothenic acid (vitamin B5) is an essential molecule required for the synthesis of coenzyme A and acyl carrier protein (ACP), two important molecules in fatty acid metabolism and other metabolic reactions [5]. The double-deletion mutant resulted in an auxotrophic and attenuated strain which conferred protection in mice challenged with virulent *M. tuberculosis* [5]. In attempts to further enhance the safety of this live attenuated *M. tuberculosis* vaccine candidate, two other mutant strains were constructed combining *panCD* deletion with either *lysA* or *leuD* deletions, involved in lysine and leucine biosynthesis, respectively. The mc26020 strain, constructed by the inactivation of the *panCD* and *lysA* genes, is strictly auxotrophic for pantothenate and lysine, severely attenuated and capable of inducing protective responses against an aerosolized *M. tuberculosis* challenge in both immunocompetent and immunocompromised mice [6].

The strain constructed by inactivation of the *panC* and *leuD* genes was shown to confer long-term protection against challenge with virulent *M. tuberculosis* in guinea pig model which is equivalent to that afforded by BCG [7]. Simian immunodeficiency virus (SIV)-positive and SIV-negative Rhesus macaques were immunized with *panCD* and *leuD* mutant strain, and safety studies, clinical, hematological and bacteriological monitoring were carried out and revealed no vaccine-associated adverse effects [9].

Another successful approach employed to rationally attenuate *M. tuberculosis* combines deletions in *secA2* and *lysA* genes. The *secA2* gene encodes a component of a virulence-associated bacterial protein secretion system involved in inhibiting the host immune system and consequently promoting the *M. tuberculosis* survival within the host. Thus, *secA2* mutant was shown to increase both host cell apoptosis and priming of antigen-specific CD8+ T cells in vivo. The *secA2* and *lysA* double-mutant strain retained the effects obtained by *secA2* single mutation, but with an improved safety profile in immunosuppressed mice [8].

Recently, the first live-attenuated *M. tuberculosis*-based vaccine, MTBVAC, entered clinical trials. MTBVAC contains in two independent deletions without antibiotic-resistance markers in the genes *phoP*, coding for a transcription factor key for the regulation of *M. tuberculosis* genes, and *fadD26*, coding for one of the major mycobacterial virulence factors [9]. First, a mutant strain containing a single mutation in *phoP* gene was constructed and showed a high degree of safety, improved immunogenicity and protective efficacy compared to BCG in several animal models, from mice to non-human primates. Then, a second independent mutation deleting *fadD26* gene was introduced to obtain MTBVAC which was shown to be functionally and phenotypically comparable to its prototype after rigorous preclinical safety and biodistribution experiments [9]. This vaccine candidate was genetically engineered to fulfill the Geneva consensus requirements for Phase I clinical trials of live mycobacterial vaccines candidates which demands improved protective efficacy and safety potential relative to BCG, and two non-reverting independent mutations without antibiotic resistance markers [10].

Overall, these multiple deletion mutants demonstrated the feasibility to obtain rationally attenuated *M. tuberculosis* strains, which so far were shown to be safe without compromising their ability to provide protective immunity, making them viable live vaccines candidates against TB.

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O6

Monoclonal antibodies for the therapy of cancer

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BMC Proceedings 2014, 8(Suppl 4):O6

Introduction: The introduction of monoclonal antibodies for the treatment of cancer: Monoclonal Antibodies (mAbs) comprise a class of therapeutic biologics that has been increasingly used over the last decades. The concept of using antibodies to selectively target tumors was proposed by Paul Ehrlich over a century ago [1]. The development of the hybridoma technology in 1975 enabled the production of monoclonal antibodies, which contain uniform var...
functionality, and antibody fragments. Advances in protein- and glyco-engineering are now enabling the production of next-generation mAbs that are potentially more efficacious compared to the first-generation versions. Although the use of antibodies to guide drugs to a specific target has been explored for over 30 years, advances in the knowledge of linker and drug properties, and antibody engineering, design and selection, have led to the development of a new generation of molecules that are demonstrating promising clinical results [9]. One example is trastuzumab emtansine, a humanized anti-HER2 antibody conjugated to DM1 that is undergoing evaluation in Phase III studies of breast cancer patients [7]. Bispecific antibodies, as the name suggests, are designed to bind to two different targets. Modifications in protein sequence have shown to extend antibody half-life and afucosylated mAbs are known to have enhanced effector functions [9]. Most anticancer mAbs kill cells directly by binding to an antigen associated with a tumor cell and inducing cell death via effector functions, cytotoxic payloads or blockade of signals required for growth. However, in the last few years, antibodies that present indirect mechanisms of action, such as the activation of the immune system to combat tumors, have been developed. Ipilimumab, a first in class T-cell potentiator was approved for treatment of metastatic melanoma and represents the first therapy demonstrated to improve overall survival in melanoma [10]. Other advances illustrate how new insights into the structure and function of receptors allow them to be targeted in novel ways, with expected improvements in the therapeutic efficacy. EGFR is the target for three marketed anticancer mAbs (cetuximab, panitumumab and nimotuzumab) (Table 1). Recently, monoclonal antibody 806 (mAb806) was shown to target a conformational epitope exposed on wild-type EGFR when it is overexpressed on tumor cells or induced by the presence of oncoegenic mutations such as EGFRVIII. The mechanism of action of mAb806, which allows for EGFR inhibition without normal tissue toxicity, creates opportunities for combination therapy and strongly suggests mAb806 will be a superior targeted delivery system for antitumor agents. This concept of conformational epitope targeting by antibodies reflects an underlying interplay between the structure and biology of different conformational forms of the EGFR family [10].

Conclusions: The number of monoclonal antibodies that have been approved or are in development for cancer treatment has been steadily increasing over the last years. Because of their high target specificity, generally low toxicity and the ability to activate the immune system, the use of therapeutic antibodies for cancer treatment is very promising. They offer a promising alternative to conventional chemotherapy, provide targeted drug delivery, prevent drug resistance and improve the therapeutic window. Several of these antibodies are currently in Phase III clinical trials. In general, mAbs are generally low toxicity and the ability to activate the immune system, the use of therapeutic antibodies for cancer treatment is very promising.

Table 1(abstract O6) Monoclonal antibodies approved for clinical use in oncology

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<th>Antibody format</th>
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References

O7 HPV vaccines in Brazil and the world
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BMC Proceedings 2014, 8(Suppl 4):O7

Human papillomavirus (HPV), the most common newly acquired, sexually transmitted infection worldwide, infects about 75% of sexually active individuals in their lifetimes. The high-risk HPV types, the most common of which are HPV 16 and 18, cause cervical and other anogenital cancers, as well as some head and neck cancers [1]. Different HPV types are related to different lesion grades. HPV16 is found in about half of cervical cancers, the majority of anal cancers and about 30% of oropharyngeal cancers, besides a significant proportion of vulvar, vaginal and penile carcinomas. The low-risk types, the most common of which are HPV 6 and 11, cause genital warts, low-grade cervical dysplasias and recurrent respiratory papillomatosis. Most of our understanding of HPV and carcinogenesis is derived from studies of
women and cervical cancer. However, it is clear that HPV causes a significant proportion of tumors in both women and men [2]. Therefore, new prophylactic HPV vaccines have the power to prevent many HPV infections, thus reducing the burden of HPV-associated diseases.

Two vaccines have been developed, a quadrivalent vaccine that protects against HPV 16, 18, 6 and 11 (Merck & Co., USA) and a bivalent vaccine that protects against HPV 16 and 18 (Glaxo Smith-Kline, Belgium). Both vaccines are composed of HPV L1 proteins that have spontaneously self-assembled into virus-like particles (VLPs). However, they have different manufacturers, valencies, adjuvants and are produced in different types of cells. Both are administered by intramuscular injection, in three doses (0, 1 or 2 years apart). In a number of clinical trials conducted in thousands of subjects in many countries since 1998, the safety, immunogenicity and efficacy of these HPV VLP vaccines have been demonstrated [3].

Prophylactic efficacy was measured considering HPV infection and disease endpoints, particularly high-grade cervical intraepithelial neoplasia or worse for the bivalent and quadrivalent vaccines, as well as vulvar intraepithelial neoplasia (VIN) or vaginal intraepithelial neoplasia and genital warts, for the quadrivalent vaccine only. For both the bivalent and quadrivalent vaccines, results of different trials allow for the examination of broad trends in efficacy in preventing HPV 6/11/16/18-related disease in several groups of patients categorized according to their HPV status at baseline. Efficacy rates were obtained in naive women for the vaccine-HPV types which led to the recommendation of the vaccine to young individuals, prior to exposure to HPV which occurs concomitant to sexual activity onset. Cross-protection against non-vaccine types was also noted, but the significance and durability of protection is yet to be defined. To increase the coverage to more than 70% of cervical cancers attributable to HPV 16 and 18, it is possible that the best and most efficient responses will be obtained with multivalent vaccines against a larger number of genital, anal and oropharyngeal HPV infections which are presently under development [4].

VLPs are noninfectious protein subunit vaccines and show safety profiles similar to other protein subunit vaccines such as tetanus or hepatitis B vaccines. Both vaccines were generally well tolerated in clinical trials and maintain a good safety profile after licensure. Since the VLP vaccines were designed primarily to protect by inducing viroin neutralizing antibodies, type specific antibody responses to the VLPs have been the primary focus of immunogenicity studies. Both vaccines were shown to be highly immunogenic in the clinical trials, resulting in essentially 100% seroconversion in the different populations studied which included both females and males. Both vaccines induce the expected B cell memory response which is a property of vaccines with durable immune responses. Although the long term persistence of stable antibody levels is an encouraging finding, the antibody levels needed to prevent infection or disease are currently unknown. However, long-term follow-up studies have shown that efficacy is maintained for at least ten years and most studies suggest protection may be much longer. Available data do suggest that HPV vaccines may provide a lengthy period of protection, likely to usher a vaccinated individual through the years of highest infection risk and beyond. Additional studies are ongoing to verify these projections.

HPV vaccines introduction and implementation. Based on its demonstrated clinical efficacy and acceptable safety profile, HPV prophylactic vaccines have been introduced in hundreds of countries around the world, including most countries in Latin America. In Brazil, the quadrivalent HPV vaccine was approved in 2006 and the bivalent vaccine in 2008 [5]. The rapid approval and launch of such vaccines are a clear indication that governments and policy makers are aware of the expected impact on the prevention of one of the most common causes of female mortality worldwide.

In 2007, Australia was one of the first countries to introduce the quadrivalent HPV vaccine in a public immunization programme for girls and young women. Four years after the beginning of the HPV vaccination program, a substantial decrease in vaccine-targeted genotypes is evident. In fact, a dramatic reduction in genital warts was observed in young women as well as a significant reduction of herpetic infections. On the other hand, older women and men, as well as men who have sex with men, show very little or no reduction of this benign tumor caused by low-risk HPV types. These results led Australia to include young men in their national immunization program which, in time, translate into reductions in HPV-related lesions in both genders. Moreover, a reduction in high-grade cervical abnormalities is being observed as a consequence of the introduction of HPV vaccination [7].

Similar results are rapidly accumulating in several countries which implemented national HPV vaccination programs including New Zealand, Sweden, Denmark, Belgium, Germany, Canada, and USA. Despite such very positive results, incorporation of HPV vaccination in the public health sector is still to be seen in the developing world, mostly due to vaccine cost. HPV vaccine implementation will also depend on local infrastructure for vaccine delivery to the initial target population during the window of highest vaccine efficacy, i.e., prior to sexual exposure. Furthermore, introducing HPV vaccines in the present cervical cancer control system is hampered by the fact that secondary screening with Pap tests (or HPV DNA testing) will still be required to detect cervical cancers and pre-cancers caused by non-vaccine HPV types [8]. Ongoing cost-benefit studies and negotiations between governments, the private sector, and non-governmental organizations, are enabling several developing countries, where the vaccine is most needed, to implement the necessary programs [9]. The Ministry of Health of Brazil has announced in June 2013 the decision to introduce the quadrivalent HPV vaccine in the national immunization program. From 2014, this vaccine will be offered to girls and adolescents 11-12 years old. The details of the program are largely unknown which includes the possibility of an extended vaccination schedule (0, 6, 60 months; in clinical trials 0, 2, 6 months).

The success of the prophylactic vaccines against HPV is clear. However, these vaccines are not available nor will be available in the short run, as public immunization program in many countries. Moreover, the coverage rates will vary at large due to economic restrictions and other issues. Vaccine acceptance is largely determined by health beliefs, such as the individual's perceived susceptibility to the disease, vaccine characteristics, such as cost and efficacy, and obstacles to obtaining the vaccine. Education of physicians, policy makers, parents and adolescents will be crucial for delivering HPV vaccines to target populations during the window of highest vaccine efficacy, prior to sexual debut. Despite all efforts and the excellent results observed in real life, it may take some time before these vaccines are administered to the general population worldwide. Moreover, women will still be at risk for developing cancers caused by other HPV types not included in the vaccine and hence screening and monitoring strategies will be required. It is important to stress that disease outcomes should be recorded since at present there is no immune correlate and the importance of serum antibody levels is simply not known. Moreover, vaccinated populations should be followed-up for long-term safety, sustained immune responses and vaccine disease efficacy [10].

References

Tuberculosis (TB) caused by Mycobacterium tuberculosis, remains a major global health concern. According to the World Health Organization (WHO), TB was responsible for the occurrence of 1.8 million deaths in 2012 [1], and currently represents the main cause of human death due to a single pathogen. Increasing HIV-TB co-infections, the emergence of multidrug-resistant (MDR) [2,3], extensively drug-resistant(XDR) [3,4], and, more recently, of totally drug resistant strains (TDR) [5] have increased the need for developing new drugs to treat TB. Globally, 3.7% of new TB cases and 20% of previously treated patients are estimated to have MDR-TB [2]. Ideally, novel anti-TB drugs, should be effective against the resistant strains, decreasing the length of treatment thereby improving patients compliance with lower dose frequency, minimal drug-drug interactions and reduced toxicity issues [1,3].

Isoniazid (INH) is the most prescribed drug for active TB and prophylaxis. It has been demonstrated that its primary target is the M. tuberculosis 2-trans-enoyl-ACP (CoA) reductase enzyme (inhA) [4]. Furthermore, INH is a pro-drug, activated by the mycobacterial KatG-encoded catalase-peroxidase enzyme in the presence of manganese ions, NAD(H) and oxygen [5]. Unfortunately, the use of INH has been allied to a series of collateral effects, especially neurotoxic and hepatotoxic side effects [6]. Our group has described a new approach to the rational design of an INH analogue based on an inorganic group (pentacyanoferrate III/II) attached to the nitrogen atom of the heterocyclic ring of INH, which inhibits the validate target inhA [6,7]. The metal center of the compound can promote an electron transfer reaction that mimics the in vitro activation of INH by KatG enzyme. In fact, our group has also proved that this new compound pentacyanoferrate(II) (named IQG-607), does not require the activation by katG or any other enzyme to bind to its primary target the M. tuberculosis inhA [7,8]; this profile might help to overcome an important mechanism of INH-resistance, the missense mutations in the katG gene. Moreover, our group has demonstrated that the compound IQG-607 is able to inhibit in vitro activity of wild-type and INH-resistant M. tuberculosis 121V,147T and S94A M. tuberculosis inhA mutant enzymes more slowly than INH [8,10]. Slow rates of dissociation are expected when we intend to reach higher inhibitory effectiveness and, therefore, the intervals between the doses administered to patients can be longer.

Noteworthy, another new compound containing a pentacalixarene and an oxadiazole moiety, pentacyanocalix[2-meritil-5-(piridin-4-il)-1,3,4-oxadiazole] ferrate(I) (II), (named IQG-639) was also found to be able to inhibit the in vitro activity of wild-type and INH resistant (S94A) M. tuberculosis inhA enzymes. Other experiments demonstrated that both IQG-607 and IQG-639 were active against culture of M. tuberculosis H37Rv and two INH-resistant clinical isolates, showing satisfactory efficacy in vitro.

Importantly, we have established toxicological parameters in mice to determine the safety of both compounds and to guide us in the next in vivo tests. Furthermore, 90 days repeated-doses toxicological studies in rats revealed a very favorable outcome for IQG-607 which was also very active in mouse macrophages infected with M. tuberculosis. It thus appears warranted to examine the potential in vivo anti-TB activity of these two compounds, IQG-607 and IQG-639. Accordingly, the activity of these compounds was evaluated by using an in vivo murine model of tuberculosis infection. Swiss mice were infected with M. tuberculosis H37Rv strain and IQG-607 and IQG-639 (250mg/kg) were administrated during 28 or 56 days. A dose response study was also performed with IQG-607 at 5, 10, 25, 100, 200 and 250mg/kg. The activity of test compound was compared with that of positive control drug INH at 25 mg/kg. After either 28 or 56 days of treatment, both IQG-607 and INH significantly reduced M. tuberculosis-induced splenomegaly, and also significantly diminished the colony forming units (CFU) in both spleens and lungs. IQG-607 or INH ameliorated the lung macroscopic aspect, reducing the lung lesions (granuloma like) to a similar extent [9]. However, IQG-639 was not capable or significantly modifying any evaluated parameters. In addition, experiments using early and late controls of infection revealed a bactericidal activity for IQG-607 in the animal model of infection. The promising activity of IQG-607 in M. tuberculosis-infected mice brings the hope that this compound might represent a good candidate for clinical development as a new antmycobacterical agent. Experiments to demonstrate efficacy and security in dogs and mini-pigs are under way.

References
nucleic acid (aptamers) selection technologies in association with nanobiotechnologies and their multiple applications in biomedical sciences. Discovery of biomarkers using combinatorial technologies: Combinatorial libraries displaying very diverse set of random peptides or very large repertoire of antibody fragments' fused to the capsid surface of filamentous phage have been successfully exploited in the discovery of novel biomarkers, and are excellent platforms for the design of high-affinity, protein-based binding reagents. Such markers are either peptide ligands that mimic epitope regions (imotopo) or monoclonal antibody fragments (Fab or scFv) against specific targets selected through cycles of biopanning (binding-washing-elution-reinfection-amplification) called Phage Display. The fundamental advantage of this technology is the direct link between the experimental phenotype and its encapsulated genotype, which allows the evolution of selected binders into optimized molecules [1,2]. Peptide ligands may be used to map immunologically active sites in proteins, carbohydrates, lipids, and also as individual epitope-mimicking antigens and immunogens [3,4]. The selected mimotopes may also be directly used in phage-based ELISA immunoassays, resulting in simple, specific, sensitive, and low-cost immunodiagnostic tests [5]. Similarly, the selection of antibodies from combinatorial libraries has also become an important tool for the generation of reagents, diagnostics, and therapeutic molecules. It is the only method to obtain specific antibodies bypassing the immunization step, which mimics the natural process of human antibody in vivo, resulting in high affinity antibody ligands, which may be suitable to human administration and potentially applicable to clinical diagnosis and treatment. We have generated successful molecules for diagnostic [5-8] and therapeutic [7,9] purposes through phage display by in vitro, ex vivo and in vivo approaches of selection. Such strategies are dependent on a very stringent subtractive selection scheme that includes well-characterized target samples and controls, target purification, elution/selection protocol with or without competitor molecules, number of selection cycles, and quality of the library and its diversity. Another important combinatorial technology is the selection of aptamers, which are random RNA or DNA sequences with conformational actions that specifically bind to a variety of targets, including proteins, small molecules, oligonucleotides, and peptides. In RNA aptamer selections, a DNA pool containing random sequences is typically synthesized artificially, which are subsequently transcribed to give the starting RNA population. Aptamer ligands are then evolved during several cycles of selection, elution, PCR amplification, cloning and transformation. Among several advantages of aptamers over other common biomarkers, we can include the similar molecular recognition of antibodies, the ability to be completely engineered in test tubes, the chemical synthesis at very low costs, desirable storage features due to their resistance to high temperatures and humidity without degradation, lack of immunogenicity, high potency and specificity. We have successfully selected aptamers for prostate cancer diagnosis, and to validate its potential use, magnetic beads were functionalized with our novel aptamers for targeted, well-recognized circulating tumor cells, and can be an auxiliary tool for molecular diagnostics. Briefly, short peptides, antibody fragments and aptamers aiming the selection of functional core sequences of epitope or paratope targets can be used in combination with nanotechnologies and biophotonics for improvement of diagnostic platforms, which will be further explored. Electrochemistry: Biosensors combine the biological nature of markers with the power of microelectronics and optoelectronics to offer powerful analytical tools with major applications in medical diagnostics. The foundations of biological sensors’ technology involve the interplay of fundamental disciplines, demanding specific knowledge on physical chemistry (nanoparticles), materials science (polymers), physics (optics and solid state), biology (antigen, antibody, biochemistry, genetics, substrates, and clinical information of diseases) and engineering (electronics and microfabrication). The specific interaction between the target analyte and the complementary recognition layer functionalized with a biomarker produces a physico-chemical change that is detected and measured by an electrochemical transducer through electric alterations as voltage (voltammetry), current (amperometry) or resistance (impedance). We discuss here developed specific and sensitive detection using electrochemistry technology for dengue virus, leishmaniasis, leprosy, breast and prostate cancer, and many other diseases [8,10]. Our aim is to translate these parameters into a universal platform using specific polymers as electrodes that not only allow prompt conjugation of biomarkers, but also mediate electron transfer kinetics to achieve a faster response in a simple, portable, specific and sensitive instrument. However, other technologies are also very competitive in the diagnostic field, due to specific functionalities and uses, such as target capture and enrichment by magnetic nanoparticles [10], imaging mediated by quantum dots, and other biophotonic technologies for the improvement of conventional assays, which is further discussed in the paper. Nanoparticles and biophotonics: Microparticle agglutination test has been used in clinical diagnostics since 1980’s. However, nanoparticles only recently became important diagnostic tools due to the development of different synthesis routes, chemical groups and polymers. Our first approaches with nanoparticles were focused on agglutination assays, specifically with modified latex Particle Gel Immunoagglutination test (PaGla) and Magnetic Microparticle-ELISA (MME) for antigen and antibody detection. The agglutination tests were performed with microparticles and nanoparticles coupled with filamentous bacteriophages displaying fused imotopes on its surface, which has favored the formation of the antigen-antibody or peptide-protein complexes, amplifying the optical detection in ELISA assays or after the chromatographic separation of the microagglutinates [10]. Different from latex nanoparticles, a new class of semiconductor nanocrystals, called Quantum Dots (QDs) has been developed with potential applications in diagnosis, drug delivery, biomedical imaging, sensors and optoelectronics. However, QDs are still considered toxic due to the use of hazardous reagents and synthesis under high temperatures, which affect specifically stability and biocompatibility. These QDs are highly luminescent and their emission wavelengths are dependent on their diameter, varying from 2 to 20 nm. Recently, our group has synthesized a novel class of ultrasmall QDs through an aqueous phase route (green chemistry), also called magic-sized QD. These extremely small nanocrystals present diameters below 2 nm, are highly stable, non-toxic, non-immunogenic, and with proper functionalization may become a powerful biomarker that may be used in many different applications, including immunohistochemistry and in vivo imaging studies, as shown with a breast cancer cell line. Interestingly, other biophotonic technologies have also the potential to be used in clinical and laboratory settings. We have explored the surface plasmon resonance (SPR) technology to improve the conventional ELISA tests of many different diseases. Surface plasmons are surface electromagnetic waves that propagate in a direction parallel to the metal/dielectric interface, usually formed with gold nanofilms. The wave is on the boundary of the gold polymer with the external medium (sample), and these oscillations are very sensitive to any changes of this boundary, such as the adsorption of molecules to the functionalized gold surface. The technology relies on reflectivity measurements used to detect DNA or proteins by changes in the local index of refraction upon adsorption of the target molecule to the functionalized gold surface without labeling. We have reached very high sensitivity using specific phage display-derived biomarkers for leprosy and dengue virus detection, which are diseases with diagnostic problems due to their nature and specific clinical conditions, and results indicate that simple SPR systems can substitute conventional ELISA tests in the near future. Conclusion and perspectives: We have successfully shown that combinatorial technologies have revealed functional determinant sites of molecules, which were combined with multiple research tools, techniques, and instruments that enabled entirely novel approaches for diagnostics and therapeutics. We believe that extensive and global analytical techniques, such as genomics and proteomics, may allow major target identification, but cross-reactions still remain as a problem, which can be solved by reducing the target site, a goal that has been promptly achieved by Phage display or Aptamer selections. The search for universal and robust diagnostic platforms and for highly specific and sensitive markers have been the highest challenges in the medical field, due to the variable disease spectra, different pathogenetic backgrounds, specific sampling, the complex interactions with vectors and environments, which may result in very diverse phenotypes [10]. Only multidisciplinary teams and novel nanobiotechnological approaches can meet the demand of smart solutions for human diseases diagnostics. Acknowledgments: The authors gratefully acknowledge the financial support of the Brazilian Funding Agencies: CAPES (Rede Nanobiotec/Brasil, Project N. 8), FAPEMIG (Pronex APQ 02413-08), and MCT/CNPq. References


O10 Unfractionated and low molecular weight heparin
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BMC Proceedings 2014, 8(Suppl 4):o10

Background: Unfractionated heparin (UFH) is mostly obtained from porcine and bovine mucosa and has been widely used for the treatment and prevention of thrombotic events. It consists of molecular chains of various lengths varying from 2000 to 40,000 Da [1]. Low molecular weight heparins (LMWHs) are smaller chains of UFH that can be obtained, from unfractionated heparin, by various chemical and enzymatic depolymerization processes and since they are produced from natural heparin, they must share structural and functional features with the parent compound, however, the depolymerization process used for their production leads to unique structural and functional characters. Apart from its noble anticoagulant properties, heparin and its derivatives can interact and modulate proteins involved in different biological processes such as inflammation [2] and angiogenesis [3]; yet, its mechanism of action are still under debate. Furthermore, heparin broader use is still impaired due to its strong anticoagulant activity and hemorrhagic complications.

Methods: Employing several physical-chemical analyses, nuclear magnetic resonance spectroscopy, scanning ultraviolet spectroscopy, circular dichroism and chromatographic techniques, coupled to various in vivo and in vitro biological and functional assays, our laboratory has been, for several decades, in the forefront of heparin and heparin-like structure/function studies as well as their distribution in the animal kingdom.

Results and conclusions: Over the years we’ve shown differences in heparin structure, molecular weight and biological activities revealing a tremendous level of variability. As a classical example, the commercial heparins from bovine lung and bovine mucosa differ in the amounts and content of their constituent disaccharides [4]. A systematic study on the structure of some mammalian and invertebrate heparins has shown that all the heparins contain two different basic regions that vary according to the tissue and species of origin. Low and Ultra Low Molecular Weight (ULMWHs) heparins have also been extensively characterised, where we’ve showed that ULMWHs are structurally related to LMWHs; however, their saccharide composition and average molecular weight differ considerably. In general, they possess higher levels of 3-O-sulfated glucosamine residues and higher amounts of unsaturated uronic acid [5].

More recently, the extraction, structural characterization and activity of heparinoids from the Pacific White Shrimp, L. vannameli, with repeating structures closely resembling those of heparin, have been reported [3,4]. These heparin-like structures, in contrast to those from mammalian heparin, have low anticoagulant and hemorrhagic potential but, exhibit potentially useful anti-inflammatory and anti-angiogenic activities. Furthermore, against the general accepted view, we’ve showed that the anticoagulant activity of heparin derivatives are related to the conformational stabilisation of the ATpolysaccharide complex, rather than the induced secondary structural changes in AT. The secondary structural changes induced by active and inactive saccharides were detectable to a high degree of sensitivity by synchrotron radiation circular dichroism (SRCD) but, they did not differ significantly [6]. Altogether, we’ve been dissecting the mechanism of action of heparin and its derivatives, bringing shedding light on the relationship between structure and function of this class of remarkable important molecules.

References

O11 Project Aedes transgenic population control in Juazeiro and Jacobina Bahia, Brazil
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BMC Proceedings 2014, 8(Suppl 4):o11

The genetic control of insects is a promising technology to be applied for the control of wild populations in urban and agricultural areas as an alternative to the widely used methods based on chemical and mechanical control. In Brazil, there is considerable concern about the occurrence of Dengue disease across the country, and a clear demand for improved vector control. A joint project among Medfly and Mosquito Facility Brazil, University of São Paulo and Oxitec was agreed upon in order to test in urban areas, social, technical and operational aspects of genetic control for A. aegypti population employing an RIDL transgenic strain. The PAT - Project Aedes Transgenic - was established in the city of Juazeiro, by a demand of Secretary of Health of State of Bahia. As the first large project on releasing transgenic insects in a human populated area, the PAT would follow all phases and steps recommended by the international community and fit into the strict Brazilian biosecurity laws.

Community engagement - From the outset the project partners have worked closely with the Brazilian regulatory system to obtain required permits for field activities. It has been a clear focus of PAT from the outset to adopt full transparency and a vigorous and proactive community engagement campaign.
Mass rearing - Critical to any SIT program is the ability to consistently rear large volumes of high quality insects. Egg production has an average of 12 million/week and 700,000 males. Field activities have concentrated on the suburb of Itaberaba. Males were marked with different colors and released from the same point in order to evaluate relative dispersal and longevity. The RIDL strain dispersed further with a mean distance travel of 75m (95% CI 30-109) compared to wild Itaberaba strain, 44m (95% CI 20-73), although this difference was not significant. Ovitrapping formed the main monitoring tool for A. aegypti - wild populations in both the treated and the untreated areas of Itaberaba. Larvae hatched from field-collected eggs from ovitraps were screened for fluorescence to determine paternity (fluorescent larvae, transgenic father; non-fluorescent larvae, wild-type father). Adult trapping was conducted periodically using aspiration surveys and BG-traps. This coincided with the release of marked cohorts of RIDL mosquitoes enabling longevity of released males to be assessed. After 12 months of twice week releases - 18 millions - the suppression reached 96%. In another district - Mandacaru - the releases started in march 2012 and with the density of 14,000 males/ha/week in 5 months, 100% of larva captured in ovitraps were fluorescent, indicating a high suppression. After the success in both districts, a larger target was aimed, a urban population of 45,000 people, city of Jacobina, where the releases started in June 2013 with a perspective to reach a large suppression in 24 months.

012
Assessing new strategies for TB diagnosis in low- and middle-income countries
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BMC Proceedings 2014, 8(Suppl 4):O12

We will report the current situation of tuberculosis globally and in Brazil, the need for new strategies towards tuberculosis control, focusing on new diagnostic technologies. Critical comments are given on the state of the art regarding the evaluation of new health technologies, degree of scientific evidence needed, evaluation of clinical impact, cost-effectiveness of incorporation into the health system and the social impact.

Currently, the given pragmatic approach indicates that it is not appropriate to conduct an investigation on the incorporation of new technologies only in a "purely experimental" manner in clinical research centers. Research and practice clinical processes become intertwined and the main outcomes to be considered are the patient’s health and actions creating a more effective health system in which the new technology will be incorporated. In this new scenario, it is essential that the academic biomedical areas reformulate their undergraduate curricula to include courses that address the development of new technologies, including the assessment of clinical impact, and economic and social incorporation of these new technologies into the current health system that will influence the future practice of their graduate students. In parallel, only through collaborative activities between academics, health service providers (public or private), producers of raw materials, laboratories and representatives of civil society will it be possible to conduct studies under routine conditions in demonstration areas to enable an analysis appropriate to the relevance of the incorporation of new technologies in the country.

References

013
Polymeric nanoparticles for the delivery of siRNA and the partnership between WSU and UNIT
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BMC Proceedings 2014, 8(Suppl 4):O13

RNA interference (RNAi) is an endogenous regulatory process whereby double-stranded RNA (dsRNA) present in the cell cytoplasm causes sequence-specific, post-transcriptional gene silencing [1]. dsRNA is first cleaved into short (19-21 nucleotides long) dsRNA fragments (siRNA) by Dicer, an endoribonuclease. The siRNA, which may be endogenous or exogenous (synthetically produced therapeutic) is subsequently loaded onto the RNA-induced silencing complex (RISC). The sense or passenger strand of the siRNA is then unwound and cleaved. The retained anti-sense or guide strand is highly specific to the target messenger RNA (mRNA) through complementary base paring. The activated RISC-siRNA complex scans, binds and degrades the complementary target mRNA in the cell cytoplasm at a highly specific position relative to the 5'-end of the anti-sense strand, thus causing degradation of the complementary mRNA, which then leads to gene silencing. This process is catalytic, allowing the same RISC-siRNA complex to cleave multiple copies of the target mRNAs, and will continue until the siRNA is degraded or diluted upon cell division.

With the decoding of the human genome siRNA has emerged as a broadly applicable and versatile therapeutic platform. The drug discovery aspect is greatly optimized for therapeutic siRNA compared to small molecular weight drugs, as one only requires knowledge of the sequence of the target gene in the case of siRNA, while trial and error through more or less random modification of the drug is required during screening of small drug molecules [2]. The specificity of siRNA is particularly relevant in the treatment of cancer, as there is the potential to selectively act only on cancerous cells, even when the therapeutic reaches non-target cell populations. siRNA therapy also holds promise in targeting the so-called 'non-druggable' diseases - those not amenable to conventional therapeutics such as small molecules, or monoclonal antibodies [3]. Another advantage of therapeutic siRNA is that gene regulation happens in the cell cytosol, and not the nucleus as in DNA-based gene therapies. This is significant, as the passive transport through the nuclear membrane is highly regulated, much more so than that across the cellular membrane, and is thus particularly relevant to postmitotic cells such as those in the airways.

The critical limiting factor in translating siRNA therapeutics to the clinic is the ability to efficiently deliver siRNA to the cell cytoplasm[4]. Free siRNA is very unstable in the extracellular milieu, with plasma half-lives of only ca. 10 min [5]. Moreover, due to its negative charge and large size, free siRNA is not readily internalized through passive diffusion, and is easily degraded in the lysosomes following cellular internalization through endocytosis [4,6]. Therefore, only a small fraction of the initial siRNA dose reaches the cell cytosol when administered as a free molecule. The use of nanocarriers for the efficient delivery of siRNA, and the ability to formulate such nanocarriers for regional delivery to the tissue of interest is thus of great relevance in developing siRNA therapeutic platforms.

Because viral carriers are potentially associated with severe immunological responses, non-viral siRNA carriers are preferred [6]. Non-viral (nano) carriers may involve complexation of siRNA with a positively charged carrier, chemical conjugation, or encapsulation [5,7]. The most widely used methods for the delivery of nucleic acids are lipoplexes, and
polypelexes, and complex/self-assembled domains. Conjugation of siRNA to singly functionalizable molecules such as PEG and cholesterol has been also explored. More recently, conjugation of siRNA to multivalent nanocarriers has been proposed, and this strategy may provide unique opportunities that may help overcome the challenges associated with the delivery of siRNA with complexes and singly functionalizable molecules. In this work we will discuss recent advances on the use of nanocarriers for siRNA delivery, with a special focus on the targeting of lung diseases.

Acknowledgements: The authors would like to acknowledge financial support from NSF (CBET Grant # 0933144), Nano@WSU (seed support), and for a fellowship from the Graduate School at Wayne State University (WSU) to Denise S. Conti.

References

O14
Transplantation of autologous bone marrow stem cells in patients with chronic spinal cord injury
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BMC Proceedings 2014, 8(Suppl 4):O14

Stem cells are being investigated by their potential use in regenerative medicine. There is currently a lack of effective clinical therapy for a number of conditions, such as severe spinal cord injury (SCI). SCI can lead to chronic paraplegia, considered an irreversible condition. The administration of stem cells has been tested as a potential therapy for SCI. In the present study we evaluated the feasibility, safety and potential efficacy of autologous mesenchymal stem cells (MSC) transplantation in patients with chronic complete SCI.

We conducted a phase I/II, non-controlled study in 14 patients of both genders aged between 18-65 years, with ASIA A classified chronic traumatic SCI. Baseline somatosensory evoked potentials (SSEP), spinal magnetic resonance imaging (MRI) and urodynamics were assessed before and after treatment. Pain rating was performed using the McGill Pain Questionnaire and a visual analogue score scale.

Bone marrow-derived MSC were cultured and characterized by flow cytometry, cell differentiation assays and G-band karyotyping, showing morphological and phenotypic characteristics of MSC, as well as genetic stability. MSC were injected directly into the lesion following laminectomy and durotomy.

Transplantation of bone marrow derived MSC was an overall safe procedure. All of the patients were discharged within 48 hours after surgery. Only one patient developed a post-operative complication, evolving a liquoric fistula that was treated by an additional surgical procedure. Nine patients had improvements in urologic function. All patients displayed variable improvements in sensitivity and 8 patients developed lower limbs motor functional gains, principally in the hip flexors. All patients had variable improvements in ASIA global scores. Additionally, 6 patients had changes in the AIS score to grade B and one to grade C. A direct correlation between motor gain and lesion level and an inverse correlation between light touch gain and lesion volume were found 6 months after transplantation. One patient presented changes in SSEP 3 and 6 months after MSC transplantation.

Although there is an abundance of studies describing the natural history of neurologic functional gains during the first year post-SCI, there is a lack of data regarding the degree of neurologic recovery that may naturally occur during prolonged follow up investigation. In the present study, we enrolled only patients with chronic and complete spinal cord injury (ASIA A) whom had previously been subjected to decompressive surgery and lengthy rehabilitation protocols without acquiring significant motor or sensory gains. Although there was not a controlled study, based on the patient profiles and the expected spontaneous gains, our study showed potential benefits of MSC transplantation treatment, in various degrees of motor and sensory improvements, clinical pain measures and urodynamics parameters.

The use of MSC presents several advantages, such as isolation from bone marrow aspirates and large-scale expansion in cell culture procedures, which are feasible to be performed in autologous transplantations. We conclude that intralesional transplantation of autologous MSC in spinal cord injury patients is safe and feasible, and may provide some progressive benefits. We are currently beginning new clinical trials based on MSC therapy for SCI.

O15
Recepta biopharma portfolio: clinical and pre clinical development
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BMC Proceedings 2014, 8(Suppl 4):O15

ReceptaBiopharma is a Brazilian Biotechnology company dedicated to the research and development of monoclonal antibodies and peptides as therapeutic agents for treatment of cancer. Monoclonal antibodies (mAbs) recognize specific targets on the surface of cells, or secreted signaling molecules, and can work as anti-tumor agents by inducing tumor cell death. mAbs act by targeting the tumor cells themselves, but can also cause tumor death by targeting endothelial cells (anti-angiogenic agents) as well as cells from the immune system, such as T-cells (immunomodulators). mAbs that target tumor cells can cause tumor cell death by several processes, including induction of apoptosis by interference with survival and proliferation pathways, cytotoxicity mediated by proteins of the complement system (CDC - complement-dependent cytotoxicity) or by effector cells (ADCC-antibody dependent cell cytotoxicity), as naked agents. mAbs can also be used as carriers, conjugated with toxins or radioisotopes, leading to a broad spectrum of therapies. Over 10 mAbs have been approved as therapeutic agents for Oncology. Before a drug can be offered to patients, there are several well-defined phases in the process of Drug Development. After discovery of a novel agent, in pre-clinical development mAbs are characterized in terms of 1) binding to the target (in vitro assays), 2) biochemical features, 3) functional activity (cell-based assays), 4) reactivity to human tissues (immunohistochemistry), 4) anti-tumor efficacy, safety and pharmacokinetics (animal studies). In order to test a novel drug in human patients, a compilation of all pre-clinical data and the design of the first clinical trial are included in a package that is submitted to the regulatory agencies. Clinical development also goes through phases: Phase I Studies evaluate safety, pharmacokinetics and dose escalation. Phase II studies evaluate safety and efficacy. Phase III Studies are designed to confirm clinical efficacy with a larger number of patients, which are critical for approval for commercialization. Recepta’s drug development program has mAbs in discovery, in pre-clinical development and in clinical trials, as well as peptides in discovery and pre-clinical development. Recepta’s portfolio and associated challenges in drug development will be discussed.

O16
Development of novel oncology biomarkers for cancer
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BMC Proceedings 2014, 8(Suppl 4):O16
**Introduction:** According to American Cancer Society projections for 2014, approximately 1.5 million new cancers are expected to be diagnosed and more than several hundred patients are expected to die of cancer, with more than 90% of these cancers being solid tumours (ACS, 2010). Epithelial cell-derived cancers comprise approximately 80-85% of all cancers, and include, amongst others, breast, bladder, lung, pancreatic, thyroid and prostate cancers [1-4]. Presently, breast cancer is the most common cancer among women worldwide as more than 1 in 4 cancers in women (about 28%) are of the breast, and thyroid cancer is the most common epithelial cell-derived malignancy of the endocrine glands [4,5]. These cancers may exhibit no signs in its early stages. Further, the aggressive cases are difficult to detect and when undetected, prognosis deteriorates rapidly. Neutralization antibodies targeted to genes associated with cancer is a good strategy to detect cancer at early stages among many treatment approaches because of its high specificity and affinity. KalGene is developing a multi-protein assay to identify aggressive epithelial cancers, including breast, colon, and prostate, thyroid cancers. We have successfully developed several monoclonal antibodies against important biomarkers. Our monoclonal antibodies have shown excellent affinity and specific reactivity to the recombinant protein and cell lysates. Purified monoclonal antibodies were tested on tissue microarrays (TMA). Tissue microarray technology allows a massive acceleration of studies correlating molecular in situ bindings with clinico-pathological information. In this method, minute tissue cylinders (diameter 0.6 mm) are removed from hundreds of different primary tumour blocks and subsequently brought into one empty ‘recipient’ paraffin block. Sections from TMA blocks can be used for all different types of in situ tissue analyses including immunohistochemistry. TMA sections were de-paraffinized and heat-mediated antigen retrieval was performed. Non-specific immunoglobulin binding was blocked with 10% normal horse serum (NHS) for 30 min at room temperature. The TMA sections were then incubated with the appropriate diluted Kalgene monoclonal antibody in NHS. Thereafter, the slides were incubated with the appropriate secondary antibody diluted in NHS. Signal from the secondary antibody can be detected, then visualized. **Results and conclusions:** The membranous KAL001 staining was calculated as a weighted average, based on results from three core samples per tumour. KAL001 expression levels were positively associated with well (versus poorly) differentiated tumours (n = 18; p = 0.05), low preoperative serum carcinoembryonic antigen (n = 76; p = 0.0002), and 5 year survival (n = 128; p = 0.01). The presence of perineural invasion and macroperforation were associated with lower KAL001 staining scores, but small sample numbers precluded statistical analysis of these results. Our study findings demonstrate that reduced KAL001

**References**


**O17**

**Pre-clinical evaluation of novel anti-tuberculosis molecules**

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BMC Proceedings 2014, 8(Suppl 4):O17

Tuberculosis (TB) currently represents a major global health concern, especially when considering the emergence of multidrug-resistant strains of Mycobacterium tuberculosis. On this regard, the identification of new effective agents to treat this infectious disease is urgently needed. Herein, we describe the pre-clinical assays on the anti-tubercular effects of a pentacyano(isoniazid)ferrate(II) compound, namely IQG-607. Previous experimental evidence [1] clearly demonstrated the in vivo effectiveness of this compound in a mouse model of TB infection. Accordingly, IQG-607 (22 to and 560 μmol/kg) was able to markedly reduce the number of colony-forming units (CFU) in both spleen and lungs of M. tuberculosis H37Rv-infected animals, following long-term schemes of oral administration, during 28 or 56 days. In this series of experiments, the effects of IQG-607 were comparable to those observed for the reference compound isoniazid (182 μmol/kg). More recently, we demonstrated that in vitro incubation of IQG-607 led to a marked reduction of CFU counts in M. tuberculosis H37Rv-infected macrophages, being this effect similar to that displayed by either isoniazid or rifampicin [2]. Concerning the mechanism of action, IQG-607 was found able to block the biosynthesis of mycolic acids, as indicated by radiolabelling studies with acetate incorporation [2], and it displayed bactericidal effects when tested in vivo [1]. The acute oral administration of IQG-607 (560 to 1120 μmol/kg) was not related to any clinical sign of toxicity, whereas the treatment with isoniazid (182 μmol/kg) led to death rates of 80% in mice [3]. The same doses of IQG-607 were also devoid of toxicity, when administered acutely by oral route to rats (unpublished data). To extend evidence on the safety profile of IQG-607, this molecule was also examined in a protocol of repeated dose 90-day oral toxicity in rats. As a main conclusion, there was no evidence of severe toxic signals, although it was not possible to accurately define the NOAEL (non-toxic adverse effect level) for IQG-607. Nevertheless, the dose of 56 μmol/kg might be assumed, once the animals treated with this dose did not show any signal of toxicity (unpublished data). Taken together, our results provide convincing evidence on the efficacy of IQG-607. It can be assumed that IQG-607 is well absorbed when dosed by oral route, being able to reach the bacilli and killing them within the phagosome of the macrophages. Furthermore, we demonstrated a favorable toxicological outcome for this compound, even when dosed acutely, or in long-term schedules of administration, for both mice and rats. Further studies are required to determine the safety of IQG-607 in non-rodent species, as well as the efficacy of this molecule against resistant strains of M. tuberculosis. Although additional experiments with this molecule are still required, it is tempting to infer that IQG-607 might well represent a lead compound for development of innovative anti-TB drugs.

**References**


**O18**

**High content screening of induced pluripotent stem cells as a model to study human brain diseases.**

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BMC Proceedings 2014, 8(Suppl 4):O18

Induced pluripotent stem (iPS) cells differentiated into neural progenitor cells (NPCs) hold great potential as a tool for modeling brain disease. When coupled to high content screening (HCS), NPCs may become a powerful tool for drug discovery. One of the goals of the new Molecular Biology and Cell Reprogramming, headed by Dr. Stevens Rehen at the D’Or Institute for Research and Education, is to develop a high content screening (HCS) strategy to study human brain diseases through the use of iPS. As a starting point, the effects of psychoactive drugs were characterized in NPCs by using HCS. Thousands of cells and single mitochondria were analyzed individually by HCS software and submitted to several sequences of morphometric analysis and fluorescence quantification. Morphological and functional alterations in mitochondria, which can be linked to energetic metabolism...
failure, a key trigger to abnormal neuronal development, were also observed. I will discuss the impact of HCS technology applied to neural stem cells as an attractive platform to drug discovery, cytotoxicity assessment and disease modelling.

Supported by: BNDES, CNPq, FINEP, CAPES and FAPERJ.

O19

Stem cells and degenerative diseases
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BMC Proceedings 2014, 8(Suppl 4):C19

Cell therapies utilizing stem cells hold a great promise for the treatment of chronic-degenerative diseases. For more than a decade now, pre-clinical and clinical studies have been using bone marrow derived cells for therapy of diverse degenerative diseases. Cardiologists have arguably performed the largest number of clinical trials and we will concentrate our presentation on the use of various cell therapies in heart diseases. The first wave of clinical trials using bone marrow derived mononuclear cells is close to an end and results are rather disappointing. Most controlled studies, using multicenter, double-blinded and placebo-controlled designs have not shown heart function improvement based on measured ejection fraction - EF - as a surrogate endpoint. This has been observed for acute and chronic myocardial infarction [1-3]. Chagasic cardiomyopathy [4] and more recently for dilated cardiomyopathy [unpublished results]. Clinical trials using mesenchymal stem cells, derived either from bone marrow or adipose tissue are underway [5]. These cells have been proved safe but efficacy trials are still underway. Similarly, trials using cardiac derived stem/progenitor cells have been initiated with either c-kit positive [6] or cardiosphere derived cells [7]. Safety trials have been concluded and point to significant reduction in scar area in infarcted patients. Efficacy trials are currently starting. Pluripotent cells, either embryonic stem cells (ESC) or induced pluripotent stem cells (iPSC), have been tested in pre-clinical models of acute and chronic myocardial infarction with reported success [8], although permanent engraftment of these cells has been questioned. Finally, direct differentiation of fibroblasts into cardiomyocytes, using transcription factors, has been accomplished both in vitro and in vivo [9,10], opening new therapeutic avenues for handling the epidemic of heart failure that has followed improved survival after myocardial infarction.

References

O20

Nitrogen fixation in grasses - gluconacetobacter activates genes in sugarcane
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BMC Proceedings 2014, 8(Suppl 4):C20

Nitrogen-fixing bacteria have been isolated from sugarcane (Saccharum spp.) and other grasses in an endophytic and beneficial interaction that promotes plant growth. In this interaction, bacteria colonize the intercellular spaces and vascular tissues of most plant organs without causing disease symptoms. The best characterized sugarcane endophytic diazotrophs are Gluconacetobacter diazotrophicus, clustered in the alpha subclass of Proteobacteria, and Herbaspirillum seropedicae, Herbaspirillum rubrisubalbicans and Burkholderia sp, clustered in the beta subclass of the Proteobacteria (Reis et al. 2000). These particular types of endophytic plant growth promoting bacteria (PGPB) can offer several benefits to host plants such as to provide nitrogen trough Biological Nitrogen Fixation (BNF) and to produce plant growth hormones (e.g. auxins and gibberellins), promoting plant development, increase in biomass, defense against pathogens and tolerance to abiotic stresses [1,2]. In Brazil, BNF plays a fundamental role in sugarcane cultivation by reduction of the use of nitrogen fertilizers, making Brazilian sugarcane culture more competitive in global markets. It has been suggested that the relatively low mineral N inputs used for Brazilian sugarcane production over the last 100 years has historically selected for varieties with a low response to applied mineral N and a high N2-fixing ability [3]. Studies on the quantification of BNF to the Brazilian sugarcane varieties, using 15N isotope dilution and 15N natural abundance, have demonstrated that the amount of fixed N2 can be highly variable. Very large BNF-inputs were observed in several sugarcane varieties, especially the wild noncommercial species Katakau (Saccharum spontaneum) used in plant breeding in Brazil, as well as the commercial varieties SP 70-1143 and CB 45-3, that exhibit high yields in low-fertility soils. SP70-1143 could obtain 72% of N-requirement from BNF, while Chunee (Saccharum barberi), a wild noncommercial species, obtained only 14% [3,4]. An important biotechnological challenge of this century is to develop tools to apply for a sustainable agriculture, that would increase productivity using less fertilizers, pesticides, water and cultivated area. The associations that occur between sugarcane and other grasses with nitrogen-fixing endophytic bacteria have raised a large interest in their use in agriculture, in view of the positive effects on root development, and the increase in biomass and productivity. Experiments carried out at EMBRAPA Agrobiologia have shown significant results in biomass increase and grain yield due the use of diazotrophic endophytic bacteria inoculation in sugarcane, maize and rice inoculants. However, studies have also shown that the plant genotype and the environment where the association is established can influence the degree of beneficial results obtained by the plant caused by the association with endophytic bacteria.

This plant / nitrogen-fixing endophytic bacteria interaction represents a novel system of beneficial plant-microorganism association, which has unique features that remain to be characterized. The studies on BNF quantification have indicated that the selection of the best combination of endophytic diazotroph strains as well as sugarcane varieties needs to be exploited to obtain the maximum benefit from this association in agriculture. The signaling pathways by which sugarcane plants can decipher
bacterial signals and respond properly for a successful association, by controlling endophyte recognition, colonization and nitrogen fixation rates, are still not clearly understood. Therefore, the major goal of our research is to develop biotechnological tools to help to obtain novel plant varieties more responsive to bacteria inoculants. The studies are based on the identification and use of plant genes that are markers and/or functionally important for the establishment of an efficient association with the endophytic diazotrophic bacteria, using genomic approaches.

We first addressed if plants were actively responding to bacteria association, mainly by studies of gene expression profiling of sugarcane plants inoculated with \textit{C. diazotrophicus} or \textit{H. rubrisulburicans}. Database analysis of the sugarcane EST sequencing project (SUCEST) revealed ETS expression and preferentially expressed in inoculated plants, which were functionally classified as members of several cellular and metabolic pathways [5,6]. Expression profiling analyses of sugarcane genes responsive to the endophytic diazotrophic bacteria revealed a high percentage of monotoc specific genes, suggesting that the plant may have evolved special molecular mechanisms to recognize and establish efficient association with the bacteria [6]. Furthermore, several genes differentially expressed in inoculated plants do not match with any sequence of non-redundant GenBank database. These findings point the association between sugarcane and endophytic diazotrophic bacteria as a model with great potential for the discovery of new genes. We also data indicated that sugarcane plant does not behave simply as a silent host for the growth of these bacteria and actively participates in the association with endophytic diazotrophic bacteria [5,6].

Another mechanism to be unraveled is how an endophytic and non-pathogenic type of association is established. First, sugarcane might recognize the bacteria, activating an intricate signaling network to manage plant defense responses. In a successful association, plants must allow bacteria colonization and, on the other side, should control bacteria numbers to avoid pathogenicity. We identified several members of one important class of receptor protein, the RLKs (Receptor Like Kinase), as candidates to be involved in sugarcane-nitrogen fixing bacteria association [5-8]. The data indicates that signaling pathways dependent on receptor proteins might represent an important mechanism for sugarcane recognition of endophytic diazotrophic bacteria. Also, our data suggests that the establishment of a beneficial and endophytic association would depend on the proper balance between defense responses that are induced and repressed. Ethylene is a phytohormone that often acts as a signal in pathogen defense as well as in plant development. Sugarcane EST database analysis indicated that ethylene signaling pathway is responsive to the association with diazotrophic endophytes [5,6]. Remarkably, gene expression analysis showed that some members of ethylene response pathway are repressed while others are activated after inoculation. The same genes had an opposite expression profile in response to pathogens, and were differentially regulated in sugarcane contrasting BNF genotypes [9]. Altogether, our results suggest that specific components of ethylene signaling pathway may identify a beneficial endophytic association, modulating plant response to diazotrophic endophytes.

An important question to be addressed is which mechanisms are involved in promotion of sugarcane growth during the association. Our data on sugarcane EST expression analysis showed that several members of sugarcane nitrogen assimilation apparatus and nitrogen metabolism are activated in plants colonized by diazotrophic endophytes [5]. Gene expression studies on Glutamine synthetase (GS) genes in sugarcane suggested that it could be important for the association with the endophytic diazotrophic bacteria [5]. We found that some GS genes are differentially expressed between the contrasting BNF genotypes, and this may explain, at least in part, the capacity of these sugarcane genotypes to grow in low nitrogen addition [10]. We also investigated the relation of phytohormone signaling elements with the promotion of sugarcane development by the diazotrophic bacteria. Several members of phytohormone biosynthesis, transport and response pathways, including auxin and gibberellin, were preferential or exclusively expressed in inoculated plants [5,6]. Also, several genes involved in plant growth and development that are regulated by hormones were also preferentially expressed in sugarcane inoculated with the endophytic diazotrophs, such as specific genes that control cell division and expansion [5]. It indicates that some basic mechanisms controlling plant growth and development might participate in this association.

This first gene profiling analyses identified several differentially expressed sugarcane genes during early stages of an efficient association between a high BNF sugarcane genotype and the diazotrophic bacteria \textit{G. diazotrophicus} and \textit{H. rubrisulburicans}. Nevertheless, the comprehension of how various sugarcane regulatory mechanisms are coordinated and connected to genotype and environmental signals, in order to control the establishment of a beneficial and endophytic type of association is still a big challenge. Currently, we are applying next generation sequencing technologies to compare expression profile in two sugarcane BNF contrasting genotypes and in response to soil conditions, such as nitrogen sources and water deficit. An integrated differential transcriptome was generated and it provided an overview of sugarcane metabolism, growth and development controlled by nitrogen, water and endophytic nitrogen-fixing bacteria during a successful association. All together, the data suggests that plant genotype, nitrogen and water soil conditions control regulatory networks that are important during the establishment of the beneficial association.

Our molecular analysis of the association is revealing sugarcane genetic controls involved in plant response to the endophytic diazotroph colonization. We propose that several levels of regulation might be operating in sugarcane during the association with endophytic diazotrophic bacteria. Signaling molecules may be involved in plant and bacterium recognition, triggering plant signaling pathways that, depending on both plant and bacterium genotypes will differentially regulate downstream mechanisms to allow or not bacteria colonization. To ensure an endophytic type of association, the plant must maintain a stringent control over the processes of invasion and proliferation of bacteria. To establish a beneficial association, several pathways controlling plant growth and development might be integrated with the plant response to the endophytic bacteria. The expression profiling analysis of sugarcane genes responsive to the endophytic diazotrophic bacteria allowed the identification of several pathways that might be involved in the association. Remarkably, several of them are reported to have a role both on plant defense and development, supporting a general coordination of both processes in order to control the establishment of a beneficial and endophytic type of association. Finally, the various sugarcane regulatory mechanisms might be coordinated and connected to plant genotype and environmental signals, in order to control the success of the association. We are currently searching for plant expression markers of genotypes and environmental conditions that would represent efficient associations. Further experiments are necessary to determine the function of these genes in sugarcane in order to identify pathways that might be crucial for the establishment of a successful association.

Acknowledgements: This work was supported by INCT, CNPq, FAPERJ & CAPES.

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O21
Ethanol from Sorghum
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BMC Proceedings 2014, 8(Suppl 4):O21

Ceres Sementes do Brasil, Ltda is an affiliate of Ceres, Inc founded in 1996 with headquarters in Thousand Oaks, California, USA, focused on development and marketing of non-food grasses for advanced biofuels and biopower with the aim of providing new opportunities for growers and a cleaner environment for us all. In 2007, Ceres founded its first Brazilian affiliate in São Paulo and initiated research in Brazil aiming to prepare the Brazilian market for a commercialization of sweet sorghum and high biomass sorghum hybrids developed for production of biofuel and bioenergy. Ceres works directly with leading mills and suppliers of biofuel technologies to facilitate the introduction of sweet sorghum into existing operations and infrastructure and also carry out tests with leader institutions and innovative producers. Ceres current products are commercialized under the brand Blade® and plans to introduce a number of high-yielding sorghum hybrids with high biomass and high sugar content to complement or expand feedstock supplies in sugarcane-growing regions. In addition to a conventional breeding program, Ceres is developing genetically modified seeds, improved for productivity and agronomic characteristics. We hold a wide collection of plant genes that we plan to introduce in our sorghum breeding program in Brazil.

The presentation will cover some considerations about the opportunities for sweet sorghum as a complementary alternative to sugarcane for production of ethanol, electric energy and, in the future, sugar production. It will also discuss some perspectives related to high biomass sorghum as a current alternative to complement biomass production before the sugarcane crop and potential markets in pellet production, heat/vapor and second generation ethanol. The challenges to the adoption of these alternative crops and to the development of new conventional as well as genetically modified hybrids will be addressed.

O22
Downstream events in the NIK-mediated defense associated with resistance to begomovirus
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BMC Proceedings 2014, 8(Suppl 4):O22

The NSP-interacting kinase (NIK) receptor-mediated antiviral signaling has been identified as a virulence target of the begomovirus nuclear shuttle protein (NSP) [1,2]. NSP suppresses the activity of the NIK receptor through specific binding to the kinase domain and hence enhances begomovirus pathogenicity [1,3]. NIK receptors belong to the plant defense group of the leucine-rich repeat (LRR) receptor-like kinase (RLK) subfamily, designated LRR-RLK II. This subfamily of RLKs from tomato and Arabidopsis is constituted by 14 proteins harboring four complete LRRs (with 24 residues) and a fifth inconsistent repeat (with 27 residues) arranged in a single continuous block within the extracellular domain [4]. Based on sequence conservation and structural features, the members of the LRR-RLK II subfamily are clustered into three distinct branches: (i) antiviral defense proteins (ii) developmental and defense proteins, such as the somatic embryogenesis receptor-like kinases (SERK-like) including SERK1 and SERK3/BAK1 and (iii) functionally unassigned proteins. The Arabidopsis NSP-interacting kinase 1, NIK1 (At5g16000), NIK2 (At3g25560) and NIK3 (At1g60800) are in the defense group I of the LRR-RLK II subfamily and are virulence targets of the bipartite geminivirus nuclear shuttle protein, NSP [1,4]. NSP from CaLCuV (Cabbage leaf curl virus interacts with all three NIKs from Arabidopsis to suppress their kinase activity [4]. The NSP-NIK interaction is also conserved among geminivirus NSPs and NIK homologs from different hosts [2]. Tomato and soybean NIK homologs also interact stably with NSP from CaLCuV [2] and with NSPs from the tomato-infecting geminiviruses TGMV (Tomato golden mosaic virus), TCyLV (Tomato crinkle leaf yellow virus) and ToYSV (Tomato yellow spot virus) [1,2]. Several lines of evidence indicate that NIK functions in defense. NSP from CaLCuV acts as a virulence factor to suppress the kinase activity of transmembrane receptor NIKs [1]. Second, loss of NIK1, NIK2 or NIK3 function in Arabidopsis is also linked to an enhanced susceptibility phenotype to CaLCuV infection [1,3]. In addition, overexpression of NIK1 from Arabidopsis in tomato plants attenuates symptom development and delays ToYSV infection [5]. NIK exhibits trans-autophosphorylation activity in vitro and substrate phosphorylation activity in vivo and in vitro, and interacts with the ribosomal proteins L10 (rpl10) and L18 (rpl18) [5]. NIK-mediated phosphorylation of rpl10 promotes translocation of the ribosomal protein to the nucleus where it may function to mount a defense response that negatively impacts virus infection. This is consistent with the notion that the regulated nuclearcytoplasmic shuffling of rpl10 links the antiviral response to receptor activation. We found that NIK1 undergoes a stepwise pattern of phosphorylation within its activation-loop domain (A-loop) with distinct roles for different threonine residues [6]. The conserved Thr-474 and Thr-469 were phosphorylated in vitro and mutations at Thr-474 impaired autophosphorylation and were defective for kinase activation in vitro and in vivo. In contrast, a mutation at Thr-469 did not impact autophosphorylation and increased substrate phosphorylation, suggesting an inhibitory role for Thr-469 in kinase function. Our results establish that NIK1 functions as an authentic defense receptor as it requires activation to elicit a defense response [6,7]. Our data also suggest a model whereby phosphorylation-dependent activation of a plant receptor-like kinase enables the A-loop to control differentially auto- and substrate phosphorylation.

To identify novel regulators of NIK-mediated defense response, we screened a two-hybrid library for partners of rpl10. We discovered a novel transcriptional factor, which interacts with rpl10 in the nucleus of plant cells to down-regulate the expression of ribosomal genes. These data are consistent with the observation that constitutive activation of the NIK receptor by replacing Thr-474 with aspartate impairs global translation in Arabidopsis and tomato transgenic lines and confers broad-spectrum tolerance to begomovirus infection. Our data also indicate that the NIK immune receptor-mediated antiviral signaling operates through a bipartite module. One defense signal-transducing branch is mediated by the regulated nuclearcytoplasmic transport of ribosomal protein to impair translation and the second branch of the antiviral signaling transduces a typical defense signal through induction of the plant immune system. We will discuss novel insights into the regulation of the NIK-mediated antiviral signaling and an efficient-acquired defense against begomovirus by modulating the activity of the immune defense receptor NIK in tomato plants.

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Nitrogen-fixing, plant-growth promoting bacteria are arguably the biotechnological tool of highest potential to improve agricultural productivity in short term. Nitrogen fixation and phytohormone production by these bacteria have been considered the most important factors for plant growth promotion. However, the underlying mechanisms responsible for productivity increases by associative bacteria are not clear. Moreover, the intensity of the plant growth promotion, including transfer of the fixed nitrogen from the bacteria to the plant, depends on an efficient interaction of the plant genotype and bacterial species. Azospirillum spp. are nitrogen-fixing, plant growth promoting bacteria that can associate with several cereals such as maize, rice and wheat, and also biofuel crops such as sugar cane and Pennisetum. Azospirillum brasilense is one of the most used plant growth promoting bacteria (PGPB), being used in Brazil, Argentina, Mexico, India and Europe. Analyses of field experiments have shown a success rate of inoculation with Azospirillum ranging from 60 to 70%, with statistically significant increases in yield varying from 5 to 30% [1]. The regulation of nitrogen metabolism in A. brasilense has been extensively studied. Here we will review nitrogen fixation regulation in this bacterium and advances in the understanding of aspects of interaction with cereal plants obtained by transcriptomic analyses.

Regulation of nitrogen fixation in A. brasilense: The transcription initiation at nif promoters is dependent on the NifA protein. The function of NifA can be regulated at two levels, nif gene transcription and NifA protein activity, and at regulation both levels occurs in response to oxygen and/or fixed nitrogen. Evidence of a nifA-like gene A. brasilense was first obtained by Pedrosa and Yates (1984) by the isolation and characterization of a nifA mutant strain (FP10) [2]. The expression of the nifA gene in A. brasilense is only partially repressed by ammonium and oxygen, being maximal under nitrogen fixation conditions. In the absence of ammonium, oxygen levels have no effect on its expression, but it is partially repressed by ammonium under limiting oxygen conditions and almost fully repressed by high ammonium and high oxygen conditions. Analysis of the nifA promoter region showed two sequences resembling the Fnr consensus sequence located downstream from the probable transcription start, but their functionality has not been proved. Regulation of nifA expression seems similar to that of Sinorhizobium meliloti in respect to oxygen repression and Azorhizobium caulinodans in respect to ammonium control of promoter activity. Indeed, there is evidence supporting the involvement of the two-component system FixJ-FixL and the FNR-like protein FixK in A. brasilense. The activity of A. brasilense NifA protein is controlled by oxygen and ammonium concentrations. Oxygen-sensitivity of NifA is probably related to a linker region between the central and carboxy terminal domains containing a conserved Cys-X4-Cys, which together with 2 cysteine residues found in the central domain, form the cysteine motif Cys-X11-Cys-X14-Cys-X4-Cys potentially involved in binding an oxygen sensitive FeS cluster. Mutagenesis analysis showed that the cysteine motif is essential for the B. japonicum and R. leguminosarum NifA activity in vivo.[3].

A functional glnB gene is required for A. brasilense NifA activity. Since an N-terminal truncated NifA protein is active independently of the GlnB and is insensitive to the ammonium levels, regulation of NifA activity by GlnB probably involves its N-terminal domain. Furthermore, A. brasilense NifA is inactive in E. coli, but expression of A. brasilense GlnB is capable to activate NifA in this background. Moreover, purified GlnB and the N-terminal GAF domain are capable to interact in vitro. The nitrogenase activity of A. brasilense is reversibly regulated by ADP ribosylation of the dinorogen reductase (NifH) in response to increase in ammonium concentration or energy depletion. This process is called nitrogenase switch-off and is catalyzed by dinorogen reductase ADP-ribosyltransferase (DraT). Upon consumption of added ammonium or restoration of energy levels, the ADP-ribose group is removed from NifH by the dinorogen reductase activating glycohydrolase (DraG) leading to nitrogenase reactivation (nitrogenase switch-on). The two enzymes are oppositely regulated in response to the stimulus: under nitrogen fixing conditions DraG is active and DraT is inactive, while addition of ammonium leads to activation of DraT and inactivation of DraG. A model for the ammonium-dependent control of DraT and DraG activities in A. brasilense has been proposed [3]. Under nitrogen fixing conditions the PI proteins GlnB and GlnZ are upregulated and soluble in the cytosol. Under this condition DraG is active and DraT is inactive, therefore NifH is not modified and active. As ammonium levels increase, the PI proteins are deuridylylated by the uridylyl-removing enzyme GlnD. Deuridylylated GlnB stimulates the transferase activity of DraT triggering ADP-ribosylation of NifH with the consequent “switch-off” of nitrogenase activity. Deuridylylation of GlnZ causes the formation of DraG/GlnZ complex, which associates with the membrane protein AmtB, thereby removing DraG from the cytoplasm and inhibiting its ADP-ribosyl glycohydrolase activity [3].

This model can explain ammonium-induced switch-off of nitrogenase but the anaerobic induced NIFH modification in A. brasilense is not dependent on GlnB, GlnZ or AmtB and must therefore occur by an independent mechanism.

Transcriptomic analyses of wheat roots colonized by A. brasilense: A. brasilense is one of the most promising plant growth-promoting bacteria (PGPB). Wheat roots colonized by A. brasilense is a good model to investigate plant-PGPB interaction including improvement in plant growth. To comprehend the molecular basis of plant-A. brasilense interaction a RNA-Seq transcriptional profiling of wheat roots colonized by A. brasilense was carried out [4]. CDNA libraries from inoculated and non-inoculated wheat roots were sequenced and mapped to wheat EST database and A. brasilense genome. The unmapped reads were assembled de novo. A total of 23,215 expressed sequences from wheat were identified and 702 gene transcripts of A. brasilense. Bacterial colonization led to differential expression of 776 wheat ESTs belonging to various functional categories, including genes involved in transport activity, biological regulation, defense mechanism, production of phytohormones and phytochemicals. In addition, A. brasilense genes encoding proteins related to bacterial chemotaxis, biofilm formation and nitrogen fixation were highly expressed in colonized wheat roots. Interestingly, several ESTs encoding regulatory proteins of the wheat cell cycle were regulated by A. brasilense, which was consistent with a higher proportion of colonized root cells in the S-phase. The results reinforce that PGPB-inoculation might be an important alternative to improve nutrient acquisition, including nitrogen, and productivity in important crops such as wheat.

Concluding remarks: The use of plant-growth promoting bacteria to improve productivity of important cereal crops such as maize and wheat is a reality. Research in this area has enhanced our understanding on the bacterial physiology and the mechanisms of bacteria-plant interaction. Among these bacteria, A. brasilense is one of most promising. Field experiments have shown consistent increase in productivity. Furthermore, Azospirillum is amenable to genetic studies and manipulation, and much is known about its nitrogen metabolism and regulation of nitrogen fixation. Finally, advances in omics sciences have allowed recent progress in the understanding of molecular aspects of plant-Azospirillum interaction. The knowledge accumulated on this system may allow future genetic manipulation to maximize the benefits of plant-growth promoting bacteria.

Acknowledgements: This work was supported by the Brazilian Program of National Institutes of Science and Technology-INCT/Brazilian Research Council-CNPq/MCT, CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), Fundação Araucária, and CAPES (Cooperação de Aperfeiçoamento de Pessoal de Nível Superior).

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RNA silencing is a biochemical mechanism that regulates gene expression by post-transcriptionally activating a sequence-specific RNA degradation by three different pathways: (i) small interfering RNA (siRNA) silencing of exogenous mRNAs; (ii) micro RNA (miRNA) silencing of endogenous mRNAs, and (iii) associated with DNA methylation and suppression of transcription [2]. These processes share three basic biochemical phases: (i) formation of double-stranded RNA (dsRNA); (ii) processing of dsRNA into small dsRNA molecules (snRNAs); and (iii) targeting of single-stranded small RNA to sequence-specific DNA or RNA molecules. Although several mechanisms can generate dsRNA, the sRNA processing and effector phases have a common biochemical core. One key-component of this system is small RNA molecules of 21 to 26 nt called siRNAs (small interfering RNAs) which originated from longer double-stranded RNA (dsRNAs) cleaved by a specific RNase endonuclease called DICER with distinctive dsRNA binding, RNA helicase, RNase III and PZI (Prial/Argonaute/Zip/krille) domains. The siRNAs strands are further relaxed (unwound) and one strand is incorporated in a RISC complex (RNA-induced silencing complex) which contains a member of the Argonaute (Ago) protein family, guiding this complex to an mRNA with a complementary sequence, which is then cleaved, leading to gene silencing. siRNAi can be triggered by double-stranded or partially self-complementary hairpin RNA formation. In addition to playing a powerful role in creating loss of function mutations in plants, siRNAi biological functions include also the regulation of endogenous gene expressions, such as micro RNA (miRNA), heterochromatin formation, transposon repression and defence against viral infection [2,3]. Several strategies have been employed for genetically engineering resistance to viruses in transgenic plants, including the expression of coat protein genes, the expression of truncated defective genes and antisense RNA. Now that we better understand the mechanisms of RNA silencing (RNAi) and its biological functions, it is possible to look back on initial experiments from a new perspective. It is now known that plants naturally process viral RNA to generate small sequences of a pathogen’s genetic material that can be specifically used against that pathogen through the RNA-induced silencing complex. It was recognized that an RNAi-silencing (post-transcriptional gene silencing or PTGS) mechanism was responsible for the resistance against RNA viruses, and that it depends on the formation of double-strand RNA (dsRNA) whose antisense strand is complementary to the transcript of a targeted gene. This discovery led to the introduction in transgenic plants of constructs to produce intracellular generation of siRNA-like species to induce targeted gene silencing and virus resistance. RNAi silencing has been an important tool to generate plants resistant to a large range of viruses. RNA sense- or antisense-mediated strategies resulted in a maximum resistance frequency of 20%, but often far lower frequencies were obtained. In addition, not all viral genes used in transgenic constructs rendered resistant plants. The use of inverted repeat constructs, resulting in dsRNA transcripts, rendered a very efficient system in which much higher frequency of transformed lines will display efficient gene knockdown or virus resistance. The probable reason is that the dsRNAs fed directly into the silencing pathway at the level of the RNase III-like enzyme Dicer, and therefore there is no reliance on the action of plant-encoded RNA-dependent RNA polymerase proteins. So far, most examples of RNAi-mediated virus resistance are related to RNA plant viruses.

Golden mosaic, caused by the Bean golden mosaic virus (BGMV), transmitted by the whitefly Bemisia tabaci Gen., is one of the most limiting diseases for bean cultivation in the American continent. Since highly resistant bean varieties are unavailable, control practices have focused on controlling the vector with high-toxicity insecticides, with cost-benefit ratio and environmental concerns. In Brazil, the golden mosaic causes annual losses in bean production ranging from 90,000 to 280,000 tons. In addition, there are approximately 180,000 hectares unsuitable for bean cropping in the dry season due to the high prevalence of BGMV. The concept of using RNA constructs was explored to generate genetically modified (GM) bean lines resistant to the BGMV by silencing the rep viral gene, which encodes the only protein strictly essential for viral genome replication [4]. Two lines (named 2.3 and 5.1) showed immunity to the BGMV upon inoculation at high pressure (>300 viruliferous whiteflies per plant during its entire life cycle). Under field conditions, no virus infection has been observed in transgenic genotypes from 2007 to 2013, even under high B. tabaci pressure (Aragão and Faria, 2009; personal communication). In contrast, non-transgenic varieties have showed up to 100% infection, presenting characteristic severe symptoms of the golden mosaic disease. In 2011, a transgenic bean event (named EMB-PV051-1; Embrapa 5.1) resistant to the BGMV was approved for cultivation and consumption in Brazil. Biosafety evaluations were carried out taking into account the proposal protocol by the Brazilian Biosafety Committee (CTNBio) to demonstrate the safety of transgenic bean event to the environment and human health. Analyses have suggested no differences between the transgenic event and its non-transgenic counterpart.

References


Monsanto: partnering for sustainable agriculture
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Monsanto is driven by a global commitment to sustainable agricultural production. This commitment includes bringing technologies to farmers that improve their lives by helping them be more productive while using fewer resources—leading to a reduced overall effect on the environment. By using this commitment to drive our actions, we believe we are creating a positive impact on our world.

But, we cannot do this alone. That’s why, in addition to developing our own products, we also partner and collaborate with others to develop technologies for farmers. We also license our biotech traits and germplasm in our products, we also partner and collaborate with others to develop technologies in the brands they choose.

External technology collaborations are a key to our promise of delivering innovative new products in the future. At Monsanto, we are focused on developing collaborative alliances that produce lasting benefits for our farmer customers and all involved [1].

Reference


Bt technology - the way forward
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Bacillus thuringiensis (Bt) is a Gram-positive soil bacterium, has been isolated from a range of habitats including soil, grain dust, phylopilae and dead insects. It is characterized by its ability to produce crystalline inclusions called “endotoxin proteins” during sporulation. The crystalline inclusions (Cry and Cyt proteins), along with the spores, have a great potential to control a
number of insect pests belonging to the order Lepidoptera, Diptera, Coleoptera, Hemiptera and Hymenoptera. Besides Bt may also control nematodes. Therefore, they represent a valuable tool for Integrated Pest Management (IPM). Bt is one of the most important microbial biopesticides produced in the world, accounting for 1-2% of the global insecticide market and is responsible for over 90% of all biopesticide sales. The widespread occurrence of this bacterium and interest in the use of Bt products as an alternative to chemical insecticides stimulated the isolation of native Bt strains in many parts of the world. The characterization may also be used to predict insecticidal activity, determine ecological distribution, and identify new cry genes. Besides being an important component of studying Bt resources, the characterization of Cry proteins and its genotypic composition may help understanding its insecticidal activity. Bt also produces other important toxins such as: phospholipases, proteases, quinases, α exotoxins, β exotoxins, VIP (Vegetative Insecticidal Proteins), and δ endotoxins (crystalline inclusions). Bt harbors more than 72 classes of Cry protein, the most common proteins used in transgenic plants. Table 1 below shows all Bt genes present today in transgenic plants in Brazil. Abroad proteins Cry3A and 35 have been used in transgenic crops. Despite the great number of Cry proteins described and more than 600 genes from cry1Aa1 to cry72, not many different proteins have been cloned in crops nowadays. There are many different perspectives in Bt technology in Brazil besides the use in programs of transgenic crops. The demand for the use of Bt based biopesticides has increased in many Brazilian regions. Some important factors have contributed as the introduction of the new pest Helicoverpa armigera that has attacked crops such as maize, cotton, soybean, millet, sorghum, beans, green peppers, tomato, okra etc. Another important factor is because of “safinha”. Due to a favorable climate, crops are planted all over the year, and the farmers plant a second crop of corn or cotton called the safinha. This new scheme of crop rotation is to first plant a crop of rain-fed such as rice, soybean or maize, and then after these crops are harvested, plant a second crop of soybean, sorghum or even maize. Safinha can also be defined as a farming strategy whereby the farmer takes advantage of a long tropical growing season to produce two crops in a single growing season, thereby maximizing revenue per acre. So, it is paramount to continue the research on Bt to find new and more efficient genes, and produce Bt based biopesticides feasible and cheap to the market.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Bt genes</th>
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<tbody>
<tr>
<td>Corn</td>
<td>Cy2Ab,1Ab,1A,1A05 (1Ab, 1Ac, 1F), Cy3Ab, 1F, VIP3Aa</td>
</tr>
<tr>
<td>Cotton</td>
<td>Cy2Ab,1Ab2Ae, Cy1Ac, 1F</td>
</tr>
<tr>
<td>Soybean</td>
<td>Cy1Ac</td>
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References


O28

The future of lab animal facilities in Brazil

Alexandre Viecili

O29

Transgenic livestock for agriculture and biomedical applications

Charles Long

The classical definition of a genetically engineered or “transgenic” animal would be “those animals modified by recombinant DNA (rDNA) techniques” according to the U.S. Food and Drug Administration (United States Food and Drug Administration, Guidance #187, 2009). Thus, the introduction or deletion of any gene by using recombinant DNA would fall into this category. Since the first reports of stable germline transmission of integrated DNA in 1982, hundreds of thousands of transgenic animals have been produced, the vast majority of which are mice used in biomedical research. However, there are also thousands of agrarian and aquaculture species that have been produced for a wide variety of purposes from production of human biopharmaceuticals, xenotransplantation and to enhance economically important production traits.

We can separate the transgenic animals produced in our laboratory into 3 major classes; production agriculture, biopharmaceutical production in milk and biomedical models of human disease. This brief review will highlight some of those projects in each category.

Transgenic livestock in production agriculture: Production agriculture projects in our laboratory have utilized RNA interference to enhance meat production through improved muscle growth. Myostatin is a negative regulator of muscle cell proliferation during fetal development. It is known that inactivating mutations in a number of species, including cattle, develop a muscle overgrowth phenotype [1]. Although the increased muscle can be considered a positive trait, the increase in dystocia related to the size of the calves at birth limit the utilization of these naturally occurring myostatin mutations in production agriculture. RNA interference has been utilized to overexpress a short hairpin RNA (shRNA) with homology to the myostatin mRNA in vivo. Direct injection into the perivitelline space of bovine zygotes with recombinant lentivarial vectors promoted the efficient integration of the shRNA expression constructs into the bovine genome. The expression and cellular processing of the myostatin shRNA led to degradation of the myostatin mRNA and an overt muscling phenotype in transgenic cattle [2]. Subsequent studies in pigs also show a significant increase in fetal pig weights compared to transgenic controls. The advantage of this approach is...
In collaboration with rEVO Biologics, Texas A&M has rederived a line of malaria vaccine antigen in the milk of goats to produce biopharmaceuticals without the hundreds of millions of dollars goats and cattle have all been utilized to produce proteins of biomedical interest, the mammary gland can be made to produce nearly any protein both animals and humans through genetic engineering. By placing a mammary gland specific promoter in a position to express a protein of interest, the mammary gland can be made to produce nearly any protein desired [4]. Production of useful biopharmaceutical proteins in the milk of several species has been demonstrated. Species such as mice, rabbits, pigs, goats and cattle have all been utilized to produce proteins of biomedical value in milk. This is a low cost, high yield system that can be implemented to produce biopharmaceuticals without the hundreds of millions of dollars necessary to build cell culture or bacterial processing facilities. In partnership with rEVO Biologics our laboratory has reinitiated a project to produce a malaria vaccine antigen in the milk of goats. In collaboration with rEVO Biologics, Texas A&M has rederived a line of goats, originally produced through pronuclear microinjection, that produce a tagged malaria parasite protein exclusively in their milk. At the beginning of this project, scientists at rEVO inserted a modified portion of a malaria parasite protein (MSP1 42) into mice and collected the milk. MSP1 42 was purified and mixed with an adjuvant to create an immune response that could mitigate a malaria exposure/challenge [5]. This vaccine was tested in non-human primates and effectively protected the animals from a lethal dose of malaria parasite. Thus, it was clear that the MSP1 42 was capable of producing effective vaccine. To increase production, rEVO inserted RNAI encoding the same protein into the genome of a dairy goat and produced larvae that can be readily purified to produce vaccine.

Transgenic livestock for biopharmaceutical production: Mammals have the remarkable ability to produce large quantities of their mammary glands and secrete these proteins during lactation. This natural protein production "factory" can be utilized to produce proteins that benefit both animals and humans through genetic engineering. By placing a mammary gland specific promoter in a position to express a protein of interest, the mammary gland can be made to produce nearly any protein desired [4]. Production of useful biopharmaceutical proteins in the milk of several species has been demonstrated. Species such as mice, rabbits, pigs, goats and cattle have all been utilized to produce proteins of biomedical value in milk. This is a low cost, high yield system that can be implemented to produce biopharmaceuticals without the hundreds of millions of dollars necessary to build cell culture or bacterial processing facilities. In partnership with rEVO Biologics our laboratory has reinitiated a project to produce a malaria vaccine antigen in the milk of goats. In collaboration with rEVO Biologics, Texas A&M has rederived a line of goats, originally produced through pronuclear microinjection, that produce a tagged malaria parasite protein exclusively in their milk. At the beginning of this project, scientists at rEVO inserted a modified portion of a malaria parasite protein (MSP1 42) into mice and collected the milk. MSP1 42 was purified and mixed with an adjuvant to create an immune response that could mitigate a malaria exposure/challenge [5]. This vaccine was tested in non-human primates and effectively protected the animals from a lethal dose of malaria parasite. Thus, it was clear that the MSP1 42 was capable of producing effective vaccine. To increase production, rEVO inserted RNAI encoding the same protein into the genome of a dairy goat and produced larvae that can be readily purified to produce vaccine.

Transgenic livestock as biomedical models of human disease: In addition to the benefits livestock animals bring to the human population in terms of food and fiber, they also represent interesting models to study diseases important to both human and veterinary medicine. Although naturally occurring genetic mutations have been discovered that produce important models of human disease, the ability to specifically modulate gene expression in livestock represents a unique opportunity to study gene function in animals. Livestock, especially pigs, share a similar size and physiology to humans and are important subjects for study of potential disease therapies prior to undergoing costly clinical trials. Our lab has been using livestock as biomedical models for human obesity and metabolic syndrome, by inducible tissue specific expression of transgenes delivered by recombinant retroviral vectors. Retroviral vectors were utilized to produce the first transgenic mice. Although these initial experiments were successful, problems with germline transmission hindered further utilization for many years. Since those initial studies, a multitude of innovations have produced a new class of recombinant, replication incompetent lentiviruses that have overcome many of the initial limitations. These highly modified and updated vectors are rapidly being utilized in human gene therapy trials with good results. Thus, lentiviral vectors are recognized as a safe and effective means for transduction of a number of cell types including gametes and embryos. Microinjection of concentrated lentiviral particles into the perivitelline space of livestock oocytes or zygotes can produce genetically modified embryos at high rates [2,6]. In the context of transgenic livestock, lentiviral mediated delivery of transgenes represents a unique regulatory obstacle due the nature of the integrated construct. Instead, in the short term, utility as a vector for agricultural applications would appear limited at best. Nonetheless, many biomedical applications can utilize the ability of these vectors to package and deliver transgenes into oocytes or zygotes to efficiently produce genetically modified embryos for subsequent transfer and production of offspring. Our laboratory is currently developing new recombinant lentiviruses capable of tetracycline inducible, tissue specific expression of transgenes in livestock species. In contrast to the murine model, there are currently limited options for this type of transgene expression systems in swine or other livestock species. These constructs can be designed to overexpress proteins, suppress endogenous translation via RNAI or both. The goal of the current work is to target stearoyl Co-A desaturase (SCD-1), an enzyme that is a key regulatory element in lipid biosynthesis [7]. By altering expression of SCD-1 in a tissue and inducible way, we can control tissue steatosis. Muscle and hepatic steatosis is detrimental to human health, but is considered a valued production trait in cattle muscle (marbling). Thus, the animals produced in these studies will have utility as biomedical models, but also could be useful in livestock production systems. The further refinement of inducible, tissue specific methods of genetic engineering offer exceptional opportunities for production of biomedical models of human disease and livestock production.

Summary: As new genetic engineering technologies develop, the ability to effectively and efficiently engineer the genome of livestock species will improve. Recently the utilization of site specific nucleases (TALEN and ZFN) and RNA guided nucleases (CRISPR/Cas9) have revolutionized the field of genetic engineering [8,9]. Application of these techniques are underway in our laboratory as well as many others in order to impart unique traits to livestock. These traits will lead to improved production of food and fiber as well as produce animals capable of making biopharmaceuticals and nutraceuticals. We are now limited in the point in livestock genetic engineering where the technology is no longer the limiting factor. We are now only limited by our imaginations and the burdensome regulatory pathways for bringing these products to the world's population.

Acknowledgements: The work described here is funded in part by the National Institutes of Health, grants number: 5R24OD011188-03, 1R1HDO-59969-01A2, 1R2HDS5631-01A2, and 3R2HDS5631-0251.

References:
Diarrhea is one of the most significant issues for global health in children under the age of five. Close to 60% of mortality in this risk group is caused by pneumonia, diarrhea or measles, which is usually associated with malnutrition. In Brazil, this scenario is no different, with the Northeast semi-arid region having alarming rates of child mortality, almost twice that of the current national mortality rate, with some cities being three times higher, placing child mortality rates in the Northeast of Brazil among those of the most problematic in the world. The oral rehydration solution, introduced by the World Health Organization (WHO), is still one of the major medical advances in the past 50 years, and is believed to save the lives of 1 to 2 million children each year. However, the oral rehydration solution simply restores body fluid normality, treating the consequences and not the causes of diarrheas. Other means to effectively address worldwide diarrheic ailments still need to be devised or improved, focusing more on preventive rather than curative approaches, to prevent or shorten the course of disease and to minimize recurrences. A great example is breast-feeding. A series of studies documented the reduction of diarrhea episodes in breast-fed children and, also, noted a faster recovery time following an infection in these children. The positive benefit of breast milk is attributed to the antimicrobial actions of human milk proteins, such as lysozyme and lactoferrin that can enhance intestinal and systemic immunological functions. Unfortunately, breast-feeding is not always an option, especially for toddlers, which is usually aggravated by undernourishment and low standard of living, reinforcing the need for alternative strategies. Consequently, to successfully combat childhood undernourishment, morbidity and mortality in the Northeast region of Brazil, the investment in approaches and models that are more suitable to the local semi-arid adapted agriculture-base is required.

The goat is an important economic asset to the Northeast region, providing milk, meat and leather, with the number of goats in the Northeast region representing more than 90% of the Brazilian herd. Historically, goats have been used as a model in biotechnological studies involving the expression of specific proteins in the milk of lactating animals. In this way, the use of goats as a human-relevant animal model (hLf) in milk is a proven reality that can be employed for the reduction of malnutrition, infectious diseases and diarrhea in children in the Brazilian semi-arid region with an extraordinary potential for success. Due to the protective nature of human milk imparted, in part, by the presence of lysozyme and lactoferrin, the generation of transgenic lines for the production of these important antimicrobial compounds in the milk of the local goat population is a unique approach for delivering the potential protective benefits of human milk. In addition, the use of rhLZ and rHF transgenic animals can be used as a research tool for studying and elucidating the individual roles of these antimicrobial proteins in gut development and their synergistic in vivo effects.

The proposed research was designed with the rational that the transgenic approach can be used to modify the milk composition of dairy animals to supply milk-borne human immunocompounds (rHfL, rHfF) and nutrients to children in a continuous manner. Therefore when consumed, this milk would impart enhanced immune capabilities that would work to benefit the growth and health of the young and also help in the management of diarrheal diseases. Genetically modified animal models by focusing on model to reduce child mortality and morbidity caused by gastrointestinal ailments (diarrhea) and malnutrition and also aiming to promote the development of the Northeast semi-arid region of Brazil. In 2012, a transgenic goat line producing rHfL was produced in Brazil at UNIFOR, with animals and milk being under investigation focusing on similar validating in vitro and in vivo studies as performed at UC Davis, translating results and adapting the strategy to the conditions of the Brazilian northeast region.

The concept of producing a milk with enhanced antimicrobial properties was first tested in mouse models in the early 1990s where milk from transgenic mice expressing rHfL was bacteriocin against cold- spoilage organisms and a mastitis-causing strain of Streptococcus suis, with results in mice suggesting that rHfL into the milk was effective in inhibiting bacterial growth. The Artemis line of rHfL transgenic goats was then established in 1999 at UC Davis and since then, extensive and multiple risk assessment studies carried out on the transgenic line have shown no negative impact of the presence or expression of the lysozyme transgene on animal well-being, health, growth, reproduction and milk production and animal health. Furthermore, below, we will present microbial and functional techniques to investigate any effects on the physiology of the host (lactating goats) at the whole animal level, no unintended effects of transgene expression were found. Milk from rHfL transgenic goats possesses enhanced antimicrobial activity over milk from non-transgenic control goats as it significantly slowed the growth of pathogenic bacteria in vitro. Milk from rHfL transgenic goats also has a longer shelf-life with fewer bacteria growing in milk of transgenic animals and milk survived at room temperature for longer periods than control milk before bacterial growth occurred. Milk from transgenic animals also had a lower somatic cell count, a measure commonly used to monitor the state of infection of the mammary gland from lactation to lactation, thereby indicating modulation by rHfL resulting in a healthier udder. Upon consumption by both ruminant and non-ruminant animal models, pasteurized milk from rHfL transgenic goats was capable of modulating intestinal bacterial. Weaning pigs served as a human-relevant animal model receiving milk from rHfL transgenic animals for 16 days had significantly lower numbers of coliforms and E. coli in their intestine than did pigs fed milk from non-transgenic control animals. Compared to control milk-fed pigs, the microbiota of rHfL-fed pigs more closely resembled that of human infants being breast-fed with the enrichment of Bifidobacteriaceae and Lactobacillaceae, both biomarkers of increased gut and host health. These beneficial changes were accompanied by the reduction of clostridia and Streptococci, which are components of the fecal microbiota of infants fed formula that lacks lysozyme as well as decreased levels of disease-related bacteria such as Mycobacteriaceae and Campylobacteriaceae. This study demonstrated that consumption of lysozyme-rich milk resulted in beneficial microbe enrichment and detrimental microbe reduction in the gut microbiome community. Altogether, these data indicate that rHfL expressed in the milk of dairy animals can indeed be biologically active in the intestine and modulate gut microbiota, much like human milk.

Bacterial challenge work was also conducted in the pig as a human-relevant medical model. To determine if lysozyme-rich milk could have clinical effect on the symptoms of diarrhea in the pig model. Finally, two animal models of malnourishment were recently used to evaluate the effect of dietary supplementation with milk from the transgenic line of goats. The pig and non-ruminant animal models, daily supplementation with rHfL goat or control milk for a period of two weeks resulted in the reestablishment of the body weight and gut morphology in malnourished rats receiving the transgenic milk to values similar to full-fed control animals, demonstrating a positive effect in the physiological recovery from malnourishment. Using a pig model for malnourishment, the supplementation of the diet with control or rHfL milk significantly improved
intestinal morphology and permeability, promoting gut recovery and demonstrating that the administration of both control and rhLF milk was beneficial in the recovery of the gastrointestinal tract. Our intention is that such milk from transgenic animals can protect and benefit malnourished children around the world.

In summary, the data obtained thus far has indicated no detrimental effects of the transgenic rhLZ goat or rhLF cattle lines on milk properties and processing, and on general well-being and animal health. In addition, milk from lysosome transgenic goats and lactoferrin transgenic cows have shown positive impacts on gut microbiota and morphology, significantly assisting in mitigating intestinal damage caused by malnutrition, and helped to resolve E. coli-induced diarrhea and clinical signs more quickly than control milk. This work has progressed from testing the use of transgenics to modify the properties of milk in a mouse model to the testing of the efficacy of the rhLZ and rhLF milk to act at the level of the intestine in the pig and rat models of human health. Work is now focusing on the mechanism of action of rhLZ and rhLF in milk, also moving to the rat model for additional pharmacological and toxicity testing as one of the last steps before human clinical trials. The ongoing studies proposed by the research consortium represents an important step towards the ultimate goal of using genetic engineering to improve the health and quality of animal-based food products designed for human consumption. The use of milk with rhLZ and rhLF represents a viable approach to reduce morbidity and mortality due to diarrhea and malnutrition, thereby resulting in the potential to promote the development and improvement in the quality of life in the semi-arid region. The availability of livestock milk with human milk properties appears to be a potential food source to enhance and prolong the protective benefits of human milk by promoting disease reduction and growth enhancement. In fact, the mammary gland transgene expression systems for the production of functional proteins in the milk of animals has already been proven as a viable technological alternative to aid in the resolution of problems of the modern world as a means of human pharmaceutical production. It is expected and anticipated that such research efforts will lead to milk that serves as food to nourish and to protect infants and children against diarrhea illnesses in unfavorable regions of the world, also providing an enhanced oral rehydration solution for diarrheal infections that each year, according to the WHO, are responsible for the death of close to a million children worldwide.
Preclinical tests in Brazil
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BMC Proceedings 2014, 8(Suppl 4):O33

Historically the Brazilian pharmaceutical industry went through different phases, which reflected, at least in part, national economic stages. Until the last decade Brazil was dominated by foreign pharmaceutical industry. This has been changing as some national companies, supported by the federal government, have started developing new molecules derived from academic research, as well as producing drugs whose patents have expired. Biopharmaceuticals are extremely sophisticated drugs, synthesized by cell manipulation and designed to treat complex diseases. This change in the industry's attitude pointed to new requirements, well known in developed countries, such as greater regulatory involvement, training people, structuring specialized centers, among others. Naturally, some centers have emerged in the last two decades and these changes have brought a new impetus to preclinical studies laboratories. This fact was evidenced by Ordinance No. 8 (of 16th June 2011), which established the Working Group for the articulation of Reference Centers in Pharmacology. Furthermore, other actions were also reinforced, such as the introduction of Good Laboratory Practice (GLP) by INMETRO, the search of bilateral recognition for toxicology studies between Brazil and OECD, the release of the Guidelines for nonclinical security studies required for drug development by ANVISA, and the establishment of the regulatory agency for animal facilities (Conselho Nacional de Controle de Experimentação Animal - CONCEA), created in 2008 with the approval of the "Atouca" Law. ANVISA, however, still has a timid performance in preclinical studies. It is based on the law of September 23, 1976, which addresses the need to prove the drug efficacy and safety, and the RDC No 136 of May 29, 2003, which mentions the need for reporting preclinical trials results for a new drug to be registered. At this point, it should be emphasized that there is a long way to go, considering that for risk and safety assessment of new drugs (especially as it relates to Biopharmaceuticals) there is often a need to apply two or three concomitant guidelines including EMEA, ICH and OECD for designing a study plan. This practice also brings with it the need for training people in the scope of quality, as well as management of this practice. Depending on their size and strategy, multinational pharmaceutical companies may conduct extensive in-house research or seek to license promising drugs from academia, other pharmaceuticals, or biotechnology companies. The Brazilian academic institutions have substantial research and publications related to new molecules, as well as new therapeutic applications for known molecules. However the practice of partnerships between the academia and industry is quite new in our country. Few industries have learned this path as a route to innovation, but those who have already tried it, are emerging as innovative companies obtaining their first products. Following the development of new drugs, preclinical studies stay in between the academic research and the clinical trials, to determine the safe use interval between the effectiveness and the therapeutic regimen. Thus, it is not uncommon that a long and laborious period is devoted to this phase, since the data obtained in the development of protocols may require changes in doses, in the molecule, in the pharmaceutical formulation or in the vehicle. Thus, the need for a well trained staff, working as a team, with knowledge of regulatory compliance practices (GLP) and good relationships with the industry that owns the investigated molecule is a must. A solid development requires the participation of leading scientists in each one of the areas involved in the discussion of the data obtained. These professionals are veterinarians, professionals of veterinary clinical analysis lab, veterinary pathologists with training in toxicology, as well as the toxicologist as the director of studies in accordance with BLP. Correct decisions about the course of development of the drugs are based on such discussions, which should also include toxicologists from the industry staff, who must have the ability to understand the importance of data and guide the necessary changes. The interaction between the laboratories that develop the preclinical protocols and the industry, tends to promote common learning, generating aggregation of skills to the research area and the overall development of the pharmaceutical industry. It is important to note in this regard that most Brazilian companies are still in this learning process and have not yet internalized all the requirements discussed above.

Biomass pretreatment: a critical choice for biomass utilization via biotechnological routes
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BMC Proceedings 2014, 8(Suppl 4):O34

Introduction: The necessary biomass pretreatment step, to render the material accessible to the relevant enzyme pool, has been under thorough investigation as the production of biomass syrups, via enzymatic hydrolysis, with high sugars concentrations and yields and low inhibitors concentrations requires the pretreatment to be both efficient and low cost. A good choice for biomass pretreatment should be made by considering: (i) the possibility to use high biomass concentration; (ii) a highly digestible pretreated solid by either increasing the biomass superficial area or decrease in crystallinity or both; (iii) no significant sugar degradation into toxic compounds; (iv) yeast and bacterial fermentation compatibility of the derived sugar syrups; (v) lignin recovery; (vi) operation in reasonably sized and moderately priced reactors and (vii) minimum heat and power requirements [1]. Considering the most known pretreatments, such as dilute acid, hydrothermal processes, steam explosion, milling, extrusion, and ionic liquids, different pretreatment methods produce different effects on the biomass in terms of its structure and composition [2]. For example, the hydrothermal, steam explosion and acidic pretreatments conceptually remove mainly the biomass hemicellulose fraction whereas alkaline pretreatments remove lignin. On the other hand the product of a milling-based pretreatment retains the biomass initial composition. Furthermore, cellulose crystallinity is not significantly reduced by pretreatments based on steam, or hydrothermal, or acidic procedures, whereas ionic liquid-based techniques can shift crystalline cellulose into amorphous cellulose, substantially increasing the enzymatic hydrolysis rates and yields. As such, the choice of pretreatment and its operational conditions as well as the concentration of the enzyme used in the hydrolysis step, determines the hixose and pentose sugars composition, the concentration and toxicity of the resulting biomass syrups. The activity profile of the enzyme blend and the enzyme load for an effective saccharification may also vary according to the pretreatment. Indeed, a low hemicellulose load can be used for a xylan-free biomass and a lower cellulose load will be needed for the hydrolysis of a low crystalline and highly amorphous pretreated biomass material.
As the pretreatment choice will also be affected by the type of biomass, the envisaged biorefinery model will need to consider the main types of biomass that will be used for the biorefinery operation so as to select an appropriate, and versatile pretreatment method [3]. Considering the biorefinery concept which broadens the biomass derived products, the C6 sugars could be fermented into ethanol, while the C5 stream could be used for the production, via biotechnological routes, of a wide range of chemicals with higher added value. To date, sugarcane and woody biomass, depending on the geographic location, are strong candidates as the main renewable resources to be fed into a biorefinery. However, due to major differences regarding their physical properties and chemical composition, the relevant pretreatments to be used in each case are expected to be selective and customized. Moreover, a necessary conditioning step for wood size reduction, prior to the pretreatment, may not be necessary for sugarcane bagasse, affecting the pretreatment energy consumption and costs. Moreover, the choice of pretreatment should take into account the foreseen utilization of the main biomass molecular components (cellulose, hemicelluloses and lignin). It is important to point out that lignin can be used as a valuable solid fuel or as a source of aromatic structures for the chemical industry.

Sugarcane is one of the major agricultural crops when considering ethanol production, especially in tropical countries. In Brazil, sugarcane occupies 8.4 million hectares, which corresponds to 2.4% of farmable lands in Brazil. The gross revenue of this sector is about US$ 20 billion (54% as ethanol, 44% as sugar, and 2% as bioelectricty) [4]. In addition, up to 50% of all vehicles in Brazil are flex fuel cars, which corresponds to approximately 15 million cars [5]. Given the above, Brazil is an important player in this scenario, and, consequently, sugarcane bagasse and straw are promising feed stocks for biomass ethanol. Brazil produced, in 2008, 415 million tons of sugar cane residues, 195 million tons of sugarcane bagasse, and 220 million tons of sugarcane straw, whereas the forecast for the 2011 sugarcane production is 590 million tons, which would correspond to 178 million tons of bagasse, and 200 million tons of straw [6]. Currently, in Brazil, R&D on the use of biomass via biotechnological routes has been focused mainly on agricultural residues such as sugarcane residual biomass.

Advantages and disadvantages of different types of pretreatments: Acid pretreatment. Pretreatment with dilute sulfuric acid has been reported as one of the most widely used processes due to its high efficiency. This pretreatment removes and hydrolyzes up to 90% of the hemicellulose fraction, rendering the cellulose fraction more accessible to hydrolytic enzymes. However, it presents important drawbacks related to the need for a neutralization step that generates salt and biomass sugar degradation with the formation of inhibitors for the subsequent fermentation step such as furfural from xylose degradation. The removal of inhibitors from the biomass sugar syrups adds cost to the process and generates a waste stream. Additionally, mineral acids are corrosive to the equipment, which make the pretreatment an option suitable only for softwoods, whereas for hardwoods (including the hemicellulosic portion), a high recovery of sugars, and the feasibility for industrial implementation. Moreover, the soluble stream rich in carbohydrates derived from hemicellulose in the form of oligomers and monomers may be easily removed and used as feedstock for the production of higher added-value products such as enzymes and xylitol. Other attractive features include less hazardous process chemicals and conditions, the potential for significantly lower environmental impact, and lower capital investment. The fact that the steam-explosion process does not require previous grinding of the raw biomass is an important feature, considering that the energy required to reduce the particle size before the pretreatment (pre-grinding) can represent up to one-third of the total energy required in the process. The main drawbacks related to steam-explosion pretreatment are the enzyme and yeast inhibitors generated during the pretreatment, which include furfural and hydroxymethyl furfural; the formation of weak acids, mostly acetic, formic, and levulinic acids, the two latter acids being derived from furfural’s and hydroxymethyl furfural’s further degradation; and the wide range of phenolic compounds produced due to lignin breakdown. Several detoxification methods have been developed in order to reduce the inhibitory effect, which represent additional costs in the overall process. Other limitations of this method include the incomplete disruption of the lignin-carbohydrate matrix.

Ionic liquids pretreatment. ILs are able to disrupt the plant cell wall structure by the solubilization of its main components. This class of salts is also able to alter cellulose crystallinity and structure, rendering the amorphous cellulose prone to high rates and yields from enzymatic saccharification. Indeed, this combination of effects generates a pretreated material that can be easily hydrolyzed into monomeric sugars when compared to other pretreatment technologies, also rendering the enzymatic attack faster as the initial hydrolysis rate is greatly increased [9,10]. Nevertheless, ILs are still too expensive to be used for biomass pretreatment at the industrial scale, as an innovative and promising biomass pretreatment technologies, the use of IL stands out. These versatile classes of chemicals can be tailored to suit the selective extraction and recovery of the biomass components, such as the recovery of a cellulose-hemicellulose rich material in an amorphous form which is prone to enzymatic hydrolysis with high yields and rates. Additionally, the possibility of recovering the extracted sugars in oligomeric form and of producing industrially attractive compounds from the lignin fraction is also promising. The major drawbacks of the ILs include the large amounts of these chemicals that are required and the high cost of their disposal. However, the use of ILs for biomass treatment is a very active field of research and there is hope that more environmentally friendly and cost-effective processes will be developed in the near future.

Mechanical pretreatments. Mechanical pretreatments of biomass aim primarily to increase the surface area by reducing the feedstock particle size, combined with debelification or reduction in the crystallinity degree. This approach facilitates the accessibility of enzymes to the substrate, increasing saccharification rates and yields. The most studied biomass mechanical pretreatment for biomass is the milling process, mainly the ball-milling, which presents a high energy consumption, and wet disk-milling pretreatments [7,8]. Another mechanical treatment to be considered is extrusion, even though this process involves additional thermal and/or chemical pretreatments.

Liquid hot water (LHW) pretreatments. The liquid hot water (LHW) is based on the use of pressure to keep water in the liquid state at elevated temperatures [11]. This process provides high energy efficiency. Besides removing lignin from the biomass native material washing also removes inhibitors, salts, furfural and phenolic acids. This pretreatment, whereby sodium hydroxide has been the most studied reagent is similar to the Kraft pulping process used in the pulp and paper industries. The main effect of alkaline pretreatments is the biomass lignin removal thereby reducing the steric hindrance of hydrolytic enzymes and improving the reactivity of polysaccharides. The addition of air/oxygen to the reaction...
mixture dramatically improves delignification. The alkali pretreatment also causes partial hemicellulose removal, cellulose swelling and cellulose partial decrystallization.

Conclusion: Several factors must be taken into account regarding the choice for biomass pretreatment regarding the most advantageous use of the biomass solid and liquid streams resulting from the subsequent enzymatic hydrolysis step. The resulting sugar syrups stream and the lignin stream, as either a solid or a liquid form must be carefully considered for the deployment of a fully integrated biorefinery, for the use of biomass as a source of fuels and chemicals in a sustainable and environmentally friendly way.

References

O35 Renewable hydrocarbons from sugarcane
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BMC Proceedings 2014, 8(Suppl 4):O35

Amyris is a renewable products company providing sustainable alternatives to a broad range of petroleum-sourced products. Amyris applies its industrial synthetic biology platform to convert plant sugars into a variety of molecules - flexible building blocks that can be used in a wide range of products.

Amyris’s initial portfolio of commercial products is based on Biofene®, Amyris’s brand of farnesene, a long-chain branched hydrocarbon, manufactured using our engineered microbes in fermentation. First generation renewable fuels have been an important part of the effort to reduce the world’s petroleum dependence and greenhouse gas emissions associated with transportation fuels. However, these first generation renewable fuels have some limitations, ranging from lower energy density than petroleum fuels and lack of fungibility with existing petroleum distribution systems.

Amyris renewable fuels are designed to be optimal transportation fuels. Specifically, our fuels are designed to be drop-in, cost competitive replacements for petroleum-derived fuels, compatible with existing engines yet with superior performance. Building on our Biofene hydrocarbon building block, we are currently selling renewable diesel in metropolitan areas in Brazil and are pursuing industry certification for our renewable jet fuel.

O36 Biotechnology strategies with industrial fuel ethanol Saccharomyces cerevisiae strains for efficient 1st and 2nd generation bioethanol production from sugarcane
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BMC Proceedings 2014, 8(Suppl 4):O36

Background: In Brazil the production of fuel ethanol is based on the fermentation of sucrose from sugarcane by selected industrial Saccharomyces cerevisiae yeast strains [1-3], a mature and highly competitive technology. Taking into account that the feedstock costs have a major role in the overall economics of the process, it is expected that more efficient conversions of sucrose into ethanol (1st generation bioethanol) will be of high economical significance. Another promising strategy to improve bioethanol production is the fermentation of the lignocellulosic material present in sugarcane bagasse and leaves (2nd generation bioethanol), a biomass containing large amounts of xylose, a sugar not fermented by S. cerevisiae yeast strains [4]. Thus, although the integration of 1st and 2nd generation ethanol production in the Brazilian industry is considered the best option for bioethanol production [5], there are still several drawbacks to fully develop an efficient industrial production system. In this presentation we will show the metabolic and genomic engineering strategies that we are introducing into industrial yeast strains to improve bioethanol production in Brazil.

Results and conclusion: We have recently show that it is possible to improve sucrose fermentation through genomic and evolutionary engineering strategies that switch the way yeast cells ferment this dissaccharide: the active transport and intracellular sucrose hydrolysis allows an increase of 11% in the ethanol yield [6]. We are introducing these modifications into the genome of diploid industrial fuel ethanol yeasts that dominate fermentation processes in Brazil [1,2], showing excellent results. In order to improve the 2nd generation bioethanol production, we have initially screen a panel of several Brazilian industrial S. cerevisiae strains for their ability to ferment xylulose (an intermediate in xylose catabolism), and the best xylulose fermenting yeasts were engineered by chromosomal integration of the xylose reductase and xylitol dehydrogenase genes (both from Scheffersomyces stipitis), and also the xylulokinase gene from S. cerevisiae, under control of strong constitutive promoters. Our results show that the recombinant yeast strains can ferment xylulose efficiently, especially under glucose-xylose and sucrose-xylose co-fermentations, highlighting the importance of modifying industrial yeast strains for efficient 1st and 2nd generation bioethanol production from sugarcane.

In an attempt to further improve the industrial recombinant yeast strains, new yeast species isolated from rotten wood in Brazil [7-9] have been also evaluated for the fermentation of xylose and cellubiose (a dissaccharide present in cellulose hydrolysates). Cellulbiose inhibits the cellulases required for cellulose hydrolysis, and the β-glucosidases used to hydrolyze this dissaccharide constitute the highest cost in the enzymatic blend. Our results show that some Spasmothora yeast strains are efficient cellulobiose fermenters due to active transport of the sugar and intracellular hydrolysis of disaccharide, which has a great potential for lignocellulose fermentation since there will not be glucose present in the hydrolysates to compete with xylose transport by the yeast hexose transporters [4,10]. Some Spasmothora yeasts are also efficient xylose fermenting strains due to xylose reductase and xylitol dehydrogenase enzymes with paired co-factor preferences, as well as high-capacity active H+-xylose symporters. We have sequenced the genome of the type strain of the xylose-fermenting S. arborareae yeast [7], and identify several candidate genes encoding for the enzymes involved in xylose catabolism, as well as genes for sugar transporters. These genes have been already cloned into overexpression plasmids for introduction into S. cerevisiae yeast to further optimize bioethanol production from sugarcane in Brazil.

Acknowledgements: The work at LBMBL is funded by the Brazilian agencies CAPES, CNPq, FAPESC and FINEP, and by the Japanese International Cooperation Agency (JICA).

References

**O37 Technology overview and perspectives on next generation technologies**
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*BMC Proceedings* 2014, 8(Suppl 4):O37

Today Brazil is the second world producer of fuel ethanol but has the best production system in terms of environmental impact and social positive impacts. This is due to the use of sugarcane as feedstock that is perhaps the world champion crop in terms of biomass productivity and photosynthesis efficiency, even not being near to the theoretical limit. In this presentation is shown how the sugarcane culture and its industrial processing co-evolved through adaptation to the production environment, in the agricultural area adaptation to the soil, climate and biotic and abiotic stresses and in the industrial area adaptation to the exposition to sugar prices volatility, to the very high interest rates that Brazil practiced for decades, together with economic uncertainties and high capital costs. The present industrial process that is very efficient, very resilient and able to adjust the product mix in an ample range, is self-regulated in its processing co-evolved through adaptation to the production environment, in the agricultural area adaptation to the soil, climate and biotic and abiotic stresses and in the industrial area adaptation to the exposition to sugar prices volatility, to the very high interest rates that Brazil practiced for decades, together with economic uncertainties and high capital costs.

This industrial process is very mature and for the last 10 years almost there was no significative gains because most of the possible gains with the present knowledge are there in operation in large scale. That means that new ideas are needed and that our Brazilian process is ready for new, revolutionary technologies as the so called second generation technologies.

CTC is developing its own cellulose ethanol production process learning from the evolution of the first generation. The idea is make possible using all carbohydrates produced by the sugarcane plant during its photosynthesis and not only the soluble sugars, sucrose mostly, as today. Since sucrose is only one third of the carbohydrates actually produced annually Brazil has the theoretical potential for tripling its output form the sugar mills. Of course it will not be possible to have 100% efficiency but very significative gains will be possible as soon as new solutions for breaking the fiber of sugarcane are available for industrial use. That means that new ideas are needed e.g. to bring the enzymes costs close to presently practiced at the starch converting units and making possible to develop microorganisms that can efficiently ferment all sugars released by the enzymes including the pentoses such as xylose, that presently our microbial fermentation workhorse Saccharomyces cerevisiae cannot ferment naturally. This fermentation process will have to be as efficient and productive as it is today in order to make possible producing the second generation ethanol as cheap or cheaper that it is today.

**O38 Solazyme: “unlocking the power of microalgae: a new source of sustainable and renewable oils”**
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*BMC Proceedings* 2014, 8(Suppl 4):O38

- **The technology platform of Solazyme**: Solazyme’s biotechnology platform transforms a wide variety of sugars into renewable oils with high added value, using microalgae, microorganisms that are naturally capable of storing energy in the form of oil. Solazyme’s technology enables the conversion of this feedstock into oils for a variety of applications including chemical, cosmetic, food and fuel.
- **Unique to Solazyme’s process is their proprietary ability to tailor oils**, meaning that for the first time in the history, Solazyme has the ability to design oil profiles rather than simply use what’s available in nature today. Through its world class tailoring capabilities, Solazyme is coupling proprietary strains of algae with standard industrial biotechnology; converting what the earth produces naturally- carbohydrate sugars- into what society needs most - oil.
- **The existing source of oils × new source of oil**: While the physical and chemical characteristics of conventional oils have traditionally been dictated by oils found in nature, or blends derived from them, this is no longer the case. Solazyme has created a new paradigm that enables the company to design and produce novel tailored oils that cannot be achieved through blending of existing oils alone. This core competency has created an incredible market opportunity.
- **Solazyme’s platform enables the production of renewable oils with different carbon chain lengths, saturation levels and profiles simply by changing the microalgae strain used in the fermenter.**
- **Solazyme renewable oils - the microalgae**: Through Solazyme’s industrial biotechnology platform, the company is able to harness the oil-producing capability of microalgae, an organism that has evolved naturally to produce oil prolifically and efficiently, making it an ideal organism for industrial fermentation. The company’s technology allows for the optimization of oil profiles with different carbon lengths, saturation levels and functional groups to modify important oil characteristics, such as flash point, pour point, cloud point, oxidative stability, smoke point, and viscosity, thus enabling much more stable and sustainable end products. Solazyme’s platform is also feedstock flexible and can utilize a wide variety of renewable plant-based sugars, such as sucrose, dextrose, and sugar from other sustainable biomass sources including cellulose. Utilizing standard industrial fermentation equipment to efficiently scale and accelerate microalgae’s natural oil production time to a few days, Solazyme feeds their proprietary, oil-producing microalgae plant-based sugars in enclosed fermentation tanks, where they are in effect utilizing “indirect photosynthesis,” in contrast to the traditional open-pond approach.
- **Markets**: Solazyme leverages their proprietary biotechnology platform to tailor oils that address major markets served by conventional oils: transportation fuels, chemicals, skin and personal care products, nutritional, among many others.
- **Sustainability and safety - breaking paradigms**: Solazyme oils address many of the challenges associated with traditional oils, such as supply constraints, volatile pricing, and potentially negative and irreversible environmental effects. The “drop in” nature of Solazyme’s...
Second-generation ethanol (GII). The contribution of CTBE
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BMC Proceedings 2014, 8(Suppl 4):O39
The Brazilian Bioethanol Science and Technology Laboratory (CTBE) 
one of the laboratories of the Brazilian Center of Research in Energy and 
Materials (CNPEM) has as one of its main goals contribute to the 
development of the process of obtaining second generation ethanol (GII) 
mainly from lignin cellulose fractions of cane sugar.
To achieve this goal leads its own line of research, develop partnerships 
with other public and private institutions or acting as national laboratory 
offers its facilities of laboratories and process development pilot plant to 
researchers involved in projects to produce ethanol, energy and 
chemicals from lignocellulosas materials.
The activities of CTBE are based on research and development line that 
integrates the current process of obtaining first-generation ethanol (GI) of the 
sugars extracted from cane sugar coupled with the second generation 
process of deconstruction of lignocellulosas fractions (bagasse and straw), 
enzymatic hydrolysis of cellulose and fermentation of pentose and 
hexoses. The production of ethanol GI although technically demonstrated, at the 
present time not achieved results that enable production at competitive 
costs.
The critical barriers to overcome to achieve this goal are:
- Pretreatment: efficient fractionation of lignocellulosas material for better 
  recovery and hydrolysis of pentose and cellullin.
- Hydrolyses: Obtaining a more efficient and productive hydrolyses complex;
- Enzymatic hydrolysis: optimization of hydrolysis of cellulose;
- Ethanol from pentose: development of pentose fermentation to 
  ethanol.
The negative impact of these barriers on the process of obtaining second-
generation ethanol will be discussed. The research lines proposed for 
overcome it and improve the performance of this process integrated with 
first generation ethanol will be presented.

Brazilian biotechnology network - the challenge to innovate 
in biotechnology in Brazil
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BMC Proceedings 2014, 8(Suppl 4):O40
One of the most important roles the Brazilian Society of Biotechnology - 
SBBIOTEC can play is to organize the Biotechnology Sector. The Brazilian 
Biotechnology Network aims at the academic sector. The academic sector 
is represented by 48 Graduate Courses registered at CAPES (Coordenação 
de Aperfeiçoamento de Pessoal de Nível Superior) /Ministry of Education, 
specifically in the area of Biotechnology. There are an even larger number of 
Graduate Courses related to Biotechnology but registered at CAPES in 
other areas such Biochemistry, Genetics. Pharmacology among others. So 
the Biotechnology academic sector is not irrelevant. However less than 1% 
of the scientific output in Biotechnology in Brazil reaches the market. The 
main goal of the Brazilian Biotechnology Network is to increase the 
visibility of the science that is produced in the Biotechnology sector in 
Brazil and related areas of application to catalyse the transfer of innovative 
scientific results to the industry. The academic and the private 
bioindustry sector do not interact. For this reason the number of scientists 
in Brazil hired by the private sector is small although Brazil graduates more than 1,000 PhDs/year. This reality differs considerably from 
the context in the United States that for this reason and others control 
70% of the Biotechnology business in the world. In 2011 [1] CEBRAP and 
BRBIOBETEC mapped 237 Biotechnology companies in Brazil. Efforts of this 
nature happen spasmodically. How was the Biotechnology sector 
structured in Brazil in 2011? 85% of the Biotechnology enterprises in Brazil 
had less than fifty employees and 56% of the revenues of these companies 
was around 1 million US $/year. Eighty % of these companies are located 
in three states: Sao Paulo, Minas Gerais and Rio de Janeiro. Most 
companies do not know that a network that I established in the North East 
of Brazil - RENORBIO deposited over one hundred and fifty patent 
applications in the last seven years and established 10 startup companies. 
This network and two others i established recently, one in the Amazonian 
region (Bioamazonia) and the other in West, the Savannah region in Brazil 
have web system and their graduate courses operate as Networks. RBB is 
tended to cover the whole country using the same software with some 
adjustments. Probably a larger number of patent applications and startup 
companies can be made visible if we extend the same software to the 
whole academic sector in Brazil thru the RBB. The software offer in 
addition: students, professors, disciplines, scientific outputs, patents and 
startup companies. Even small companies might be stimulated to hire 
PhDs holding patent applications. The software not only cites patent 
applications but the development of process in the patent Agencies. The 
RBB will be funded by the FAP/DF ( Fundação de Amparo a Pesquisa do 
Distrito Federal), not only the software to be developed by Hiraigy Tl but 
the monitoring of the system at SBBIOTEC - www.sbbiotec.org.br.
During the last three years I have contributed to the Bioentrepreneur 
blog of Nature Biotechnology: http://blogs.nature.com/tradesecrets/ 
author/Ibarret. In my last contribution published in September 26 I state 
that the efforts to develop biotechnology in Brazil now exceeds three 
decades. This analysis that deals with Opportunities and Limitations for 
Biotechnology Innovation in Brazil is part of an eBook published by 
Bentham Books [2] I have written this e-book now because Brazil has 
emerged financially and now has more opportunities in biotech than 
previously. The book is intended for those who want to know some of 
the history of biotech in Brazil, and to ponder the power of this 
technology and the opportunities we now have in our hands. Brazil may 
become a relevant actor in this area internationally, taking advantage of 
some circumstances here that are not available in other countries, 
particularly our biodiversity.
Since the seventies Brazil started training young scientists in plant genetic 
engineering and molecular biology, hoping to incorporate this nascent 
technology into EMBRAPA’s (Brazilian Enterprise for Agricultural Research) 
plant breeding efforts. This work I started in the 80s took place at The 
National Center for Genetic Resources and Biotechnology (CENARGEN) 
and added to the efforts of a dozen excellent geneticists. The soybean 
revolution in Brazil was brought about by Romeu Klih; aluminum-resistant corn 
developed for the acidic “cerrado” soil was created by Ricardo Magnavacca; 
and the foundations of maize breeding had been previously done by the 
late Ernesto Paterniani. Eleuzio Curvello did cotton, and Alcinio Carvalho, 
coffee, for 52 years of his life. Dalmo Giacometti and Silvio Moreira, citrus. 
Marcilio Dias and Hiroshi Ikuta are the fathers of the vegetable genetics;
Raul Moreira tackled banana; Ady Raul da Silva, wheat. Finally Frederico 
Menezes Veiga, sugarcane in the North of Rio de Janeiro. Through all this, 
plant breeding has continuously built cultivars to feed our seed industry. 
High-tech seeds and low-cost farming practices were the result of seed 
laws and plant breeder’s law in Brazil. The end result is that Brazil can 
competitively produce nearly 200 million tons of grain because it is cheap 
for the farmer. Brazil tried to follow the growth of commercial biology. 
In the ’90s, Brazil introduced protections for intellectual property around 
genetics and plant breeding rights as aid before, but that efforts were not 
very successful. Brazil suffered through rampant inflation for decades 
even as it invested in Biotechnology. The e-Book describes adjustments that must be made to assure the 
success of our investments, particularly to the laws and regulatory 
framework. The book investigates the immense possibilities of agricultural 
bioindustry in Brazil, partly because the mechanisms of public private 
interactions are well designed and in operation. The public perception in
Brazil and many other countries has turned against genetic engineering for political and ideological reasons: when the first engineered soybean resistant to glyphosate was released commercially a campaign against transgenic plants prevented the application of this technology in agriculture for almost a decade in Brazil. However, the world adopted recombinant DNA technology in the pharmaceutical area and most products utilized internationally by the public in this industry (including Brazil) are genetically engineered. There is a twisted public perception problem in the Ag Biotech that has to be faced globally, particularly in Europe. The only solution is to focus biotech on the issues surrounding poverty (discussed in one of the e-book’s chapters).

In the pharmaceutical industry in Brazil the context is quite different. Brazil lags behind many developed and emerging countries, and does not have an equivalent to EMBRAPA in the pharmaceutical area. The area is growing, however (in 2011, up 14%), because the market is a demanding one. We import active principles from other countries and commercialize foreign products for our market here. So we are growing without innovation. There are initiatives that pull large, nationally funded companies together as consortia, and which propose “biosimilars” for monoclonal antibodies with expiring patents, but there is no innovation as an initiative. Brazil has no contract manufacturing organization, and not one CMO or cGMP-facility. Because of this, Brazil cannot scale up its pharmaceutical products, nor can it properly conduct the clinical studies needed for registration at ANVISA (http://portal.anvisa.gov.br/wps/portal/anvisa-ingles). Brazil has never produced a block buster or registered a pharmaceutical product at the FDA. Large corporations in general do not invest in Brazil, claiming Brazil’s patent laws are not adequate. And finally, Brazil has no risk capital funds (Burrill & Co is a solitary actor).

There is a movement in the right direction, however. The government is supplying loans for biotech projects, allowing repayment in 10 to 15 years, with subsidized interest and the absence of capital payment for 3 to 5 years. Also, the federal government and private sector are funding scholarships to train 100,000 students in the next five years at all levels from high school to PhD. This is great news, but there are many more problems that need to be resolved in the coming decade. In 2005 Brazil sanctioned an Innovation Law. We estimated that in ten years 10 billion US $ would flow to the Biotechnology sector. The decade is almost over and games never finished.

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O42 Graduate program in biotechnology and biodiversity - “Pro-Centro Oeste Network”
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The Graduate Program in Biotechnology and Biodiversity (PGBB) is a consortium of public and private education institutions of the Center-West Region of Brazil committed to offer academic studies at the doctorate level. The Program was created in 2012 as an initiative of the “Pró-Centro Oeste Network”. The coordination of the Program belongs to Universidade de Brasília. The following institutions participate in the Program:
- Universidade de Brasília (UnB) - DF
- Universidade Católica de Brasília (UCB) - DF
- Universidade Federal de Goiás (UFG) - GO
- Pontifícia Universidade Católica de Goiás (PUC-Goiás) - GO
- Instituto Federal Tecnológico Goiano (IFGoiano) - GO
- Universidade Federal de Mato Grosso do Sul (UFMS) - MS
- Universidade Católica Dom Bosco (UCDB) - MS
- Universidade Federal da Grande Dourados (UFGD) - MS
- Universidade do Estado de Mato Grosso (UNEMAT) - MT
- Universidade Federal de Mato Grosso (UFMT) - MT

The course has a duration of 48 months and currently there are 50 students enrolled. The faculty is composed of 70 investigators. The Program encourages students from multiple disciplines to experience research in close relation to several fields of biotechnology.

Goals of the program:
1. To provide graduate education at the doctorate level
2. To promote innovation in the fields of basic science and technology in order to contribute to the sustainable development and to improve the quality of life of the Center-West Region of Brazil;
3. To contribute to the local bioindustry through the development of products, processes and services based on the rational use of the regional biodiversity.

Lines of research:
1. Science, technology and innovation for the sustainability of the Center-West Region.
2. Bioeconomy and conservation of natural resources.
3. Development of biotechnological products, processes and services.
More information on the Program can be found at http://redeprocentrooeste.org.br/

O43 Developing GM insects for sustainable pest control in agriculture and human health
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BMC Proceedings 2014, 8(Suppl 4):S43

With increasing demand for effective control of insect pests coupled with environmental sustainability, farmers and disease vector control authorities are facing enormous challenges to, respectively, maintain food production and protect human health. Pest control has long relied upon insecticides, which can be very effective, but continued reliance is hampered by several factors. Concerns over their potential impacts on the environment and human health have led to implementation of restrictions on residues on food, the number of sprays per season, implementation of spray-free pre-harvest periods, and withdrawal from the market of some modes of action. Insect populations develop resistance to insecticides, and there are a limited range of modes of action available. These issues have driven the sterile insect technique (SIT) to other pest control tools - such as mating disruption using sex pheromones, release of natural predators, spraying biopesticides (eg. Bt) and cultivation of insect-resistant transgenic crops - which are employed together to form integrated pest management (IPM) strategies. We propose a new pest control approach, called RIDL (Release of Insects carrying a Dominant Lethal) [1], as a potentially valuable new IPM component for agriculture and public health.

RIDL utilizes transgenic technology to engineer novel traits in pest insects, for application against the wild pest population. We have generated RIDL strains in several insect species: in the dengue vector mosquito Aedes aegypti [2], for example, when larvae are reared in restrictive conditions, male and female offspring do not survive to adulthood due to overexpression of a lethal effector gene, TTA. Permissible conditions are provided by adding tetracycline to the larval medium; the "Tet-off" genetic system, and therefore expression of TTA, is suppressed and the insects survive as normal. Releasing these insects into the wild over a sustained period leads to mating between released males and wild females, resulting in population suppression as their progeny do not survive in the absence of tetracycline. In the factory, however, the RIDL strain can be reared as normal with tetracycline. These transgenic insect strains also express a fluorescent protein marker, which is heritably screened under specialised filters and robust in field conditions.

This approach is similar in effect to another mating-based pest control strategy, the Sterile Insect Technique (SIT), in which mass-reared insects are sterilised by radiation prior to release into the field. SIT (and RIDL) offers pest control that is highly species-specific, with consequently minimal ecological impact; and as it relies upon the mate-seeking instincts of male insects, it is highly effective against low-density or difficult-to-reach pest populations, and can provide highly effective local pest eradication and a barrier to reinvasion. SIT has been used with success against a number of important pest insects, notably in eradicating the New World Screwworm (Cochliomyia hominivorax) from North and Central America. Despite its success, wider SIT implementation is constrained by several inherent limitations. The use of radiation to sterilise the insects also compromises their performance in the field, and the requirement to invest in costly radiation sources and facilities generally restricts SIT application to large-scale programmes that justify the investment. Release of both sexes of sterile insects reduces SIT efficiency - with Mediterranean fruit fly (Medfly, Ceratitis capitata), male-only releases are 3-5 x more efficient per male than are bi-sex releases [3]- and generating sexing strains that permit efficient, large-scale sex-sorting is technically challenging by conventional chromosomal translocation methods. Rearing and releasing large numbers of a pest insect requires a reliable method of marking the methods, to distinguish between wild and sterile, and presents the risk of accidental escapes of fertile, mass-reared pests.

RIDL overcomes these challenges, providing an alternative to sterilisation by irradiation, built-in bio-containment, and a heritable visible genetic marker. Moreover, a female-specific variant of RIDL, called fsRIDL, offers a means of producing male-only cohorts of the insects on a large scale. These have been developed in the Tephritid fruit flies olive fly (Bactrocera oleae) and Medfly, and the Lepidoptera diamondback moth (Plutella xylostella) and pink bollworm (Pectinophora gossypiella), using sex-alternate splicing sequences from sex determination genes (transformer and doublesex in the Tephritid and Lepidoptera, respectively) to regulate female-specific expression of the TTA effector gene, conferring tetracycline-repressible lethality in females only [4-6]. After fsRIDL males are released into the field they find and mate with wild females, the female progeny of which do not survive: as with RIDL and the SIT, with sustained releases the reproductive capacity of the wild population crashes.

Several of these RIDL/fsRIDL strains have undergone further assessment for potential application in the field. Laboratory experiments have been conducted to characterise traits relevant to future field performance, such as longevity, male mating competitiveness and penetrance of the engineered trait when reared on natural host plants (compared to artificial diets). Protocols for these experiments have typically been developed and validated for the SIT, particularly with Tephritid fruit flies. On-crop survival and mating competitiveness trials have been conducted in field cages. Later experiments, conducted in large cages in greenhouses, have sought to investigate whether releases of fsRIDL insects will suppress a target population, as designed. In these cages, wild-type populations of the target pest - olive flies [6], Medfly or diamondback moth (manuscripts in preparation) - were established and stabilised, after which weekly releases of fsRIDL males were initiated, with population size monitored relative to those in untreated cages. In all instances, caged populations treated with fsRIDL males crashed to extinction.

In addition to directly suppressing a wild pest population, fsRIDL also provides a powerful insecticide resistance management benefit [7]. Survival of fsRIDL males results in the background genetics of the mass-reared insect population introgressing into the target population. If the mass-reared fsRIDL colony comprises insecticide susceptibility alleles, the resulting introgression into the wild pest population leads to a powerful reduction in the population’s resistance to a given chemical mode of action. There is, therefore, scope for synergistic use of fsRIDL and insecticides, with potential to reduce overall insecticide use and protect efficacy of valuable and effective modes of action.

In the open field, the first trials with a transgenic strain of insect was with a fluorescent protein-marked strain of pink bollworm, irradiated and used for conventional SIT in Arizona, USA [8]. This trial provided evidence that genetically engineered insects can perform well in the field compared to wild-type insects, and assessment of the strain for programmatic SIT use is ongoing.

In a small town in the Cayman Islands, releases of male RIDL Ae. aegypti mosquitoes (strain ‘OX513A’) were conducted in 2009 and 2010 [9,10]. Relative trap captures of RIDL and wild mosquitoes, together with larvae of each genotype hatching from eggs collected from the field in ovitraps, indicated that they could perform strongly in terms of finding and competing for wild mates in the field. Male-only releases, principally to avoid additional biting by releasé male mosquitoes (male mosquitoes do not blood-feed), were facilitated by a pupal sex-sorting method that separates males and females by size (females are bigger). In 2010, releases of RIDL males were conducted over a more prolonged period, resulting in suppression of the wild population by 80% relative to nearby untreated areas. Following this first demonstration of RIDL efficacy in the field, OX513A has undergone open field trials in Malaysia and Brazil, where further success has been demonstrated. This genetic technology shows great promise for species-specific and powerful control of the dengue vector mosquito, Ae. aegypti, and other difficult-to-control and important pests such as Medfly, olive fly, pink bollworm, diamondback moth and Ae. albopictus. Furthermore, cross-species function of RIDL and fsRIDL systems - in Tephritid fruit flies, mosquitoes and Lepidoptera - demonstrates that this technology should be relatively easily transferred to other target species in the future, offering a new pest control tool for wider implementation of IPM in agriculture and public health.

References


O44 Northeast network in biotechnology (RENORBIO)

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BMC Proceedings 2014, 8(Suppl 4):O44

The Northeast Network in Biotechnology (RENORBIO) was established in 2004 by the Federal Government that identified the demand for this type of professional and signals a great opportunities them to act on the growing industry of biotechnology in Brazil. It is a network of research, development and innovation, which base is the Post-Graduation Program (PGP-RENORBIO) that have 36 institutions of education and research in the nine states of the Northeast, besides Espírito Santo, which also integrates this initiative. Started in September 2006, it represents a new model of Post-graduate program - a network program - fully supported by CAPES direction. The PGP-RENORBIO specifically aims to qualify researchers, in a doctorate level, with a solid technical-scientific basis, able to act on several markets, such as teaching, research, services and industry. This way their specific objectives are: 1) Training qualified people for the activity of research and of higher teaching in the field of Biotechnology; 2) Encourage research in the area of Biotechnology, under the multi e interdisciplinary perspective; 3) Producing, expanding and applying knowledge to Biotechnology to the economical and cultural reality of the Northeast region.

In the long run, it is hoped that the PGP-RENORBIO, as it make possible the interaction of institutions of research and teaching, national and international, and the best use of the critical mass existing in the Northeast region, contribute to the process of consolidation of the Northeast Biotechnology Network and to the effective to and systematic development of Biotechnology in the Country. The RENORBIO’s PGP assumes that it is possible to obtain growth with quality through means of integration strategies which promote development with Competitiveness, according to a world tendency. Hence, the integration of consolidated and emergent groups constitutes the basis of the strategy of the RENORBIO Program. Take part in this effort about two hundred Doctors connected to the thirty-six existing institutions in the nine states of the Northeast, besides Espírito Santo. This is being made possible because of an explicit institutional support, in a form of an Institution’s Consorcia. Besides supporting the strengthening of the existing programs in the institutions that integrate this initiative, by the input of additional resources to them, this strategy discourages the disordered creation of new programs in Biotechnology in Region, avoiding the lavishness of resources and competencies. On the other hand, taking in account that the program works by adhesion, according to rules of defined institutional association in its regiment, allows the participation of a great number of national and international institutions, such as EMBRAPA and FIOCRUZ, which generally stay aside from the human resources formation efforts in the Country, considering they do not constitute themselves as Institutions of Higher Learning. By means of that integration strategy, all participating institutions, even those of less developed states, may offer a broad resume academic curriculum related to four areas of concentration where Biotechnology offers its most important applications: Health, Agriculture and Animal Raising, Natural Resources and Industrial Biotechnology.

Science, technology and innovation are essential factors for the development and competitiveness of nations. Countries without scientific competency cannot promote their development based on their own technologies and innovations have to pay to use the innovations developed in other countries. For the last twenty years, Brazil has quadrupled its scientific contribution in the global context. It is notorious this fact that results in the consolidation on the Post-Graduation in the Country, under the leadership of CAPES, since the scientific production and the post-graduation have a direct relation. However, enhance the Brazilian scientific contribution on the international context it is still necessary and, in this sense, it is opportune to stimulate the participation of young scientists in the Brazilian post-graduation system.

Also, to stimulate the participation and insertion of Brazil in the use of the advances of bioscience in order to reduce hunger and minimize the serious problems in public health, especially those related to childhood mortality. So, in addition to being an opportunity, it is a mission of science. Such advances cannot preclude Biotechnology, a relatively young branch of bioscience, whose full development depends on the elucidation of the main dogmas of biology, which demand high competence and excellence.

Brazil is one of the largest detainers of biodiversity. Northeast Region alone, holds 42% of its area constituted by semi-arid; a region with unique climate and biodiversity in the whole world. That and other characteristics make the scenario for Biotechnology very promising. The progress in this area may be accelerated if there are joined efforts conjugated among government, scientific and corporative community and in the development of joint projects, in the formation of productive partnerships, in the training of human resources, in the creation of a favorable environment to new investments and in the development and/or adaptation of technologies with the objective of broadening Competitiveness and making the market of biotechnological products more dynamic.

The opportunities for alliances and new partnerships between the knowledge generating sector and the goods and services one, represented predominantly by small and medium size companies, constitute focal point of the Biotechnology and Genetic Resources Program, from the Ministry of Science and Technology, which adopts among its strategies of action and organization of network projects to enhance the flow of innovation and channel the production and the commercialization of its results for the benefit of society.

In the long run, it is hoped that RENORBIO constitutes itself into a Center of Excellence in Biotechnology, which will internalize and develop as more advanced technologies for a wide application in all areas of Biotechnology, with adequate levels of excellence and relevance.

Considered a successful program, the PGP-RENORBIO had its General Coordination in the State University of Ceará (UECE) during the period from 2006 to 2012 and now its Coordination is in the Federal Rural University of Pernambuco (UFPE), Currently PGP-RENORBIO has 246 professors and 520 students; oversees 15 post-doctorates, and has trained 281 doctors and completed the supervision of eleven postdocs. The results obtained with the development of the student research made possible to, in five years, increase the number of patent applications from 8 to 216, the number of professors involved in patent rose from 6 to 70; addition to the considerable increase on the production of papers from its researchers and students, having also been a significant increase in publications in journals with high impact factor. For a biotechnology program so young, these data are of great relevance, showing that there is a substantial change in the culture of generation of products / processes in the region.

There is also a concern of PGP-RENORBIO is the transfer, effectively, of the processes and innovative products generated in their laboratories in order to produce wealth, life quality and social inclusion in the Northeast Region. This challenge gets to be conquered by the creation of the first 11 companies from students, graduates and professors, as well as the licensing of 17 inventions and 17 other negotiations. Thus, PGP-RENORBIO has established close collaboration with industry and confirming your main
goal to train qualified personnel in close relation to the needs of the productive sector.

O45
A network website - The RENORBIO experience
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BMC Proceedings 2014, 8(Suppl 4):O45

The Brazilian Northeast, occupying an area of 1,554,387,725 km², with approximately 3,000 km of coastline, has 56.46 % of its territory (877,565,831 km²) in a semi-arid zone [1], a region with unique climate and biodiversity in the world, with a very promising scenario for Biotechnology.

However, it is a very poor region, concentrating 61.3 % of its municipalities with Low Human Development Index, the lowest rate in Brazil [2]. It is also a region with low capacity to generate wealth, contributing with only 13.5 % of the Brazilian GDP [3] and 7 % of the biotech companies in the country [4]. In terms of skilled manpower [5], the Northeast is the third region with the lowest number of PhD researchers (18,433) and the second in numbers of PhDs per 100,000 inhabitants (34.77).

Nowadays, Biotechnology, besides the sustainable use of biodiversity, can make valuable contributions to today’s solutions and breeding, including to genetically change the existing life forms, thereby leading to improved technology for the development and installation of the human species on the planet. Thus, this area progress can be accelerated if the government, the scientific community and the business community combine efforts in the development of joint projects, formation of productive partnerships, human resources training, creation of favorable environment for new investments and development and/or adaptation of technologies aiming to increase the competitiveness and to boost the market of biotech products. That is what the Northeast Biotechnology Network (RENNORBIO) seeks.

Founded in 2004, the Network proposal is to integrate the scientific competence of the Northeast in biological sciences, based on a PhD course in Biotechnology (PPGB-RENNORBIO), in order to enable a gradual rise of the competence dispersed in smaller universities that isolated, in the short or medium term, would not be able to create competitive graduate studies programs. The dispersed competences were replaced by a participatory model that promoted the integration of the critical mass of Northeast professors and researchers to qualify PhDs in Biotechnology, with the formal support of the institutions involved and the Science and Technology Departments of the States of the Northeast and Espírito Santo. Therefore, several structural actions have been performed, some already established, others in progress, combining cost reduction with parallel and redundant investments and consequent gradual consolidation of Centers of Excellence in Biotechnology in the Northeast.

In 2006, together with the structure of the PPGB-RENNORBIO, the construction process of a Website (http://www.renorbio.org.br), which would meet the needs of a network program, involving, at the time, 28 institutions distributed in 10 states, also began. During its first five years, PPGB-RENNORBIO academic process, of all its students, was through this website: from the selection process, subject offerings and enrollment, subject consultations, to reports and data collection of professors and students from Lattes CV. The Website was therefore an important tool in the construction of RENORBIO identity, meeting the network basic principle, i.e., to eliminate institutional, local visions, in favor of a more comprehensive regional vision, in order to enable the Northeast to grow as a whole through Biotechnology.

RENNORBIO today includes 36 institutions, 246 professors and 520 students; supervises 15 post-doctorates; 281 PhDs were qualified. Five years after it started, the number of requests for patent applications increased from 8 to 216; the number of professors involved with patents increased from 6 to 70; in addition to the considerable increase in the production of scientific papers by its researchers and students; as well as the significant increase of publications in higher impact journals. For such a young biotechnology program, these data are of great importance, as it shows that there is a substantial change in the culture of creation of products/processes in the region. Now, RENORBIO is concerned with the effective transfer of innovative processes and products created in its laboratories in order to produce wealth, life quality and social inclusion in the Northeast. A challenge that has just began to be conquered by the creation of the first

11 companies constituted by students, graduates and professors, as well as the licensing of 17 inventions. RENORBIO website greatly contributed to obtain these promising results in several ways: through its integrating role, giving a unique identity to RENORBIO; its management potential, providing data for management; and its disclosure ability, giving visibility both nationally and internationally to the program. The website integration can be best understood by considering the program logic. RENORBIO chose to use its own system, secure, with a design that emphasizes its logo and completely different from any of the participating institutions website. The idea was to eliminate any merely institutional or multifaceted vision of the Network and to introduce the concept of a single regional program with a roving coordination. Thus, all academic procedures of the students, from 2006 to 2011, were conducted in RENORBIO Website and the data transferred to the institutions responsible for the certificate issuance. As of 2012, new procedures were adopted, as described below.

As an excellent tool used in multi-institutional projects, the website consists of records of professor and student and administers the selection process, online registration of students, subject offers, academic transcripts, records of professors, student reports, among others, additionally to the Center for Teaching Support (NAE). NAE is the space where professors and students interact. RENORBIO Graduate Academic Center. There, each subject consists of a community of practice, unique, that integrates all students and professors connected to it with a set of opportunities available that support the professors’ various tasks, such as issuance of grades and attendance, paper request and receipt, bulletin board, conduction of surveys, among others. To students, in addition to the features related to the classroom, NAE provides means to interact with peers and the professor, enabling communication among them - chat room and messaging tools - as well as providing information on the academic life.

As a management tool, the website is a database containing all the information about the students and those relevant for the professor’s program. The manager has full access to the Website, he/she may enter any information or modify any data whatsoever, and therefore, the PPGB-RENNORBIO Executive Secretary was the only one with this access. However, all information can be removed through specific reports, without interfering with the quality of the data, thus allowing all further members of the program management to have the information they need directly from the Website.

The difficulty of gathering information from such a large group, about 600 people active per year, needs to be facilitated for the manager. Thus, mechanisms to collect data from professors and students were created, and, among them, the capture of information of interest from the curriculum vitae on CNPq Lattes stands out. Throughout time, other requirements not initially foreseen were identified and some of them have already been developed, while others are currently under development.

As of 2012, a new enrollment procedure was established and each student started to enroll directly into the website of the institution where he/she belongs. However, in case the student wishes to attend subjects offered by other institutions of the Network, RENORBIO Website should still be used for enrollment. The new challenge is to maintain, in this new structure, RENORBIO identity/unity already conquered. Some important actions may be to provide a copy of each student’s updated academic transcript, to give continuity and to improve the students’ semi-annual reports and to create a space for annual recertification of the professors, as well as to maintain and improve internal demand mechanisms.

In addition to the strong use of the website by RENORBIO members, it seems that it has promoted good visibility to the Program and provoked the interest not only of national groups, but also international. From mid-2010 until the present time, 145,814 people visited RENORBIO website, accessing an average of 5 pages per visit, with an average duration of 4 minutes and bounce rate of 46.54%. The percentage of new visitors was 43.8 %, and the return rate was 56.2 %. At a national level, the website was visited by more than 250 municipalities from several Brazilian states, but mainly by the northeastern capitals and the states of Rio de Janeiro, Minas Gerais and Sao Paulo. Internationally, countries such as the United States, Germany, Colombia, Spain, France, Argentina and Italy are among the 10 who accessed RENORBIO website the most over the last month, with a median of 232 visits, 67.76 % new visits, median rate rejection of 49.96 %, an average of 4 pages per visit, with an average duration of 3 minutes. Portugal and the United States accounted for 1430 and 1370 visits, respectively, but with
the highest rejection rates (73.85% and 64.45%, respectively). Colombia had 242 visits, with 68.60% new visits, the lowest rejection rate (38.02%) and access to a high number of pages (6.86), with a visit average duration of 7min. These data are consistent with the increasing number of foreigner interested in applying to the PhD at the PPGB-RENORBIO. In 2012, three Colombians and one French were accepted.

RENORBIO website also has space to publicize the labs that integrate the Network, the biotechnologies developed by students and/or professors and the opportunities of scholarship/jobs/internships, which, still, need to be better explored.

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O46
The bionorte network for biodiversity and biotechnology RESEARCH in the brazilian amazon states
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BMC Proceedings 2014, 8(Suppl 4):O46
Created in 04/12/2008 by the Ministry of Science, Technology and Innovation (MCTI) in collaboration with the State Secretaries for Science and Technology (SECTs) from the nine States of Legal Amazon by MCTI decree n° 901. The BIONORTE consortium has the objective of integrate expertise for research, development, innovation and also to graduate personnel at doctorate level. The initiative is focusing in biodiversity and biotechnology and aims to generate knowledge, process and products which can contribute to the sustainable development of the Brazilian Amazon region.

The BIONORTE is lined by MCTI, and managed by a Director Council, composed by representatives of MCTI, MDIC, MI, CONSECTI, CONIFAP, CNPq, FINEP, CAPES, FOPROP, Institutions of Research and Education, Entrepreneurs and Companies. BIONORTE is instrumented by a Scientific Committee, composed by representatives from the academic community from all the member States and by their local Scientific Committees and managed by a Executive Coordination in a MCTI division (CGEC/SPEED). So far the BIONORTE developed two main initiatives that resulted in:

-Graduate program of biodiversity and biotechnology (PPG-BIONORTE): This graduate program was approved by CAPES in October of 2011 with concept 4 (in a scale of seven) and initiated its classes in March of 2012. By now the BIONORTE program has one hundred fifty professors from eighteen main Amazonian institutions. It has thirty eight courses (five as obligatory and thirty three non obligatory) working in two major areas with three research lines as followed:

  -Major area: Biodiversity and Conservation
    -research line 1: Knowledge of Amazon Biodiversity
    -research line 2: Sustainable Use and Conservation of Amazon
  -Major area: Biotechnology
    -research area 1: Bioprospection; Bioprocesses and Bioproducts Development

At the moment, by the end of its second year, the BIONORTE counts with one hundred eight two graduate students. For 2014 more hundred seats are available and the selection process for new doctorate grad students is in line.

**Integrated research projects:** Even before PPG-BIONORTE was created, the Amazonian network was establishing its activities. A public call for projects was launched by CNPq (MCT/CNPq/FNDCT/CT-AMAZÔNIA/BIONORTE - Nº 66/2009) for integrated R&D projects in a framework requirement: the candidates should be from Amazonian States and the subjects need to cover the fields of biodiversity, conservation and biotech. The total number of subscriptions reached seventy projects from which, twenty four were recommended and twenty were hired. The financial resource was provided by the Sectorial Funding from MCTI, SECTs and State Research Funding Agencies (FAPs) from the Amazonian member States. The hired projects have been granted for equipments, consumables and scholarships. Due to its successful results, Brazilian government launched the second version of this public projects call, also through CNPq with the Sectorial Funding (MCTI/CNPq/FNDCT - Ação Transversal - Nº 79/2013), contemplating once again the BIONORTE network. This call was opened until November 4th and there is chance for hiring new R&D projects yet this year.

Along with all these results and activities we can state the BIONORTE initiative is in the right track for its successful consolidation! If you want to know more about us, please go to the BIONORTE site at www.bionorte.org.br.

**Acknowledgements:**
The BIONORTE Amazonian network initiative has financial aid from MCTI (Sectorial Funding), from SECTs and FAPs of each State member, from CAPES, CNPq, FINEP and SUFRAMA. We thank Dr. Luiz Antônio Barreto de Castro very much for the idealization of BIONORTE Research Network and for his constant encouragement.

O47
The context of biotechnology - The biotechnology sector in Brazil. Going up?
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BMC Proceedings 2014, 8(Suppl 4):O47
The presentation will discuss the status of the biotechnology sector in Brazil. We see a lot of attention in the last months to the Health Ministry effort to decrease the health trade deficit by establishing technology transfer and production agreements involving international and local companies and public institutions. The emergence of companies as Orygen and Bionovis and the announced projects conducted by Biomm and Recepta are part of this strategy.

On the other hand, we also see a complex scenario for start-ups in the bio field. In fact, it is even more difficult for entrepreneurs or researchers to start a company in Brazil due to the lack of programs to support them.

On the second stage, start-ups have to deal with an unbelievable regulatory environment where companies have to wait two or more years to start operations. And no financing; non-refundable resources are scarce at FINEP, the brazilian innovation agency. It is also hard for a start-up to find investors, as there are few funds investing in life sciences projects. So, companies are financed by a combination of research/scientific programs, state level programs and, in some cases, angels investors.

Management is also a trouble for the start-ups. Only a few have dedicated business teams, scientific interaction is small and collaborations with local or international companies are rare. Whether these are causes of consequences of the financial situation has still to be determined.

Also, the environment for biotechnology has to be analyzed. We see a lack of crucial components in the product development chain in the country, from pre-clinical labs, translational centers to biologics manufacturing facilities. There are not many professional business incubators and technology parks too. And also, it is not easy to find trained people in product development.

In the short term, the development of the bioindustry in Brazil will depend on how these big projects will perform. In case of success, they could create a positive cycle, encouraging new projects and creating a positive legal and regulatory environment. Will all the other companies have to wait? Or can we use different strategies together?
O48

Intellectual property on life sciences and biotechnology: state of
affairs, impacts and perspectives

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BMC Proceedings 2014, 8(Suppl 4):O48

Everywhere in the world, it has been a major challenge to properly protect inventions and other innovation-oriented achievements in the fields of Life Sciences and Biotechnology. Many aspects make these fields different and probably harder to protect than general inventions on more traditional fields, such as mechanics or chemistry.

First, there are several ethical considerations, which have been taken into account by legislators and judges everywhere. The appropriation of knowledge related to living forms is sometimes seen as illegitimate, particularly that related to the human body. Some legislations ban patents in those fields explicitly. In other cases, courts may rule them unlawful based on ethical considerations not directly linked to any of the traditional patentability criteria. In Brazil, law takes into consideration the rights of traditional peoples on their traditional knowledge, and even on the knowledge that may be developed from genetic resources assumed to belong to traditional communities. This requirement, particularly when they are difficult to accomplish with as they are in Brazil, may stand also in the field of ethical obstacles to knowledge appropriation and commercialization.

A second source of uncertainty in the protection of intellectual assets in those fields has to do with the use of the flexibilities present in the Treaty on Trade-Related Intellectual Property Aspects, generally referred to as “TRIPS.” That treaty requires that patents be granted in every technology field but in the field of Life Sciences. With the exemption of genetically modified microorganisms (autonomous, cells not included), living forms may be put out of the general rules for patent granting, and even be non-patentable at all, as it happens in Brazil and in many developing countries. Last, but not least, the application of the patentability criteria itself for life forms may be trick, leading to conflictive decisions on courts, which may only be pacified in the highest level, as happened recently in the United States.

There are several ways to address the theme of adequate or proper protection. The best approaches, in my opinion, are the ones, which correlate objective and well defined ways of protection to rates or speeds of knowledge disseminations and innovation. Such approaches are applied transversally, among countries or jurisdictions, and may be insightful provided the sample does not comprise countries where jurisprudence on Life-Sciences and biotech patents is not consolidated yet.

Indeed, one of the worst things in terms of the impact of patent systems on innovation seem to be the insufficient consolidation of the understanding of the laws by the courts. Probably that is the reason why many countries follow the North-American example and create specialized courts for IP matters, together with a continuous effort in order to better describe in law the criteria for obtaining a patent. Ethical considerations, for their inherent interpretative nature, probably add to law uncertainty.

Brazil clearly stands in such a bad situation. Several laws rule the appropriation of knowledge and the patentability on Biotech/Biodiversity areas, there is no clear way to fulfill all requirements regarding traditional knowledge, genetic resource definition is unclear and ownership on it is subsequently problematic, as well as on the knowledge which can be developed from it and on the products thus developed. ANVISA “anuência” adds the final touch to uncertainty on those patents when they relate to pharmaceutical products. The result is uncertainty and low investment in all fields related to the Brazilian Biodiversity, and to any pharmaceutical oriented patent. The contradiction between the enormous potential for innovation coming from the spectacular Brazilian Biodiversity and the low rate of product development and patenting may be enough to demonstrate that such uncertainty is clearly detrimental for innovation.

When a country chooses not to protect a particular field of technology knowledge, it clearly avoids investments in that field. It is not clear why Brazilian legislators have decided to ban from patentability insulated natural substances with a known and well defined industrial application. Some say it was due to a perception that there was no endogenous technology capacity, which would be necessary to write the patents and negotiate them. If this is really the case, maybe it is now time to reconsider, not only in Brazil, but also in the countries which have not adopted such protections for the sake of providing no more than the minimum requirements of TRIPS. Of course, minimal requirements are minimal, they are a floor and can never be imagined as a ceiling, as this would confine legislation to the standards established internationally and prevent the country legislators to evaluate and decide with sovereignty the ways the knowledge about the country biodiversity and the results of research on Biotech and Life Sciences should be protected.

In the international comparison, the United States seems to offer adequate conditions for Biotech, to which their patent system helps for sure. Nonetheless, even there some uncertainty results from court decisions, especially when related to genes. This probably suggests that the international community might develop some kind of “suí generis” protection system for segments of genetic sequences with well-defined function and potential industrial application or use.

O49

Sapiens Parque: the innovation park of the technology pole of Greater Florianópolis for the promotion of sectors that propel the future

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BMC Proceedings 2014, 8(Suppl 4):O49

Technology pole of Greater Florianópolis: Competitive companies are one of the bases of a nation’s economic development. Among the proven success strategies in this field are Scientific and Technology Parks, whose mission is to promote intelligence that expands corporate competitiveness.

In this context, Florianópolis stands out in Brazil for its development based on the promotion of innovation. This strategy was materialized by a policy called TECNÓPOLIS that induced the creation of companies by establishing facilities that support innovation like the CELTA incubator, ParTec Afia and the innovative Sapiens Parque.

Sapiens and its concept: Sapiens Parque is the fruit of research and development. It is characterized as an Innovation Park, which at the time of it launching in April 2003 was a globally unprecedented concept that is described below:

An Innovation Park is a Cluster of Innovation Clusters - an environment fortified with infrastructure and systems to attract and develop talents and enterprises that are capable of generating ideas and knowledge and transform them into new products and services for society, promoting the region’s sustainable socio-economic development.

The key instrument for Sapiens’ planning, implementation and operation is its Conceptual Model, which defines and integrates all the critical elements needed for guaranteeing the proper implementation of the development, as illustrated: (Fiates, Fiates 2011).

1. “Sapiens Assets” - these are the “pillars” of Sapiens, upon which the other subsystems are structured. The modules include: Scientia, Artis, Naturlarium and Gens.

2. “Sapiens Clusters” - The Sapiens clusters establish its identity and “thematic territory” and operate in sectors that are strategic to the region, including Technology, Tourism, Services and the Public Sector.

3. “Sapiens Structure” - includes the physical, human and financial elements that support the operation of the clusters such as: the Park Infrastructure, Regional Infrastructure, People and Capital.

4. “Sapiens Actors” - these are the “Actors” from whom the Park should interact and for whom the results of the development are generated. They include: government, companies, research and education institutions and society.

1. Formation of Innovation Clusters: Considering local and regional factors based on state and national studies, four priority areas of operation were defined for Sapiens: Clean Energy & Technologies, Health & Biotechnology, Creative Economy and ICT & Mechatronics. These are the embryos of the so-called Innovation Clusters at Sapiens.

Final considerations: Sapiens Parque is a large and long term development conceived to establish a new growth cycle in Greater Florianópolis’ Innovation Pole. It is organized from a legal and business perspective to permit agile and consistent public and private investment, facilitate the
transformation of knowledge and ideas into successful outcomes in the market and consequently promote economic development throughout the region.

References

POSTER PRESENTATIONS

P1

Array comparative genomic hybridization in confirmation of the deleted genes in a patient with subtelenchial deletion of the long arm of chromosome 10 associated with sagittal craniosynostosis and dysmorphic features
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Background: Craniosynostosis results from premature ossification of one or more cranial sutures and leads to alterations in the shape of the skull and/or premature closure of cranial fontanels, causing impairment of brain perfusion, vision and hearing, airway obstruction, learning difficulties, severe cosmetic deformities and high intracranial pressure [1]. To date, the genetic mechanisms leading to sagittal craniosynostosis are poorly known. The identification of candidate genes underlying this condition may contribute to elucidation of the etiology of this common malformation. The aim of this study was to associate genotype-phenotype of a patient with a deletion in the long arm of chromosome 10 and craniosynostosis.

Methods: A newborn female born at term (39 weeks) with birth weight 3.405 kg and length 45 cm, an occipitofrontal head circumference (OFC) of 36.5 cm, 2nd pregnancy of a mother at 39 years old and a father at 41 years old, not consanguineous and without family history of congenital anomalies was examined by neonatologists using the Merck protocol (2003) for early identification of major and minor anomalies. GTG Banding analyses were performed on metaphase obtained by stimulating peripheral blood lymphocytes from patient and their parents in accordance with standard procedures. The technique Multiplex ligation-dependent probe amplification (MLPA) was performed using kits for the subtelomeric regions of all chromosomes (P036 and P070-E1-B2 - MRC-Holland). Array Comparative Genome Hybridization (aCGH) (OGT CytoSure IScera 6x60k) was performed according to the manufacturer’s recommendations.

Results and conclusions: The newborn had sagittal craniosynostosis (scaphocephaly), microcephaly, facial asymmetry, short forehead and bitemporal narrowing, microtia, hypoplastic and low-set ears, thin lips, strabismus, retromicrognathia, anteriorized anus, right single palmar crease, short stature and conductility of 2-3’rd fingers and congenital heart disease. The karyotype was normal and MLPA detected a de novo deletion in region 10q26.2-q22, confirmed by aCGH that showed a 12.9 Mb deletion (122085501-135053489) (The UCSC Human Genome Working Draft Build 36/hg 18). Mutations in the FGFR2 gene cause several syndromes that result in craniosynostosis (Crouzon, Pfeiffer, Apert, Jackson-Weiss, Beare-Stevenson Cutis Gyrate) and familial scaphocephaly [2]. The newborn does not have any features suggestive of these syndromes, which may be due to the fact that loss of one allele results in FGFR2 haploinsufficiency instead of gain of function. The PTTPRE gene (receptor tyrosine phosphatase type E) is involved in the formation and differentiation of osteoblasts and its allelic absence may explain the craniosynostosis phenotype [2]. In addition, hemizygosis for ATE1 gene may explain heart disease, as it encodes an arginyltransferase, enzyme responsible for posttranslational arginylation, a crucial molecular mechanism for the development of heart failure. Our results are concordant with studies in mice reporting that deletion of arginyltransferase promotes congestive heart failure [3]. Other phenotypic signs found in this newborn may be related to the absence of BNIP3, MSMB, DOCK1, ADAM8 and ADAM12 genes, most of which are responsible for cell signaling, proliferation and differentiation. Reports of craniosynostosis patients with de novo deletions are important to characterize the patients phenotypes, and associate them with specific genes, which will contribute to the improvement of genetic counseling for this condition [4,5].

Acknowledgements: We thank the Institute of Biosciences of USP for technical support and PPSUS - SESAFAPES/CPNPq for financial support.

References

P2

The influence of Escherichia coli cultivation temperature on interferon alpha 2a expression (IFN-α2a)
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Background: The Hormone Group of IPEN develops researches on recombinant pituitary hormones produced in Escherichia coli at laboratory scale, including human growth hormone (hGH) and prolactin (hPRL). The best results were obtained using the L.P. promoter and the signal peptide DsbA, and so the protein of interest is secreted into the bacterial periplasmic space, in its authentic form without an initial methionine [1,2]. The expression, under control of the L.P. promoter, regulated by the thermosensitive repressor, is used for large scale production in E. coli. Thermal induction presents a great advantage over chemical inducers like IPTG or nalidixic acid, that are expensive and dangerous for manipulators and environment, but it presents the disadvantage that proteins particularly thermolabile like hPRL could suffer proteolysis and aggregation, influencing negatively the production. Considering the limitations on the use of IPTG and the periplasmic secretion, successfully obtained with our hGH expression vector using L.P. promoter and W3110 strain, we decided to use this system for IFN-α2a production. The goal of this work was the construction of the IFN-α2a vector, obtaining an the IFN-α2a periplasmic expression.

Methods: The DNA sequences corresponding to the Ndel restriction site, start codon, signal peptide DsbA, cDNA of the IFN-α2a, stop codon, and the restriction site BamHI were inserted into the plasmid, pJL-DsbA-mPRL [3]. This plasmid contains the L.P. promoter and the gene for ampicillin resistance [1]. The new vector was called pJL-DsbA-IFNα2a. The IFN-α2a
sequence was synthesized by the GenScript Corporation (Piscataway - NJ - EUA) in the pUC57 plasmid. The vector obtained was amplified in E. coli DH5a strain and then introduced into the expression W3110 cells. After selection, the best clone was used to test different cultivation temperatures (32°C, 35°C, 37°C and 42°C). Analyses of the periplasmic fluid obtained by osmotic shock based on Western Blotting and SDS-PAGE were carried out.

Results and conclusions: Construction of pL-DbSa-IFNα2a plasmid was confirmed by restriction analysis. IFN-α2a secreted into the periplasmic space of E. coli was obtained, as shown in Western Blotting and SDS-PAGE. We can observe that the best growth temperature for the IFN-α2a was 37°C probably because at this temperature the bacteria presented a better growth and the production of aggregates was lower. The production yield was of ~0.1 μg/mL/mg.

Acknowledgements: This work was supported by FAPESP, São Paulo (Project n. 2007/59540-3) and by CNPq, Brasilia (Projects GD 141221/2011-9, PQ 303839/2008-2, PQ 3000473/2009-5 and DT 310512/2010/7).

References

P3

Anti-tuberculosis activity ofoleanolic and ursolic acid isolated from the dichloromethane extract of leaves from Duroia macrophylla

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Methods: Software P3 was used for analyzing 48 positive clones, methotrexate being used for gene amplification based on methotrexate resistance.

Results: Antibiotics resistance patterns of the isolate showed a high frequency of resistance to isoniazid (INH), rifampicin (RMP) and ethambutol (EMB), as well as to streptomycin (SM), kanamycin (Km), and cycloserine (Cs). The MICs were determined after 48 h of incubation at 37°C. The MICs of the isolate were as follows: INH 0.2 μg/mL, RMP 1 μg/mL, K m 2 μg/mL, Cs 1 μg/mL, and SM 8 μg/mL. The isolate was sensitive to ethambutol (EMB) and susceptible to ciprofloxacin (CFQ) and amikacin (AMK). The isolate was also resistant to streptomycin (SM) and kanamycin (Km).

Conclusions: The isolate showed a high level of resistance to isoniazid (INH), rifampicin (RMP) and ethambutol (EMB), as well as to streptomycin (SM), kanamycin (Km), and cycloserine (Cs). The MICs were determined after 48 h of incubation at 37°C. The MICs of the isolate were as follows: INH 0.2 μg/mL, RMP 1 μg/mL, K m 2 μg/mL, Cs 1 μg/mL, and SM 8 μg/mL. The isolate was sensitive to ethambutol (EMB) and susceptible to ciprofloxacin (CFQ) and amikacin (AMK). The isolate was also resistant to streptomycin (SM) and kanamycin (Km).

References

P4

Expression and characterization of mouse prolactin (mPRL) in CHO dfr6 cells

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Expression and characterization of mouse prolactin (mPRL) in CHO dfr6-cells

Methods: mPRL gene was obtained by PCR reaction from a pUC57 plasmid introducing the human signal peptide and the XbaI restriction enzyme sequence. After purification, the gene was introduced into the correct DNA sequence, Lipofectamine was used to introduce the plasmid pEDdc-mPRL into CHO dfr6-cells. For selection, cells were cultivated in alpha MEM medium without nucleosides with 10% dialyzed fetal bovine serum and antibiotics. Dot blot Western blotting with rabbit anti-mPRL antisera (NIDDK-NIH, USA) were used for analyzing 48 positive clones, methotrexate being used for gene amplification. Cultivation was carried out in serum free media CHO-S-SFM II
Involvometry Metal Ion Affinity cells/mL, which is much higher than Proceeding National Academy of Sciences Pouteria caimito In larvicidal activity assays, fourth stage 8(Suppl 4): 32(Pt 2) 4 larvae were organisms, among (Linnaeus, 1762), a mosquito that is the main target for the disease Insect feeding mobilizes a Aedes Dengue is a viral systemic disease caused by an arboviral of 2014, A. aegypti μ The A. sisalana In vitro A. aegypti Candida is a plant that is produced in several states in the Brazilian northeast Agave A. aegypti Structural variants of prolactin: occurrence and physiological The biological action of saponins 2014, experimental study, conducted at the Research Endocrinol "a. The disease is transmitted by Aspergillus brasiliensis The Lancet Infectious Diseases against larvae of Aedes aegypti mosquito, Endocr Rev Journal of chromatography A

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Acknowledgements: This work was supported by FAPESP, São Paulo (Project n. 2007/59540-3) and by CNPq, Brasilia (Projects PQ 303839/2008-2, PQ 3000473/2009-5 and DT 30152/2010/7).

Evaluation of antimicrobial potential and cytotoxicity of Pouteria venosa species
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Background: The use and research of medicinal plants in Brazil have as allies the great plant diversity and low cost associated with treatment [1]. The popular usage relates to the species of the family Sapotaceae which reports antibacterial, antifungal, antiviral, antineuralgic, antipyretic, anti-inflammatory, and others [2]. It was shown that the species of genus Pouteria, belonging to the Sapotaceae family, have proven biological activities, it is cited Pouteria camito with antioxidant and photoprotection activity against UVA and UVB, Pouteria ramiflora with antineoceptive, anti-inflammatory, antioxidant, photoprotection activity (against UVA and UVB), antimicrobial activity and toxicity against larvae of Brine Shrimp, and Pouteria torto with antimicrobial and antifungal activity [3]. Phytochemical studies performed with species of Sapotaceae have revealed the presence of alkaloids, flavonoids, terpenoids, benzenoids, phenylpropanoids and Lapachol, responsible for a large spectrum of biological activities [4]. Since these were proposed with effective biological activities of the genus Pouteria, belonging to the Sapotaceae family, and their constituents, and considering that the species of Pouteria venosa, known as "tuturubá, leiteiro, Bapeba, Sapota black", has not yet defined its antimicrobial activity, aimed to evaluate the antimicrobial and cytotoxic potential in view of the bacterial and fungal infections control.

Methods: In vitro experimental study, conducted at the Research Laboratory of Wound Care at Federal University of Alagoas. It was evaluated four fractions and crude extract parts of the species Pouteria venosa named as samples A, B, C, D and E. Antimicrobial activity was determined by microbial sensitivity tests, the method of disk diffusion and broth microdilution method for determination of minimum inhibitory concentration (MIC). It was used 13 strains of microorganisms, among them Gram-positive and Gram-negative bacteria and fungi like Candida albicans, Saccharomyces cerevisae and Aspergillus brasiliensis. Distributed by American Type Cell Collection. Was obtained to evaluate the cytotoxicity by means of Metiltetrazolium colorimetric method which investigated the cell viability of the samples tested.

Results and conclusions: The samples demonstrated antimicrobial activity in eight of the fifteen microorganisms evaluated in the disk diffusion test. Three of Gram-positive bacteria: Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, and five Gram-negative bacteria: Pseudomonas aeruginosa, Shigella flexneri, Proteus mirabilis, Acinetobacter calcoaceticus and Enterobacter aerogenes. Samples A, B and C showed high antibacterial potential front S. aureus, S. epidermidis, S. pneumoniae and constituting an effective alternative and economically feasible for the dengue vector combat. The outcomes of our research resulted in the patent.

References

Background: Dengue is a viral systemic disease caused by an arboviral of Flaviviridae family, affecting about a 700 thousand cases per year in Brazil. It is endemic in several regions such as Southeast Asia, South Pacific, East Africa, Caribbean and Latin America. The disease is transmitted by Aedes aegypti (Linnaeus, 1762), a mosquito that is the main target for the disease control through strategies ranging from the larval to the adult combat. The larvicides commonly used to combat the vector, besides being toxic, presents drop in larvicide efficacy since the larvicides commonly used to combat the vector, besides being toxic, are resistant to insecticides. Aedes aegypti is a mosquito that is the main target for the disease.
P. aeruginosa (inhibition zones ≥14). The results obtained by determination of the MIC of these strains showed that the fraction of sample C was considered with better antimicrobial activity, inhibiting microbial growth at concentrations between 1000 and 250 μg/mL-1. These findings corroborate with the literature, since the species Pouteria torta, Pouteria pallida e Pouteria ramiflora, also showed antimicrobial activity against these microorganisms [3,5]. The fungi evaluated were not sensitive to the Pouteria venosa samples. Sample C was considered non-toxic at the concentration of 200 μg mL-1, and is considered a promising route of pre-clinical in vivo. It emphasized the importance of the outcomes from the perspective of development and innovation of new therapeutic alternatives in infection control.

Acknowledgements: Brazilian Ministry of Science and Technology, Federal University of Alagoas, University Tiradentes.

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P7
Cytotoxicity activity of glycoalkaloids extract from fruits of Solanum lycocarpum A. St.-Hil.
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Background: Phytochemical analysis of the fruits of Solanum lycocarpum, popularly known as “fruta do lobo”, showed high concentrations of steroidal alkaloids, such as solanine and solamargine which are substances with potential cytotoxic activity [1]. The cytotoxic activity displayed by these compounds may be due to presence of sugar moieties (rhamnose). Since there is interaction of glycoalkaloids with plasma membrane cholesterol to lose its function, resulting in alterations in its permeability which induces cell death [2]. The aim of this study was to evaluate the cytotoxicity activity of the glycoalkaloids solamargine and solasoline purified from S. lycocarpum and commercial α-solamargine and solasonine from potato sprout toward cell lines MCF-7 (human breast adenocarcinoma cell line), B16 (murine skin) and 3T3 (normal mouse embryo fibroblasts) by MTT.

Methods: The cells lines were cultured at 37°C in a humidified atmosphere containing 5% of CO2 supplemented with 15% fetal bovine serum, using Dulbecco's modified Eagle's medium (Sigma). Penicillin (100 U/mL), streptomycin (100 μg/mL) and ciprofloxacin (100 μg/mL) were added to the medium to prevent bacterial growth. A stock solution (10 mg/mL) of glycoalkaloid was prepared in 5% DMSO. Solamargine, Solasoline purified from S. lycocarpum and α-Solamargine (Sigma) was directly diluted in the medium to obtain concentrations ranging from 5.75 to 5.75 μM. The final concentration of DMSO was less than 0.5% and it had no negative effects on the cell lines. Cells were trypsinized (0.15% trypsin and 0.02% EDTA), counted in a hemocytometer (2.5x105 cells/well), and incubated in a 96-well plate for 4 h. After addition of the alkalioc acid or vehicle dissolved in fresh medium, the cells were cultured at 37°C in a 5% CO2 atmosphere for 48 h, and cytotoxicity was analyzed by the MTT assay. For this purpose, 20 μL MTT/well (5 mg/mL in Hanks solution) were added to the 96-well plate, and the assay was incubated for 4 h under the same conditions. Thereafter, the plates were measured through 550nm wavelength analysis, using an ELISA reader. Treatments were compared to negative control (medium with 0.5% DMSO) and positive controls Dorxorubicin (0.258 μM) and actinomycin D (0.119 μM). Cytotoxicity was calculated by the formula: percent cytotoxicity = [(1-absorbance of experimental wells/absorbance of control wells)x100%. IC50 values were also determined. Data were analyzed by the Sisvar software.

Results and conclusion: For the cell line MCF-7 the IC50 of Solamargine, solasonina and α-solalnine were 13.55, 14.57 μM and 51.23 μM, respectively. The 3T3 cell line showed IC50 value of 20.11 μM, 13.47 and 49.79 of Solamargine, solasonina and α-solalnine, respectively. Tested compounds (solamargine, solasonina and α-solalnine) showed higher cytotoxicity to B16 with IC50 values of 34.075, 22.611 and 57.380 μM, respectively. Finally the tested glycoalkaloids showed pronounced cytotoxicity activity and may be further explored for the development of potential lead compounds active against cancer cells.

Acknowledgements: This study was supported by grants from Fundação de Amapá à Pesquisa do Estado do São Paulo (2012/06889-7) and CAPES through fellowship granted to FRB.

References

P8
Antibacterial evaluation silver of nanocomposite on Staphylococcus aureus and Escherichia coli
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Background: The treatment of bacterial diseases has become an increase public health problem, since the conventional antimicrobial substances lose their effectiveness due to the microbial resistance phenomenon [1]. Silver is a metal with potential antibacterial activity, and showed significant efficacy against several species of bacteria. The silver activity to prevent microbial growth is enhanced when the metal is found as nanoparticles [2]. Silver nanoparticles (NPAG) have their bactericidal and bactericidal activity based on their ability to kill or inhibit growth of bacteria. Pathogenic microorganisms, both gram positive and negative were sensitive to NPAG. The NPAG may be employed in conjunction with the biopolymers forming a nanocomposite that emerge as a promising therapeutic alternative. The xanthan gum is a biopolymer with interesting therapeutic activity based on their ability to kill or inhibit growth of bacteria. Pathogenic microorganisms, both gram positive and negative were sensitive to NPAG. The NPAG may be employed in conjunction with the biopolymers forming a nanocomposite that emerge as a promising therapeutic alternative. The xanthan gum is a biopolymer with interesting therapeutic activity based on their ability to kill or inhibit growth of bacteria. Pathogenic microorganisms, both gram positive and negative were sensitive to NPAG. The NPAG may be employed in conjunction with the biopolymers forming a nanocomposite that emerge as a promising therapeutic alternative. The xanthan gum is a biopolymer with interesting therapeutic activity based on their ability to kill or inhibit growth of bacteria. Pathogenic microorganisms, both gram positive and negative were sensitive to NPAG. The NPAG may be employed in conjunction with the biopolymers forming a nanocomposite that emerge as a promising therapeutic alternative.
spectrometric. The bacteria used in this study were *Staphylococcus aureus* and *Escherichia coli*. The evaluation of antimicrobial activity was assessed by the method of holes in wells, based on Mueller-Hinton agar. The abovementioned microorganisms were scattering from a microbial solution for 10⁶, and the holes were subsequently filled with 50 µL of a silver nanoparticles (test) solution and 50 µL of a xanthan gum alone (control) solution. All experiments were conducted in triplicate. An adaptation of the Kirby & Bauer technique using microbial suspension of 10⁶ bacteria was also used for observing possible inhibition zones after incubation in bacteriological incubator at 37°C for 24 hours.

**Results and conclusion:** The analysis of transmission electron microscopy showed NAG about 3 ± 0 mm in size medium. The atomic absorption spectrometry showed a concentration of 49, 2 mg/g of metal per gram of NAG. The antimicrobial activity of silver NAG showed inhibition zones of 12.2 ± 0.3 mm and 11.6 ± 0.5 mm for *Escherichia coli* and *Staphylococcus aureus*, respectively. Both microorganisms were evaluated at a concentration of 5 mg NAG. The disk diffusion assay indicated that the sensitive zone of inhibition was 9.6 ± 0.4 mm and 9.7 ± 0.3 mm respectively for these pathogens, the control xanthan gum was not able of inhibit the growth of any pathogens tested. The results, it was concluded that the nanocomposite studied in this work presented a broad spectrum of antimicrobial activity, suggesting that use can be a viable alternative for the treatment of pathogenic organisms.

**Acknowledgements:** Authors would like to express their gratitude to CNPq, CAPES and FAPITEC for the financial support and scholarships.

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**Background:** The hyperglycemia characteristic of diabetes can cause cellular and tissue damage due to the biochemical alterations that lead to the formation and accumulation of advanced glycation end products (AGEs) [1]. Aminoguanidine (AG) prevents the formation of AGEs by reacting with the initial glycation products, proving to be effective in improving proteinuria and vessel elasticity, in the prevention of diabetic retinopathy, and in the treatment of patients with diabetic nephropathy [2]. Guanidine derivatives have demonstrated various biological activities, such as antihypertensive and antidiabetic effects. It has been proposed that aminoguanidine and some of its derivatives (DAGs) may increase sensitivity to insulin [3]. The aims of this study were to investigate the effect of AG and two of its derivatives (DAG11 and DAG15) on the regulation of blood glucose and on the insulin tolerance test (ITT) in normoglycemic and diabetic rats, and the possible toxic effects of these derivatives.

**Method:** Wistar rats, 2 months of age, which had diabetes induced by Alloxan (40 mg/kg, i.v.), and their controls were administered the vehicle. At 21 days after the induction of diabetes, the animals were intraperitoneally treated for 7 consecutive days with saline (SAL), AG (10 mg/kg), DAG11 or DAG15 (10 mg/kg). On the 28th day the animals were anesthetized (ketamine, 80 mg/kg and xylazine, 12 mg/kg) and the ITT was performed with the administration of insulin (0.25 IU/kg, i.v.) for the analysis of insulin sensitivity between the experimental groups, there was an increase of 55% and 67% in the model used. Despite having no action on basal glucose, the diabetic animals treated with AG and DAG15 presented increased (p < 0.05) KITT. The results were expressed as mean ± SEM and were compared using ANOVA, with Student-Newman-Keuls post hoc test (p < 0.05). The toxicological evaluation was performed using human lymphocytes[4]. The study was approved by the Ethics Committee for the use of animals of the Federal University of Alagoas (LEAL 01/2012).

**Results and conclusions:** The basal blood glucose of the animals treated with SAL, AG, DAG11 and DAG15 presented no significant differences. In the normoglycemic animals, although no significant difference occurred between the experimental groups, there was an increase of 55% and 67% in the KITT of the AG and DAG15 rats, respectively, compared to the SAL animals. The diabetic animals treated with AG and DAG15 presented increased (p < 0.05) KITT (150% and 81%) compared to the SAL animals. No toxic effects were observed for any of the substances tested, at least not in the model used. Despite having no action on basal glucose, AG and DAG15 may be promising prototypes for diabetes treatment drugs, in view of their increased insulin sensitivity action in diabetic animals.

**Acknowledgements:** National Council for Scientific and Technological Development (CNPq Grant No. 563660/2010-4)

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BMC Proceedings 2014, 8(Suppl 4):P11

Evaluation of antifungal activity of glycoalkaloids from the Solanum lycocarpum St. Hil (Iboreia) in the cell membrane of dermatophyte of Trichophyton rubrum

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BMC Proceedings 2014, 8(Suppl 4):P12

Degradation of Aa and Bj chains from bovine fibrinogen by serine proteases of the Amazonian scorpion Brotheas amazonicus

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BMC Proceedings 2014, 8(Suppl 4):P12

Background: Proteolytic enzymes within venoms from different scorpion species belong to serine and metalloproteases class, and some isoforms of these enzymes show proteolytic activity over Aa and Bj subunits of fibrinogen [1-3]. Brotheas amazonicus is a non-lethal amazonian scorpion, belonging to a different group from Tityus genus, and there are scarce studies about its venom in literature [4]. Enzymes acting over fibrinogen can lead to the creation of more efficient antithrombotic drugs, and the low lethality of B. amazonicus venom is a potential factor for developing a better drug.

Material and methods: Venom was early incubated with metallo or serine proteases inhibitors (PMSF and EDTA), and proteolytic activity was evaluated by zymogram in SDS-PAGE using as substrate bovine fibrinogen. After electrophoresis, gel was rinsed with 2.5% Triton X-100. Gel was incubated in humid chamber for 24 hours at 37°C, in 0.1M Glycine pH 8.3 solution. Dye was prepared using 0.02% Comassie Blue R-250 solution. Proteolytic activity of B. amazonicus venom over bovine fibrinogen was tested, by mixing 200 μL of fibrinogen solution (2μg/μL) in 0.01M PBS pH 7.4 with 50 μg of B. amazonicus venom, for 12 hours at 37°C. After this process, no clots were observed, so 5μg of Bothrops atrox venom [5] was incorporated to the system, but also no clotting was observed, suggesting B. amazonicus venom had activity over fibrinogen, but with no clotting formation. Changes induced by B. amazonicus venom on bovine fibrinogen were evaluated by 12% SDS-PAGE electrophoresis stained with silver nitrate. Inhibition efficacy of fibrinogenolytic activity of B. amazonicus venom by anti-scroptic serum was tested, by adding different concentrations of anti-venom in 200μL of bovine fibrinogen (2μg/μL) plus 5μg of B. amazonicus venom. This system was incubated for 24 hours at 37°C, and after this process clotting induction by 5μg of B. atrox venom was performed.

Results and conclusion: Proteolytic activity of B. amazonicus venom over bovine fibrinogen was only inhibited by PMSF - specific inhibitor for serine proteases. B. amazonicus venom degraded bovine fibrinogen without fibrin clot formation, confirmed by clots absence when B. atrox venom was incorporated to the system. In SDS-PAGE electrophoresis of degraded fibrinogen, it was possible to detect that B. amazonicus venom degraded Aa and Bj subunits of fibrinogen, and anti-scroptic venom specific for Tityus species shows great neutralizing efficacy when 1:1 proportion, suggesting that B. amazonicus toxins show similar antigenic properties of serine proteases from Tityus genus venom. Results suggest a serine protease with bovine fibrinogen affinity, able to degrade different regions from this molecule unlike thrombin and with a high similarity of proteases from Tityus sp. Such characteristics, plus the fact that this venom has a low
toxicity, make these proteases inside *B. amazonicus* venom as candidates for antithrombotic drugs or even vaccines against scorpionic accidents.

**Acknowledgements:** Amazonas State Government, Brazilian Ministry of Science and Technology and National Council of Development in Research and Technology (CNPq)

**References**


**P13**

**Study of the biological potential in vitro extracts for Zeyheria tuberculosa (Bignoniaceae)**

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**Background:** The indiscriminate use of antibiotics, associated with the emergence of microorganisms human resistant pathogens to major classes of antibiotics, has caused many clinical problems in the treatment of infectious diseases. Plants used in order to medicinal purpose for treatment, healing and/or prevention is one of the oldest forms of medicinal practice of humanity [1]. Biomonitored studies are developed and refined, relating popular knowledge to realization of bioassays that confirm the therapeutic efficacy and the low toxicity, as the plants with efficacy proven for a particular purpose. *S. aureus* and *S. tuberculosa* have been used in various types of injuries and different stages of the healing process. Therefore, this study aims to develop, characterize and physically evaluate the effect of collagen films containing EFA on wound healing of second intention.

**Results and conclusions:** All tested samples showed moderately active against the strain of *Staphylococcus aureus* (ATCC 25923) using the agar diffusion method by the well technique with percentage of inhibition >25% and <75%. The *X*5 extract showed the most significant antimicrobial activity in MIC, inhibiting the strain growth of *S. aureus* with 1000 until 125 μg mL⁻¹ concentration. Identified the absence of toxicity in all samples, as the mortality percentage rate was ≤30% (CL50 < 1000 μg mL⁻¹). In the antioxidant test, all samples were considered inactive with CL50 > 200 mg/mL. Phytochemical studies previously performed with Z. *tuberculosa* showed the presence of isolated flavonoids justifying the antimicrobial activity found [3]. These results represent the primary indications security plant species for performing in-vivo bioassays with potential in the infection control.

**Acknowledgements:** Brazilian Ministry of Science and Technology, Federal University of Alagoas, Center for Information Technology (CIT-PROPEP), Research Laboratory of Wound Care, Laboratory of Biomaterials, Tiradentes University.

**References**

Results and conclusion: The FEFA50 and FEFA100 films formed transparent, continuous, homogeneous and easy to use. With the addition of EFA50 EFA100 and movies, these had the thickest, stretching, tension and lower Young’s modulus. In biological assay (scarring) not macroscopic signs of abscesses or hypertrophic scarring in either group were observed. At 7 days, the IWC observed in FEFA50 was higher compared to the control group, while at 14 days, and FEFA50 FEFA100 these indices showed increased compared to control and movies only COL. FEFA50 FEFA100 and promoted increased neutrophil infiltration in 3 days, but the lifocítico infiltrate was reduced to 14 days. It was also observed the early development of granulation tissue, re-epithelization of dermal annexes and better collagen organization. This study suggests that films of collagen as an additive containing the essential fatty acids, particularly FEFA50, are promising for use as roofing or wound dressings for dermal enhancement of scar repair process biomaterials.

Acknowledgements: Authors would like to express their gratitude to CNPq, CAPES and FAPITEC for the financial support and scholarships. References


P15
Development of fermented and flavoured kefir milk
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BMC Proceedings 2014, 8(Suppl 4):P15

Kefir is a dairy beverage, obtained from lactic acid and alcoholic fermentation of its grains, which resembles yoghurt in its flavour, aroma and consistency. It is composed of approximately 15-16 lactobacilli, approximately 7-9 streptococci/lactococci, 8 yeasts and 2 acetic bacteria (acetobacter). The fermenting action of Kefir bacteria and yeasts increases the biological value of milk, increasing synthesis of the B group vitamins, which help in the digestive process. The abundance in Calcium, Phosphor, and Magnesium is another of Kefir’s characteristics, as for all other dairy products. It is an easily digested product that eliminates harmful bacteria and yeasts from the intestine, increases beneficial and protective bacterial population, and as a probiotic food has many therapeutic applications. This study had as its objective the production of fermented milk based on Kefir, when different flavours were tested, to have it sensorial tested to find the one with the highest acceptance. Firstly, an analysis of the total coliform content was performed, followed by a microbiological quantification analysis of the colony-forming-unit per gram (CFU/g), to ensure the quality of the product to be offered to tasters. Secondly, the Kefir milk was flavoured with 4 different flavours (passion fruit, strawberry, grape and mango), where juice from concentrate pulp and fruit bits were added to the milk. Kefir, when different flavours were tested, to have it sensorial tested to find the one with the highest acceptance. Firstly, an analysis of the total coliform content was performed, followed by a microbiological quantification analysis of the colony-forming-unit per gram (CFU/g), to ensure the quality of the product to be offered to tasters. Secondly, the Kefir milk was flavoured with 4 different flavours (passion fruit, strawberry, grape and mango), where juice from concentrate pulp and fruit bits were added, used to 60 tasters (students, professors and staff from UNISOS), according to the model for rank-preference test (164/IV/ Testes Efetivos - Testes de preferência' Effective Test - PreferenceTests) according to the Instituto Adolfo Lutz. This study suggests that films of collagen as an additive containing the essential fatty acids, particularly FEFA50, are promising for use as roofing or wound dressings for dermal enhancement of scar repair process biomaterials. References


P16
Evaluation in gene polymorphic α-actin3 at blumenau in individuals for better performance in sport
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BMC Proceedings 2014, 8(Suppl 4):P16

Background: 3 α-actin (α-ACTN3) isoform is characteristic of fast fibers, expressed only in type II fibers, which plays structural and regulatory functions in cytoskeletal organization and muscle contraction. Stabilizes sarcomeres during fast and forceful contraction of muscle fibers used during sports activities that require explosion, such as race. Gene polymorphism was identified in the α-called ACTN3 R577X results in exchange of C to T at nucleotide position 1747 of exon 16, that is, a mutation resulting in the conversion of arginine (R) allele by premature termination codon (allele X) at amino acid 577. This change leads to lack expression of α-ACTN3 in individuals homozygous for the allele X, which supposedly causes decrease in muscle mass and affect the performance activities that require muscle contractions with high levels of strength and / or speed. The deficiency of α-ACTN3 does not result in a pathological phenotype as muscular dystrophy or myopathy. We analyzed the frequency and estimated genotype α-ACTN3 in individuals from Blumenau to guide them in the performance physical activity.

Methods: For analysis, was used for DNA extraction blood sample by phenol-chloroform method, followed by PCR and Real-Time results analyzed by the graph generated. Protocol was used reagents as recommended by Applied Biosystems®.

Results and conclusions: We obtained the number of 23 subjects, 12 females and 11 males, aged 22-60 years. The distribution of genotypes was 39.1% for genotype RR, RX, and 47% to 13% to XX. The result for females was 50% for RX, followed by 33.3% 16.7% for RR and XX. For males was obtained a number of 45.5% for RR, 45.5% for RX and a small number for XX 9.1%. The result of the polymorphism has examined the possibility of directing sports practices specific to individuals according to their genetic sensitivity training. For individuals that express α-ACTN3 gene (genotype RX or XX) may have advantage in ways that require explosion and muscle strength when compared with individuals with XX genotype.

Acknowledgements: Laboratório Genolab, Blumenau/SC.

References


P17
PncA gene expression and prediction factors on pyrazinamide resistance in Mycobacterium tuberculosis
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BMC Proceedings 2014, 8(Suppl 4):P17

Background: Mutations in the pyrazinamide (PZA) coding gene, pncA, have been considered as the main cause of pyrazinamide (PZA) resistance in Mycobacterium tuberculosis. However, recent studies suggest there is no simple mechanism of resistance to PZA. The pyrazinamide (PZA) efflux rate is the basis of the PZA susceptibility Wayne test, and its quantitative measurement has been found to be a highly sensitive and specific predictor of PZA resistance. Based on biological considerations, the POA efflux rate is directly determined by the PZA2ase activity, the level of pncA expression, and the efficiency of the POA efflux pump system. This study
analyzes the individual and the adjusted contribution of PZase activity, pncA expression and POA efflux rate on PZA resistance. Methods: Thirty M. tuberculosis strains with known microbiological PZA susceptibility or resistance were analyzed. For each strain, PZAse was recombinantly produced and its enzymatic activity measured. The level of pncA mRNA was estimated by quantitative real time PCR, and the POA efflux rate was determined. Mutations in the pncA promoter were detected by DNA sequencing. All factors were evaluated by multiple regression analysis to determine their adjusted effects on the level of PZA resistance. Results and conclusions: Low level of pncA expression associated to mutations in the pncA promoter region was observed in pncA wild-type resistant strains. POA efflux rate was the best predictor after adjusting for the other factors, followed by PZase activity. These results suggest that tests which rely on pncA mutations or PZase activity are likely to be less predictive of real PZA resistance than tests which measure the rate of POA efflux. This should be further analyzed in light of the development of alternate assays to determine PZA resistance.

References

P18
Brain-Computer Interface (BCI) combined with Virtual Reality Environment (VRE) for inferior limbs rehabilitation in post-stroke subjects
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BMC Proceedings 2014, 8(Suppl 4):P18

More than 10 million people in the world live with some kind of motion handicap caused by a Central Nervous System (CNS) dysfunction. Stroke is the major cause of this disability in the adult population. Due to the increase of elderly people in the world’s population, such index tends to increase. The proposal of this research is to provide a tool for rehabilitation, useful for subjects that suffer from lower limbs movement handicap, acquired by stroke. This tool carries a 3D Virtual Reality Environment (VRE), which emulates the movement of a heath person, using the immersion of the subject through an avatar. The subject’s brain generates commands to control the avatar while conducting the rehabilitation process. The brain waves are captured by an Electroencephalography (EEG) equipment, that information is sent to a computer for processing, which sends the information to the virtual environment to control the avatar, completing, thereby, the Brain-Computer Interface (BCI) tool. This system asks two different tasks for the subject: move the left or right leg, stimulating brain’s areas responsible for each one of those motor activities, implying thereby, in the rehabilitation process. The VRE provides, for the subject, a feedback of his/her motion intentions. The system works as an attractive environment, which motivates the subject to use it, and, at the same time, is useful to evaluate his/her treatment evolution.

References

P19
Production of virulence factors by species of Candida albicans isolated from urine culture
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BMC Proceedings 2014, 8(Suppl 4):P19

Background: The candiduria can be defined as the observation of yeasts in urine examination [1]. The ability of yeast to adhere to, infect and cause the disease in the set is defined as a potential virulence or pathogenicity [2]. Virulence factors expressed by Candida species may vary depending on the type of infection, the location and the stage of the infection, and the nature of the host response. The production of proteases and phospholipases enzymes and biofilm production of some virulence factors are expressed by the Candida species that degrade host tissues [3]. Thus, this study aimed to evaluate the production of proteinase, phospholipase and biofilms of Candida species isolated from patients at the university hospital in Dourados - MS.

Methods: In this study we used samples of Candida albicans from samples of urine cultures of patients admitted to the University Hospital of Dourados, from June 2010 to June 2012. The yeasts were isolated and identified according to the conventional method. The phospholipase production was verified by the method of egg yolk agar plate, the proteinase production by the method of agar containing bovine serum albumin by the method of biofilm and adherence to polystyrene. Tests to evaluate the enzymatic activity were performed in duplicate in three different times. During the study period, 24 yeasts were obtained from patients hospitalized.

Results and conclusions: The samples analyzed in the phospholipase test, two (8.33%) were moderate with enzymatic activity and 23 (91.66%) showed no enzymatic activity. The proteinase test, 15 (50%) samples showed strong enzymatic activity. Seven samples (41.66%) showed moderate enzyme activity and two (12.5%) samples showed no enzymatic activity. The biofilm test, 22 (91.66%) samples showed biofilm formation and two (8.33%) did not showed biofilm formation. As shown, most of the samples showed no enzymatic activity in phospholipase production, but most isolates showed proteinase production and biofilm indicating the high virulence of these isolates. The values found, refers to the importance of the studies since the action of these enzymes in the host organism.

Acknowledgements: The Universidade Federal da Grande Dourados and FUNDCT-MS for financial support.

References

P20
Lipid-binding allergens from Dermatophagoides pteronyssinus mites extract isolated by liposomes
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Background: House dust mites (HDM), including Dermatophagoides pteronyssinus (Dp) and Dermatophagoides farinae (Df), are one of the commonest aeroallergens worldwide eliciting allergic manifestations [1]. Allergic phenomenon is tightly associated both with the mites themselves and with ligands derived from mite-associated bacterial/fungal products. Some Dpt allergens belong of lipid-binding proteins, including...
Der p 2 (protein with myeloid differentiation protein 2 (MD-2) related lipid recognition domain), Der p 7 (structurally homologous to lipid binding protein family) and Der p 13 (lipid transporter molecule) [2]. Thus, in this study we aimed to evaluate the ability of liposomes to adsorb lipid-binding proteins from Dermatophagoites pteronyssinus mites extract.

Methods: Liposomes were prepared by ethanolic injection using dipalmitol phosphatidylcholine/cholesterol or dipalmitol phosphatidylethanolamine/cholesterol diluted in ethanol. To Poll-down assays, Dpt allergens were incubate with liposome preparations and then washed three times with PBS solution. The adsorbed proteins on liposome surface were removed by SDS treatment and then analyzed by SDS-PAGE. Additionally, the lipid-binding proteins were analyzed by ELISA to evaluate the immunoreactivity of Dpt-specific IgE and IgG1 antibodies [3].

Results and conclusions: Several proteins ranging from 21 to 205 kDa were enriched in poll-down assays, including a polypeptide with high molecular weight (>205 kDa). In addition, Dpt-adsorbed on liposomes were reactive to IgE and IgG1 antibodies from allergic patients analyzed by ELISA. Further analysis using mass spectrometry will be conducted to identify the proteins adsorbed on liposome surface. Liposomes might be used to produce enriched fractions of lipid-binding proteins from Dermatophagoides pteronyssinus for further studies in allergic diseases.

References

P21
Bioprospecting of Ocotea minarum (Laurales: Lauraceae) by ethanol extract in control of strains of gender Candida
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Background: The Ocotea minarum is a plant native to Cerrado and found in abundance in this Brazilian biome. Belonging to the family Lauraceae is popularly known in the region as “Shin-broom”. It is a medium-sized tree, occurring in several areas, among them, the Mato Grosso do Sul. Due to the presence of some compounds such as tannins, steroids, triterpenes and flavonoids in its shell, it may have antifungal activity [1]. There are records of the popular use of its bark in the form of infusion and aqueous extract for vaginal congestion, edema, necrosis, degeneration or inflammation. On the other hand, symptoms of toxicity, following 14-days treatment with PIC were not observed. Indeed, there was no significant change in the animals weight and pharmacological potential. Quim Nova 2002, 25(3):449-454.

Antifungal activity detection of mangrove plants extracts against Candida albicans

Acknowledgements: To Federal University of Dourados Region (UFGD) and FUNDECT-MS.

References

P22
In vitro antineoplastic activity in triple-negative breast cancer cell line and in vivo
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Background: Triple negative breast cancer (TNBC) is a heterogeneous subgroup (ER-, PR, and HER2-) of invasive breast cancer, associated to poor prognosis, partially due to its resistance to available drugs. Therefore, it is imperative to discover new treatment options for the disease. In this context, we have synthesized and screened novel naphthoquinone-derived drugs (patent-protected), rationally designed to act through multiple pathways to avoid tumor chemoresistance.

Methods: Drugs antineoplastic efficacy (AE) was assessed in the cloudin-low TNBC cell line, MDA-MB231, by cellular metabolic viability (CMV) and ICSO calculation (MTT method: GraphPad Prism version 5.1). Drugs toxicity was studied in healthy mice, following the Guideline 423 (for test of chemicals) of OECD; blood cells and tissues were analyzed by a Pathologist. Computational molecular dock studies were conducted to investigate the molecules tridimensional conformation and binding energy to toposisomerase 2 (TOPO) and P53 (Autodock Vina software).

Results and conclusions: We screened the AE of 43 novel drugs in MDA-MB231 (CMV≤50% with 7 drugs). Of these, the most promising drugs PIC20 (IC50 1.38x10^-6 M; CMV = 10%) and PIC21 (IC50 0.08x10^-6 M; CMV = 30%) showed significantly higher AE than cisplatin (IC50 1.56x10^-6 M; CMV=90%), doxorubicin (IC50 1.76x10^-6 M; CMV = 62%), and paclitaxel (IC50 5.05x10^-6 M; CMV = 80%). None of the treated mice died, neither demonstrated symptoms of toxicity, following 14-days treatment with PIC. Indeed, there was no significant change in the animals’ weight and general activity/behavior. Major organs showed no significant morphological changes, congestion, edema, necrosis, degeneration or inflammation. On the other
hand, there was a 48.98% decrease in their hematocrit count. Finally, based on the crystalline structure of proteins deposited on PDB (1QZR, TOPO; 1E7U, PI3K), and PIC20 and PIC21 tridimensional structures, we concluded that the novel molecules bind to the ATP domain of the proteins with similar interaction energy (E) than the TOPO - Doxorubicin (E = 5.6) and Etoposide (E = 5.7) - or PI3K Inhibitors - LY294002 (E = 9.5) and Wortmannin (E = 8.8): PIC20: E = 5.3 and -8.9; PIC21: E = 5.7 and -8.2, for TOPO and PI3K, respectively. In conclusion, we present novel and potentially safe drugs to treat TNBC, in an innovative and economically viable approach.

Acknowledgements: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Espírito Santo (FAPES).

**P23**

**Purification of prothrombin complex proteins from human plasma in anion exchange resin using pseudoaffinity chromatography**

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**Methods:** Plasma was directly applied to the ANX Sepharose FF column, previously equilibrated with citrate buffer 25 mM containing NaCl 85 mM and CaCl₂, pH 6. The unbound proteins were washed out with the same buffer. After a washing with citrate buffer containing NaCl 200 mM, elution was carried out in the same buffer with a linear calcium gradient from 2.5 mM to 25 mM. Three different buffers were tested: citrate, Bis-Tris and MES. Finally, column was washed with citrate buffer containing 500 mM NaCl. Chromatographic fractions were analyzed by: activity of Protein C using the chromogenic method as representative of the prothrombin complex proteins, protein content by the Bradford method, and SDS-PAGE.

**Results and discussion:** Protein C eluted within the CaCl₂ concentration range studied (2.5 to 25 mM). Using NaCl, this protein eluted only with a much higher salt concentration (> 250 mM), confirming that the mechanisms of elution with these 2 salts are different. It was also observed that a wash with 200 mM NaCl improved the purification. Therefore, the method combines conventional anion exchange with pseudoaffinity chromatography. Chromatograms of the experiments presented different profiles: citrate buffer presented 2 peaks, while Bis-Tris and MES presented only one, indicating that citrate buffer led to a better separation of the proteins. The SDS-PAGE gels showed that contaminant proteins coeluted with the prothrombin complex proteins, but in comparison to purifications profiles: citrate buffer presented 2 peaks, while Bis-Tris and MES presented different elution of proteins by variation of calcium concentration is called chromatography of pseudoaffinity [2]. In this study we exploit this property of the vitamin dependent coagulation factors to develop a new method for purification of prothrombin complex proteins from human plasma using an anion exchange resin.

**Acknowledgements:** NERORIO, FUNCAP and CNPq for financial support.

**References:**


**P24**

**Cloning and expression of the NS1 protein of dengue virus in a prokaryotic system**

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**Background:** Dengue virus nonstructural protein 1 (NS1) is a highly conserved glycoprotein involved in the production of infectious virus and in the pathogenesis of dengue disease [1]. Serum or plasma DENV NS1 level has been found to correlate with viremia titer and disease severity. It can be found in the peripheral blood circulation for up to 9 days from illness onset, but can persist for up to 18 days from illness onset in some cases. Thus NS1 detection offers a larger window of opportunity for diagnosis of dengue compared with virus isolation, RT-PCR or NASBA [2]. In this context, the aim of this work has been the establishment of conditions for expression and purification of the recombinant protein NS1 of dengue virus serotype 2 produced in E. coli for further development of a serological diagnostic method at low cost.

**Methods:** The gene encoding the NS1 protein was amplified through RT-PCR and subcloned into the expression vector PET-28a for expression of the recombinant protein. The corresponding recombinant plasmid was transformed into the NS1 protein in bacteria E. coli strain BL21 (DE3) and the clones obtained were expanded and induced with different concentrations of IPTG at 37°C for analysis of the expression and purification of recombinant proteins. After lysis of the bacterial culture, the protein fractions collected (supernatants and precipitates) were analyzed by SDS-PAGE and immunoblotting with monoclonal antibodies (anti-His6).

**Results and conclusions:** The SDS-PAGE and immunoblotting analyses revealed the presence of proteins of approximately 45 kDa in the precipitates. This result indicated the formation of inclusion bodies, which is commonly found for proteins produced in prokaryotic system. Thus, one of the perspectives of this work is to standardize methods for the obtaiement of soluble proteins under suitable conditions for immunological studies.

**Acknowledgements:** RENORIO, FUNCAP and CNPq for financial support.

**References:**


**P25**

**Cell therapy in renal ischemia/reperfusion experimental model using recombinant G-CSF**

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**Methods:** Male Wistar rats (n = 18), 200g approx. (CEUA050/2013), divided into 3 groups (6 animals each) : control group (C) 5% glucose solution (solvent) groups treated with G-CSF at a dose of 10 (G10) and 50 (G50) mg / kg / per 5 days. After treatment, the rats were placed in metabolic cages for urine...
collection and obtaining urine volume. Values were obtained from creatinine, proteinuria, urea and number of circulating leukocytes. The results were expressed as mean ± SEM. The averages of values between groups were calculated using one - way ANOVA followed by post hoc Fischer for comparison between different groups.

Results: A significant increase in the number of circulating leukocytes in animals treated with G-CSF (C = 9687 ± 899 / mm3; 14375 ± 1697/mm3 G10, and G50 = 19670 ± 1663/mm3, p < 0.05). There was not a significant increase in urine volume after 24 hours treatment with G-CSF. There was no significant difference between the values of creatinine clearance, proteinuria and urea, among groups C, G10 and G50.

Conclusion: There was no impairment of renal function in animals treated at doses of 10 and 50 mg / kg / per 5 days.

Financial support: FAPES

References

P26
Identification of Klebsiella pneumoniae that produces β-Lactamase blaKPC gene
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Background: Resistance of Klebsiella pneumoniae to carbapenems is mainly associated with acquired carbapenem-hydrolyzing β-lactamases [1]. These β-lactamases can be metallo-β-lactamases (IMP, VIM, VIM, expanded-spectrum oxacillinases (OXA-48), or Ambler class A enzymes (NMCA, IMI, SME, GES, and KPC) [1,2]. The most common class A carbapenemases in K. pneumoniae are the K. pneumoniae carbapenemases (KPCs) [2]. Adequate detection of carbapenemases resistance genes, such as blaKPC, is crucial for infection control measures and appropriate choice of antimicrobial therapy [3]. In Dourados/MS there are no reports on the monitoring of KPC-producing multidrug-resistant strains of clinical interest. The aim of this study was to identify the gene blaKPC in strains of Klebsiella pneumoniae.

Methods: The samples were collected during February-May/2012 from patients attended at University hospital in Dourados/MS and identified by classical bacteriological methods. The strains were most frequently obtained from the urinary tract, vaginal secretion, nasal and rectal swab. Antibigrams were realized by using the disk-diffusion method on Mueller-Hinton agar (Bio-Rad Laboratories), and susceptibility break points were determined as previously described and interpreted as recommended by the Clinical and Laboratory Standards Institute [4]. All strains with reduced susceptibility to imipenem or meropenem (MIC ≥ 2 µg/mL) were screened for carbapenemase production by the modified Hodge test. The presence of gene coding for KPC, were assessed by PCR as described by Cuzon et al., (2010) [5].

Results and conclusions: From October 2012 to April 2013, 26 strains of K. pneumoniae carbapenemase from patients were isolated. Among the wards, those that had a higher incidence of samples were recovered from intensive care units (ICUs) of the hospital, probably due to immune deficiency of patients, submitted to invasive therapeutic procedures. The strains identified as producing carbapenemases were evaluated by PCR amplification using primers specific for blaKPC gene. Five K. pneumoniae carbapenemase strains were positive in PCR. Eighteen strains were positive to modified Hodge test, but were PCR negative. This profile difference could be due to the presence of other classes of carbapenemases. Thus, these eighteen strains of K. pneumoniae carbapenemase need to be tested for the presence of β-lactamases such as IMP, VIM, OXA, and NDM-1.

Acknowledgements: This work was supported by the Foundation Support the Development of Education, Science and Technology of the State of Mato Grosso do Sul (FUNDECT, 05/2011 and 04/2012).

References

P27
Evaluation of the cytotoxic and mutagenic potentials of ethanolic extract of Baccharis gaudichaudiana DC. (Asteraceae).
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Background: Evidenced by their traditional use and through scientific studies, natural products have been important participants in drug discovery, providing novel structures that can be used as potential drugs. Among the plants investigated to date, those of the Baccharis genus are important sources of natural medicinal products. Comprising more than 500 species distributed throughout the North and South American continents, this genus is commonly used in folk medicine as antipyretic agents, antirheumatic and to control hyperglycemia. B. gaudichaudiana, popularly known as “carquejeira-doce” is found in grasslands, Brazilian savannas, and less pronounced in tropical humid lowland. Many secondary metabolites have been characterized and isolated, such as flavonoids, diterpenes, tanins, saponins and essential oils.

Methods: In order to evaluate the cytotoxic and mutagenic potentials of the ethanolic extract of B. gaudichaudiana, the Somatic Mutation and Recombination Test (SMART) in wing somatic cells of Drosophila melanogaster, was performed employing two genetic markers located on the left arm of chromosome 3: multiple wing hairs (mwh, 3-0.3) - a homozogous-viable recessive mutation that produces multiple thichomes per cell instead of one trichome; and flare3 (Flr3, 3-38.8) - a recessive mutation that produces flare-shaped wing hairs. Three D. melanogaster strains were used: 1) multiple wing hairs; y; mwh j; 2) flare3: Flr3/In (3LR)TM3, ri pepse (f3) 89Aa bx34ee Bd5; and 3) ORR: flare-3: ORR; Flr3/In (3LR)TM3, ri pepse (f3)89Aa bx34ee Bd5. Two different crosses were carried out: Standard (ST) cross and High-Bioactivation (HB) cross. For the ST cross, virgin flare-3 females were mated with mwh males. For the HB cross, which is characterized by an augmented level of CYP 450, virgin ORR, flare-3 females were mated with mwh males. Third instar larvae obtained from both crosses were fed chronically (48 h) with ethanolic extract of B. gaudichaudiana (5, 10, 20, 40 or 80 mg/mL). Ultrapure water (MilliQ) was used as negative control and urethane (10 mM) as positive control.

Results and conclusions: Results from both crosses were rather similar. Concentrations between 5 to 40 mg/mL of the extract did not show cytotoxic activity. On the other hand, concentrations higher than 80 mg/mL significantly reduced the survival rates. The concentrations tested are not mutagenic in ST cross. However in HB cross, at higher concentration (40 mg/mL), the frequency of mutant spots was statistically increased. These results suggest that, under these experimental conditions, the ethanolic extract (at higher...
concentrations) might have compounds that, when metabolized by CYP450, can be mutagenic, and at high concentrations (>80 mg/mL), cytotoxic. However, further studies are needed to identify the constituents of this extract and confirm the efficacy and/or risks of its use, as well as to encourage the rational use of natural resources.

Acknowledgements: FAPEMIG; CNPq; UFU; CAPES.

References


P28

Exosomes as a predictor tool of acquired resistance to melanoma treatment

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BMC Proceedings 2014, 8(Suppl 4):P28

Background: Exosomes are small endosome-derived vesicles ranging from 30-120 nm in diameter secreted from cells through exocytosis and, in cancer context, are able to prepare metastasis niche and suppress host’s immune system. Despite these pro-tumorigenic characteristics, exosomes can be useful for several Biotechnological applications such as biological anticancer vaccines, cancer diagnosis and prognosis. Due to its property of carrying parental cell’s protein and active nucleic acids there is an increasing interest to use exosomes to track cancer metabolism alterations in different conditions, for example, during drug administration [1].

One of the most common types of cancer that develops drug resistance during treatment is Melanoma. The majority of drugs developed against melanoma targets the oncogenic mutations in BRAF (BRAF V600E) that are present in nearly 60% of cutaneous melanoma tumors. Resistance to BRAFV600E inhibitors are classified in two main groups, Intrinsic Resistance and Acquired Resistance, the second is developed after treatment starts that happens mainly by MAPK reactivation and RTK upregulation [2].

Various methods are being studied to overcome drug resistance in melanoma, but there is a need to detect when the resistant mechanism is arising to orientate which approach to choose. This early detection has demonstrated to be a difficult task especially during advanced stages where there are several metastatic niches.

With this aim, our research project was designed to create a method to follow tumor’s status through melanoma-derived exosomes. As most types of melanoma overexpress MART-1 we checked its expression, as well as PDGFR-B and others proteins, in PLX4032 (V600E mutated BRAF inhibitor) resistant and sensitive melanoma cell lines to start to understand their potential to be exosomes-associated proteins in the development of acquired resistance detection method[2].

Methodology: Cells were cultured in exosome depleted media by FBS ultracentrifugation during 70 min at 100.000xg. Sensitive (M229, M238, M249) and resistant (M229AR, M238AR, M249AR) melanoma cell lines to PLX 4032 ( vemurafenib) were cultured until 75% of confluence and the supernatant was processed to obtain the exosomes following 15000 g - 10 min, 17000 g - 15 min and 160,000xg - 1 hour.

Results: To confirm this protocol it was done a Transmission Electron Microscopy (TEM), followed by Flow Cytometry against CD63, an exosome molecular marker. TEM showed an uniform size for exosomes ranging from 60-90 nm in diameter and Anti-CD63 Flow Cytometry showed a positive population of 99.1%. Western Blots were done with the cells lines cited previously demonstrating that the exosome’s protein content of MART-1, PDGFR-B, FGFR3 and others, corresponds to the parental cell.

Conclusion: Preliminary results show that exosomes can be useful tools to predict cell’s metabolism. Here we present that protein differential expression between sensitive and resistant cell lines can be detected by exosome profile such as PDGFR-B increased expression in resistant lines.

References


P29

The green synthesis of gold nanoparticle using extract of Virola oleifera

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BMC Proceedings 2014, 8(Suppl 4):P29

Background: The green synthesis consists of an environmentally friendly method of producing gold nanoparticles (AuNPs). Physical and chemical syntheses have energy intensive and may involve toxic chemicals, while biological techniques are cost-effective, clean, non-toxic and environmentally appropriate.

Virola oleifera is widely used in folk medicine. The bark of the trunk when scraped produce a resin rich in phenolic compounds, which is used against bleeding hemorrhoids, cramping, and also has healing action of chronic wounds and ulcers, diarrhea and counter hemoptysis. It is known that the presence of phenolic extract gives the reducing action, but studies need to be done to understand what substances are involved and what the mechanisms of formation of newly synthesized nanoparticles.

Thus, the aim of this study was to describe a new route for the synthesis of AuNP’s using resin of Virola oleifera with future application in nanoscience from the synthesis of nanoparticles for applications as nano biosensors.

Methods: To check the effect of variables on conversion of the reaction, as well as finding the conditions that maximized the synthesis of nanoparticles, one factorial design (3 2) with 3 levels and 2 variables was done. The concentration of the reducing agent (0.5, 1.0 and 2.0 mL), and the synthesis time (5, 10 and 15 min). These intervals were defined based on the literature.

To prepare nanoparticles, was used the gold precursor solution (HAuCl 4 with 2.5 × 10 –5 M) and as reducer agent the resin lyophilized of Virola oleifera (1mg/mL), both diluted in distilled water. Based on the experimental design, the solution of the reducing agent was added to the gold solution and stirred for predefined times.

AuNP’s samples were collected after the synthesis step and had their optical properties assessed by spectrophotometry UV-visible (SHIMADZU).

The size and morphology of AuNP’s were examined by transmission electron microscopy (JEM-1400, JEOL Inc, USA).

Results and conlusions: The absorption spectra of UV-Vis showing that the synthesis resin of Virola oleifera leads to the formation of nanoparticles with different optical properties according to the synthesis time and concentration of reducing agent.

The results of the electronic spectra of the solutions obtained and the analysis in the transmission electron microscope showed the difference in the absorbance spectrum of the particles. It was observed that the concentration of the reducing agent was significant in the synthesis process, and that the absorbance peaks were found in the highest concentrations of reducing agent, with consequent increase in nanoparticle size.

References


Characterization of cytotoxic activity of compounds derived from anacardic acid, cardanol and cardol in oral squamous cell carcinoma

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BMC Proceedings 2014, 8(Suppl 4):P30

Background: Cancer is the second leading cause of death worldwide, and oral cancer ranks tenth among all types [1]. Chemotherapy, radiotherapy and surgery are current therapeutic options; however, these are not fully efficient. Permanent functional impairment and aesthetic scars are frequent [2]. In this scenario, it is crucial to find therapeutic alternatives, including those derived from the flora, which currently provides about 1/3 of all new medicines. Anacardic acids, cardanols and cardols are the main constituents of the cashew nut shell liquid (herein referred to as "LCC") and together, account for 90% of its composition. The liquid is an industrial by-product, with low economic value prior to processing. The nut, the proper fruit from the plant *Anacardium occidentale*, is edible. Anacardic acids, cardanols and cardols are made of a phenolic ring connected to a long side chain (usually C15H31-n) that can bear several to numerous substituents. Additionally, a methyl group can be found in the phenolic ring [3]. Apart from current industrial uses, it has been demonstrated that some of these compounds may exert microbicidal and anti-oxidative activities. Anacardic acid has been shown to be cytotoxic to lung, liver and gastric tumor cells through epigenetic mechanisms by inhibiting histone acetyl-transferases (HATs) [4] and in a caspase-independent manner [5]. However, given the possible molecular diversity obtained from LCC constituents, not all distinct LCC derivatives have yet been fully analyzed or characterized.

Aim: The aim of the present study was to screen for compounds with cytotoxic activity in oral cancer cells and characterize the observed effect.

Methods: Constituents of LCC were extracted, purified and subjected to chemical reactions to generate new compounds. Oral squamous carcinoma cells (OSCC-3) were treated with the parental and derived compounds (total of 8) at 25ng/mL for 24h, 48h and 72h, as well as with staurosorpine (300nM) and ethion (diluent of compounds); as positive and negative controls, respectively. Cytotoxicity and cell viability were measured by spectrophotometry and crystal violet assays. To investigate dose-dependency, treatment with compounds with promising results was additionally carried out, at concentrations of 0, 5, 10, and 25ng/mL, and cell viability was measured. In order to identify the type of cell death, DNA fragmentation studies and Western Blot for caspases were performed.

Results: Of the eight compounds tested, four showed initial cytotoxic activity at 25ng/mL, at all incubation times analyzed. When tested for dose-dependency, two compounds induced, at concentrations between 10 and 25ng/mL, a marked decrease in cell viability, which dropped from approximately 70% to less than 20%. DNA fragmentation assay showed that one compound induced apoptosis, whereas its saturated counterpart did not. These results were further expanded by western blot analyses.

Conclusion: Compounds derived from LCC have considerable cytotoxic activity towards oral cancer cells. Because of their versatility, it is possible to identify new therapeutic motifs that may mediate these effects. In this light, new therapeutic agents may be developed from the compounds tested.

Acknowledgements: Capes, CNPq and DPP/ UnB.

References

C.L.O. low concentrations with significant growth inhibition in vitro of cell lines derived from solid tumors [5].

Acknowledgements: Brazilian Ministry of Science and Technology, Federal University of Alagoas, Center for Information Technology (NIT-PROPEP), Research Laboratory of Wound Care, Laboratory of Biomaterials, University Tiradentes.

References

P32
Antifungal activity of essential oil of Amyrisbalsamifera against Cryptococcusneoformans
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BMC Proceedings 2014, 8(Suppl 4):P32

Background: Cryptococcus neoformans can cause infection in immunocompromised individuals, especially in patients with acquired immunodeficiency syndrome, in which meningoencephalitis is the main clinical manifestation [1]. There are few available antifungals for cryptococcosis treatment and all of them present high toxicity, besides the reports of resistance. In this context, the natural products from plants are an important source in the search for new antifungal compounds. In this study, it was evaluated the antifungal activity of the Amyrisbalsamifera essential oil (EO) against C. neoformans. The determination was made by minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) using the microdilution broth method.

Methods: Broth microdilution protocols based on the CLSI reference document M27-A3 [2], were used to determine MIC values for 15 isolates of C. neoformans. Briefly, twofold serial dilutions, in Roswell Park Memorial Institute (RPMI 1640) broth, with final test concentrations ranging from 2 to 1024 μg/mL for A. balsamifera was tested, and the inocula was prepared in the same broth with 107 yeasts/mL. Results were read after about 72 h, and MICs were defined as the lowest test concentrations causing complete growth inhibition. Quality control determinations of the MIC values of fluconazole were performed by testing Candida parapsilosis ATCC 22019 and the results obtained were within the recommended limits. To determine minimum fungicidal concentration (MFC) [3] values, after reading the corresponding MIC values, 10 μl samples from all optically clear tubes (complete growth inhibition) plus the last tube showing growth were subcultured on Sabouraud Dextrose Agar Petri dishes. The dishes were incubated at 35°C for for 3 days, until growth was clearly visible in the control samples, and MFC values were determined as the lowest concentration EO which there was no visible growth.

Results and conclusions: A. balsamifera EO exhibited wide-spectrum antifungal activity. Evaluation of MIC and MFC values showed that the EO was active against all the tested strains. MIC values ranged from 128 to 256 μg/mL against Cryptococcus, and MFC values were between 128 to 512 μg/mL. According to Scorzoni [4] compounds with MICs ≤ 256 μg/mL are considered relevant in the investigation of substances for therapeutic purposes, so in our study we can conclude that the EO showed antifungal activity against yeasts of the complex C. neoformans.

Acknowledgements: UFG, Embrapa Arroz e Feijão e Embrapa Cenargen.

References

P33
Effects of hecogenin on Larvicidal activity against Aedes aegypti mosquito, the dengue vector
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Background: Dengue is a viral disease, caused by the dengue virus, a Flaviviridae family virus. Dengue is transmitted by several species of mosquito within the genus Aedes, principally A. aegypti. The dengue control is based on the mosquito combat, most of time through chemical insecticides. Hecogenin is a sapogenin present in the leaves of species from the Agave genus, with a wide spectrum of reported pharmacological activities. The present study was undertaken to evaluate the effect of hecogenin in Aedes aegypti mortality. Currently, several studies have shown the increase of the insect resistance for various chemical pesticides. In this way, the aim of this study was verify de larvicidal activity of the hecogenin acetate against A. aegypti larvae (L4).

Methods: Twenty larvae in the fourth stage (L4) were exposed to the concentration (20 mg/mL) of the hecogenin acetate for 5 days. The Hecogenin Acetate was dissolved in water and Tween 80. The control group consisted of 20 larvae in the fourth stage, exposed to tap water plus tween 80 for 5 days.

Results and conclusions: As results we found that the hecogenin acetate doesn’t killed larvae in the first 24 and 48 hours, killed 10% of larvae after 72 hours, 80% of larvae after 96 hours and 95% of larvae in 120 hours in the concentration. This results confirm that the hecogenin have larvicidal activity against A. aegypti. As mechanism of action, is possible that the acetate mimics the insect growth hormone, stopping its development and causing him to death.

Acknowledgements: Biotechnology Center and Federal University of Paraíba.

P34
Profile susceptibility to fluconazole and voriconazole antifungals by species of Candida albicans isolated from urine culture
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BMC Proceedings 2014, 8(Suppl 4):P34

Background: The term candida can be defined as the observation of yeasts on direct examination of urine through the presence of pseudo-hyphae and fungal growth in urine culture [1]. The yeast Candida can be detected in the urine in patients who have bladder colonization and in patients who have urinary tract infection [2]. Currently, there are few options in the medicine antifungal market for the treatment of urinary tract infections. And the most used are amphotericin B, ketoconazole, fluconazole and voriconazole. Therefore, the determination of the profile of antifungal susceptibility among isolates of C. albicans urine in patients, it is so important as epidemiological marker that serves to guide therapeutic procedures [3]. Thus, many methods have been tested as alternative to the antifungal susceptibility, as the method of agar disk diffusion M44-A12. So,
this study aimed to evaluate the antifungal susceptibility profile of Candida albicans isolated from the urine of patients admitted at the university hospital in Dourados - MS.

**Methods:** In this study we used 24 samples of Candida albicans from urine cultures of patients hospitalized at the university hospital of Grande Dourados, from June 2010 to June 2012. The yeasts were isolated and identified according to the conventional method. The antifungal susceptibility of isolates of Candida albicans was evaluated using the disk-agar diffusion method, according to the standards of the Clinical and Laboratory Standards Institute (CLSI) M-44 [4], using as antifungal fluconazole and voriconazole. The tests were performed in duplicate and the reading of the plates was performed using the methodology described by Demitto, 2012 [5].

**Results and conclusions:** The results obtained in testing susceptibility to the antifungal fluconazole showed that 54.16% of the samples were sensitive and 45.83% were resistant to the same drug. The antifungal voriconazole showed that 54.16% of strains were sensitive and 45.83% were resistance to the same drug. According to the results obtained by Demitto et al. 2012, the antibiotics fluconazole and voriconazole showed equivalent efficacy in vitro. So, this may be related to cross-resistance due to the similarity of the chemical structure of these azoles, because strains resistant to fluconazole also showed resistance to voriconazole.

**Acknowledgements:** In the University Federal of the Grande Dourados and FUNDECT-MS, for financial support.

**References**


**P35**

Effects of α-amirona on inflammation in topical edema ear model in mice

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**BMC Proceedings 2014, 8(Suppl 4):P35**

**Introduction:** In vivo models of inflammation are definitely the best tools to study the biological role, because no other approach will allow the simultaneous investigation of cells and mediators that interact during the inflammatory process. Several reports in the literature have shown the efficacy of α, β-amyrin, the main component of the resin plant of the genus Protium in studies with in vitro models of inflammation. Another component of this resin with anti-inflammatory activity is the α-amirona, but this is still less studied.

**Objective:** To evaluate the topical anti-inflammatory activity of the compound isolated α-amirona in models of skin inflammation in mice.

**Methods:** We used 30 male mice of the Balb-c strain weighing 30-35g were divided into 6 groups (control phenol, treated with the extract at concentrations of 0.6 mg/ear, 0.3 mg/ear, 0.1 mg/ear and control ears treated with dexamethasone). Edema was measured by the technique of 10% phenol in acetone (20 mL/ear) on the right ear of all groups. The extract (0.6, 0.3 and 0.1 mg/ear) or dexamethasone (0.1 mg/ear) was used as positive control, were applied directly after phenol. The left ear of each animal was used as a control for evaluation of edema. One hour after treatment, the animals were anesthetized with thiopental 80mg/kg and sacrificed by intracardiac injection of potassium chloride 3M, 5mm circles were taken from their ears with the aid of a punch biopsy and weighed on an analytical balance. Evaluation edema was taken as the difference in weight of the tissue samples.

**Results:** The group of animals that received topical treatment with solution of α-amirona exhibited a decrease in edema induced by phenol when compared to the control group. However, the positive control group that was treated with dexamethasone showed a sharper decrease edema.

**Conclusions:** The compound herbal α-amirona showed topical anti-inflammatory action, in the model of ear edema induced by phenol in mice, but at a lower power action of dexamethasone.

**References**


**P37**

Electrochemical evaluation of anti-CRP/CRP interaction for the molecular diagnosis of the cardiovascular risk
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**Background:** The C-reactive protein (CRP or PTX1) is an acute phase protein, expressed by hepatocytes, that is regarded as a clinical marker for infection and has been increasingly used as a risk indicator in chronic inflammatory diseases [1]. Atherosclerosis is an inflammatory disease that remains a major cause of morbidity and mortality. Its progression is associated with the accumulation of lipoproteins in the endothelium, establishing plaques whose rupture may result in the formation of a thrombus, arising several cardiovascular complications [2]. Studies show there is a correlation between the risk of cardiac events (such as myocardial infarction or stroke) and increasing levels of CRP [3]. Currently, there are several methods to detect CRP, including immunoassays and agglutination. However, these methods are not sensitive enough, time-consuming or cost-ineffective [4]. Therefore, new diagnosis methods are being developed. Electrochemical biosensors are small devices that combine the selectivity of biochemical molecular recognition with the sensitivity of electrode transducers, being remarkable for their specificity, speed, portability and low cost [5]. In this work, a graphite electrode surface is modified with poly(3-aminotriphenol) and a specific antibody (anti-CRP), aiming the detection of its antigen (CRP), through electrochemical methods.

**Methods:** The monomer solution (3-aminotriphenol) was prepared in 0.50 mol.L⁻¹ sulfuric acid solution for electropolymerization by potential cycling between -0.4 V and +1.0 V vs. Ag/AgCl at 50 mVs⁻¹. This solution was performed freshly, before each electropolymerization and deaerated with ultra pure nitrogen before for 4 minutes. After electropolymerization, the modified electrode was rinsed in deionized water to remove unreacted monomer. The electrochemical polymerization was performed through cyclic voltammetry in a three-compartment cell using a potentiostat from NCCLS between -0.4V and +1.0V vs. Ag/AgCl at 50 mVs⁻¹. After confirmation of binding sites for sugar D-galactose, purifications were performed by affinity chromatography and biological assays with the fraction ligand and the void as well with the crude extract of the latex for stimulation of macrophages in culture cytototoxicity assay, and evaluation of the effect of the fractions in the evolution of the tumor (breast adenocarcinoma) in Balb/C mice for the evaluation of animals.

**Results:** The results of the experiments show that the crude extract of E. tirucalli components has cytotoxic features that promote the lysis of red blood cells in high concentrations, however, at higher dilutions was not shown a cytotoxic effect, however, when carried immunofluorescence microscopy on the surface of tumor cells which could interfere with the tumor microenvironment. Therefore, the anti-CRP/CRP interaction on the modified electrode surface is a promising platform for the molecular diagnostic of the inflammatory process and cardiac diseases.

**Acknowledgments:** PROPP-UFU, FAPEMIG and CNPq.

**References:**

**P38**

Evaluation of the antitumor effect of lectin obtained from the latex of Euphorbia tirucalli against tumor cells of ehrlich
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**BMC Proceedings 2014, 8(Suppl 4):P38**

**Introduction:** According to the World Health Organization, cancer is the second leading cause of worldwide death and breast cancer is among the most frequent types. Conventional therapies show many side effects, high cost and sometimes are inefficient in the total eradication of tumors. Looking for new alternatives to cure this disease a lot of studies have been developed in the field of medicinal plants, due to the vast distribution and use of folk medicine by patients as a single treatment or as a supplement to conventional treatment, obtaining positive effects in several cases. Currently, research has been directed for the characterization and purification of molecules and substances in order to identify the responsible components for therapeutic activity and minimizing side effects. Euphorbia tirucalli popularly known as Labyrinth, is a frequently cited plant in traditional medicine for its use as an antitumor agent. The present study aims to purify the lectin fraction obtained from the latex of E. tirucalli, and verify the relation and contribution of this bioactive substance, as well as the nonbonding fractions and crude extract, the activity against tumor Ehrlich. Methods: We performed tests of hemagglutination and hemagglutination inhibition with different carbohydrates to the identification of lectin activity. After confirmation of binding sites for sugar D-galactose, purifications were performed by affinity chromatography and biological assays with the fraction ligand and the void as well with the crude extract of the latex for stimulation of macrophages in culture cytotoxicity assay, and evaluation of the effect of the fractions in the evolution of the tumor (breast adenocarcinoma) in Balb/C mice for the evaluation of animals. Results: The results of the experiments show that the crude extract of E. tirucalli components has cytotoxic features that promote the lysis of red blood cells in high concentrations, however, at higher dilutions was identified lectin activity. From the SDS-PAGE, we observed the protein profile of the crude extract and purification efficiency of affinity column fraction lectin (binder). Regarding lectin's feature, this was evidenced in hemagglutination tests subsequent to the exclusion chromatograms of the hemolytic effect. MIT assay in front of tumor cells, the fraction lectin have not shown a cytotoxic effect, however, when carried immunofluorescence slides, it was found that the fraction of E. tirucalli interact with receptors present on the surface of tumor cells and lymphocytes. Conclusion: The chromatographic method was efficient in the purification process of the fraction with lectin activity from the latex of E. tirucalli eliminating the cytotoxic effects attributed to the crude extract. From this separation was observed the interaction of fraction lectin with receptors surfaced tumor cells which could interfere with the tumor microenvironment. Although the production of TNF-α by macrophages in test stimulation may be related to the antitumor activity and suggest a mechanism of action for this biomolecule by inducing inflammatory response.

**References:**


P39

**Identification of carbapenemase genes in *Serratia* spp.**

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**Background:** Reports of nosocomial infection due to carbapenem resistant *Serratia* spp. have become significantly more common. This resistance may be due to production of distinct carbapenemases, such as KPC [1,2]. This enzyme, initially described in *Klebsiella pneumoniae* isolates, has also been detected among other organisms, such as *Serratia marcescens*, emphasizing the global risk of interspecies spread of resistance genes [2,3]. The aim of this study was to identify carbapenemase genes in *Serratia* spp. Isolated of nosocomial infection.

**Methods:** The samples were collected during May/2012 to May/2013. The strains were recovered from urinary tract, tracheal aspirate and blood culture from patients hospitalized Dourados/MS hospital. The identification of *Serratia* spp. and the sensitivity test was carried out using a Vitek (BioMérieux) automated system. All strains with reduced susceptibility to imipenem or meropenem were screened for carbapenemase production by the modified Hodge test as recommended by the Clinical and Laboratory Standards Institute [4]. The presence of KPC coding gene was assessed by PCR as described by Cuzon et al. [5] (2010) [5].

**Results and conclusions:** From May/2012 to May/2013, fifty strains of *Serratia* spp. isolated. The wards that had the highest incidence of *Serratia* spp. were the intensive care units (ICUs). The strains identified as producing carbapenemases were evaluated by PCR using primers specific for *bla*<sub>ve</sub> gene. Fourteen *Serratia* spp. strains were positive in PCR. This work describes the first report of KPC gene in *Serratia* spp. isolates in Mato Grosso do Sul, Brazil and confirm the high level of resistance of *Serratia* spp. against carbapenemases. The clinical importance of detecting carbapenemases producing *Serratia* spp. is to contribute to hospital infection control, reducing the spread of multidrug-resistant microorganisms and providing results that help in choosing the most appropriate antimicrobial, prolonging the life of patients.

**Acknowledgements:** This work was supported by the Foundation Support the Development of Education, Science and Technology of the State of Mato Grosso do Sul (FUNDECT, 05/2011 and 04/2012).

**References**


P40

**Evaluation of in vitro biological potential of plant species *Sesbania corniculata* (Euphorbiaceae).**

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**BMC Proceedings** 2014, 8(Suppl 4):P40

**Background:** Medicinal plants with antimicrobial properties built into the problem of multidrug deserve investment in research, by guiding the discovery of herbal medicine effective against emerging pathogens and related bacterial and fungal infection. Brazil has a great biodiversity of plants, which are popularly used for medicinal form. Vegetables have been widely used in health care due to its medicinal properties, such as antibacterial, antifungal and immunomodulatory activities [1]. Species of the family Euphorbiaceae are popularly used against diseases of viral, antimicrobial, anti-inflammatory, antitumor, antihypertensive, muscle relaxant [2], including *Sesbania macrocarpa*, *Sesbania hispida*, *Sesbania commersoniana*. However, works that related biological potential of *Sesbania corniculata* species are scarce. Through this, the objective of this study was to evaluate the antimicrobial activity and toxicity of *Sesbania corniculata*, guanxuma-de-chifre, popularly used as antidiarrheal, antibacterial and elimination of kidney stones [3].

**Methods:** Experimental in vitro study, was conducted at the Laboratory of Wound Care at Federal University of Alagoas. Were evaluated two fractions of the species *Sesbania corniculata* extract, X<sub>1</sub> and X<sub>2</sub>. 14 microorganisms were used, standardized between bacteria and fungi which were distributed by American Type Cell Collection. Antimicrobial activity was determined by microbial sensitivity tests, the method of disk diffusion (DD) and the method of broth microdilution for determination of minimum inhibitory concentration (MIC). All extracts were tested against *Artemia salina* Leach. For the study of cell viability, only the X<sub>1</sub> extract was tested.

**Results and conclusions:** The extracts showed moderately active for *Staphylococcus aureus* and *Pseudomonas aeruginosa* in the DD test (percentage inhibition >28.6 to <51.38), confirmed by the results of CMI. The extract X<sub>2</sub> is the fraction of the best antimicrobial potention, inhibiting the growth of *P. aeruginosa* lineage, concentration of 1000 to 125 μg mL<sup>-1</sup> by the result of the MIC. These results corroborate previous studies since species of the family Euphorbiaceae [3], *Heterocalyx coront* and *Euphorbia hirta* [4], *Paldillus croton* and *Croton eriogon*, that also showed antimicrobial activities against these organisms [4]. Was identified the absence of toxicity in all samples, since the percentage of mortality was <30 %. This finding dismissed the realization of the quantitative assay. All extracts showed inactive against fungii tested, because it inhibited their growth. The low activity and/or the absence of antifungal activity may be due to the plant extracts tested did not damaged the membrane permeability to allow fungal cell. The evaluation of cytotoxicity by MTT, X<sub>2</sub> extract showed significant cytotoxicity (p < 0.0001). These results cooperates with MTT describes a study significantly increased compared to the antitumour agent 5-fluorouracil [5]. In vitro studies are the basis for further research of technological advancement involving the use of *S. corniculata* for therapeutic purposes, including as antimicrobial.

**Acknowledgements:** Brazilian Ministry of Science and Technology, Federal University of Alagoas, Center for Information Technology (CIT-PROPEF), Research Laboratory of Wound Care, Laboratory of Biomaterials, Tiradentes University.

**References**


P41

Synthesis and activity of conjugates Gallic acid - GnRH-III
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BMC Proceedings 2014, 8(Suppl 4):P41

Background: Most of scientific studies with peptide have aim to therapeutically. Nowadays, few compounds have the ability to activate or inhibit biological processes. The combination of anticancer drugs with a molecule which recognizes tumor specific connections, through a receiver, may provide an effective anticancer agent. The hormone GnRH-III, analogue of the hormone GnRH (Gonadotropin releasing hormone), has been described as inhibitor of tumor growth, and has the possibility of use in cancer therapy. In addition, the gallic acid and its derivatives show great potential biological, inducing apoptosis in tumor cells [1]. The aim of this work was to study the feasibility of synthesis of conjugated containing Gallic Acid and the hormone GnRH-III in order to obtain anticancer molecules and available the activity

Methods: GnRH-III (Gip-His-Trp-Ser-Asp-Lys-Pro-Trp-Gly-NH2) was synthesized using a peptide technique called solid-phase peptide synthesis (SPS) [2]. The coupling of Fmoc-amino acid and gallic acid were performed by activation of carboxyl groups with disopropylcarbodiimide (DICI)/N-hydroxybenzotriazole (HOBt). We used the protector Trt (trityl) to protect the ε-amino group of Lys, which allowed selective deprotection of this group for subsequent coupling in the side chain of Lys. Purification of the crude material was carried out in a system of high performance liquid chromatography (HPLC). The purity of the fractions was determined on an analytical HPLC and confirmed by mass spectrometry. Mosman colorimetric test was performed in order to detect biological activities

Results and conclusions: The synthesis of the conjugate containing gallic acid proved to be difficult, but using optimized conditions of deprotection of the ε-amino group of Lys, the conjugated GnRH-I/gallic acid was obtained. According to the results, it can be noted that the entrance of gallic acid in question was not a major problem encountered in synthesis, but the removal of the protecting group of Lys. The results showed, as expected, that the IC50 of gallic acid was 14 ug/mL. The conjugate GnRH-III/Gallic Acid showed IC50 much smaller than gallic acid. Apoposis was induced low concentrations, showing an interesting compound for the anticancer research.

Acknowledgements: FAPESP, CNPq and CAPES

References

P42

G-CSF prevents cerebral infarction and maintain muscle strength in experimental model of ischemic stroke
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BMC Proceedings 2014, 8(Suppl 4):P42

Background: Cerebral infarction is an ischemic stroke resulting from a disturbance in the blood vessels supplying blood to the brain, being the leading cause of physical and cognitive disabilities in adults [1]. The currently approved administration of thrombolytic agents is effective only within about the first 3 hours poststroke [2]. Recent studies have demonstrated that administration of growth factors can reduce stroke size or functional defects [3]. Among the factors, the granulocyte colony-stimulating factor (G-CSF) demonstrated ability to promote differentiation of hematopoietic cells, as well as neurogenesis and promoting formation of new synapses [4,5]. Therefore, the aim of this study was to evaluate if the protective role of G-CSF in experimental ischemia is associated with the maintenance of muscle strength.

Methods: Swiss webster mice (Mus musculus) males (n = 16), weighing approximately 30 g, underwent global cerebral ischemia. Was occluded common carotid arteries for 80 minutes, and after this period the blood flow of the common carotid artery was released, while the arterial blood supply to the left remained interrupted. The stroke animals received vehicle (5% glucose solution) or were treated with G-CSF at a dose of 100 mg/kg/day, administering it after 24 hours of treatment. All the experimental procedures were performed in accordance with National Institutes of Health (NIH) guidelines, and study protocols were previously approved by the Institutional Animal Care and Use Committee (CEUA Protocol # 011/2011). The measurement of the strength of the mice was performed in the pre and post surgery through software coupled to a force transducer. The quantification of the area of cerebral infarction using 2,3,5-triphenil tetrazolium chloride was established by morphometric analysis using Image J program (NIH). The data are presented as means ± SEM. Statistical analysis was performed using Student’s t test for comparison of groups using the software Prism® 5.0 (GraphPad, San Diego, CA, USA). p values <0.05 were considered to be statistically significant.

Results and conclusions: A significant increase in the number of circulating leukocytes in the animals treated with G-CSF (= AVE + vehicle 2,550 ± 283/mm3 vs. G-CSF + AVE = 15,650 ± 1,294/mm3, p <0.01) was observed. The strength after surgery was significantly higher (p <0.05), in the group treated with G-CSF (88 ± 4 g; t value = 0.0473) when compared with vehicle group (71 ± 5 g). The areal extent of cerebral infarction was significantly lower (p <0.05) in animals treated with G-CSF (0.205 ± 0.03 cm2), Student t value = 0.0331 compared to the control group (0.401 ± 0.07 cm2).

Our results demonstrate the neuroprotective effect of G-CSF in mice undergoing ischemic brain, thereby contributing to the reduction of neurofunctional impairment caused by stroke, as the maintenance of strength in the treated group. 


References

P43

Expression of proteins Heat-Labile (Lt-1) and Heat-Stable (Sta) Escherichia coli expression of proteins Heat-Labile (Lt-1) and Heat-Stable (Sta) Escherichia coli
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BMC Proceedings 2014, 8(Suppl 4):P43
Introduction: Escherichia coli has a direct relationship with the diarrheagenic diseases, which occur quite in developing countries due to poor sanitation system and public health. It mainly affects children, causing a rate of morbidity and mortality worrying.

This bacterium has a versatility of strains expressing different mechanisms and virulence factors, thus the importance in studying the taxonomy of these cepas. Classified into six categories: enteropathogenic E. coli (EPEC), enterotoxigenic E. coli (ETEC), enteroinvasive E. coli (EIEC), enterohemorrhagic E. coli (EHEC), enterogaegrettive E. coli (EAEc) and diffusely adhering E. coli (DAEC). Among them, ETEC is the most common etiologic agent of infantile diarrhea related and has the characteristic resist peristaltic movements and adhere to the small intestine produce enteroxin. The enterotoxins are proteins encoded by plasmids and occurring in two forms: one stable toxin (ST) which may be of the type Sta and Stb or a labile toxin (LT), which also can be of two types LT and LT-1 or LT-2. Strains of ETEC causes diarrhea when adhere to enterocytes of the small intestine, mediated by one or more adhesins called CFA (colonization factor antigens).

The development of antisera and specific antibodies identification of species and subspecies of Enterobacteriaceae prevent the misuse of antibiotics, will enable the taxonomic study and facilitate medical diagnosis.

Methods: This study samples of enterotoxigenic E. coli (ETEC) are used the bank’s sample Instituto Leônidas and Maria Deane (ILMD-FIOCRUZ). After extraction of genomic DNA samples, was made the standardization of PCR. The result was visualized on 1.5% agarose gel and bands for the purification of LT-1 and Sta genes was used QIAquick Gel Extraction Kit QIAGEN. Then, the preparation of competent cell was made with top 10 for cloning and subcloning, DHu5 plyS and BL21 for expression. Purified fragments were ligated to the vector pGEM T Easy cloning and transformation was performed. The white colonies obtained after transformation of bacteria with binding were subjected to screening by PCR and sent for sequencing. Plasmids were digested by the restriction enzymes EcoRI and BamH1 cloning vector and pQE (Invitrogen) was digested using enzymes SpH1 and Pst1. Fragments linked to expression vectors were inserted by transformation into BL21 plyS plasmid and subjected to extraction. Confirmation of the expression of target proteins was performed by SDS-PAGE and confirmation of gene expression was used Western blot (Invitrogen).

Results: Confirmation of cloning was performed by PCR with sense and antisense primer pGEM of the genes chosen allowing increasing the size of the fragment. In the subcloning, we used the restriction enzyme EcoRI digested that the correct gene Sta, however digested LT-1 gene sequences not expected. Due to this gene for the expression of LT will use an expression plasmid (pQE) different. Protein expression heat-stable Sta using plasmid pRSETC was seen in Western Blot presenting highest expression pellets and size of 4 KDA.

References:

Production of MLM-Type structured lipids from fish oil catalyzed by Thermomyces lanuginosus lipase

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BMC Proceedings 2014, 8(Suppl 4):P44

Background: Lipases are powerful tools for the syntheses of structured lipids (SL) which are triacylglycerols (TAG) having particular fatty acid residues at specific positions. The production of TAG with medium chain fatty acids (MCFA), in positions sn-1 and sn-3, and a long-chain fatty acid (LCFA), in the internal position, has recently increased due to its nutritional interest in applications as reduced calorie fats [1].

Molecular structure of TAGs influences the fatty metabolism in organisms. Consequently, it is possible to control and improve the nutritional and pharmaceutical properties of TAGs with a correct design of SL chemical structure. The dietary reference intake of long-chain n-3 PUFA is based on current intake of a healthy population, and consists of 135-270 mg a day of EPA-DHA [2]. The intake of reduced levels of long-chain n-3 fatty acids have negative consequences in human health, and might occur relatively frequently in situations of irregular fish consumption. So, the aim this work is to produce food oil with a low calorific value, with high content of eicosapentaenoic (EPA) and docosapentaenoic (DHA) acids from fish oil which, is useful for people who suffer from obesity or metabolic disorders caused by lack of such polyunsaturated fatty acids (PUFA) in the metabolism.

Methods: Acidolysis reaction: A fixed amount of Thermomyces lanuginosus immobilized (Lipozyme TL IM) and free lipase (Lipozyme TL 100L) (2% wt% of total substrates) was used for the acidolysis reactions that were performed at 40 °C, in solvent-free media, at a molar ratio 1:2 (fish oil: free fatty acid) in thermostated-capped cylindrical glass vessels under magnetic stirring, for 24 h.

Analysis of products: The product mixture was separated by thin-layer chromatography (TLC) on silica gel plates and developed with n-hexane/ethyl ether/acetnic acid (70:30:1). After this procedure, TLC plates were air-dried, and sprayed with 0.2% (w/v) 2,7-dichlorofluorescein in 95% ethanol, and the bands were visualized by ultraviolet light. The various groups of compounds (triacylglycerols, free fatty acids, diacylglycerols and monoacylglycerols) were identified by comparison with standards. The bands corresponding to TAG were scraped from TLC plates methylated and analyzed [3].

Results: It was possible to obtain TAG of MLM type by acidolysis of fish oil with caprylic acid. Higher incorporations of caprylic acid (50%) into the TAG in these conditions were attained with both forms of lipase. The oils generated in this process provide about 88 to 92% of the mixture of EPA-DHA per g of oil using free (TL 100L) and immobilized (TL IM) forms of Thermomyces Lanuginosus lipase. This amount was adequate for daily intake of EPA/DHA besides generating oil with low caloric level.

Conclusions: The both forms tested of Thermomyces lanuginosus lipase was able to catalyze the incorporation of caprylic acid into fish oil. The oil generated is process could be used as a dietary supplement for specific clinical situations, once it has been possible to produce of nutritionally valued oils, rich in EPA and DHA with low caloric level.

Acknowledgements: The authors are grateful to financial support received from FAPERJ, CNPq and CAPES (Brazil) is greatly acknowledged.

References:

P45 Development of artificial blood vessels through tissue engineering

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BMC Proceedings 2014, 8(Suppl 4):P45

Background: Cardiovascular diseases are one of the main causes of mortality in many parts of the world, with atherosclerosis figuring as the principal cause of coronary occlusion, stroke and aortic aneurysm [1]. Saphenous vein is the most commonly used vascular prosthesis for small-caliber (<6 mm) vascular grafts, however, 10%-40% of patients do not have a saphenous vein suitable for prosthetic replacement due to size mismatch or venous disease [2]. Tissue engineering approaches are being used to develop palliative methods for these pathologies such as the construction of artificial blood vessels. The purpose of this work was to biosynthesize artificial vessels using Gluconacetobacter hasenii's bacterial cellulose (BC) as scaffolding [3]. Functional and structural characteristics of the vessels were evaluated, as well as the coating of the cellulosytic tubular scaffolding with human aortic smooth muscle cells (HASMC). The vessels obtained exhibited appropriate mechanical properties and their histology showed connective nogorous and proper phases as a basis to mimic, respectively, the intimal and medial layers of a blood vessel.
In vitro cultures of HASMCs in the presence of tubular scaffolds demonstrated their ability to support colonization by human aortic smooth muscle cells.

Methods: BC vessels were developed using G. hansenii (ATCC 2376) grown in a mannitol, yeast extract and peptone medium, in an Erlenmeyer flask, containing a silicone tube (4 mm inside diameter) connected to a circulation air pump. Bacterial cells were cultured for 12 days under static conditions and allowed BC biosynthesis around the template tube. The artificial vessels were then washed with NaOH (0.1 mol L\(^{-1}\)) sterilized by autoclaving. To assess the effect of the artificial device on human cell viability, primary cultures of HASMC were grown over the BC vessels, in a 231 medium at 37°C with 5% of CO\(_2\). Vessels' morphology was evaluated by scanning electron microscopy (SEM) and the mechanical properties of the vessels assessed by tensile tests. For the viability assays, cells were seeded at a concentration of 15 x 10\(^{5}\) cell/vessel, grown in the nonporous side of the membrane and analyzed by MTS colorimetric assay (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium), after 24, 48, 72h and 7, 14 days of culture. The absorbance was quantified at 490 nm in a microplate reader.

Results and conclusions: SEM analysis showed that BC vessels are composed of fibers with a nonporous surface formed at the liquid/solid/air interface (inside the vessel), followed by a porous layer (outside the vessel). Tensile tests showed similar burst strength of the cellulose vessel when compared to a native blood vessel (224 ± 41 kPa). Human cells remained viable over the BC scaffolding for up to 14 days, the total duration of the experiment indicating that BC vessels represent a suitable support for cell colonization. Results presented here highlight the potential of BC as a biomaterial for vascular tissue engineering and its applications as a model for artificial blood vessels.

Acknowledgements: CNPq, CAPES, FINEP, LCME/UFSC.

References

P46
Adaptation of glucocerebrosidase-producing CHO cells to serum-free suspension culture
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Background: Deficiency of the lysosomal glucocerebrosidase (GCR) enzyme results in Gaucher disease, the most common inherited storage disorder [1]. Current treatment consists of enzyme replacement therapy by administration of exogenous GCR. Although effective, it is exceptionally expensive [2]. In Brazil, the public healthcare system provides the drug free of charge for all Gaucher’s patients, which reaches the order of $84 million per year. However, the production of GCR by public institutions in Brazil would reduce significantly the therapy costs. With this purpose, we have previously developed a cell line producing recombinant GCR using anchorage-dependent CHO cells [3]. Recent advances in cell culture technology have allowed the elimination of serum along with the growth of cells in suspension mode. This mode is preferred in industrial production due to the well-understood principles of scaling parameters and the ease of process control. Additionally, the serum is expensive and the process of adapting CHO cells producing recombinant GCR to growth in suspension culture and serum-free medium was successful. Protein bands of 66-69 kDa corresponding to secreted and glycosylated GCR were detected by western blotting analysis. Surprisingly, direct adaptation was more effective than sequential adaptation in obtaining high-producing clones. However, an unexpected decreased in the GCR expression was observed in long-term culture (40 days), indicating the presence of unstable cells producing GCR. In addition, the secreted protein was not active in the serum-free suspension culture, although glycosylation analysis showed a properly glycosylated GCR containing complex- and hybrid-type oligosaccharides, consistent with a functional enzyme. These results suggest that the stable production of an active GCR by CHO cells may be serum-dependent. The next steps include the addition of serum in the cell culture adapted to suspension. To our knowledge, this is the first report describing an adaptation process of CHO cells producing GCR for serum-free suspension culture.

Acknowledgements: This work was supported by grants from FAPESP, CNPq, and Fundação Butantan.

References

P47
Chromosome instability and expression of Braf, Tert and P53 in macrophage murine cell line (J774-1)
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Background: Cancer origin is closely linked with mutations and changes in the chromosome structure, making important such cytogenetics studies [1]. Macrophage murine cell line J774-1 is derived from a reticulum cell sarcoma and presents various characteristics of primary macrophages as synthesis and lysosome secretion, phagocytosis, Fc receptor expression (2,3). This work aimed to analyze the fundamental number and the constitutive heterochromatin using the staining with the fluorochrome Hoechst 33258. In order to understand possible changes in gene expression pattern, it was also quantified the levels of expression of the following important classes of genes: (A)-Genes responsible to promoting cell proliferation as B Raf and Telomerase (TERT); (B)-The P53 gene responsible by tumor suppressing.
Methods: To obtain mitotic chromosomes, cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics. Culture bottles were kept at 37 °C in a CO2 incubator. After reaching approximately 80% confluence, cells were treated with 0.01% colchicine solution for three hours and trypsinized. The sample was treated with 0.275 M KCl hypotonic solution and fixed in Carnoy (methanol:acetic acid:1:3 v:v). The cells were transferred to histology slides, stained with 5% Giemsa and examined under an optical microscope. To analyse constitutive heterochromatin, slides were stained with the A-T specific fluorochrome Hoechst 33258. For analysis of genomic expression, a real time PCR was performed for each of the manufacturer’s instructions.

Results and conclusions: The chromosome fundamental number of J774-1 cell line was very variable, ranging from 32 to 79, with the majority of metaphases with chromosome numbers concentrated between 72 and 73. This result indicates that J774-1 cell is a heterogeneous cell line. Hoechst staining showed that the constitutive heterochromatin of this cell line is A-T “rich” and it is concentrated in the pericentromeric region, corroborating with data observed in other murine cell lines and normal murine cells. Using a real time PCR, it was detected a higher expression of BRAF and TERT genes in relation to control (whole blood cells) but comparing the expression of BRAF and TERT genes of J774-1 cells with other murine cell lines (S180, B16F10, MEF, NIH-3T3), the analysed cell line showed lower levels of expression of these genes. In relation to PS3 gene, it was detected higher expression levels in relation to control and all the other cell lines. High PS3 expression was not expected in tumors or immortalized cells, once that its role is proliferation inhibition, but according to Campsi (2005) [4], even presenting intact function of tumor suppressors as P16 and P53 cells can acquire immortalized phenotype having an indefinite proliferation potential[5]. Cytogenetic results indicate high chromosome instability, with the presence of numerical and structural chromosome aberrations. The high fundamental number suggests a polyploidization event, probably due to endoreduplication, once many metaphases with diplochromosomes were found. Polyploidization may be the event that triggered genetic instability, generating chromosomal abnormalities and changes in gene expression, both events very important in the carcinogenesis.

Acknowledgements: FAPEMIG, UFU, CAPES and CNPq.

References:

P49
Antimicrobial activity of nano cerium oxide (IV) (CeO2) against Streptococcus mutans
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BMC Proceedings 2014, 8(Suppl 4):P49

Background: Cerium oxide (CeO2) is a technologically important material due to its properties and applications in several areas that range from engineering to biological sciences. Despite the hydrothermal microwave method versatility, its crystallization kinetics has low speed when the processing temperatures are below 300°C which are the desired conditions for soft chemistry processes [1,2]. At lower temperatures it was found that this material has antimicrobial activity against several bacteria, including E. coli [3,8]. The CeO2-mediated photodynamic killing of microorganisms was attributed to CeO2 (I) according to the metal-oxygen (Ce-O) and other results. Their surface areas were approximately 200m²/g with around particle sizes of 5nm. Micrographs showed different sizes and shapes of clusters. Microbiological tests against Streptococcus mutans for the containing nano cerium oxide (IV) composite in ZnO at 10% (diluted in BHI- broth) showed antibacterial effect in minimum inhibitory concentration of 0.22 mg / mL.

Conclusions: The results showed the direct influence of the synthesis method employed on the morphology and size of nanoparticles cerium oxide (IV) obtained. Nano cerium oxide (IV) seems to be a material of great potential for use against S. mutans (a dental bacteria) responsible for cariogenic process due to its purity and particle size. The matching results among the tests employed in biological assays showed that the techniques of fluorescence, spectrophotometry, resazurin and fluorescence did not suffer reagent interference as may eventually occur with some products of natural origin. The dental composite consisting of odontologic ZnO and nanometer CeO2 in the proportion of 10% showed superior antimicrobial properties when compared to the standard used (dental zinc oxide + eugenol).

Acknowledgements: Laboratory of Oral Biology.

References:

P48
Antimicrobial activity of nanocerium oxide (IV) (CeO2) against Streptococcus mutans
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BMC Proceedings 2014, 8(Suppl 4):P48

Background: Cerium oxide (CeO2) is a technologically important material due to its properties and applications in several areas that range from engineering to biological sciences. Despite the hydrothermal microwave method versatility, its crystallization kinetics has low speed when the processing temperatures are below 300°C which are the desired conditions for soft chemistry processes [1,2]. At lower temperatures it was found that this material has antimicrobial activity against several bacteria, including E. coli [3,8]. The CeO2-mediated photodynamic killing of microorganisms was attributed to CeO2 (I) according to the metal-oxygen (Ce-O) and other results. Their surface areas were approximately 200m²/g with around particle sizes of 5nm. Micrographs showed different sizes and shapes of clusters. Microbiological tests against Streptococcus mutans for the containing nano cerium oxide (IV) composite in ZnO at 10% (diluted in BHI- broth) showed antibacterial effect in minimum inhibitory concentration of 0.22 mg / mL.

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Acknowledgements: Laboratory of Oral Biology.

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areas. Thus, the study of squamous cell carcinoma of the oral cavity and oropharynx should consider the anatomical site, size and pattern of tumor infiltration as characteristics that may influence the biological behavior of the tumor [2]. This study aimed to evaluate the clinical and pathological aspects related to human papillomavirus (HPV) in squamous cell carcinoma of oral and oropharyngeal.

Methods: Clinical data from 99 patients, from the Hospital Santa Rita and the Hospital Universitario Cassiano Antonio Moraes, diagnosed with squamous cell carcinoma of the oral cavity and oropharynx were obtained through interviews and analysis of records. The study population was categorized using criteria such as age, gender, history of smoking and alcohol and HPV infection. The clinical parameters of the location of the primary lesion, tumor size (T), regional lymph node involvement (N) and tumor invasion pattern were obtained by physical examination and pathology.

Results and conclusions: The study population had an age range 30-93 years (mean 57.6 years), 80.8% of cases were male. Tobacco consumption was observed in 84.8% of patients, and alcohol consumption in 77.7%. High risk HPV types was detected in 4.04% of cases. Using the TNM system, it was observed that 27.27% of the cases had stage I and II and it were considered early stage, while 65.65% were in an advanced stage (stages III and IV). Most of the tumors had size T3 and T4 (57.57%). The most affected anatomical site was the oral cavity (83.83%), followed by the oropharynx (16.17%). The tongue was the anatomic site more affected, fact that are compatible with the data found in the literature [3-5], which can also be related to a higher incidence of lymph node metastasis. These data reveal late diagnosis. There was no association between HPV infection and clinical aspects analyzed.

Low frequency of HPV infection can be justified by the majority of cases presenting primary lesion in the oral cavity and not in oropharynx, where frequency has been higher. However, in cases where human papillomavirus infection has been detected, greater impairment was observed with stages III and IV and primary tumor size T corresponding to T4, which means invading tumor tissues. In addition, many patients were heavy smoking and drinking, which are risk factors that increase the mutation of cells and may contribute to a worse prognosis.

Acknowledgements: CNPq, FAPES, CAPES, PPGBiotec-UFES.

References

P50

Vacuum dried membranes of poly (l-lactic acid) and bacterial cellulose for biomedical applications

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BMC Proceedings 2014, 8(Suppl 4):P50

Background: Periodontitis is the inflammation of tissues supporting the tooth when gingivitis is not treated [1]. The treatment should reduce/eliminate inflammation of the tissue, which is induced by the biofilm with its byproducts and correct anatomic defects caused by the disease [2]. Guided tissue regeneration is a technique that applies membranes that form a blood clot that may allow the growth and differentiation of cells and regenerate the periodontal tissues. This should be able to block and isolate the area and contribute passively to tissue integration with guaranteed protection, including possible bacterial contamination [3,2]. This work aimed to obtain biocomposites of poly-l-lactic acid (PLLA) with bacterial cellulose (BC) by different methodologies to be applied as membranes for the treatment of periodontitis. The characterization was performed by scanning electron microscopy, cell adhesion and contact angle. It was observed that the membranes 80/20 prepared by casting prior to vacuum drying of BC exhibited better adhesion. This membrane proved to be non-cytotoxic and more hydrophilic.

Methods: Biocomposites of PLLA/BC were obtained from different four methods (M1-M4). M1: solvent exchange with BC; M2: Vacuum dried BC, with ratio of PLLA/BC 80/20; in M3 and M4 we used BC lyophilized membranes. In the third method PLLA/CHCl3 solution was poured over the BC membrane and in the fourth method PLLA/CHCl3 solution was filtered on a Gooch funnel. The evaluation of the composite membranes was performed by the techniques of scanning electron microscopy, cell adhesion (Vero cell line, ATCC CCL 81, passage 192) and contact angle.

Results and conclusions: By visual inspection and the cell adhesion assay it was clear that the most satisfactory results were obtained for the membranes made with vacuum-dried BC/PLLA. On those membranes (M2 method), non-toxicity of the membrane was verified through the formation of a monolayer of adhered cells. Membranes obtained by this second method also showed more favorable results in decreasing the value of the contact angle, a consequence of the increasing hydrophilicity of the sample [5]. This membrane has a smooth surface and did not show toxicity to the cells by the addition of BC. These requirements are important for a biomaterial to be used for medical purposes. In this work BC and pure PLLA membranes were prepared and combined to form composites aimed to biomedical applications. Particular attention was given to the resulting biomaterial so as to guarantee that it could be used in the human body, therefore meeting the necessary initial requirements, such as non-cytotoxicity, biodegradability and biocompatibility. Taking into account that PLLA is a biodegradable and bioreabsorbable polymer and BC is highly hydrophilic, through the analysis of cell adhesion and contact angle we showed that the second preparation method was the most appropriate, that is, the one using vacuum dried BC. The seeded cells formed a monolayer, indicating satisfactory adhesion; on the other hand, no cytoxicity of the membrane and enhanced hydrophilicity of the composite membrane were observed, allowing the use of this membrane for biomedical applications.

Acknowledgements: To CNPq, CAPES, FAPESC, FINEP, FAP/UNIVILLE.

References

P51

Linear B-cell epitopes in BthTX-I, BthTX-II and BthA-I, phospholipase A24, from Bothrops jararacussu snake venom, recognized by therapeutically neutralizing commercial horse antivenom to poly-l-lactic acid scaffolds reinforced with bacterial cellulose nano-fibres.

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BMC Proceedings 2014, 8(Suppl 4):P51

Background: The benefits from treatment with antivenom sera are indubitable. However, the mechanism for toxin neutralization has not been completely elucidated. A mixture of anti-thrombotic and anti-crotaline horse antivenom has been reported to be more effective in neutralizing the
effects of *B. jararacussu* snake venom than anti-bothropic antivenom alone. This study determined which regions in the three PLA₂s from *B.jararacussu* snake venom are bound by antibodies in tetravalent anti-bothropic and monovalent anti-crotalic commercial horse antivenom.

**Methods:** The epitopes recognized by therapeutic horse antivenom sera in the three major PLA₂s present in the venom of *B. jararacussu*, BthTX-I, BthTX-II and BthA-I, were mapped using the parallel Spot-synthesis strategy. Two peptide libraries were designed to more precisely define the epitopes recognized by anti-bothropic and/or anti-crotalic horse antivenom. Each consisted of 69 peptide sequences of fourteen amino acids each that overlapped by nine amino acids and covered the entire protein sequences of the three PLA₂s. The oligomeric structure of PLA₂s, proteins were solved by X-ray crystallography and are available in the protein data bank. The sequences of fifty PLA₂s were selected and grouped into three sub-groups. Shared amino acids sequence from the 12 epitopes recognized by the reaction between the *B. jararacussu* PLA₂s and anti-crotalic/anti-bothropic horse antivenom were analyzed by a multiple sequence alignment.

**Results and conclusions:** Mapping experiments of BthTX-I, BthTX-II and BthA-I using two small libraries of 69 peptides each revealed six major IgG-binding epitopes that were recognized by both anti-bothropic and anti-crotalic horse antivenom. Two epitopes in BthTX-I were only recognized by the anti-bothropic horse antivenom, while anti-crotalic horse antivenom recognized four unique epitopes across the three PLA₂s. Our studies suggest that the harmful activities of the PLA₂s present in the venom of *B. jararacussu* are neutralized by the combinatorial treatment with both antivenom sera through their complementary binding sites, which provides a wide coverage on the PLA₂s. In conclusion, the peptide arrays formed directly onto cellulose membranes allowed the identification of the major antigenic determinants in the three most important PLA₂s (BthTX-I, BthTX-II and BthA-I) isolated from *B. jararacussu* snake venom recognized by commercial anti-bothropic and anti-crotalic horse antivenom. The cross-reactive epitopes located in the Lys49-PLA₂, the major protein of this venom, recognized two specific epitopes located in a region of the enzyme responsible for the myotoxic action, which contributes to the deleterious effects of snake venom. In addition, the ability of the anti-crotalic horse antivenom to neutralize the anticoagulant activity was most likely associated with the acidic Asp49-PLA₂. This study provides proof that the mixture of anti-crotalic and anti-bothropic horse antivenom is qualitatively more effective in neutralizing the effects unleashed by *B. jararacussu* snakebite. Regions recognized by the protective antivenin sera are prime candidates for improved venom cocktails or a chimeric protein encoding the multiple epitopes to immunize animals as well as for designing future synthetic vaccines.

**Acknowledgements:** CNPq, FAPERJ, CAPES, FIOCRUZ (PROEP)

**References**


**Cytotoxic activity evaluation of chalcones on human and mouse cell lines**

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*BMC Proceedings* 2014, 8(Suppl 4):P52

**Background:** The Brazilian National Cancer Institute (INCA) estimated last year almost forty thousand new cancer cases of laryngeal carcinoma, melanoma, tracheal, bronchial and lung cancer. It is known that multidrug resistance and unspecific toxicity are the major challenges for commercial anticancer drugs [1]. In this regard, natural compounds and derivatives are considered as a source of novel antitumor drugs. Among these molecules, chalcones have gained pharmacological importance due its mechanisms of cytotoxicity of four chalcones toward three tumor cell lines: Hep-2 (human laryngeal squamous carcinoma cells), B-16 (mouse melanoma), A549 (human lung adenocarcinoma epithelial cells) and one normal cell line: 3T3 (mouse fibroblasts).

**Methods:** The cytotoxicity of chalcones was evaluated by the colorimetric MITT assay, which determines the reduction of tetrazolium into insoluble formazan by mitochondria of viable cells [3]. For this purpose, four chalcones: *trans*-chalcone; Licochalcone A; *4*-Methoxychalcone and *3*- (trifluoromethyl)chalcone were tested against four different cell lines: Hep-2, B-16, A549 and 3T3. The cell culture was carried out by using Dulbecco’s Modified Eagle Medium for A549 and 3T3, RPMI 1640 for Hep-2, and F-10 for B-16. All medium were supplemented with fetal calf serum. Each cell line was plated in a 96-well plate (3x10⁴ per well) for 24 hours before adding the medium with chalcones in a 5 different concentration treatment (25, 20, 15, 10 and 5 μg/mL). After 48 hours, compounds were withdrawn and a solution of fresh medium containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MITT) was added. After 4 hours of incubation, the product (formazan) was solved by DMSO. Hereafter, the plates were measured through 550nm wavelength analysis, using an ELISA reader. Treatments were compared to negative control (medium with 0.25% DMSO) and positive control (2.5 μg/mL doxorubicin). Cytotoxicity was calculated by the formula: percent cytotoxicity = (1-absorbance of experimental wells/absorbance of control wells) x 100% [4]. IC₅₀ values were also determined. Data were analyzed by the software Sisvar.

**Results and conclusions:** The IC₅₀ values for A549 were 81.29 μM, 81.34 μM, 85.40 μM and 46.13 μM for *trans*-chalcone, *3*- (trifluoromethyl)chalcone, *4*-Methoxychalcone and Licochalcone A, respectively. Hep-2 showed up the most susceptible cell line with IC₅₀ values below 10 μg/mL for *trans*-chalcone, Licochalcone A and *3*- (trifluoromethyl)chalcone. IC₅₀ values for Licochalcone A, *trans*-chalcone, *4*-Methoxychalcone and *3*- (trifluoromethyl)chalcone for tumor mouse cell line B-16 were, respectively, 25.89 μM, 45.42 μM, 50.15 μM and 61.54 μM; for non-tumor mouse cell line 3T3 were 33.42 μM, 48.40 μM, 64.34 μM and 43.44 μM. All IC₅₀ values for *4*-Methoxychalcone were higher than 50 μM. Thus, among the chalcones tested *4*-Methoxychalcone was the least cytotoxic and Licochalcone A the most effective for all cell lines, followed by *trans*-chalcone and *3*- (trifluoromethyl)chalcone.

**Acknowledgements:** This project is financially supported by “Fundação de Amparo à Pesquisa do Estado de São Paulo” (FAPESP), process number 2012/15862-5.

**References**


**Antifungal activity of flavonoids and modulations of expression of genes of fatty acid synthesis in the dermatophyte Trichophyton rubrum**

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*BMC Proceedings* 2014, 8(Suppl 4):P53

**Background:** Dermatophytes are fungal infections caused by keratinophilic fungi known as dermatophytes and classified in three genera: *Trichophyton*, *Epidermophyton* and *Microsporum*. *Trichophyton rubrum* is the most frequent species associated to dermatophytosis worldwide [1]. The infections caused by dermatophytes are not lethal, but are difficult to treat and uncomfortable. In the case of *T. rubrum*, they tend to be chronic, and although the superficial infections are more common, cases of deep infection have been reported in immunocompromised patients [2][3]. The number of antifungal drugs are still limited, and the
acquired resistance for some of clinical antifungal have been shown as well as the side effects that have been promoted by them. Reasons for the challenge in development of new antifungal drugs are the similarities shared by fungal and mammalian cells and the lack of knowledge about the biology of these pathogens. Recent evidences have shown that the fatty acid synthase (FAS) is an interesting antifungal target [4] because of marked differences between human and fungal cells. The aim of this study was to evaluate the antifungal activity of four flavonoids described as FAS inhibitors and verify the modulation of genes in the pathway of fatty acid synthesis in T. rubrum growth in presence of the most effective one as FAS inhibitor.

Methods: The susceptibility assay was carried out using the microdilution test in RPMI medium in 96-well plates with the flavonoids quercetin, ellagic acid, galangin and genistein in a range of 1000-1.9μg/mL toward the strain ATCC MYA-3108 of T. rubrum [5]. The modulation of genes fatty acid synthase (FAS) 1p, FAS 2p subunits), acetyl-CoA carboxylase 2p subunit and fatty acid transporter protein (FAT) was analyzed by quantitative PCR using Sybr Green after 16h of incubation of strain of T. rubrum with MIC of quercetin in liquid Sabouraud medium.

Results and conclusion: Quercetin showed the most effective antifungal activity with MIC of 125 μg/mL, ellagic acid presented MIC of 250 μg/mL, galangin and genistein were ineffective against T. rubrum (MIC > 1000 μg/mL). The positive controls fluconazole and cerulenin presented MICs of 63 and 125 μg/mL, respectively. The analyse of gene expression of the fatty acid synthetic pathway showed the majority of the genes were downregulated by quercetin, fluconazole and cerulenin. However, cerulenin caused a low upregulation of FAS2p gene. Thus, the results suggest the activity of quercetin could be due the modulation of genes of the pathway of fatty acid synthesis, which is a fungal specific target for development of antifungal drugs.

Acknowledgements: This study was supported by grants from Fundação de Amparo a Pesquisa do Estado de São Paulo (2012/02920-7 and 2009/12419-0) and CAPES.

References

PS5
Repositioning of new potential schistosomical drugs using chemogenomic strategy
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Background: Schistosomiasis remains a severe problem of public health in developing countries [1]. Several reports show that praziquantel, the drug of choice for treatment, is failing, which allows the emergence of new therapeutic approaches. Schistosoma mansoni, the causative agent of human schistosomiasis, is highly resistant to praziquantel in Brazil [2]. In this study, compounds involved in the metabolic pathways of S. mansoni were identified and evaluated for antischistosomal activity.


References

PS4
Antimicrobial activity of the ethanolic extract of Guettarda sericea against oral streptococci
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Background: The genus Guettarda (Rubiaceae) comprises plants widely distributed in tropical areas [1]. Regarding the Guettarda sericea species, the literature shows that there is a lack of botanical and phytochemical studies [2]. Thus, the present study aimed to evaluate the antibacterial effect of ethanol extract of leaves of G. sericea (EEFS) on the growth of Streptococcus oralis ATCC 10557 and S. salivarius ATCC 7073 in both the planktonic and biofilms states.

Methods: Different methods were employed to verify the antimicrobial potential. Among these are the determination of minimum inhibitory concentration (MIC) determination of the death curve and evaluation of minimum bactericidal concentration (MBC) [3]. Furthermore, quantification of biomass and the number of viable cells of the biofilm were performed [4]. The negative and positive controls used in all assays were respectively 4% DMSO and chlorhexidine gluconate. To determine the toxicity of EEFS, it was used the toxicity test on Artemia nauplii [5].

Results and conclusions: The data showed that the extract has a remarkable antimicrobial effect, able to inhibit the growth of planktonic and development of biofilms of S. oralis strain until the concentration of 0.25 μg/mL. Regarding the toxicity, it was observed that death of Artemia nauplii occurred at higher concentrations than those that exhibited antibacterial effect. It can be concluded that EEFS can be used as agents for the control of biofilm formation of S. oralis. In addition, complementary methodologies that seek purification of the active compounds and their cytotoxic effects on eukaryotic cells need to be held, aiming its use as an herbal agent.

References
NADH-ubiquinone oxidoreductase mitochondrial precursor, and glutamate metabolism (glutaminase). One of these targets was associated with thibendazole, whose activity has been previously evaluated against *S. mansoni*. [3]. However, 18 drugs were predicted to have activity against other targets and have never been evaluated against *schistosoma* parasites (e.g., acetazolamide, doxorubicin, morantel tartrate, axantel pamoate, thibendazole, and menthol). Our next step is to experimentally screen these drugs against *S. mansoni*. Being a cost and time saving route, drug repositioning is expected to accelerate the discovery of new anti-schistosomum chemotherapies.

Financial support: FAPEG/Goiás.

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P56
Bacterial cellulose membranes and spheres composite with poly (l-lactic acid) through in situ polymerization
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BMC Proceedings 2014, 8(Suppl 4):P56

Background: Since poly(l-lactic acid) (PLLA) shows great biocompatibility as well as biodegradability [1] and bacterial cellulose (BC) is a high purity biomaterial with unique structural and mechanical properties, composed of cellulose and water [2,3], both are biopolymers with great potential for biomedical applications. This work had the purpose of producing biocomposites with properties that bring together the interesting BC and PLLA attributes. With these in mind, an in situ polymerization in BC membranes and spheres was made by L-lactic acid condensation. The presence of toxic residues in the composite was avoid by not using a catalyst [4] since it was possible to solubilized lactic acid in the BC hydrogel.

Methods: BC membranes and spheres were produced by cultivating Gluconacetobacter Hansenii ATCC 23769. For polymerization, commercial L-lactic acid was dehydrated at 105 °C under vacuum, followed by a three hours pre-polymerization step at 150 °C, also under vacuum [4]. When temperature decreased to 75 °C, BC membranes and BC spheres were added. Polymerization took place under vacuum, at 90 °C and agitated at 300 rpm for 48 hours. Samples were taken each 24 hours, submerged in methanol, washed in distilled water at 30 °C and 120 rpm for 24 hours and then dried at 45 °C. Microstructure was analyzed by SEM. To evaluate the cell viability, cultures of mouse fibroblasts L929 were grown in Dulbecco's Modified Eagle Medium (DMEM) in a humified atmosphere, at 37 °C with 5 % CO2. Cell viability was determined using the colorimetric assay MTS [3-(4,5-dimethylthiazol-2-y)-5-(3-carboxymethoxyphenyl)-2(4-sulphonyl)-2H-tetrazolium]. Cells were seeded in a concentration of 105 cell/membrane and analyzed after 24 hours. Absorbance at 490 nm was quantified in a microplate reader [2].

Results and conclusions: Macroscopically, several properties of the composite biomaterial were observed. Compared with BC control all samples seemed more rigid, showed a white color when hydrated and a slight brightness when dried. Those properties were more evident within the 48 hours treatment. The SEM images showed evidences of integration between the BC and PLLA. MTS assays showed that there were cell adhesion on the 48 hours samples, but it also showed that there was a significant decrease of cell viability compared with the BC control, what suggests that cells do not have the same affinity for the biocomposite compared with the pure BC. Besides, shape of the samples was also an important factor since the difference in cell viability was significant between the spheres and the membranes.

Acknowledgements: To CNpq, CAPES, FAPESC, FINEP, LCME/UFSC.

References

P57
Use of gold nanoparticles on graphite electrodes functionalized with poly (4-aminophenol) in the development of a bioelectrode for hepatitis B
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BMC Proceedings 2014, 8(Suppl 4):P57

Background: Hepatitis B is an infectious disease of the liver, highly prevalent in society, caused by hepatitis virus (HBV), which affects approximately 350 million individuals worldwide [1]. It is estimated that the number of deaths from the disease is in the range of 2000 to 4000 per year, mainly by hepatic cirrhosis and hepatocellular carcinoma [2]. Due to the fact that in adults in most cases the initial infection shows no symptoms, the diagnosis is difficult and late.

The development of biosensors for disease diagnosis has been a much valued currently, and in this area there are already several innovative research. Among the various detection techniques used in these sensors, due to the low cost and high sensitivity, electrochemical detection has been widely used [3]. Some polymers have high applicability in this area, because through chemical affinity can immobilize the molecule that will bind to its target. Studies realized by our group [4] indicates that the poly (4-aminophenol) electrodeposition in acid medium is interesting for the immobilization of oligonucleotides. Polymer embedded with metal nanoparticles provided the suitable microenvironment for biomolecules, since improved the electron transfer with the electrode surfaces, resultant in enhanced sensing performance [5].

In this work, specific graphite electrode modified with poly(4-aminophenol)/gold nanoparticles was sensibilized with specific oligonucleotide for the detection of hepatitis B virus and compared to the bioelectrode in the absence of the gold nanoparticles.

Methods: The monomer solution was prepared in 0.5 mol L-1 HClO4, and the electrodepolymerization was carried out by cyclic voltammetry in a three compartment cell using a CH Instruments potentiotstat model 420A. Gold nanoparticles (1:3 solute/solvent) was added onto graphite electrode containing poly(4-aminophenol). Then, the solution containing the HBV oligonucleotide (0,414 mg.mL-1) was added onto poly(4-aminophenol) with gold nanoparticles. Other experiments were conducted in the absence of the gold nanoparticles. The detection of the immobilization of oligonucleotides was carried out by differential pulse voltammetry in a electrochemical cell of
one compartment. The oxidation peak of guanosine present in the oligonucleotide was monitored in the potential of +0.9 V (vs. Ag/AgCl).

Results and conclusions: It was possible the immobilization and detection of the specific probe for the hepatitis B virus onto graphite electrode modified with poly(4-amino-phenol)/gold nanoparticles using linear voltammetry. It was observed a peak in +0.94V attributed to guanosine monophosphate, an usual biomarker for biosensor. The amplitude of the current signal increased about 100% when compared to the modified electrode in the absence of gold nanoparticle. From these data, it was concluded that the use of these nanoparticles was able to increase the reactivity between the polymer and the specific oligonucleotide, which demonstrates a potential use of this platform for the development of biosensors.

Acknowledgements: This work was supported by PROPP-UFU, FAPEMIG, CNPq and CAPES.

References

Table 1(abstract P58) Association of TCF7L2 gene polymorphism and inflammatory markers in DM2

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P60
Cytotoxicity of Agave sisalana in hemocytes of the mosquito Aedes aegypti, which transmits dengue
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BMC Proceedings 2014, 8(Suppl 4):P60

Background: The dengue is a viral disease transmitted by a mosquito from the Culicidae family from Africa, the Aedes aegypti. Most common in tropical and subtropical regions, this mosquito has diurnal activity and is difficult to control due to the high resistance of its eggs and its quick transformation into larvae. The severe illness may cause death, most commonly presenting symptoms such as high fever, pain behind the eyes and headaches.

Methods: Fifteen larvae (L4) were exposed in a 6.5 mg/mL concentration of A. sisalana juice, during the periods of 3, 6, 12 and 24 hours, completing 4 experimental groups and one control group for each, with means, 4 control groups.

Results and conclusions: The A. sisalana had a cytotoxic necrotizing effect on A. aegypti hemocytes, showing that after 12 hours of exposition the larvae in juice had a 21% hemocytes mortality compared with the control group. After 24 hours, a cell mortality of 16.5% in relation with the control group, as well the growth of hemocytes’ size and granularity, being compatible with the noted necrosis. In fact, this effect was acute, because, after 24 hours, the majority of larvae were dead even when a sub-lethal concentration was used, what suggest that this is the major sial juice action mechanism.

Acknowledgements: Biotechnology Center and Federal University of Paraíba, João Pessoa, Paraíba, 58037760, Brazil.

P61
Viability of human dermal fibroblasts cultured on bacterial cellulose and Aloe vera composites
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BMC Proceedings 2014, 8(Suppl 4):P61

Background: Development of multifunctional scaffolds has allowed restructuration and improvement of native tissues, presenting characteristics that mimic complex tissues formation [1]. An ideal scaffold should provide enough transport of nutrients and also adequate mechanical support, allowing the incorporation of many cell types. Likewise, biocompatibility, mechanical properties and water retention are other important characteristics of biomaterials used for tissue regeneration [2]. In this perspective, searching for a biomaterial that promote healing and cell development, fractions of Aloe vera combined with bacterial cellulose (BC) are being intensively studied in our laboratory. BC hydrogels present characteristics that provide an optimal environment for cell culture [3,4]. So the combination of biological components of a natural plant and the unique properties of BC is believed to promote desirable hydrogel-cell interactions and also improvement of the healing dynamics of injured tissues [5]. The objective of this study was to evaluate the viability of human dermal cells when in contact with the novel BC-Aloe vera composites.

Methods: BC-Aloe vera composites were developed using G. hansenii bacterial strain ATCC23769. The culture medium used for the production of BC membranes consisted of mannnit, yeast extract and peptone. For the composites, fractions of Aloe vera (gel, total gel and polysaccharide fraction 1) were added to the membranes in a concentration of 40%, at temperature of 25 °C under static conditions for 10 days. After that, membranes were washed with solution of NaOH (0.1 mol L⁻¹), sterilized by autoclaving and stored in cool and dry place until use. In order to evaluate the cell viability, primary cultures of Human Dermal Fibroblasts adult cells (HDFa) were grown in Dulbecco’s Modified Eagle Medium (DMEM) in a humidified atmosphere, at 37 °C with 5 % of CO₂. Groups of investigation consisted of pure BC and BC composed with fractions of Aloe vera extract Cell viability was determined using the colorimetric assay MTS (3-(4,5-dimethylthiazol-2-yl)-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium). Cells were seeded in a concentration of 10⁴ cell/membrane, grown in the porous side of the membrane and analyzed at 24, 48, 72 h and 7 days of culture. Absorbance at 490 nm was quantified in a microplate reader. In parallel to the MTS assay we also performed qualitative analysis of cell viability by Live/Dead® test.

Results and conclusions: Results indicated a good interaction between the cells and the BC composites, which was indicated by the stable cell viability observed over time. In 24 hours, we could observe an increase of cell viability in all groups when compared the control. After this time point, there was a slight decrease in cell viability, possibly caused by natural cell senescence, but remained cells were still viable. Those findings could be confirmed by the qualitative analysis (Live/Dead®), which showed good cell adhesion and viability over all time points. Interestingly, cell spreading was even observed at day 7. Considering these initial results, we could concluded that the formulated composites have a great potential to be used in several biomedical applications, especially for epithelial tissue repair.

Acknowledgements: CAPES, CNPq,FINEP.

References

P62
Proposal of methodology for analysis of stress level based on EEG signals
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Stress can affect all people, regardless of age, gender or ethnicity. The human body utilizes stress as a response in three different situations, classified according to the way it generates physical, mental or emotional stress. It is important to notice that the nervous system evokes the same physiological responses, no matter what type of stress, and those responses cause a change of level in physical and cognitive performance. This paper presents an analysis methodology of the stress level using brain signals captured by Electroencephalogram (EEG). Professionals from Vitoria’s Fire Department, members of Projeto VIDA participate as volunteers. The EEG signals are captured through a cap placed over the volunteer’s head in order to capture the brain’s signals, using electrodes specifically placed in the frontal cortex at positions Fp1, Fp2, F3, F4. The results are analyzed together with the peripheral physiological signals, such as: heart beat rate (EEG), peripheral blood flow (at fingers and toes), skin conductance, breath rate and body temperature. A validation study is conducted through a comparison of data available in literature, as well as with evaluation conducted by a psychologist.

The results are analyzed in order to get a correlation between EEG signals and physical mental or emotional stress.

References


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Antiviral effects of Scaptotrigona postica propolis and their fractions

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BMC Proceedings 2014, 8(Suppl 4):P63

Introduction: Studies about viral infections have a great importance in human and veterinary health, and the number of medications available to treat these diseases is very reduced, making the search for antiviral molecules an important focus for scientific research. Propolis is a bee material manufactured by the mix of exudate of plants, saliva and bee wax. This product is used to seal the hive and involve dead invaders. Their chemical profile is very variable, and depending on the geographic origin and plant conditions of growth. The use of propolis (bee-glue) for various purposes has reports at before Christ. In Egypt, propolis was used in the preservation of bodies, performing a function of balsam, and its use persists to today in folk medicine to treat various pathologies, being widely used around the world. It is known that propolis of Apis Mellifera has compounds with antiviral activity on virus like Influenza A and B, Vaccinia virus, Hepatitis virus, HIV, Herpes virus, HIV and Poliovirus. In Brazil exists a subfamily of bees named Meliponinae, the stingless bees mixing the resins collected of plants with wax and ground, producing a different type of propolis, named geopropolis, but not all bees of this family produce this type of propolis, like Scaptotrigona postica. The biological activities of Scaptotrigona postica propolis remain unknown, the little information about this product is concentrated in antibacterial and anti fungi actions, but don’t has related of antiviral action.

Objectives: Purify, isolate, and characterize substances with antiviral activity of propolis from Scaptotrigona postica.

Material and methods: The propolis collected in Barra do Corda, city of Maranhão state, was partitioned with hexane, ethyl acetate and a solution of water/methanol (1:1). The propolis crude, as well its purified fractions, were tested by viral titer reduction technique, and determination of viral mRNA against measeles, picornavirus, influenza virus and rubella virus.

Results and discussion: Experiments with the purified fraction led to a 64-fold reduction of picornavirus production, 32-fold reduction in influenza virus production and 8-fold reduction of measles virus. Assays using RTPCR, to determine viral mRNA present in the treated and infected cells, was also performed. The purified antiviral fraction was able to reduce at 103 times the replication of rubella virus. At the moment, we are performed the optimization of the purification process.

References


P64

Partial characterization of two novel monoclonal antibodies for Listeria spp. Immunodetection

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BMC Proceedings 2014, 8(Suppl 4):P64

Background: The Listeria genus comprises ten different species, however, only two of them - L. monocytogenes and L. ivanovii - are pathogenic. Although being a well-known pathogen, its detection methodologies are still limited. The gold-standard technique involves enrichment, isolation, and biochemical characterization, which can take about 7 days [1]. In addition, since both pathogenic and non-pathogenic species are present in contaminated food, it is important to detect them both [2]. This way, new methods, such as the immunodetection using monoclonal antibodies (MAbs), have been developed to reduce the detection time of Listeria spp.. Recently, our group described a hybridoma clone secreting a MAb of the IgM isotype, called MAb-3F8, that reacts exclusively with an antigen of 30 kDa (P30) present in all Listeria spp. evaluated [3]. In the present work, we produced and initially characterized, by ELISA and Western blot, other two novel antibodies against P30 to be used in Listeria spp. immunodetection assays.

Materials and methods: A female BALB/c mouse was initially inoculated intraperitoneally with 100 μg of recombinant P30 (rP30) with complete Freund’s Adjuvant (FA). Fifteen days after the first injection, a series of 13 doses of rP30 with incomplete FA were performed weekly. Then, the mouse spleenocytes were obtained and further fused with Sp2/O cells. The resulting hybridomas were cultured and screened by ELISA using both rP30 and formalin-killed L. monocytogenes strain ATCC 19117 (Lm19117) as antigens. Thereby, two hybridomas were selected and used to produce ascites in mice [4]. MAbs isotyping kit (Sigma Aldrich) was used to determine the isotype of each MAb. For the ELISA, rP30 and formalin-killed Lm19117 and L. innocua (Linn) were used as antigens. For the Western blot (WB), Lm19117 cell-wall fraction (S) and rP30 were transferred to a PVDF membrane. In both assays, the ascites and anti-mouse/peroxidase (Sigma Aldrich) were primary and...
secondary antibodies, respectively. In addition, 3F8 (positive control) and a non-immunized mouse serum (negative control) were used. Absorbance results at least 3 times higher than the negatives were considered positives. **Results and conclusions:** The titrating assay determined that one MAB was IgG1 (MAB-G2A) and the other, IgM (MAB-MAA). In the ELISA, MAB-G2A reacted with rP30 (OD$_{450}=0.346$), but not with Lm19117 (OD$_{450}=0.020$) or Linn (OD$_{450}=0.018$). However, MSA reacted with rP30 (OD$_{450}=0.160$), Lm19117 (OD$_{450}=0.229$), and Linn (OD$_{450}=0.199$). Negatives for rP30, Lm, and Linn were 0.026, 0.048, and 0.062, respectively, while the positives were 0.418, 0.237, and 0.211, respectively. In the WB, the same pattern seems to occur with MAB-G2A, since it reacted only with rP30, while MAB-MSA presented no reaction. The fact that MSA had no reaction in WB, but showed promising results in ELISA indicates this MAB binds to a conformational epitope of P30 that is displayed on Listeria surface. Considering this, further studies will be conducted with a larger panel of bacteria to better characterize these MABs and determine their sensitivity and specificity in detecting Listeria spp. in food samples. **Acknowledgements:** We would like to thank CAPES, CNPq and FAPERGS for the financial support and scholarships. **References**


**P66**

Rutin reduces oxidative stress in animals with renovascular hypertension

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**BMC Proceedings 2014, 8(Suppl 4):P66**

**Introduction:** Rutin has been shown to be a potent antioxidant that restores impaired vascular reactivity and baroreflex sensitivity in hypertensive rats, mainly by decreasing oxidative stress.

**Objective:** This study aimed to evaluate the effect of rutin administration on the baroreflex and oxidative stress in serum of rats with renovascular hypertension (2K1C model) and their nonhypertensive controls.

**Methods:** Twenty-four rats were divided into 4 groups: sham + saline, 2K1C + saline, 2K1C + rutin. Six weeks after 2K1C surgery, animals presented hypertension compared to the control group ([42.4 ± 2 mmHg versus 121 ± 2 mmHg, n = 8, p < 0.05]), while there was no change in heart rate. After six weeks, animals were treated with saline or rutin (30 mg/kg/day) by gavage for 14 days. The baroreflex sensitivity was evaluated using intraesophageal injection of phenylephrine (8 mg/kg) and sodium nitroprusside (25 mg/kg). Lipid peroxidation was measured in serum by thiobarbituric acid reactive species assay (TBARS).

**Results:** Chronic treatment with rutin produced an improvement in baroreflex sensitivity in the 2K1C group. In the sham group, the absorbance values were 2.52 ± 0.52 vs. 2.70 ± 0.20 mmHg, 0.053 respectively, and higher compared to 2K1C + saline group (-2.55 ± 0.15 vs. -1.74 ± 0.10 mmHg, n = 6, p = 0.05). Serum levels of lipid peroxidation in 2K1C rats were higher than in the sham group (5.29 ± 0.93 vs. 4.00 ± 0.01 nmol de MDA/ml respectively, n = 6, p < 0.05). Chronic administration of rutin reduced serum levels of lipid peroxidation in hypertensive rats compared to 2K1C + saline group (2.24 ± 0.59 vs. 0.93 ± 0.55 nmol de MDA/ml, respectively, p < 0.05). No changes were found in the sham group.

**Conclusion:** These results suggest that rutin restores baroreflex sensitivity and reduce oxidative stress in rats with renovascular hypertension.

**References**


Frequency of Human Papillomavirus infection in squamous cell carcinoma of the oral cavity and oropharynx
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BMC Proceedings 2014, 8(Suppl 4):P67

Background: Squamous cell carcinoma of the oral cavity and oropharynx is a public health issue, with over 200,000 new cases worldwide each year [1]. Studies show that the incidence is higher in male individuals, aged between 40 and 55 years. The consumption of tobacco and alcohol are well-established risk factors for developing head and neck cancer, and studies have been shown synergism between these substances, which contributes to the increased risk [4]. Recently, persistent infection with high risk Human Papillomavirus (HPV) has been identified as a potential risk factor for the development of these tumors [2]. HPV is a DNA virus that features tropism to epithelial cells. The high risk HPV types, such as types 16 and 18 have the ability to integrate their DNA into the host cell DNA, immortalizing keratinocytes, with great possibilities of causing a cancer by infecting cells [3]. This study aims to evaluate the frequency of HPV infection, smoking and alcohol consumption related to the development of squamous cell carcinomas of the oral cavity and oropharynx.

Methods: 99 frozen tumors samples, classified as squamous cell carcinoma of the oral cavity (n = 83) and the oropharynx (n = 16) were analysed. The polymerase chain reaction was performed after the DNA of the samples, using the Human β-globin genes. After, electrophoresis was performed in 1% agarose gel containing ethidium bromide, to confirm the amplification and quality of extracted DNA, then the DNA samples were subjected to nested PCR with primers MY09/11 and GPS + /GP6 + for viral DNA detection of HPV subtypes 16 and 18. Samples known to be positive for both subtypes of HPV 16 and 18 were used positive controls. Subsequently, the amplified samples were submitted to electrophoresis on a polyacrylamide gel. History of exposure to tobacco and alcohol was investigated through interviews.

Results and conclusions: Patients with tumors were mostly male (80.81%) aged 30-93 years (average 57.6 years). The use of tobacco was observed in 84.8% of cases and the alcohol abuse of 77.7%. HPV infection was detected in 84 of 99 samples, which corresponds to a low prevalence (4.04%). All of patients, since there is patients that consume alcohol and tobacco concomitantly, which are also risk factors for developing the disease.

Acknowledgements: CNPq, FAPES, CAPES, PPGBiotec-LIFES.

References
Background: Increasingly infectious agents change, which is why there is a failure in the treatment of microbial infections, above all, the emergence of resistance to existing antibiotics [1]. Therefore, the search for new antimicrobial substances from natural products has increased the interest of researchers. Brazil is considered the country has one of the greatest biodiversity on the planet, where the popular use of medicinal plants has become an important source for the discovery of new active compounds [2]. However, for the safe use of these plant species, popular knowledge must relate to the performance of bioassays that demonstrate the therapeutic efficacy and low toxicity. Cordia species belonging to the family Boraginaceae, are used in folk medicine to treat gastric ulcers and proven front possess antimicrobial potential Escherichia coli and Staphylococcus aureus [3]. Phytochemical studies carried out with species of this family revealed the presence of alkaloids, naphthoquinones, saponins, tannins, flavonoids, among others, responsible for this spectrum of biological activities. Cordia nodosa species has proven antifungal activity but the work as they relate to the antibacterial activity are scarce [4]. The aim of this study was to evaluate the antimicrobial activity and toxicity of Cordia nodosa.

Methods: The experimental research was conducted in the Research Laboratory of Wound Care, Federal University of Alagoas. Four fractions were tested in different parts of Cordia nodosa, A, B, C and D. Antimicrobial activity was determined by microbial sensitivity tests, the method of disk diffusion (DD) and broth microdilution method for determination of minimum inhibitory concentration (MIC). The percentage inhibition of bacterial disk diffusion test was calculated by dividing the average inhibition hundred times the sample by the average zone of inhibition positive control [5]. 8 were used bacterial strains, among them Gram-positive and Gram-negative bacteria, distributed by American Type Cell Collection. The toxicity evaluation of the samples was measured by testing toxicity on Artemia salina.

Results and conclusions: Samples B and C were considered moderately active against the strain of Staphylococcus aureus, with a percent inhibition of 29.2% (inhibition = 8 mm) and 55.6% (inhibition = 15 mm), respectively, when compared with the mean of the positive control used Gentamicin (about 27 mm). Both samples were also moderately active against the strains of S. epidermidis, involving inhibition percentages of 33.4%. The sample C presented little active front of the line of antimicrobial activity but the work as they relate to the antibacterial activity are scarce [4]. The aim of this study was to evaluate the antimicrobial activity and toxicity of Cordia nodosa.

Acknowledgements: Brazilian Ministry of Science and Technology, Federal University of Alagoas, Tiradentes University.

References
used in brazilian folk medicine. Among the various medicinal properties attributed to the plant stands out its use to treat diseases of infectious etiology as well as inflammation [3]. The purpose of this study was to evaluate the antibacterial and antifungal activity of the leaf extract Cochlospermum regium against Gram-positive Staphylococcus aureus ATCC 29213, Enterococcus faecalis ATCC 51299, Gram-negative bacteria Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853, and against the yeast Candida albicans ATCC 90028, Candida tropicalis ATCC 750, Candida glabrata ATCC 2001, Candida krusei ATCC 6558.

Methods: To microbiological evaluation, the techniques used were Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC) and Minimum Bactericidal Concentration (MBC). The leaves were collected at farm Santa Madalena (S 22 ° 08' 13.3' / W 055 ° 08' 23.7') in December 2012. Then were evaporated in rota-evaporator and lyophilized preparation of the ethanol extract. The minimum inhibitory concentration (MIC) of the extract was determined by broth microdilution according to the recommendations of CLSI (Clinical and Laboratory Standards Institute) [2]. For evaluation of the fungicide was used Sabouraud Dextrose Agar (Difco) and bactericidal Mueller Hinton Agar (Difco). It is considered that the activity of the extracts tested in MIC is less than 100 μg/mL, antimicrobial activity will be assessed good; if for the antimicrobial activity of 100 to 500 μg/mL is considered medium; from 500 to 1000 μg/mL the antimicrobial activity is considered weak; and over 1000 μg/mL the extract is considered inactive [1].

Results and conclusions: Based on these criteria, the ethanol extract of leaf showed good activity against the C. krusei (64 μg/mL), Enterococcus faecalis (14 μg/mL), Escherichia coli (8 μg/mL) and an acceptable effect to Staphylococcus aureus (512 μg/mL) and inactivates action for C. glabrata (1024 μg/mL). But no effect was opposite C. albicans, C. tropicalis and Pseudomonas aeruginosa. C. krusei is a yeast with great medical relevance and cause of candidemia in adults, especially in hospitalized patients / debilitated. The results are interesting as they show that C. krusei shows a greater intrinsic resistance to the drug fluconazole, which is considered a standard medication and one of the most prescribed antimicrobial [4], and showed a reasonable result of the antimicrobial potential against the bacteria Enterococcus faecalis and Escherichia coli may be an alternative for treatment of infections caused by them. This study confirms the antimicrobial activity of Cochlospermum regium and indicates that it can be a potential candidate for the development of new therapies for the treatment of infections.

Acknowledgements: To Federal University of Grande Dourados (UFGD) and Foundation Support to Development of Education, Science and Technology of the State of Mato Grosso do Sul (FUNDECT-MS).

References
Background: The development of biosensors for the diagnosis and monitoring of diseases, drug discovery, proteomics, and the environmental detection of pollutants and/or biological agents is an extremely significant problem. Fundamentally, a biosensor is derived from the coupling of a ligand-receptor binding reaction to a signal transducer. In this study we used as a model a peptide marker of DENV (dengue virus) type 1. The electrochemical technique of cyclic voltammetry was performed to detect the signal generated by the interaction between the peptide and antibody from patients’ blood samples. Graphite and gold electrodes modified with chitosan film were employed to evaluate the interaction antigen-antibodies. The construction of this immunosensor, able to identify in real time circulating antibodies or antigens, can be applied in the diagnosis of dengue and other infectious and parasitic diseases.

Methods: The synthetic peptide LBPP-D1A (modified or not the N and C-terminal) corresponding to a specific epitope of DENV1 was obtained by F-moc strategy synthesis. This epitope was identified by Spot-synthesis assay using sera of patients. To develop our immunosensor printed electrodes of gold, silver and carbon were used as supports to adsorbed antigenic peptides and capture antibodies from human sera. However as this interaction is electrochemically inert, different chemical conditions and concentrations of peptides were evaluated to obtain a measurable signal. The technique involved the immobilization of the peptide with chitosan film and detection of the signal by cyclic voltammetry under condition of potential -1.0 to 1.0 V, speed of 0.1 V/s and using 200 scan cycles. Other studies involved the modification of the graphite electrode with a film of chitosan prepared in solution of glutaraldehyde and insertion of gold nanoparticles for signal optimization [1].

Results and conclusions: The best condition of immobilization/detection of the peptide was basic pH and concentrations of 1.0 mg/mL PBS buffer, pH 7.4, was used as the electrolyte and later added to this serum PBS. Studies with graphite electrode and film of chitosan prepared with glutaraldehyde although useful have shown the need for protection of the carbosylc hydroxyl group. Studies are in progress, with satisfactory results, using cross-linking epichlorohydrin. Studies on gold electrode were also performed, but with unsatisfactory results, although gold is considered a noble metal, this situation was not a good choice, unlike the graphite electrode, a lamellar structure facilitates the recticulum formation of the network more suitable immobilization. Significant results were obtained, however to guarantee the stability and storage of the disposive sensor, other additional immobilization techniques condition need be evaluated. This study shown that the construction of portable electrochemical immunosensors using specific peptides may be a sensitive, specific and alternative technique for the diagnosis of several infectious and parasitic diseases.

Acknowledgements: Brazilian Ministry of Science and Technology - CNPq, FAPEJ, CAPES, FIOCRUZ (PROEP, Programa de Inovação Tecnológica - INOVATEC)

References:

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Proteome investigation of an organellar fraction of Toxoplasma gondii: a preliminary study
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Background: Toxoplasma gondii is a ubiquitous Apicomplexan parasite responsible for systemic diseases in both humans and animals. Toxoplasmosis is a major public health problem, infecting one-third of the world’s human population and leading to abortion in domestic animals [1]. The search for new chemotherapeutic targets is imperative, due to its increasing resistance to the drugs currently available for combating this parasite [2]. Recent high-throughput proteomic approaches have provided a wealth of protein expression data on Apicomplexan parasites (e.g., T. gondii, Plasmodium falciparum), while a number of smaller-scale studies have examined specific drug-related hypotheses. Proteomic methods can be applied to study sub-cellular localization, cell function, organelle composition, changes in protein expression patterns in response to drug exposure, drug-protein binding, and validation of data from genomic annotation and transcript expression studies [3]. Organellar structures have therefore become potential targets for the parasite life cycle to control the levels of nutrients or salts that surround them [4]. The aim of this study was to perform a proteomic analysis of an organellar fraction of this Apicomplexan protozoan based on the structural and metabolic aspects.

Methods: An acidocalcisome (AC) organellar of T. gondii RH strain tachyzoites was obtained by an iodixanol density gradient [4]. The AC fraction was digested with trypsin and further analyzed by liquid chromatography-mass spectrometry (LC-MS) due to its potential for metabolite screening in proteomic studies. LC-MS analysis was conducted using a nanoHPLC coupled to an ESI-TOF-MS instrument. Trypsin-digested samples were loaded into a pre-column (Reversed phase C18) and desalted with 0.1% formic acid. Peptide separation was performed in a capillary column ( PepMap, C18 5 μm 300 Å, 75 μm I.D., 15 cm). Elution was carried out using a linear gradient (5-40% of solvent B) for 200 min (solvent A = 0.1% formic acid; and solvent B = 50% acetonitrile/0.08% formic acid). The eluate was introduced into the nanospray source set with 5.3 kV of ionization energy and a desolvation temperature of 150°C. Scans were acquired every second throughout the 400 to 2000 a.m.u. range. The precursor (double- and triply-charged) ions with intensity above 10 counts were dynamically selected and subjected only once to collision-induced dissociation for 4s, using the default automatic rolling collision energy. The MS/MS scans were acquired throughout the 50 to 1800 a.m.u. range. 108 proteins were confidently identified by data-mining T. gondii-specific databases.

Results and conclusions: Although some proteins may indicate contamination from other cell compartments, around 50% of the proteins were exclusively identified from the AC fraction (e.g., kinases, an ATP/ATP-carrier protein, a GTP-binding-like protein, lysophosphatidase) and other proteins with no annotated function or homology. Although preliminary, these organellar proteins may represent data to be analyzed and converted into metabolic information in future studies for experimental investigation, and potentially suitable targets for the development of therapeutic strategies, in addition to the role of proteomics approaches in the biomedical area [35].

Acknowledgements: FAPITEC for financial support and fellowship.

References:
The use of polyoxamers (PL) have been one of the most widely strategies used for drug delivery systems. The copolymer Poloxamer 407 is a non-ionic surfactant consisting of polyoxyethylene (POE) and polyoxypropylene (POP) that has many pharmaceutical applications due to its thermoreversible properties. PL are biocompatible and for this reason, are very attractive as carrier systems for administration by different routes, including topical.

This work aimed the development with emphasis on physicochemical characterization of PL hydrogels for quercetin, being a useful strategy to expand the therapeutic application of these compounds as active ingredients in pharmaceutical formulations designed for topical use. 1-2 In the first stage this work was carried out to study the physico-chemical characteristics of quercetin and evaluation of formulations containing PL gels 407 (28% to 30%) and PL 403 (2%) associated to solubilizing agents polyethylene glycol 600 (PEG 600) and propylene glycol (PPG). Systems with higher linearity suggest that PL hydrogels were efficient for permeation across artificial membranes. Besides, thermodynamic profile was obtained from Differential Scanning Calorimetry (DSC) assays, best values of enthalpy (0.580 J/g) and Gibb’s free energy (-12245.95 J/K mol) were observed for the system PL 407-PEG 600, showing that PL system present spontaneous micellization, stability and thermoreversibility for quercetin delivery.

References:

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BaCarb™: anovel bioinorganic matrix for local drug delivery
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3Biotechnology Progress, Journal of separation science 2014, 8(Suppl 4):P77

Background: Treatment of localized tumor by chemotherapy is mostly used as an adjuvant to surgery to protect against or delay the progression of disseminated metastatic diseases or for treatment when other local therapies, such as surgery are not feasible. Local drug delivery offers high potential as a therapeutic choice against premature oncological stages or with isolated cancers since it is more effective in reducing cancer recurrences and avoiding the undesirable side effects of drug spreading along the body [1]. Several applications of the bacterial cellulose in the biomedical field were proposed, like wound healing membranes based on the similarities to the human skin, artificial blood vessels, and as a support and a biological scaffold for tissue formation. Addition of Carr to the biomedicalengineering applications being synthesized on bacterial cellulose, a biomaterial broadly used in many selection schemes necessary for transgenic plants production. It also represents a handy and fast method to evaluate different gene constructs.

Acknowledgements: FAP-DF, CAPES, CNPq, Embrapa.

References:

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Transient expression of the cancer/testis cancer antigen NY-ESO-1 in Nicotiana benthamina using a PVX-based viral vector
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BMC Proceedings 2014, 8(Suppl 4):P76

Background: Cancer/testis antigens is a group of proteins which expression is usually restricted to the human germ line but is also present at high levels in several types of cancer cells [1]. The antigen NY-ESO-1 is one of these antigens showing high immunogenicity and, therefore, holding great potential for a cancer vaccine [2]. Its heterologous expression is being tested in different expression systems, although the success has been limited [2,3]. In this report we used a transient expression system-based on a PVX viral vector to evaluate the expression of NY-ESO-1 in leaves of Nicotiana benthamina.

Methods: The NY-ESO-1 antigen coding sequence was synthesized based on a plant codon optimized sequence. The correspondent fragment was cloned in a pSoup derived vector containing a hexa-histidine tag (6His) and signal peptides for the endoplasmic reticulum (ER), apoplast or chloroplast. These vectors were recombined to a Gateway®-compatible PVX-based vector using the LR clonase, and transferred to Agrobacterium tumefaciens strain GV3101 containing the pSoup vector [4]. Nicotiana benthamina plants were inoculated with Agrobacterium suspensions through vacuum infiltration. After 4-5 days, leaves were collected and macerated in phosphate saline buffer (PBS) at a 1:2 (w:v) ratio and analyzed by Western blot. To further characterize and quantify the expression levels of recombinant NY-ESO-1, leaf extracts were analyzed by nanoUPLC-MSE5 [5]. To this end, leaves were grinded and proteins were extracted using 50mM Tris-HCl pH 8.0 in 1:20 (w:v) ratio. Proteins were precipitated with acetone, resuspended in water and quantified using the Qubit® fluorometric assay.

Results and conclusions: The expression of cancer/testis NY-ESO-1 antigen was detected in N. benthamina leaves using a PVX-based transient assay. Different constructs were tested containing signal peptides for ER, apoplast and chloroplast. No signal was detected when the NY-ESO-1 antigen was targeted to chloroplast or to the apoplast and detection was only possible when the protein was targeted to the ER, indicating that NY-ESO-1 accumulation in leaf tissue is influenced by the subcellular localization.

NANOPLC-MSE analysis confirmed the presence of heterologous NY-ESO-1 in a level of approximately 0.1 % of the total soluble protein extract. This assay allows protein to be detected after only 4-5 days after inoculation thereby eliminating the need of long and cumbersome tissue culture and selection schemes necessary for transgenic plants production. It also represents a handy and fast method to evaluate different gene constructs.

Acknowledgements: FAP-DF, CAPES, CNPq, Embrapa.

References
Psidium guajava which represents a fungus An araginase glycidic portion in its in vitro Staphyloco 6(1) are used as medicine to treat acute lymphocytic using the ANOVA method, considered PNGase F at 37ºC for 3 hours and Nanocellulose patents trends: A Gram negative: Streptococcus pneumoniae 24(31) inactivated from the anionic exchange Psidium guajava of the antimicrobial potential was Journal of Clinical Oncology Candida albicans Candida 2014, Escherichia coli Recent Patents on ter aerogenes, Escherichia coli, μ of the antimicrobial potential was Journal of 2012, The homogeneous fraction obta

**P79**

Evaluation of antimicrobial activity of psidium guajava species

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**Background:** The *Psidium guajava*, commonly known as the apple guava, has shown various biological activities related to its main constituents, such as flavonoids, carotenoids, terpenoids and phenolic compounds. Studies emphasize anti-spasmodic and antimicrobial properties in the treatment of diarrhea and dysentery. It also has hypoglycemic, antioxidant, hepatoprotective, anti-allergic, antimicrobial, antigenotoxic, antispasmodic and anti-inflammatory action [1]. In the light of so many activities, it is suggested that this plant can help in controlling health-related infection, especially in areas of high microbiological risk, such as in the hospital environment. This work’s objective, therefore, was to evaluate the antimicrobial activity of the *Psidium guajava*.

**Methods:** *An in vitro* experimental study which evaluated the antimicrobial activity of leaves of the species *Psidium guajava*. After being dried at room temperature and ground, the vegetable material was extracted in ethanol (70%) at the Natural Resources Research Laboratory of the Federal University of Alagoas (UFAL), and the evaluation of the antimicrobial potential was undertaken at UFAL’s Laboratory for Research in Wound Treatment. The antimicrobial activity was determined by microbial sensitivity tests, with the Agar perforation test and microdilution in broth for determining the Minimum Inhibitory Concentration (MIC). Nine lines of microorganisms were evaluated, including the Gram positive bacteria: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*; Gram negative: *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Escherichia coli*, *Acinetobacter calcoaceticus*, *Salmonella enterica*; and the fungus *Candida albicans*, all distributed by the American Type Cell Collection (ATCC). For the analysis of the Agar perforation test, two methodologies were used [2,3]. The statistical analysis was undertaken using the ANOVA method, considered significant when *p* < 0.5.

**Results and conclusions:** In the Agar perforation test, the sample demonstrated antimicrobial activity against the lines *S. aureus* (Inhibition zone: 12 mm) and *P. aeruginosa* (Inhibition zone: 09 mm), evidencing a moderately active potential for the two methodologies, with bacterial inhibition of 42.85% and 32.72%, respectively, when compared with the inhibition of the positive standard control, ciprofloxacin (Inhibition zone: 28mm and 27.5mm respectively). The results obtained with the determination of the MIC demonstrated that the ethanolic extract of the leaf of *Psidium guajava* presented the lowest inhibitory concentrations, between 3.125 and 6.250 μg/mL, against the lines of *S. aureus*, *S. pneumoniae*, A. calcoaceticus and *Candida albicans*. These are the first reports of the moderate antimicrobial activity of this part of the plant against the lines which already have strains which are resistant to the currently-used substances. In the light of this, it is important to invest in a phytochemical study of this raw extract, so as to isolate and identify its secondary metabolites, the aim being to strengthen this action. This evaluation could predict the chemical constituent(s) responsible for the biological activity [4], thus promoting the development of new alternatives in the control of infection, and the development of a purification level of 10.9 and an enzyme activity recovery of 51.3% as compared to the crude extract. After electrophoresis in reducing and denaturing conditions two bands were observed with a estimated molecular mass of 48 and 46 KDa. It was verified that these two bands correspond to asparaginase II from *S. cerevisiae* and that both are glycosylated. The native enzyme molecular mass was estimated to be 136 KDa suggesting that the enzyme is an oligomer. Two optimum pH values (7.2 and 9.0) were observed. Optimum temperature at both pH, 7.2 and 9.0, was 46ºC nevertheless the activity at 37ºC was 92% of that at 46ºC. After enzyme deglycosilation both bands migrated as a single one in SDS-PAGE, indicating that the main difference between them is the glycosylation level. Isoelectric point was estimated at approximately 4.55.

**Conclusion:** These results support further research aiming at the use of the recombinant yeast asparaginase as an antileukemic medicine. The pre-clinic in vitro studies have already been initiated.

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**P78**

Purification and characterization of asparaginase II from *Saccharomyces cerevisiae* cloned in *Pichia pastoris*: a study on a possible antileukemic drug

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BMC Proceedings 2014, 8(Suppl 4):P78

**Introduction:** Bacterial asparaginase obtained from *Escherichia coli* and *Erwinia chrysanthemi* are used as medicine to treat acute lymphocytic leukemia and non-Hodgkin lymphoma. Despite the therapeutic properties of such enzymes there have been reports on adverse reactions, eventually so severe as to impede some patients of using the medicine. Besides, the only drug Brazil used to import is no longer produced. Considering these two factors our proposition is to produce non-bacterial asparaginase.

**Objective:** Purify and characterize asparaginase II from *Saccharomyces cerevisiae* cloned and expressed in *Pichia pastoris* aiming to produce an antileukemic drug.

**Methodology:** The established protocol to purify the recombinant asparaginase involved three steps: ultrafiltration using a 50 kDa Amicon membrane, molecular exclusion chromatography in Superdex200 and anion exchange chromatography in Mono-Q column. Homogeneity was confirmed by SDS-PAGE in reducing conditions and by MALDI-TOF/TOF mass spectrometry; enzyme activity was determined by the hydroxylaminolysis reaction. Isoelectric point was estimated by two-dimensional electrophoresis using the image analysis software ImageMaster (GE Healthcare); molecular mass was determined using 12% SDS-PAGE and molecular exclusion chromatography. The recombinant asparaginase glycidic portion in its homogeneous analyzed by SDS-PAGE in reducing conditions followed by periodic acid/Schiff stain. An enzymatic cleavage of N-linked oligosaccharides was performed with PNGase F at 37ºC for 3 hours and subsequent analysis by SDS-PAGE. Enzyme optimum temperature and pH were also characterized.

**Results:** The homogeneous fraction obtained from the anionic exchange chromatography showed a specific activity of 204 IU mg-1 which represents a purification level of 10.9 and an enzyme activity recovery of 51.3% as compared to the crude extract. After electrophoresis in reducing and denaturing conditions two bands were observed with a estimated molecular mass of 48 and 46 KDa. It was verified that these two bands correspond to asparaginase II from *S. cerevisiae* and that both are glycosylated. The native enzyme molecular mass was estimated to be 136 KDa suggesting that the enzyme is an oligomer. Two optimum pH values (7.2 and 9.0) were observed. Optimum temperature at both pH, 7.2 and 9.0, was 46ºC nevertheless the activity at 37ºC was 92% of that at 46ºC. After enzyme deglycosilation both bands migrated as a single one in SDS-PAGE, indicating that the main difference between them is the glycosylation level. Isoelectric point was estimated at approximately 4.55.

**Conclusion:** These results support further research aiming at the use of the recombinant yeast asparaginase as an antileukemic medicine. The pre-clinic in vitro studies have already been initiated.
product which could help in antimicrobial treatment based on a natural product.

References

P80
Production of dengue 2 envelope domain III in plant using CPMV - based vector system
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Background: The envelope protein (E protein) of dengue virus is responsible for binding the virus to the host cell. This protein is considered an important immunogen for neutralization of the virus, the only able to induce the production of neutralizing antibodies [1]. The aim of this study was to use the Cowpea Mosaic Virus (CPMV) as a vector to express, on cowpea plants, Vigna unguiculata L. (Walp) the gene fragment that encodes for domain III of the E protein of dengue virus.
Methods: The cloning of inserts in non commercial plasmid (NCP) specific sites containing the RNA-2 of CPMV, its subsequent introduction into competent cells of Escherichia coli (DH10B) and the purification of these proteins from the transformed cells was made through conventional molecular biology techniques. The chimerical virus was inoculated on cowpea plants, and the symptomatic leaves were processed for further purification of the recombinant protein. The protein was purified by a precipitation process described by Florindo et al. (2002) [2].

Results and conclusions: The SDS-PAGE electrophoretic profile of EDIII protein revealed the migration of a protein fraction having a molecular mass corresponding to the E protein band from recombinant CPMV. Different sowing dates were evaluated by REDCap. Different studies have shown an increased vulnerability of the incarcerated population to syphilis, associated to other factors like high-risk sexual behavior. The quality of incarcerated women's life could be improved by routine infection diagnosis, the implantation of a screening program for the health problems and systematic education.

Acknowledgements: This work was supported by the PROEXP 2013 (MEC/SESu) and Foundation Support the Development of Education, Science and Technology of the State of Mato Grosso do Sul (FUNDEC, 04/2013).

References

P81
Prevalence of syphilis diagnosed in female inmates of city Ponta Porã
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BMC Proceedings 2014, 8(Suppl 4):P81
Background: Syphilis is a growing public health problem in several countries. The infection is systemic, usually involving mucocutaneous ulcers and rashes in the early phases, and a range of serious complications including cardiovascular and neurological disease in later phases [1,2]. Global control of syphilis is hampered by slow and insensitive diagnostic methods, particularly for risk population like prison inmates [3,4]. This study aimed to analyze the prevalence and socio-demographic, behavioral and institutional factors associated with Treponema pallidum infection in prison women in Ponta Porã city, Mato Grosso do Sul.
Methods: The study was conducted from January to September 2013 and the sample size was calculated based on the prevalence of syphilis in Brazil. The sample was randomly selected and included 74 female inmates in Ponta Porã. To determine the prevalence of Treponema pallidum infection, blood samples were collected for serological tests using a treponemal test named enzyme-linked immunosorbent assay (ELISA) and a non-treponemal test e venereal disease research laboratory (VDRL). Each test was performed in accordance with manufacturer’s recommendations. Socio-demographic and clinical information, as well as variables related to transmission were collected in a standard questionnaire. Research Electronic Data Capture (REDCap) is being carried out to store data. The study was approved by the Research Ethics Committee of the Universidade Federal da Grande Dourados, Brazil.

Results: During the study, 11 cases of Treponema pallidum infection were identified, which represents a prevalence of 14.8 %. The serology analyses showed that the prevalence was higher than other studies, where the prevalence ranged from 0.5% and 7.5% [5]. The risk factors are still being evaluated by REDCap. Different studies have shown an increased vulnerability of the incarcerated population to syphilis, associated to other factors like high-risk sexual behavior. The quality of incarcerated women’s life could be improved by routine infection diagnosis, the implantation of a screening program for the health problems and systematic education.

Acknowledgements: This work was supported by the PROEXP 2013 (MEC/SESu) and Foundation Support the Development of Education, Science and Technology of the State of Mato Grosso do Sul (FUNDEC, 04/2013).

References

P82
Effect of chalcones in the modulation of Trichophyton rubrum cell wall synthesis genes
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BMC Proceedings 2014, 8(Suppl 4):P82
Background: Trichophyton rubrum is a dermatophyte that causes mostly superficial mycoses in skin, hair and nails, but an invasive course has been described in immunocompromised patients [1]. The resistance to usual antifungal drugs has been observed in Trichophyton what drives an increasing
demand for new antifungal drugs. Chalcones are flavonoids found in plants that exhibit pronounced antifungal activity, most likely acting on the cell wall [2,3]. The aim of this study was to evaluate the modulation of expression of genes involved in cell wall synthesis of T. rubrum in the presence of chalcones.

Methods: The T. rubrum strain H6 (ATCC MYA3108) was submitted to standard techniques for fungal manipulation and growth for 15 days as described previously by Fachin et al. [4]. The solution containing 5.10^6 conidia/mL of H6 T. rubrum strain was inoculated into 50 ml of liquid Sabouraud medium in the presence of 1.95 mg/ml of trans-chalcona metoxichalcona and control acualcin (0.24 mg/ml) and amphotericin (3.9 mg/ml) in a rotary shaker at 28°C. After 8 hours of antifungal exposition, the RNA was extracted, converted into cDNA and was used in the experiments of real-time PCR. Expression levels were calculated by the comparative Ct method using 18S rRNA as normalization gene and untreated mycelia as reference, according Bicentourc et al. [5].

Results and conclusion: The genes evaluated were DW699324 encoding the catalytic subunit of beta 1,3-glucan synthase in Aspergillus fumigatus [6]. The gene DW703091 (β-1,3-glucanosiltransferase) that is involved in the remodeling of 1,3-glucan in yeast [7]. The DW687782 is the gene encoding structural proteins of the membrane and the cell wall and is essential for the growth of Candida albicans [8]. The three genes of T. rubrum were induced in the presence of trans-chalcone, metoxichalcona and control acualcin (acts on the wall) and were repressed in the presence of amphotericin (acts on the membrane). Thus, the antifungal effect of chalcones against T. rubrum may be related to modulation of these genes involved in cell wall synthesis.

Acknowledgements: This study was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (2011/06124-8) and fellowship granted to TAB (2012/02920-7) and LFBB (2012/15862-5); CNPq (PBIC fellowship granted to MHA), PET MEC (fellowships granted to TRM, BAMC, YDC) and CAPES through fellowship granted to FRB and TTK. We thank the staff of the Biotechnology Unit, UNAERP.

References
osteoelastic differentiation of mesenchymal stem cells, through activation of osteelastic related genes. In order to ensure proper glycosylation and conformational folding and to prevent immunogenicity, we elected a mammalian cell expression system to produce these BMPs aiming at bone regeneration, stem cell proliferation and differentiation and their application in human and veterinary cell therapy.

Methods: BMPs 2 and 4 cDNAs were amplified from an in-house constructed cDNA Bank and cloned into the pGEM®-T-Easy vector. E. coli transformants were screened by colony PCR. Upon DNA sequencing, the BMP 2 and 4 inserts were transferred to a lentiviral expression vector. HEK293 cells were co-transfected with a lentiviral plasmid containing both BMP 2 or 4 and eGFP cDNAs, co-transfection, at a 4:1 ratio, with a Hygro® vector for clone selection. Cell clones were selected using 100 ug/mL hygromycin. Several cell clones were characterized and highest overproducing ones were selected for each protein. BMPs expression was analyzed by qRT-PCR, Western blotting, and in vitro biological activity by alkaline phosphatase activity in 2C212 cells during 7 days. Recombinant proteins were purified using heparin affinity chromatography.

Results and conclusions: Upon cell cloning, most of the cells present in the selected clones were positive for GFP, indicating that a high transfection efficiency was achieved. BMPs 2 and 4 were continuously secreted to the medium even after 120h of serum starvation. Purification of rhBMP2 and 4 from the conditioned medium resulted in more than 90% purity. The rhBMPs 2 and 4 bound to the resin were eluted in 450mM NaCl buffer, with a single dimeric 30-37 kDa band being observed in the eluates. In vitro studies showed that the purified rhBMPs 2 and 4 displayed high osteogenic activity. The in vivo osteobioactive analysis of purified proteins by ectopic bone formation using Rowett rats is underway. Glycosylation analysis using exoglicosidase digestion and structural analysis of the purified proteins is underway. The use of these biopharmaceuticals in bone Tissue Engineering is likely to allow accelerated recovery to both human patients and animals.

Acknowledgements: BNDES, CAPES, CNPq, FAPERJ, FAPERJ, FINEP, MCTI and MS-DECIT.

References

P85

EEG analysis and mobile robot as tools for emotion characterization in autism

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BMC Proceedings 2014, 8(Suppl 4):P85

Background: Autism or “Autism Spectrum Disorder” is characterized by manifestations of impairments in social behavior, stereotyped movements, difficulty in communication and interaction with people. This paper presents a system composed of a mobile robot to generate interactive tasks with autistic children, and an EEG (electroencephalography) equipment to get brain information to characterize their emotions.

Methods: The mobile robot is equipped with a laser sensor to detect and locate the position of the child, in order to obtain distances to her/him, and a computer for brain signal processing and to schedule rules for the interaction. In addition to that, an wireless EEG cap is placed over the child’s head in order to capture the child’s brain signals during the interaction process, through electrodes specifically placed on the frontal cortex at positions Fp1, Fp2, F3 and F4, according to the international system 10-20, one of the most widely used electrode placement system on the skull. The robotic implementation allows two modes of interaction, depending on the interaction level with the child. Through the “dog” mode and “follower” mode, the robot identifies the position of the child and moves to where he/she is, keeping a safe distance of interaction. When the child has little interest in what happens around him/her, the robot (“dog” mode) approaches and moves away from him/her, attracting his/her attention. When the child shows some interest in interacting with the robot, this (“follower” mode) follows her/him if she/he moves away from it. On the other hand, if the child approaches the robot, this will move away, keeping the distance of interaction, and stops in order to start the interaction. During the whole process, the child’s brain signals are captured by a wireless EEG cap and analyzed by the computer. The methods to evaluate autistic child during the interaction with the robot involve the Goal Attainment Scale (GAS) and a behavioral evaluation, which takes into account the child’s emotional state.

Results: The system of detection and location of the child is effective to obtain his/her coordinates. Thus, a safe distance is defined in order to preserve the physical integrity of the child during her/his interaction with the robot. Often the difficulty in communication and interaction with people prevents knowledge about feelings and emotions that individuals with autism have. The use of EEG allows monitoring the child’s brain signals and making a characterization of emotions linked to aspects of the interaction with robots.

Conclusions: The implementation of the two robotic modes of interaction (“dog” mode and “follower” mode) assists directly the process of social evolution of autistic children, as a teaching tool for parents, teachers, carers, therapists and researchers. By evaluating the detection of autistic child’s mental states by EEG along with a behavioral assessment, it is possible to establish the corresponding emotions and analyze the evolution of the interaction between the child and the robot efficiently.

Acknowledgements: To CNPq, CAPES, UFES, PPGBiotec.

References

P86

Evaluation of potential antimicrobial chlorhexidine digluconate microencapsulated in model experimental dental biofilm

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BMC Proceedings 2014, 8(Suppl 4):P86

Background: The formation of biofilm on the tooth surface is a result of the process of colonization of cariogenic bacteria, especially Streptococcus mutans, the main responsible for cariogenic (acid production) of dental biofilms [1]. The removal and chemical control of dental biofilm with chlorhexidine digluconate 0.12% (CLX) has been the most effective antimicrobial agent of choice clinic. However, despite its indisputable efficacy, no side effects such as darkening of the teeth, tongue and restorations, burning mouth and changes in taste. The formation of inclusion compounds CLX for the controlled release of the compound in a lower concentration could be a viable strategy to reduce these side effects without diminishing the therapeutic efficacy of the product [2,3]. Against this background, this study aimed to develop nanocapsules CLX (processing aid) (1.2%) in model spray drying using modified starch as the encapsulating agent, tested in biofilm model using bovine teeth.

Methods: The microbiological evaluation of in vitro antimicrobial activity of different formulations was made against S. mutans strain UA159 grown in artificial saliva, a model of biofilm produced in the flow cell. The biofilm on the teeth was exposed to nano-CLX, CLX Periogard * and standard
laboratory for 2 minutes. Cell viability was measured by fluorescence LiveDeadBacLightTM Kit with dyes Syto *9 and Propidium Iodide.

**Results:** The showed equivalence between efficiency and CLX laboratory Periogard 4, featuring both 0% percentage of S. mutans after treatment. The CLX nanocoted (1.2%) showed significant efficiency at 90% cell death indicating controlled release of the drug. The model CLX nanocoted by spray drying, and modified starch as encapsulating agents when tested in a model of biofilm using bovine teeth showed good response when used as antimicrobial.

**Conclusions:** The molecular nature of chlorhexidine ensures its effectiveness in combating bacterial biofilms, and such activity is in fact already enshrined. However, the alternative formulation nancapsulada allows an effective control of its release, ensuring control of the main process cariogenic bacteria, thereby reducing their undesirable aesthetic point of view, representing new alternative for the application and release of a single antimicrobial without the immediate need for new drugs.

**Acknowledgements:** Laboratory of Oral Biology.

**References:**

**P87**

**BUL: a novel lectin from Bauhinia unguiflora, seeds with fungistatic and antiproliferative activities**

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**BMC Proceedings 2014, 8(Suppl 4):P87**

A new galactose-binding lectin, termed BUL, has been purified from seeds of *Bauhinia unguiflora* (Caesalpinioideae) by precipitation with solid ammonium sulfate followed by agarose-lactose affinity chromatography. B. unguiflora lectin strongly agglutinated rabbit erythrocytes, both native and treated with proteolytic enzymes, and was inhibited by D-galactose and D-galactose-derived sugars, especially N-acetyl-D-galactosamine. BUL was shown to be a stable glycoprotein, maintaining its hemagglutinating activity after incubation at wide ranges of temperature and pH, but not after incubation with EDTA. By SDS-PAGE analysis under reduced conditions, purified BUL showed an electrophoretic profile consisting of a single band with apparent molecular mass of 30 kDa. BUL showed intrinsic fluorescence typical of folded globular proteins, and circular dichroism spectra of lectin in the native state showed a predominance of β-sheet secondary structure. The N-terminal amino acid sequence of 19 residues showed a high sequential similarity to other galactose-specific lectins from the *Bauhinia* genus. In addition, BUL showed antifungal activity against phytopathogenic species and showed in vitro antiproliferative activity against the HT-29 cell line of human colon adenocarcinoma in a dose-dependent manner.

**P88**

**Recombinant human proinsulin expression in Pichia pastoris using PGK promoter**

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**BMC Proceedings 2014, 8(Suppl 4):P88**

**Background:** There are indications, according to WHO, that diabetes will reach 333 million people in 2025, and already is among one of the main death causes in the world. In 2030 in Latin America, diabetes mellitus will be the second cause of deaths [1]. The actions to increase the production of insulin are strategic to respond the high demands for what we have to face in the near future [2]. *P. pastoris* methylotrophic yeast is being very used in the last decades to express heterologous genes to biotech uses [3]. The main expression vectors to *Pichia pastoris* are based in the strong promoter from AOX1 gene which codifies alcohol oxidize enzyme. However there is a search for new promoters than AOX1 due to certain disadvantages as the use of methanol as inducer that is toxic and originates undesirable subproducts during its metabolism [4]. This work has the main objective in to express in high levels the human proinsulin in *Picha pastoris* using PGK promoters.

**Methods:** The human proinsulin codifying sequence was chemically synthesized (Genone inc.) with *P. pastoris* optimized codons. The synthesized sequence was subcloned under PGK promoter control in expression vector together with the codifying region from *Saccharomyces cerevisiae* factor-a signal peptide in order to have proinsulin expression/secretion. After linearization the vector containing the expression cassette was introduced in *P. pastoris* GS115 by electroporation. To select clones, with multycopies of expression cassette, was realized a zeocin resistance test in plate [5]. Resistant clones to 100 μg/mL of zeocin were selected and submitted to crescent concentrations of the antibiotic: 500, 1000 and 2000 μg/mL. The recombinant clones that produced/secreted more proinsulin was detected by imunodot procedure using antibody anti-insulin/proinsulin (Pierce-D3E7/5B6/6). Selected recombinant clones were cultivated in YPD medium for 24 hours in 30°C at 200rpm and the supernatants were clarified by centrifugation and analyzed by ELISA and Western-blotting.

**Results and conclusion:** From 200 zeocin (100 μg/mL) resistant *P. pastoris* clones 25 were able to grow in 2000 μg/mL which indicates that they have integrated several expression/secretion cassettes in their genomes. From those one was notable for its higher human proinsulin expression/secretion in both experiments (imunodot and ELISA). The proinsulin overexpression of this selected clone was confirmed by PAGE and Western-blotting with anti-insulin/anti-proinsulin antibody. Additional experiments are in process in order to standardize the fermentative process for expression and secretion of human proinsulin as well as confirm recombinant protein amino acid sequence.

**Acknowledgements:** This work was supported by CNPq, CAPES, FAPESP and FAPDF.

**References:**

**P89**

**Antimicrobial activity of the trans-cinnamaldehyde on nosocomial enteric bacilli producers of extended spectrum β-lactamase (ESBL) Vicente Pinto1, César Barbosa, Pedro Magalhães2, Camila Coelho1, Joseires Fontenelle1, Genardo Cristino-Filho1, Helliada Chaves2, Antonio Silva2, Alrieta Teixeira3**

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**BMC Proceedings 2014, 8(Suppl 4):P89**

**Background:** The extended spectrum β-lactamases (ESBL) are enzymes that produce resistance to β-lactam antibiotics, including penicillins, wide
spectrum cephalosporins and aztreonam, by cleavage of $\beta$-lactam ring (BUSCH & JACOBY, 2010). Since ESBL-producing bacteria are frequently associated with nosocomial infections, treatment options are becoming increasingly limited (RAWAT & NAIR, 2010). In this context, the discovery of compounds which can inhibit the growth of micro-organisms which produce these enzymes becomes increasingly important.

**Methods:** In this study we evaluated the antimicrobial activity of trans-cinnamaldehyde by microdilution technique and also determined its minimum bactricidal concentration (MBC) on nosocomial enteric bacilli $\beta$-lactamases producers. We analyzed 45 bacterial species, 36 to the Enterobactericeae family and nine of the other species of Gram-negative bacteria. The most prevalent species ESBL-producing was Klebsiella pneumoniae (70% of isolates of this species). The detection of ESBL was performed by phenotypic testing (approximation discs, combination discs and minimum inhibitory concentration - MIC - using E-test).

**Results and conclusions:** The trans-cinnamaldehyde showed antibacterial activity and promote inhibition of growth for all planktonic microorganisms ESBL positive tested, with MIC ranging between 0.95 mM and 1.90 mM. Bactericidal activity was detected at a concentration of 1.90 mM, regardless of the species analyzed in this study. Our results suggest that trans-cinnamaldehyde is a compound with potential antimicrobial against ESBL-producing bacteria and can be employed both in preventing infection through their application in solutions used in the processes of disinfection of hospital instruments and equipment but also in drug development for topical action.

**References**


**P90**

Molecular dynamics simulation of dopamine and ascorbic acid amid tetrafluoroborate 1-butyl-3-methylimidazolium compared to conventional solvents

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BMC Proceedings 2014, 8(Suppl 4):P90

Dopamine (DA) is an important neurotransmitter in the Central Nervous System (CNS) of mammals being found in significant quantities in the brain. In a low concentration is associated with Parkinson’s disease [1], which makes it important detection. However, a major problem in determining DA is its joint resolution with coexisting species such as ascorbic acid (AA).

In traditional solid electrodes, AA is oxidized at a potential close to that of DA, resulting in an overlapping voltammetric response [2]. Therefore, improving the selectivity of the monitoring techniques of AD has been the focus of much research. [3] Thus, ionic liquids at ambient temperatures (RTILs) tetrafluoroborate and 1-butyl-3-methylimidazolium (BMI.BF4) can be used as electrolytes for different electrochemical reactions, which have important properties, the selectivity of them, making the technique voltammetric more efficient. Our focus as this work is to analyze the behavior of these molecules (DA and AA) amid BMI.BF4 compared to conventional solvents, using a method known as Molecular Dynamics (MD), being a powerful tool for obtaining properties structural and thermodynamic properties of these substances.

MD simulations were carried out for systems in DA [BMI.BF4] and AA [BMI.BF4]. For comparison, simulations were also carried out in water. All simulations were performed using the AMBER 9 package. We also used PTRAJ to perform analyses on the data we collected, such as calculating density, total energy, RDFs and self-diffusion coefficients, and we used xmgrace and gnuplot to aid us in visualizing the data.

As part of evaluating the interference between molecules (AA and DA), we show the results of analyzes of AA. In this case the average density is 1.0768 cc / mol, while the water density is 1.027 cc / mole. As the total energy of the two systems have different values and constants (-19.5 kcal/mol in the midst of water and -12.5 kcal / mol among LI) along the dynamics. This difference in energy is given by unfavorable interactions that AA has with the molecules of the LI. For a better understanding of the systems were calculated the radial distribution functions for each of statepoints. The results presented in both solvent were similar, demonstrating that the AA is added in both LI well as for water, i.e, they have good solvation.

Regarding the results of diffusivity, the 298K and 1 atm the coefficient of self-diffusion (MSD) showed lower values compared to the water in the same conditions. This result reveals the MSD in a higher viscosity of the LI and so may be an important property for separation of molecules (AA and DA) and therefore important in electrolysis process.

**References**


**P91**

BAP effect in vitro germination of soybean cultivars UFUS Xavante an UFUS Carajás

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BMC Proceedings 2014, 8(Suppl 4):P91

Background: Plant tissue culture is a biotechnology particularly relevant to plant genetic transformation. The ability of in vitro regeneration is one of the requirements for the production of usable plants in breeding programs [1]. As each genotype has a specific regeneration potential, several protocols have been developed with the aim of developing methodologies that can speed up the germination process [2]. In vitro, culture media for plants, not only provide macro and micronutrients, but also carbohydrates, usually sucrose as a carbon source. Better results are obtained by adding organic compounds such as vitamins, amino acids and growth regulators [3].

**Methods:** This study evaluated the germination of seeds of two soybean cultivars on MS medium with different concentrations of 0, 1, 3 and 5 μg / L BAP. The cultivars used were UFUS Xavante and UFUS-Carajás from the Breeding Program Soybean by Federal University of Uberlândia. Seeds were placed in a solution of alcohol/water 70% (v/v) for 1 min and then immersed in 0.5% sodium hypochlorite (w/v) for 30 min. After surface sterilization, the seeds will be washed with distilled water and autoclaved. After desinfection (quoting the type of disinfection) seeds of cultivars were soaked in distilled water autoclaved for a period of 1 and 7 days. Subsequently, the seeds were inoculated in test tubes with the medium remains under dark for 7 days. The evaluations were done every 7 days for 28 days [4].

**Results and conclusions:** We evaluated the occurrence of rupture of the radicle and seedling formation. After 7 days it was observed that the cultivar UFUS-Xavante that were soaked in water for one day and inoculated in medium with 3 μg / L BAP showed 100% of germination. On the other hand, the soaking for 7 days led to a decrease in germination percentage (80%) independent of the hormone concentration. At 14 days, there was the emergence of the cotyledons for both cultivars in medium with 3 μg / L BAP and with 21 days, there was the formation of seedlings in this regulator concentration. It was observed that increasing the regulator concentration associated with prolonged soaking conditions were not favorable for both varieties. New regulators combinations are necessary to assess the effect of the growth regulator and the time soybean’s formation seedlings in vitro. Keywords:...
P92

Investigating the expression pattern of the OsAPx1 gene promoter in rice
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BMC Proceedings 2014, 8(Suppl 4):P92

Background: Ascorbate peroxidase (APx) is a key enzyme of the antioxidant metabolism, catalyzing the decomposition of hydrogen peroxide (H₂O₂) in water, using ascorbate as an electron donor. The H₂O₂ is a reactive oxygen species (ROS) produced constantly by aerobic metabolism. Under biotic and abiotic stress the level of H₂O₂ increases and, in large quantities, can cause cellular damage. In rice, there are eight APx genes that encode products targeted to different subcellular compartments: cytosol, peroxisoma, mitochondria and chloroplast. OsAPx1 gene encodes a cytosolic isofrom of APx. The study of promoters is an important tool that allows to analyze the overall expression pattern of genes in plants.

Methods: A sequence of approximately 2kb preceding the translation initiation site of the OsAPx1 gene was isolated, cloned into pENTR vector and recombined in pHGWFS7 vector, which allows the fusion of the promoter sequence with two report genes, Gfp and Gus, and confers resistance to hygromycin. The construction was named pROM1. The transformation of rice calli, originated from nipponbare cultivar seeds, was performed using Agrobacterium tumefaciens. The transformed calli were grown in selection medium with hygromycin, regenerated into plants, acclimated in a greenhouse and the confirmation of transgene was verified by PCR using specific primers for the Hpt and Gus genes. For visualization of expression pattern of the promoter, by GUS histochemical assay, samples of plants were collected and analyzed by x-gluc histochemical assays. The segments were incubated in 1 mM x-gluc solution at 37°C for 16h. After reaction, green tissues were incubated in 70% ethanol for chlorophyll discoloration. In the in silico analysis of cis-elements in the promoter region of OsAPx1 was used the following databases available online: PlantPan (plantpan.mbc.nctu.edu.tw) and PlantCare (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

Results and conclusions: Nine lines of transgenic plants expressing Gus under the control of the OsAPx1 promoter were obtained. The Gus expression was observed in leaf (especially in leaf mesophyll), ligule and in wounded regions. These results show that OsAPx1 gene seems to be expressed in green tissues and to respond to damage. Apparently, there is no change in the expression pattern during different development stages. The in silico analysis demonstrates the presence cis-elements responsive to hormones, drought and light.

Acknowledgements: CNPq, FAPERGS, CAPES and ICGBE.

References

P94

Identification of a new binding protein in the insect-pest midgut Heliotis virescens that interacts with Cry1A toxins
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Bacillus thuringiensis crystal protein family (Cry) consists of a diverse group of proteins with activity against insects of different orders, such as the Lepidoptera members. Their primary action is to lyse midgut epithelial cells by inserting into the target membrane and forming pores. Among this group, members of Cry1A family are used worldwide for insect control, and their mode of action has been characterized in some detail, although it is not completely known. Cry1A-binding proteins detected on ligand blots of insect brush border membrane vesicles (BBMV) have been identified as members of the aminopeptidase N and catherin families, although the relative role of the two putative receptor molecules in insects has yet to be conclusively determined. Moreover, it seems that there are other proteins in the midgut cell surface of insect-pests that can interact with Cry1Ac, leading to cell death. Therefore, in this report, we identified the gamma region of a G-protein from Heliotis virescens (HvgGP) as a potential receptor for Cry1Ac. Hence, using in silico analyses, we determined the structure of HvgGP and its interaction region with Cry1Ac. The binding sites was confirmed through Phage Display assays, using both Cry1Ac and HvgGP as templates. Fluorescence analyses indicated that HvgGP interacts with Cry1Ac in a specific region. Although
the mode of action through membrane pore formation was already confirmed by several in vivo and in vitro assays, the mechanism through inhibition/activation of signalling pathways by the interaction with G protein complexes is still not clear. Considering the importance of G proteins on the activation of several signalling pathways and the role of Cry toxins in the agribusiness, we also propose a new mechanism of action for Cry1Ac, using HvgGP as the binding protein.

References

P95
Cellular wall of Eucalyptus grandis under influence of growth regulators - chemical-anatomical aspects
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BMC Proceedings 2014, 8(Suppl 4):P95

This research was carried out to understand the behavior of growth regulators (giberellic acid (GA3) and 6-benzilinobinapin (BAP) on lignification system of Eucalyptus grandis (FORMER Hill Maiden). After GA3 (alone) and BAP (combined) applications, analyses were carried out. These regulators were applied by a atomization system at different concentrations levels. Analomical analysis, including Måue and Wiesner test as well as chemical analysis such as insoluble (Klason) and dioxane lignin were carried out as well. Infrared spectroscopy, methoxyl determination (Seizel Method) and gelatinization of downstream applications on palm genetics and genomics. For instance, we're using such markers to perform a large genetic diversity study of the Brazilian E. oleifera germplasm. As we are also sequencing the whole genome of the species [5], we plan to map the polymorphic reads to the reference genome assembly to assess the maker distribution on the genome and its co-localization with predicted gene models, as well as the physical distance between adjacent markers. The genomic position of the polymorphism is also expected to help the designing of probes for target-enrichment of polymorphic sites.

Acknowledgements: The Brazilian Ministry of Science, Technology and Innovation (MCTI) supported this work through a FINEP grant (PRODENCE).

References

P96
Advancing palm genomics by developing a high-density battery of molecular markers for Elaeis oleifera for future downstream applications
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BMC Proceedings 2014, 8(Suppl 4):P96

Background: Although (Elaeis guineensis) is planted on only 5% of the total world vegetable oil acreage, it accounts for more than 30% of world vegetable oil production [1]. In Brazil, the Federal Government launched a plan to boost Oil Palm production as a way to meet the biofuels market increasing demand for vegetable oil. Nevertheless, the Oil Palm expansion areas in Brazil coincide with the area of occurrence of bud rot [2], a major disease that is decimating plantations already established in the area. In a way to circumvent such problem, breeders are now using E. oleifera germplasm in Oil Palm breeding programs, generating inter-specific hybrids not only resistant to bud rot, but with higher unsaturated fatty acid content, lower height [3]. E. oleifera, however, lack the genomic resources currently available for Oil Palm [1], hampering many possible studies that could potentially help breeding. Based on the foregoing, we started a project to develop a large set of molecular markers for the species based on the DA'Tseq platform [4].

Methods: We collected 552 leaf samples from 206 accessions of the EmbraPA Elaeis oleifera germplasm collection. DNA was extracted and purified and sent for DART-Pty for DA'Tseq development. A combination of restriction enzymes (RE) was used to reduce the complexity of the samples that were later pooled sequenced in the Illumina’s HiSeq 2000 platform. After barcode sorting, sequences were trimmed and used to construct a reference sequence from all unique fragments. On average we obtained more than 2 million reads for each genotype. The individual reads were then aligned to the reference sequence and silico-DArTs scored binning nearly identical sequences so that the presence of an SNP would not hinder the scoring. Using the alignment of the reads to the reference sequence, SNP markers were also discovered based on a pipeline that uses bowtie as a component.

Results and conclusions: We’ve obtained using the DA’Tseq platform over 7.000 silico-DArTs markers. Once we applied a stringent selection criterion (Call Rate >0.90, MAF >0.05, and Q Value >2), 3.187 markers survived. Based on the alignment of the reads to the reference sequence we’ve also discovered 5.500 high-quality SNP loci, and other 1.950 lower-quality SNPs. This set of polymorphic markers may now be used for a series of downstream applications on palm genetics and genomics. For instance, we’re using such markers to perform a large genetic diversity study of the Brazilian E. oleifera germplasm. As we are also sequencing the whole genome of the species [5], we plan to map the polymorphic reads to the reference genome assembly to assess the maker distribution on the genome and its co-localization with predicted gene models, as well as the physical distance between adjacent markers. The genomic position of the polymorphism is also expected to help the designing of probes for target-enrichment of polymorphic sites.

Acknowledgements: The Brazilian Ministry of Science, Technology and Innovation (MCTI) supported this work through a FINEP grant (PRODENCE).

References

P97
Identification of Brassica oleracea proteins during early infection by Xanthomonas campestris pv.campestris
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BMC Proceedings 2014, 8(Suppl 4):P97

Background: The family Brassicaceae comprises several important crops cultivated in Brazil, including broccoli, cauliflower and cabbage. One of the main diseases that affects all cruciferous plants is black rot, caused by the bacterium Xanthomonas campestris pv. campestris (Xcc). This bacterium causes serious damage to the plant, leading to severe yield losses. The disease control is extremely difficult since the seeds are the main source of
bacterial dissemination. The aim of this work was to identify proteins from Brassica oleracea during early infection by Xcc, in an attempt to identify proteins related to resistance.  

**Methods:** Plants from the resistant (União) and susceptible (Kenzan) cabbage genotypes were inoculated with the bacterium and leaves were collected at 24 hours after inoculation (hai). Approximately 0.1 g of tissue was used for protein extraction using phenol. The proteins were quantified and approximately 600 μg of total protein was subjected to two-dimensional electrophoresis (2-DE). The analysis of the 2D maps were performed with the ImageMaster 2D Platinum v7.0 software (GE Healthcare) using gels from each condition.  

**Results and conclusions:** A comparison between the 2D maps of the inoculated plants and the control condition of the resistant and susceptible genotypes was performed. The analysis of the resistant genotype revealed 22 differential proteins, including 2 exclusive to Xcc inoculated plants and 2 to the control condition. In the resistant interaction, most proteins showed decreased intensity in response to Xcc. On the other hand, in the susceptible interaction, most differential proteins were increased upon Xcc infection. One of the proteins identified was a peroxiredoxin precursor, which was decreased in the susceptible genotype inoculated with Xcc. Proteins involved in photosynthesis were also modulated by Xcc infection. The results obtained may help better understand the susceptible and resistant interactions of B. oleracea-Xcc.  

**Acknowledgements:** Embrapa, CNPq, CAPES and UnB.  

**References**

P98  

**Characterization by surface plasmon resonance of electrochemical biosensors developed with organophosphorusbased hydrolase enzymes applied to detection of neurotransmitters**  

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*BMC Proceedings* 2014, 8(Suppl 4):P98  

The biosensor devices were first researched by Clark and Lyons in 1962, aiming to measure the blood glucose level. Currently, there is a popularization of blood glucose meters and various technologies have been developed mainly for applications in medical diagnostics and environmental monitoring. However, there is a wide range of applications for biosensors not yet available. The immobilization of bioactive species that interact directly with the analyte, called bioactive surface, is often considered the most difficult step in the biosensors development for new applications. The objective of this work is to demonstrate the characterization of the bioactive surface developed by self-assembled monolayers technique using the electrostatic adsorption principle. For this, we used three different concentrations of solutions composed of covalent bonds between carbon nanotubes and three different biomaterials, Polyethyleneimine, Deoxyribonucleic Acid and Organophosphorusbased Hydrolase enzymes. These enzymes catalyze the hydrolysis of phosphorus compounds such as organophosphorus neurotoxins. The different polyelectrolyte solutions were characterized by the technique of surface plasmon resonance, and simultaneously, self-assembled monolayers were formed on the surface of a glassy carbon electrode. The enzymes activity was analyzed by cyclic voltammetry for the different concentrations of the solutions used. The results showed that the surface plasmon resonance characterization was interesting to promote the control of electrostatic adsorption. The variation of the surface plasmon resonance angle corresponds the intensity and saturation of electrostatic interactions between the layers. Biosensors developed with solutions of higher concentration, consequently, widened the detection limits, and increased the resolution of the electrical signal due to the lower saturation level, and higher slope signal. In applications to measures of low concentrations of analyte, adequate control of the concentrations of polyelectrolytes solutions reduces costs in the development of biosensors especially, the cost of isolated enzymes.  

**References**


P99  

**Selection and evaluation of citrus phloem specific promoters**  

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*BMC Proceedings* 2014, 8(Suppl 4):P99  

**Background:** The citrus industry worldwide has faced major economic losses due to the occurrence of Huanglongbing (HLB), a disease caused by *Candidatus Liberibacter* spp., Gram-negative bacteria restricted to the phloem of their hosts. Due to the lack of commercial varieties that could resist to these bacteria, genetic transformation comes as an important alternative for improving these varieties and managing the disease. Most of the work with citrus genetic transformation uses constitutive promoters, but recently studies have intensified the search for tissue-specific promoters, making possible to direct the transgene expression to the phloem and reduce it in other undesired parts of the plant, such as the fruits. It is possible that tissue-specific promoter sequences obtained from the same plant species have better acceptance by the customers and this was the objective of our work: prospect phloem-specific promoters from the citrus genome that can be used for citrus transformation with the objective to deliver transgene expression directly to the tissue of *Ca. Liberibacter* spp. accumulation.  

**Methods:** Promoters prospection was made from available citrus EST databases (iREST/HarvEST, available locally), and from the complete genome sequences of *Citrus sinensis* and *C. clementina* (available at www.phytozome.net). Five selected sequences, identified with the program PLACE (www.dna.afccr.org.jp/place/), were cloned into pCambia 2301 containing the uidA gene and inserted into *Agrobacterium tumefaciens* EHA 105. The genetic transformation was performed according to Miya et al. [1].  

**Results and conclusions:** Epicotyl segments obtained from Carrizo citrange and Hamlin sweet orange seedlings were used as source of explants for genetic transformation. From each promoter, three independent experiments were made. In each experiment an average of 300 explants were introduced and approximately 200 buds regenerated. The GUS histochemical test was performed and confirmed the transformation of 2 to 24% positive plants, depending on the construction. The preliminary results exhibit preferential expression in phloem cells. The transgene presence is being confirmed by PCR. A comparison among the GUS expression induced by the promoters will be conducted in order to determine which of them is more efficient in driving the transgene expression to the phloem cells. Such promoter will be further used in expression cassettes constructed with candidate genes for the control of HLB.  

**Acknowledgements:** INCT-Citrus (Fapesp and CNPq), Embrapa-Capes grant and EMBRAPA-Monsanto agreement (02.08.05.004.000.00).  

**Reference**  

One of the main consequences of population growth is that an equivalent food production increase is needed. To meet this basic need of society and ensure growth in food production, it is necessary the use of agrochemicals such as herbicides, preventing competition between crops and weeds for soil nutrients. This growth in use of agrochemicals has historically had undesirable consequences due to their indiscriminate and sometimes reckless use, with health problems for farmers and environmental damage. One of the possible solutions to increase agricultural production without these consequences would be the application of nanotechnology to allow a safer use of pesticides and lessen health and environmental side-effects. However, the use of nanotechnology demands an investigation of possible toxic effects of this technology, mainly in relation to contamination of soil and water. The study we performed aimed to produce nanoparticles containing the herbicide parathion and to analyze its possible genotoxic effects. The technique used was cytogenetic test of Allium cepa treated with nanoparathion, conventional parathion, triprophosphate chitosan nanoparticles, which were made in duplicate with and without humic substances. All concentrations were 0.38 mg.mL⁻¹, and negative control was made with ultrapure water and humic substances for comparative purposes. Initial results indicated less chromosome damage in nanoparathion treated samples compared to conventional parathion herbicide, indicating that nanoencapsulation is a viable option as an attempt to minimize damage caused by parathion.

References

P101
Obtaining new cultures of microorganisms that produces cellulases and xylanases from the sugarcane bagasse
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Background: The international energetic system strongly depends on fossil fuels, which causes negative effects in the environment, such as the global warming. Biofuels appear as an environmental and economic alternative for the energetic industry because of their potential source of renewable energy. Several studies are based on sugarcane culture and its derivatives, as bagasse, the sugarcane residue. Bioethanol can be produced by the fermentation of sugar or by the hydrolysis of cellulolic biomass [1]. The plant cell wall is constituted of cellulose (40-50%), hemicellulose (15-30%) and lignin (10-30%), forming the vegetal biomass. Cellulases are enzymes that form a complex that hydrolyses cellulolic materials, releasing sugars [2]. The main component of hemicellulose is the xylan, which is hydrolyzed by xylanases [3]. Cellulases as xylanases have a great biotechnological potential, they can be used in a variety of field: food, animal feed, textile and paper recycling industries. The sugarcane bagasse (SCB) is the most studied lignocellulosic waste for bioethanol production, because it is a by-product of conventional ethanol and can be find in large amount in Brazil [4]. Nowadays, the process of bioconversion of biomass has high cost and low specific activity of the enzymes that are necessary for the cellulose saccharification [5]. The aim of this research is to obtain microorganisms that hydrolyze the sugarcane bagasse and to quantify the sugar production.

References

P102
Microprojectile plant transformation for sugarcane giant borer pest management
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Background: The sugarcane giant borer, Telchin licus licus, is the major insect pest of crops in northern and northeastern regions of Brazil. This insect has a long life cycle, which lasts about 160-190 days and presenting four major life stages, egg, larva, pupa and moth [1]. During larval stage, the insect penetrates the plant as soon as they hatch and starts feeding of the midgut of the plants, releasing sugars [2]. The main component of hemicellulose is the xylan, which is hydrolyzed by xylanases [3]. Consequently, the insect penetrates the plant as soon as they hatch and starts feeding of the midgut of the plants, releasing sugars [2]. The main component of hemicellulose is the xylan, which is hydrolyzed by xylanases [3]. Consequently, the insect penetrates the plant as soon as they hatch and starts feeding of the midgut of the plants, releasing sugars [2]. The main component of hemicellulose is the xylan, which is hydrolyzed by xylanases [3].

Methods: Sugarcane variety R885156 was used for this work, as it represents one of the most cultivated sugarcane plants in Brazil. After the selection process, five dsRNA I, two dsRNA II and eight Cry plants survived. All plants confirmed to be PCR positive for the presence of the transgenes, presenting an average transformation efficiency of 0.02% plants/callus. This study was the first to be conducted in order to improve sugarcane resistant against T. licus licus. Southern blot experiments are being carried out to confirm the introduction of the expression cassette into the plants genome. The best events will be tested in bioassays to determine resistance levels against sugarcane giant borer larvae.

References
The microorganisms present in the SCB were isolated from three preparations: fresh SCB, SCB buried in soil for about 45 days and humid SCB - collected from two cane fields and stored in refrigerator. To obtain microorganisms, salin solution (NaCl 0.15 M) and rich medium (5 g/L peptone, 5 g/L NaCl and 10 g/L of SCB, pH 5.0 to 6.0) were used, followed by serial dilution. The selection medium contained cellulose and xylan and the enzymatic activity was visualized as a halo of hydrolysis around the culture, using congo red 1%. Submerged fermentation in minimum medium (MM) was used to induce cellulases and xylanases. The determination of enzymatic activity was measured by dinitrosalicylic acid (DNS), using the supernatants of culture as enzymes and Xylan birchwood-Sigma\(^*\), CMC- Sigma\(^*\), Avicel- Sigma\(^*\) and Whatman paper filter as substrate for each enzymatic dosage.

**Results and conclusions:** Seven cultures were selected (A3, B3, M2, M3, X7, F4 and D2) according to the halo of hydrolysis diameter to determine the enzymatic activity. The culture A3 proved to be a good producer of xylanase. The culture M3 produced cellulases with FPase and CMCase activity, showing that it is good for cellulose hydrolysis. The culture X7 simultaneously produces cellulases and xylanases, which favors the hydrolysis of cellulose and hemicellulose using SCB as substrate. Although the activity of xylanase has no results, we cannot conclude that the enzyme was not produced; the microorganisms need to be induced by different substrates.

**Acknowledgements:** We thank our professor Fabricia Paula de Faria, who led us in this research, professor Américo José dos Santos Reis from School of Agronomy and Food Engineering, who gave the sugarcane bagasse, and CNPq, which invested in this project.

**References**

collected in a Brucker Daltonics equipment and analyzed with the Flex Analysis 3.0 software.

Oligosaccharides with m/z 701 and 833 predominated the spectra from AEH/ Pentonop Mono BG® for all varieties of sugarcane studied. The peak of m/z 701 was confirmed to be a xylopentose (Xyl5) in MS2 mode. The peak of m/z 833.3 may represent a hexa-oligosaccharide (Sorensen et al., 2007) [2]. BCWR/Pentonop Mono BG® allowed for the detection of many feruloylated oligosaccharides. In addition, the analysis of BCWR/Driselase allowed for the detection of arabinof-xilo, feruloyl-arabinof-xilo and acetylated feruloyl-arabinof-xilo oligosaccharides. In this experimental, the oligosaccharide of m/z 745.2 was abundant and possibly represents a feruloylated tetra-oligosaccharide. The RB867515 and Saccharum sinensis BCWR/Driselase digestions exhibited abundance of the oligo structure with m/z 613, which may correspond to a feruloylated trisaccharide (Xyl2AraFeA). Acetyl substitutions (increases of 42 Da) upon the oligo structures of m/z 613, 745 and 2507,8 may have rendered the m/z shifts of 655, 787 and 2549,9 detected.

The xylopentose (Xyl5) predominated the oligosaccharide profiles for AEH digested with Pentonop Mono BG® for all varieties of sugarcane studied, suggesting that the synthesis of xylan in Saccharum may conserve a core pentasaccharide structure (Xyl5) eventually modified with acetyl and feruloyl substituents.

References

P105
Expression, purification and analysis of the anti-HIV Cyanovirin-N produced in transgenic soybeans seeds
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BMC Proceedings 2014, 8(Suppl 4):P105
Background: Human immunodeficiency virus (HIV) is infecting over 34.0 million people worldwide, being responsible for one of the major current pandemics [1]. The most severely affected region is the Sub-Saharan Africa where nearly 1 in 20 adults is infected with HIV, accounting for 69% of infections worldwide [1]. Several strategies to halt HIV spread are currently being pursued, including the use of ectopic microbiocides. This approach is particularly important for women, among which the infection rate can be almost three times more than men. Cyanovirin-N (CVN) is a lectin-like protein isolated from blue algae that can bind virally infected protein to viral infection [2,3]. In this report we describe the use of transgenic soybeans plants as a potential platform to achieve large scale, cost-effective production of CVN.

Methods: The fragment of 306bp corresponding to CVN coding region was amplified by PCR from pET30b-CVN (BioSyn) vector and cloned into bombardment vector pCBong. This vector contains the promoter and the complete signal peptide of the soybean b-conglycinin gene with a CaMV35S terminator. The final vectors pCBongCVN and pAC231 (containing imazapyr herbicide resistance gene) were co-bombarded in a 1:1 ratio into the apical meristem of somatic embryonic axes from mature soybean seeds cv. BR-16, utilizing a particle bombardment device [4]. Transgenic R0 plants were cultivated under green-house conditions to produce seed. R1 seeds were analyzed by PCR and ELISA-gp120 followed by nanoUPLC-MS® [5] to characterize and quantify the expression levels of recombinant CVN. Seeds were graded and proteins were extracted using 50mM Tris-HCI pH 8.0 in 1:20 (w:v) ratio. Total soluble proteins (TSP) were separated using gel filtration sephadex x200 followed by HPLC C4 reversed phase. Fraction containing semi-purified recombinant CVN were used for anti-HIV assay.

Results and conclusions: From 1.000 embryonic axes used in bombardment, 20 plants were recovered from herbicide selection and only 8 contained CVN transgene. After ELISA-gp120 two plants showed CVN binding to gp120 HIV glicoprotein. NanoUPLC-MS® results from TSP indicates a expression level of 1-5%. Structure characterization showed correct sequence which was possibly glycosilated. Anti-HIV assays from semi-purified SOY-CVN indicates microbical activity that was 10 folds less effective than the CVN control produced in Escherichia coli. Mass spectrometry quantification of this fraction confirms 10 fold dilution with other soybean seeds proteins, possibly due to a high affinity binding of CVN for glicynin and b-conglycin which are abundant in soybean seeds. Therefore, production of pure recombinant CVN from soybean seeds is currently being optimized, and may contribute the development of an important HIV infection prevention method.

Acknowledgements: FAPDF, CNPq, Embrapa.

References

P106
Intragenic antimicrobial peptides from Theobroma cacao
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BMC Proceedings 2014, 8(Suppl 4):P106
Background: It is well known that many bioactive peptides (intragenic) are encrypted in source proteins and that they can exert their function once released by proteolytic cleavage; e.g. hypotensive, opioids and antimicrobial peptides. However, other bioactive peptides may be “stuck” on a polypeptide chain with no cleavage sites for its release. These “non-obvious” intragenic peptides are also of interest in the search for new biologically active peptides, mainly antimicrobial peptides, in an alternative way for new drug discovery and for the control of different phytopathogens, mainly fungi), that can cause several losses to different crops of interest; e.g. rice, soybean, common bean, cocoa. In Brazil, Theobroma cacao production can be decimate by the basidiomycetes Moniliophthora perniciosa, the causative agent of cocoa witches’ broom disease. In this report we present preliminary results of the search, synthesis and activity of intragenic antimicrobial peptides (IAPs) selected from Theobroma cacao genome.

Methods: In this study, we performed a search of putative IAPs using Theobroma cacao Matina 1.6 genome [1]. Search was performed using the software Kmal v1.0 alpha [2] with user-created parameters. Eleven peptides out of 70000 filtered peptides were selected for in-house solid-phase synthesis. DSS0 [3] and Ascaphin-8 [4] were also synthesized as positive controls. Peptides were purified by RP-HPLC in a preparative C18 column. The purity and molecular mass of peptides were evaluated by MALDI-TOF MS (UltraFlex III, Bruker Daltonics). Peptide fragmentation was obtained by MALDI-TOF MS/MS experiments and the resulting data were analyzed manually using Flex Analysis 3.0 (Bruker Daltonics) software to confirm synthetic peptides amino acid sequence. The minimum inhibitory concentrations (MIC) of the synthetic peptides for Candida albicans ATCC
90028 and *Cryptococcus neoformans* ATCC 28957 was determined by microdilution broth method, according to CLSI M27-3a document [5] and were evaluated at concentrations between 25 µM-0.5 µM.

**Results and conclusions:** Pep2, Pep5, Pep6, Pep8 and Pep10 showed MICs against *C. neoformans* ATCC and *C. albicans* ATCC. The data obtained for DS01 and Ascaphin-8 for *C. albicans* showed MICs of 6 and 8 µM, respectively, in agreement with the literature [3,4]. Pep5 and Pep10 showed MICs of 126 and 64 µM, respectively. Pep6 and Pep8 inhibit *C. albicans* and *C. neoformans* growth at 4 µM and 8 µM. Pep2 was able to inhibit and kill both fungi at 2 µM. Pep2, 6 and 8 showed lower MIC values than DS01 and Ascaphin-8 for *C. albicans*. Synthetic peptides 2, 6 and 8 showed promising results against human pathogenic fungi highlighting the importance of this approach to search for new drugs. To evaluate if this approach can render promising results for agriculture, which is the main goal of our study, MICs assays are being performed with some fungi phytopathogens. This approach can be used as an alternative to the transgenic technology as the plant own information, not an exogenous one, could be used for the control of phytopathogens, as proposed for soybean [2].

**Acknowledgements:** CNPq, Embrapa, UnB and UFG.

**References:**
PEG and inoculated with *H. seropedicae* and/or *A. brasilense*. The controls consisted of wheat plantlets with and without PEG in the same condition. After 5 days, plantlets and bacteria were harvested and the co-culture medium was taken to determine the concentrations of IAA (Glickmann & Dessaux, 1995) [1], AKB (Penrose and Glick, 2003) [2], and total protein (Hartree, 1972) [3]. The treatments of CD120 without PEG and with *H. seropedicae* showed the highest IAA concentration. In Frontana the amounts of IAA are lower as compared to CD120, however, treatments under the stress condition (with PEG) and *A. brasilense* stood out. Treatments with PEG and both bacteria showed similar performances associated with CD120. The amounts of AKB increased in the presence of PEG and *H. seropedicae* for both cultivars, probably by action of ACC deaminase activity. The total protein increased in the presence of PEG comparing the two controls for both cultivars. The total protein in CD120 was greater only when bacteria were present compared to their controls indicating a plant cell death for CD120 and Frontana. It is possible to conclude that the association of *H. seropedicae* is more effective with CD120 and indicated the reduction of ethylene. Frontana associates better with *A. brasilense* Possibly PEG causes cell death in the system or exudation of proteins by the cultivars tested. These data corroborate with the literature where plant vs. bacteria interaction is genotype and strain dependent.

**References**

**P109**

**Genetic transformation of sweet oranges to over-express *SABP2* gene**

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**Background:** The world's citrus crop industry is marked by a series of diseases caused by different etiologic agents. Several of them are characterized as biotrophic pathogens like *Xanthomonas* citri subsp. citri from *Citrus* and *Xanthomonas* citri subsp. *citrulli* from *Citrullus* and *Solanum* species. The *SABP2* gene (salt activated basic peptide 2) is the main candidate for *Xanthomonas* citri virulence. The transcription of *SABP2* gene indicates pathogen association and disease development (Glickmann & Dessaux, 1995) [1]. However, the mechanism of disease development is not well understood.

**Methods:** The aim of this study was to produce ‘Hamlin’ sweet orange (*Citrus sinensis* L. Osb.) transgenic plants, via *Agrobacterium tumefaciens*, over-expressing the *SABP2* gene from sweet orange driven by the constitutive promoter ubiquitin (Ubq10). The *SABP2* gene was cloned into pCambia 2301 and inserted into A. *tumefaciens* EHA 105. The genetic transformation was performed using epicotyl segments from seedlings [2].

**Results and conclusions:** A total of 620 explants in three independent experiments were introduced and approximately 336 shoots were regenerated. The GUS histochemical test was performed and confirmed the transformation of 30 positive shoots. These shoots are being grafted onto Carrizo citrange (*Citrus sinensis* × *Poncirus trifoliata* (L.) Raf.) seedlings grown in test tubes containing MS culture medium. The presence of the transgene will be evaluated by PCR using specific primers that amplify part of the ubiquitin promoter and part of the gene. *SABP2* expression levels in transgenic plants will be assessed through qPCR. After bud multiplication, transgenic plants will be evaluated for their response to citrus canker, HLB and leprosis.

**Acknowledgements:** INCT-Citrus (Fapesp and CNPq), Embrapa-Capes grant and EMBRAPA-Monsanto agreement (02.08.05.004.00.00).

**References**

**P110**

**Characterization, isolation and cloning of sugarcane genes related to drought stress**

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**Background:** Sugarcane is a major crop worldwide as raw material for sugar and ethanol production. Drought is one of the most limiting factor that affects sugarcane productivity. In order to understand the mechanisms of drought response, field and greenhouse assays were conducted with two drought-contrastng sugarcane genotypes (IACSP94-2094/tolerant and IACSP97-7065/sensitive), and several genes up/down-regulated under drought stress identified by microarrays and RNAseq analyses. Ten differential expressed genes in both assays were evaluated by qPCR [1], and three of them showed the transcriptional profile related drought tolerance: a- Lipoxygenase (SLOX), acting in the biosynthesis of the jasmonic acid precursor, and recent studies showed their role in defense against drought stress [3]; b- Dehydrogen, correlated to drought stress and associated to maintenance of turger cells, [2]; c- Dirgent-jacalin, associated to resistance disease and abiotic stress tolerance [4] and also related to jasmonic acid, an important hormone on plant defense. These genes were chosen as target for functional analyses in rice and sugarcane transgenic plants.

**Methods:** The full-length sequences of the coded sequences of SLOX, dehydro in dignent-jacalin genes were accessed by SMARTer RACE CDNA Amplification Kit (Clontech) using the tolerant genotype IACSP94-2094 mRNA. The cloning was performed using the vector pGEM-T Easy (Promega) and E. coli DH10B lineage. The clones were sequenced. After, the sequences were subcloned into pDONR 211 gateway vector and subsequently cloned in the overexpression and silencing vectors constructions to transformation plant via Agrobacterium.

**Results and conclusions:** The coding sequence of SLOX is incomplete at SUCEST (Sugarcane Functional Genomics Database). After many attempts of full-length transcripts SLOX amplifications, only 3’ RACE fragment from five clones were identified as the tag gene. On the other hand, the sequences from the 5’ RACE amplification clones matched with others members from Lipoxigenase gene family. Nevertheless, the dehydroin and dirigent-jacalin were successful isolated and cloned as full-length coded sequences. From the 10 clones sequencing, it was found one allelic variant with a frameshift mutation and one as same identify of dehydro in dirigent-jacalin sequences from the nucleotide databases queried. From 16 clones sequencing for dirigent-jacalin, it was found one allelic variant with frameshift mutation and one sequence matching the sequences from the database queried. The allelic variants, representing apparently nonfunctional alleles, were one of most difficulties in cloning the genes coded regions. This difficulty is attributed due the polyploidy and complexity genome of sugarcane. The allelic variant choice for the functional analysis was based on amino acid sequences.
alignment among sugarcane and related plants, e.g. rice, sorghum and maize, available in Phytozone and NCBI databases. The isolated coding sequences were engineered in plant overexpression and silencing construction vectors. Thereafter, rice and sugarcane embryogenic callus were transformed via Agrobacterium tumefaciens and regenerated plants are under evaluation. The steps of identification, isolation and characterization of genes associated with response to stress are crucial for the development of transgenic plants improved for drought tolerance.

Acknowledgements: Texas A&M Agrilife, FAPESP, CNPq.

References:

**P11**

**Diversity of glycosid hydrolase family 18 genes in environment isolates of the entomopathogenic fungus *Metarhizium anisopliae***

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BMC Proceedings 2014, 8(Suppl 4):P111

**Background:** *Metarhizium anisopliae* is a model for host-pathogen studies due to its ability to infect several different arthropods. The first barrier to accomplish successful host-infection is transverse the host cuticle, which is a rigid chitin-rich structure. To surpass this barrier, the fungus produces several hydrolytic enzymes, among which are chitinases and endo-(1→3)-acetylglucosaminidases, glycosid hydrolase 18 (GH18) members [1,2]. In fungi, GH18 enzymes have nutritional importance and exhibit morphogenetic and autolytic functions, acting at different processes of fungal development and life cycle maintenance. Assigning functional role for these genes in each process is one of the goals in entomopathogenic fungi study [3]. A genomic analysis performed in our laboratory, in *M. anisopliae* E6 strain, identified twenty-three GH18 putative genes (3-5). Considering this variety, this study aims to evaluate the diversity of these genes amongst *M. anisopliae* strains, to access their distribution in environmental isolates of the fungus. DNA samples from 23 *M. anisopliae* strains [CG291, NORDESTE, CARO7, CG125, CG343, CG374, CG46, CARO12, CARO14, CARO19, CG30, CG97, CG320, AL, MT, MS, CARO11, CARO15, CARO16, CG47, PL57, CG87 and CG491] isolates from different arthropods and places were subjected to PCR analysis to evaluate the presence of each of the 23 GH18 putative genes found in the genome of strain E6.

**Methods:** All strains were grown on Cove’s Complete Medium (MCc) agar plates at 28°C until sporulation. Spores were harvested with 0.01% Tween solution and inoculated into liquid MCC cultured in a rotatory shaker at 28°C, 180 rpm for 48 hours. After growth, DNA was extracted using lysis solution and phenol/chloroform method. The presence of GH18 genes was detected by PCR, using primers designed for *M. anisopliae* strain E6 genes.

**Results and Conclusions:** From the analysis it is possible to suggest that these GH18 genes sequences are well conserved in other strains. We identified 17 *M. anisopliae* strains with possible absence of one or more GH18 genes. Amongst the most prominent are strains lacking five genes. *Chimaena*, *cauda2*, *cauda4*, *chima48*, *chima48*, *chima28*, *chima20* putative genes were detected in all strains studied. Furthermore, the *chima27* gene was not detected in eight strains and the *chima28* gene was also not detected in six strains. Strains with the initials “CARO” from Mexico displayed higher number of absences, compared to E6 isolated in Brazil. Geographical distance is one of the possible factors that could contribute for the divergence in these strains. Although the absence of genes in some strains does not necessarily imply ortholog absence, because the primers used in this work were constructed based on E6 strain sequences, our results suggest diversity of GH18 members amongst *M. anisopliae* isolates. The possible absence of GH18 genes may result in differences in development, morphology and pathogenicity in the fungus that are now under study.

**Acknowledgements:** This work was supported by grants from CNPq (Conselho Nacional de Desenvolvimento Científico Tecnológico, Brazil), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil), FAPERGS (Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul) and LNCC (Laboratório Nacional de Computação Científica).

**References:**

**P112**

**Recombinant biosynthesis of functional human growth hormone and coagulation factor IX in transgenic soybean seeds**

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BMC Proceedings 2014, 8(Suppl 4):P112

**Background:** Plants constitute promising systems for the alternative production of valuable recombinant proteins. Recently, ELELYSO™, an enzymatic drug developed by the Israeli company Protalix Biotherapeutics to treat Type 1 Gaucher's disease, became the first plant-derived therapeutic product to reach marketable status [1]. This confirms the inherent potential of plant systems for the large-scale production of pharmaceuticals and industrial proteins with high quality and at competitive costs. Soybeans (Glycine max (L) Merril) provide a potentially economically viable platform for the large-scale production of different therapeutic molecules. Plant seeds are specialized in the stable accumulation of proteins at high levels, representing an excellent source of abundant and cheap biomass. Additionally, the vegetative growth of plants can be significantly extended under a daily photoperiod of 23 h of light, inducing more than a tenfold increase in seed production when compared with plants cultivated under field conditions [2]. In this report we present the recombinant biosynthesis and the molecular and functional characterization of two important therapeutic proteins in transgenic soybean seeds: the 22 kDa human growth hormone (hGH) and the 56 kDa human coagulation factor IX (hFIX), a vitamin K-dependent serine-protease glycoprotein utilized to treat Type B Christmas disease, the second most frequent haemophilia variant [3,4].

**Methods:** A biologic process was used to introduce the plasmids pCANGF3GH (4,786 bp) and pbcNGF3FX (5,406 bp), respectively carrying the hGH and hFIX coding sequences under the transcriptional control of the alpha prime (α') subunit of β-conglycinin tissue-specific promoter from Glycine max (L). Merrill and the c-Coixin signal peptide from Coix lacryma-japonica. In soybean embryonic axes from mature seeds (cv BB-16). Independent transgenic lines were analyzed by PCR and characterized by Southern blot. The transcription levels of the corresponding coding sequences were evaluated by RT-PCR of immature seeds and the accumulation of the proteins in the protein storage vacuoles (PSV) of the seeds was detected by Western Blot and ultrastructural immunocytochemistry assays. Partial N-terminal sequencing of both proteins was determined by nano-Lc mass.
spectrometry assay. The biological activities of the molecules were evaluated by somatogenic activity bioassay (for the hGH), and by activated partial thromboplastin time assay (for the hFIX).

Results: Expression levels of bioactive hGH and hFIX were, respectively, of up to 2.9% of total soluble seed protein content (corresponding to approximately 9 g kg\(^{-1}\)) and up to 0.23% (0.8 g kg\(^{-1}\)). Immunocytocchemistry assays indicated that both molecules were efficiently directed to protein storage vacuoles in seed cotyledonary cell. The recombinant hGH and hFIX protein sequences were confirmed by mass spectrometry characterization and showed no post-translational modifiotions on the spectra covered by the assays. The somatogenic activity bioassay demonstrated that the hGH expressed in soybean seeds is fully active. Protein extracts from transgenic seeds containing the hFIX showed a blood-clotting activity of up to 1.4% of normal plasma.

Conclusions and perspectives: Soybean seeds seem to be promising vehicles for the stable accumulation of the recombinant hGH and hFIX, once it was possible to detect biologically active molecules in grains stored up to six years at room temperature.

References

P113
Selection of plant growth-promoting bacteria in sweet sorghum (Sorghum bicolor (L.) Moench) under the effects of salinity
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1
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Background: Sorghum, a grassy non-halophyte, is both drought and salinity tolerant, and is considered a promising crop for semiarid regions. During the last 20 years, its culture has expanded in Brazilian production by 780%, reaching 1,928,970 tons in 2009 [1]. Salinization of the soil and lack of rain are increasing constraints in semi-arid regions. They predominate in Brazilian irrigated regions. They predominate in Brazilian irrigated regions. They predominate in Brazilian irrigated regions. They predominate in Brazilian irrigated regions. They predominate in Brazilian irrigated regions. They predominate in Brazilian irrigated regions.

Methods: The experiment used a factorial arrangement (2\(\times\)10) with two blocks. The salinity conditions were 8 and 75 mM NaCl (Hoagland solution - SH). The isolates tested were Herbaspirillum seropedicae (Hs08 and Hs09), Burkholderia sp. (Bp16), xylanase producing strains (Bp12), 8. phymatum (Bp16), H. seropedicae/ Burkholderia spp. (Hs/B30), and Burkholderia spp. (Bp16). All were tested with inorganic nitrogen (N) at 4 mM, and un-inoculated treatments of inorganic N at 2, 4, 8, and 16 mM. The bacteria were isolated in the state of Paraíba - Brazil. Disinfected seeds were sown in sterile sand + SH (pH 6.5), and inoculated with standardized bacterial suspensions (A\(_{560}\) = 0.500). The plants were grown (35 days) in a growth chamber under controlled conditions.

Results and conclusions: Compared to the controls (4 mM N), significant increases in shoot biomass SB (3x), transpiration T (3x), and root biomass RB (2x) were observed (when N increased under non-saline conditions). Plant height PH response was lower (1.2x). However, under saline conditions, the inorganic N induced significant and varied reductions (0.2x in PH, 0.3x for SB, 0.6x for RB) and 0.4x for T. We found specific relationships between the bacteria used and both the plant’s growth, and sensitivity to salinity. The isolate Hs09 in non-saline conditions provided an T and an SB equivalent to those obtained at 8 mM N. However, the greatest benefits were seen for PH and RB. Under saline conditions, Bp16 induced 2x higher SB, BR, and T, and 1.2x higher PH than in the control. Inoculations with Hs08, and Bp16 inhibited improved SB, and thus the WRay salinity tolerance. Perhaps the action of hormones, improved nutritional status, or osmo-regulation induced by the bacteria-plant interaction may have contributed to this performance [2-4]. The stability of the PGPB action for Hs09, Hs08, and Bp16, and their attenuation of stress (Hs08 and Bp16) must be tested in other varieties, and saline soils to validate the use of this biotechnological factor for sustainable production of sorghum in saline environments.

Acknowledgements: Brazilian Ministry of Science and Technology - CNPq - Brazil, Coordination of Improvement of Higher Education Personnel - CAPES - Brazil.

References

P114
Marine natural compounds can be efficient toward aflatoxigenic Aspergillus flavus strain
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Background: The presence of high levels of aflatoxin is a serious problem to the production of raw peanuts and peanut crumbs. The high incidence of aflatoxin in peanuts in our country is mainly due to problems in primary production. High humidity and temperature conditions increase the likelihood of Aspergillus development and aflatoxins production, which is worsened during rainy weather [1]. Aflatoxins may remain in the food after the death of fungus without visible alterations [2]. The effects of aflatoxins on human and animal health, besides resistance of fungi to conventional antifungal agents has motived the search for new inhibitors. The research of marine natural products from sponges has been considered as a promising source for the development of new antifungal agents in order to discover compounds more effective and less toxic [3]. The objective of this study was to evaluate the antifungal activity of 21 marine natural compounds toward an aflatoxigenic A. flavus ATCC strains.

Methods: Aflatoxin producing (CCCT 7836) and non producing (ATCC6433) A. flavus strains were purchased from Fundação André Tosello, Campinas, Brazil and were kept in Sabouraud media at 35 °C. The minimum inhibitory concentration (MIC) of 21 sponge marine natural products, identified as SM1 to SM21, toward the two strains of A. flavus was determined by microdilution assay in 96-well plates using RPMI medium according to the protocol NCCLS M-38 [4] for 7 days at 37°C, using commercial antifungal cercobin as control.

Results and conclusions: The marine product 5MS (CIM = 3.9 μg/mL) was more effective than cercobin (CIM = 7.8 μg/mL) against A. flavus.
control aflatoxin producer strain. Moreover, marine product SMS (MIC = 1.9 μg/mL) was also a more effective antifungal agent than cercobicin (MIC = 3.9 μg/mL) against the A. flavus ATCC strain. The findings suggest that natural marine products are a promising source of new molecules for the development of antifungal compounds against aflatoxigenic fungi that affect public health and the food production.

Acknowledgements: This study was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo, CNPq, CAPES and PET-MEC.

References

P115
Insecticidal and repellent activity of typical monoterpenes from plant essential oils against Callosobruchus maculatus (Fabr. 1775)
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BMC Proceedings 2014, 8(Suppl 4):P115

Synthetic insecticides and repellents have been broadly used worldwide for the control of crop pests and insects acting as vectors of human disease. Despite effectiveness, synthetic insecticides or repellents may cause serious damages to the environment and consequently to humans and animals. The growing biotechnological investigation for alternative insecticides and repellents, particularly of natural ones from plants, may conduce to higher safety and efficiency to the control of insects. Callosobruchus maculatus (Fabr. 1775) (Corylinaeidae) is a bruchid beetle that has been considered an important crop pest and also useful as model organism for development of new insecticides/repellents due several advantages such as a quick reproduction, sexual dimorphism and easy conditions of maintenance. As a crop pest, this insect, whose control by synthetic pesticides has been not straightforward, may represent an important threat as bean (Vigna unguiculata L.) production in Brazil as well as other stored-grains cultivars in agriculture. Considering the need of new alternatives for C. maculatus control and the importance of monoterpenes in plant resistance against insects, the aim of this work was to evaluate the insecticidal and repellent activity of typical monoterpenes (geraniol, citral, (±) citronellal, citronellol and eugenol) commonly found in plant essential oil against C. maculatus. To date, all of tested compounds presented a significant insecticidal and also repellent activity against C. maculatus in a range of doses from 1 to 64 μL. The best tested compound as insecticide was the eugenol, while the best performance as repellent was accessed using eugenol. Thus, these data showed that evaluated monoterpenes presented significant insecticidal and repellent effects, which are of high biotechnological interest and useful towards the growth of agriculture worldwide.

References

P116
Development of Phaseolus vulgaris endogenous reference and Embrapa 5.1 event-specific assays for quantification of Embrapa 5.1 GM common bean using real time PCR
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BMC Proceedings 2014, 8(Suppl 4):P116

Background: Brazil is the second largest producer of genetically modified (GM) crops with 37 GM crop varieties approved by CTNBio for commercialization. One of these varieties is the Embrapa 5.1 common bean, resistant to the bean golden mosaic virus (BGMV). The Embrapa 5.1 event was developed using the RNAi concept to induce the silencing of the AC1 viral gene [1]. Labelling of food products containing GMOs in Brazil is mandatory [2], so methods for quantification of GMOs are required. Real time PCR methods have been used as the gold standard for GMO quantification. In a previous work, we have developed a construct-specific PCR detection assay for Embrapa 5.1 [3]. In this study we developed an event-specific PCR assay for Embrapa 5.1.

Methods: Genomic DNA was extracted from leaves using two different protocols, a CTAB method and the DNeasy Plant Mini Kit with modifications [3]. Quantification of the endogenous reference was performed using primers and Taqman probe targeting the lectin gene present in common bean (Phaseolus vulgaris) genome. The reaction parameters efficiency and limit of detection (LOD) were determined using conventional and GM varieties Perola and P ontoal by 10-fold serial dilution of the genomic DNA ranging from 10⁴ to 10⁶ genome copies. The assay specificity was performed using 50 accessions of Phaseolus vulgaris and 13 different crop species including maize, GM maize varieties, soybean and GM soybean RR. Quantification of the event-specific fragment was performed using primers and probe targeting the event-specific junction of the Embrapa 5.1 event. The reaction parameters efficiency and limit of detection (LOD) were determined for the variety Perola GM by 10-fold serial dilution of the genomic DNA ranging from 10⁴ to 10⁶ genome copies. The specificity assay was performed using the Embrapa 3.2 event, also resistant to the BGMV. Primers and probe concentrations were tested in order to determine the more suitable reaction efficiency.

Results and conclusions: The endogenous reference presented an efficiency of 96% and a LOD of 10 genome copies. This target was specific for the quantification of Phaseolus vulgaris varieties and no amplification was observed in 10 out of thirteen negative controls. The remaining three negative controls presented late Ct (Ct>34). Also, this assay presented similar Cts among the fifty accessions of Phaseolus vulgaris. The event-specific detection presented efficiencies ranging from 104 to 111%. A LOD of 1 genome copy was obtained. Also, this assay was specific for the Embrapa 5.1, although four out of twelve reactions were positive for the Embrapa 3.2 event. Even so, these four amplification presented late Cts (Ct>34). The primers and probes developed in this work are suitable for Taqman real time PCR quantification of Embrapa 5.1.

Acknowledgements: The work was financially supported by CNPq grant 471401/2012-9. D.T. is the recipient of a CNPq Master fellowship; G.L.V., a CAPES Master fellowship and F.C.A.B. a CAPES PNPD fellowship.

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**P117**

Functional characterization of castor bean (Ricinus communis) DGAT3 and DAcT enzymes in Arabidopsis thaliana

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BMC Proceedings 2014, 8(Suppl 4):P117

**Background:** Triacylglycerols (TAGs) are the main seed storage lipids of plants. TAGs chemical properties are largely dependent on their fatty acid composition. Diacylglycerol Acyltransferase (DGAT) genes encode the main enzymes needed for TAG biosynthesis. Different types of DGAT genes (named DGAT1, DGAT2, DGAT3 and DAcT) have been identified in plants. DGAT1 and DGAT2 are well characterized, but little is known about DGAT3 and DAcT genes in most plant species. The understanding of TAG biosynthesis enzymatic steps and its transcriptional regulation in plants is important to improve the content and composition of nutritional and industrial oils. DAcT or Diallylglycerol-acetyltransferase was previously identified in *Eunymus alatus* and it seems to be related to DGAT enzymes. This enzyme catalyzes the condensation of acetyl-CoA to diacylglycerol for the formation of 1,2-diacyl-3-acytely-sn-glycerol or simply “ac-TAGs”. These sn-3 acetylated diacylglycerol oils are abundant in *Eunymus* and display a 30% reduction on viscosity. DGAT3 enzymes were first reported in peanut and are unique due to their cytoplasmic localization due to the lack of transmembrane domains.

**Methods:** Our goal is to identify and characterize DGAT3 and DAcT genes of castor bean (*Ricinus communis*). The cDNA sequences from *Eunymus alatus* and *Arachis hypogaea* were used as queries in the blastx and tblastx programs to search for DAcT (Diacylglycerol acyltransferase) and DGAT3 (soluble Diallylglycerol acyltransferase) from different plant species. The sequences identified as RdDGAT and RdDGAT3 in the *Ricinus communis* genome were used to design RT-qPCR primers to evaluate their expression profile in developing seeds. For the functional characterization of these proteins, full cDNA sequences were amplified with gene-specific primers and cloned in pENTR-D. These entry clones were C-terminally fused to the YFP and CFP coding sequences. *Arabidopsis* mesophyll protoplasts were transformed with the YFP construction to identify DGAT3 and DAcT subcellular localization. Via Floral-dip, we transformed castor bean DGAT3-CFP in *Arabidopsis* to characterize its role in lipid metabolism and to obtain a purified protein for enzymatic assays. We also transformed yeast lipid synthesis mutants to confirm the role of these proteins in TAG accumulation by complementation assay.

**Results and conclusions:** We have successfully identified 4 different DAcT paralogs and one DGAT3 ortholog in castor bean. We have already characterized the expression pattern of the DGAT3 gene in castor bean in five different stages of castor bean seed development where we verified an active expression of this gene; however, by RT-qPCR approach, it was not possible to identify substantial expression for DAcT genes in castor bean developing seeds. The DAcTA-YFP fusion protein seems to be localized to the ER membrane as the other previously described DGATs are, on the other hand, DGAT3-YFP seems to localize to cytoplasmic structures which we believe to be oil bodies. Currently, we are characterizing *Arabidopsis* transgenic lines overexpressing castor bean DGAT3-CFP and evaluating the capacity of DGAT3 and DAcT genes to complement yeast lipid-synthesis mutants.

**PI118**

Effect of different concentrations of growth regulator on callus induction of the rice lineage AB11047

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BMC Proceedings 2014, 8(Suppl 4):P118

**Background:** The lineage AB11047 (*Oryza sativa* L.), developed by the rice breeding program of Embrapa, has a 126-day biological cycle, large grains with high ratio starch/husk and high productivity. It is resistant to the main rice diseases and degrain. Produces an average weight of 25g, while the majority of irrigated rice cultivars presents an average weight of 25g. This lineage represents a new source to ethanol production and/or animal feed, since the size of the grain is twice as big as the regular one, and it can be used for human consumption. It is observed that both economic and social demands of the rice sector, as the ethanol production chain indicate that there is an urgent need for cultivars that meet the agro-energy market.

In vitro vegetal tissue cultivation is an important tool in the search for the expansion of plant genetic variability, being essential to all kinds of plant breeding programs. An efficient plant regeneration protocol is necessary for the plant genetic transformation success. In the rice cultivation case, the process can be initiated by callus induction from mature seeds, which will directly interfere in the regeneration success, due to differences in the regenerative potential between different cultivars. This process is a prerequisite for the success of rice biotech [1]. In theory, every seed that can germinate can be used, however, the response of seeds to callus induction is highly dependent on the genotype of rice [2].

This study aimed to establish the optimal concentration of the growth regulator 2,4 dichlorophenoxyacetic acid (2,4D) to promote potentially organogenic callus from lineage AB11047 mature seeds.

**Methods:** Rice seeds were manually depucked and disinfected by immersion in solution of 70% alcohol (v/v) under slightly agitation for 2 minutes, followed by rinse with sterile distilled water, and immersion in solution of sodium hypochlorite 3% (v/v) under slightly agitation for 25 minutes. The Murashige & Skoong (MS) medium plus 3% (w/v) of sucrose, 7 g L⁻¹ of Agar and different concentrations of growth regulator 2,4D was used. The treatments consisted in a control group, without the addiction of the growth regulator; treatment 1, with 2 mg.L⁻¹ of 2,4D; treatment 2, 2.5 mg.L⁻¹ of 2,4D; and treatment 3, 3 mg.L⁻¹ of 2,4D. The cultivars were in the incubator BOD at 28°C, in the dark. The experimental delineation was completely randomized, knowing that for each genotype were used 8 repetitions, with 10 seeds each. After 7 days, the frequency of the callus formation was assessed. The data underwent variance analysis and the averages compared by the Scott-Knott’s test, at 5% probability.

**Results and conclusions:** A significant difference between the obtained averages in the different concentrations of 2,4D used and the treatment control was observed. The treatments T1 and T2 showed an average frequency of 60% and 61.2% of callus, respectively, with no statistic difference between them. The T3 was lower than T1 and T2 showing 46.2% of callus. The treatments T1 and T2 proved to be the most appropriate ones to the induction of callus from the lineage AB11047.

**References**


**P119**

In situ localization of mRNA of resembling the dirigent protein in sugarcane stems

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BMC Proceedings 2014, 8(Suppl 4):P119

**Background:** The dirigent proteins (DP) families and resembling the DP (DP-like) are exclusive in land plants and related to plants defense and forming phenylpropanoids compounds optically active, mainly pinoresinol [1]. Pinoresinol is converted into a variety of lignans. The dirigent protein
involvement is not confirmed in lignin formation, a biopolymer which is a negative factor for the ethanol cellulosic production. However, recently it was demonstrated Arabidopsis DP-like, AtDRI10, localization in the lignin polymerization site and a determinant role in the formation of a lignin specific root structure, named as Casparian strip [2]. Despite of the controversy of the DP forming-lignin its substrate diversity is consistent. Since DPs and DPs-like are represented by numerous members of gene families with high diversified sequences and with unknown functional role for most of them [3]. One of the sugarcane DP-like, named as ShDP1-like, showed special interesting due its high level of expression in the pith of mature stem [3] coinciding with higher level of the sinapyl (S) unit forming lignin [4]. Moreover, the DP expression in particular cell and tissue types is a necessary prerequisite in understanding the biological role of this gene.

Methods: In situ hybridization of mRNA: To examine the localization of ShDP1-like mRNA, the 191 pb PCR product obtained from the stem cDNA of IACSP04-063 sugarcane variety using the ShDP1-like specific pair primers was cloned at plGM easy vector (Promega). The linearized constructed vector was used for synthesis of digoxigenin-labeled antisense and sense RNA probes with the DIG RNA Labeling Kit (SP6/T7) (Roche). 20-μm sections of paraformaldehyde-embedded sugarcane stem were subjected to in situ hybridization as previously described [5]. Photomicrographs were captured with an Olympus BX 51 photomicroscope equipped with an Olympus DP71.

Results and conclusions: The ShDP1-like hybridization signals were detected in the vascular bundles and the fibers surround the vascular bundles in the young internode rind of the sugarcane stem, coinciding with the lignin accumulation. On the other hand, the stronger signals were detected in the parenchyma cells of mature internode pith and less pronounced signal in the vascular bundles and fibers of pith and rind cells. Interestingly, unlike the parenchyma cells of young internode, the lignin accumulation appears in parenchyma cells of mature pith, and probably with a S unit-rich formation [4]. Moreover, it is noteworthy that the sucrose accumulation also occurs on the parenchyma cells of mature pith. The results suggest a direct or indirect relation between ShDP1-like expression pattern and lignin accumulation. In order to access the ShDP1-like biological role, its coding sequence was isolated and cloned in overexpression vector. Currently, the transformed rice callus carrying the ShDP1-like overexpression cassette are regenerating.

Acknowledgements: FAPESP sponsors post-doctorate fellowships to PMN, MSB, JLSM. FAPESP project Grant 2010/08232-0 and 2008/58035-6. References


Background: The use of 8t crystal protein as a natural insecticide, applied directly to crops has been true for many years. Cry genes codifying these proteins are already are being used in transformation plants that become resistant to certain insects. The aim of this study was to compare the amino acid sequences that encode the Cry protein to insects of the orders Lepidoptera, Diptera and Lepidoptera / Diptera. We evaluated 15 sequences that were deposited in the GenBank and using bioinformatics tools to align and compare these sequences that present three domains (I, II, and III). It is assumed that changes in these domain influence the toxicity of the protein and her order. The results presented indicate the presence of specific regions and that may be related to the specificity of protein toxicity.

Methods: The search and download all the amino acid sequences by domains: I, II and III, of the GenBank and of the program to consult "The Blast" - Basic Local Alignment Search Tools (Altschul et al., 1997).

The sequences were divided into the following insect orders: Coleooptera, Diptera, Lepidoptera, Lepidoptera and Coleoptera, Lepidoptera and Hemiinoptera.

To compare the sequences based on similarities between the genes that act on a particular species we selected 5 genes described that attack insects of the order Lepidoptera: Cry1Ba1, Cry1Ba2, Cry1Bb1, Cry1Ca1, Cry1Ca2; 5 genes of the order Diptera: Cry8Ba1, Cry4Ba2, Cry10Aa1, Cry16Aa1, Cry19Ba1 and 5 genes, whose toxin attacks insects of the order Lepidoptera and Diptera: Cry1Aa1, Cry1Aa2, Cry1Ab1, Cry1Ab2, Cry1Ac1 and to by align these sequences using the software CLUSTAW v1.81 (Thompson et al., 1997).

Results and conclusions: This analysis revealed the occurrence of identical sequences in the 3 domains, I = 16, II = 3 and III = 6 and 14 positions. Most of these amino acids belong to the group R nonpolar and aliphatic amino acids and not were occur amino acids positively charged R group in Domain I. In the domains I, II and III was found that 8, 5 and 7 positions, respectively, were changes between amino acids of the same group (preserved), representing 40, 25 and 35.

In the field I was exchanging groups of amino acids conferring differences in behavior and stability of the molecule. The exchange of amino acids at positions 192, 226 and 171 provided a more hydrophobic molecule while the exchanges of amino acids at positions 142, 49 and 87 provided a more hydrophobic molecule.

Position 224 in domain II, and domain III at position 84 was exchanged amino acids increasing the stability of proteins by promoting hydrophobic interactions in the you interior.

When has been change in the amino acid change from one group to another modifying the chemical structure of the protein, its polarity and consequently their solubility in water and its interactions with other amino acids.

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P121

Molecular diagnosis of Fusarium graminearum and Pineapple mealybug with associated virus

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BMC Proceedings 2014, 8(Suppl 4):P121
Background: The state of Espirito Santo has in the fruit growing, one of its main economical activities. One of them, the pineapple growth, represents a good economic potential to increase income and job opportunities in the state. However, diseases such as fusariosis and mealybug wilt of pineapple are responsible for the yield losses of up to 80% [1]. The mealybug wilt of pineapple is caused by the complex Pineapple mealybug wilt-associated virus (PMWav-1, PMWav-2, PMWav-3 and PMWav-5), a single strand RNA [2], while fusariosis on pineapple is caused by the fungus Fusarium guttiforme [3]. This study aimed to develop methodologies for detection of these etiological agents in the pineapple plant.

Methods: For the analyzes, healthy and diseased pineapple plant were collected at the Experimental Farm of Incaper in the city of Sooretama, in the state of Espirito Santo. The samples were submitted to a superficial disinfection process, followed by nucleic acid extraction protocols with Trizol® and SDS buffer [4]. The nucleic acids were treated with DNase and Reverse Transcriptase enzymes for samples infected with PMWav. A RAFase enzyme was used for samples infected with F. guttiforme. The diagnosis was realized by molecular biology techniques, conventional PCR and real time PCR with specific primers for each strain of PMWav [5] and H3 primer for F. guttiforme. The samples were visualized by electrophoreses on 1% agarose.

Results and conclusions: Through these techniques were diagnosed the F. guttiforme in fruits, and detected the PMWav in different plant organs. Conventional PCR does not proved to be a good diagnostic tool due to poor sensitivity, the need for visualization on gels and the high risk of contamination. The technique of Real Time PCR can generated diagnosis with greater sensitivity, reproducibility, accuracy and speed and allowed the detection in diseased plants and plants considered healthy. Therefore, this study created molecular tools for a precise and rapid diagnosis of the etiological agents of the fusariosis disease and mealybug wilt of pineapple that allow indexing plants and propagative material the spread of disease to new areas. It is hoped that this study contribute to raising the standards of quality and competitiveness of Brazilian fruit growing, especially pineapple, the level of excellence required by national and international markets, as well as implement actions for incorporation of technological methods, techniques and processes based mainly on concepts of integrated disease management and food security, with a view to the expansion of this crop, increased productivity and income generation for farmers.

References


P122

Growth of a Saccharomyces cerevisiae strain lacking hexose transporters in different sugars after transformation with a Scheffersomyces stipitis genomic library

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BMC Proceedings 2014, 8(Suppl 4):P122

Background: Brazil is the biggest ethanol producer in the world using sugarcane as substrate. From this process, bagasse is the main by-product which can be used to produce second generation ethanol. Although Saccharomyces cerevisiae is the ideal yeast for the fermentative process, it cannot ferment xylose, one of the most abundant sugars in lignocellulosic biomass. Different yeast can ferment xylose, including S. stipitis [1], but not with the same efficiency rate as S. cerevisiae. Genes from xylose-fermenting yeasts can be used to genetically engineer S. cerevisiae to improve bioethanol production. Since it is widely known that the transport of sugars inside the cells is a limiting factor for the fermentation of sugars, and several reports have demonstrate that an increase in transport activity also increases the fermentative capacity of yeast cells [2], in the present work we have used a S. cerevisiae strain lacking hexose transporters (hxt-null) to screen a S. stipitis genomic library to identify putative xylose transporter genes.

Methods: The genomic library was constructed using DNA from S. stipitis by a private company (requested by Dr. Matsuhi from AIST, Japan) and the fragmented DNA was cloned into the overexpressing plasmid pPGK. These plasmids were used to transform a S. cerevisiae hxt-null strain (which can grow only in maltose) previously transformed with the integrative plasmid pAUR-XXXDHXR [3] that confers the cells the capacity to metabolize xylose. The clones were first select in solid synthetic medium with 2% xylose. Growth in micro-scale using synthetic medium with 2% maltose, xylose, glucose, fructose, galactose or mannose as carbon sources was performed using a Tecan Microplate reader (Tecan Echito Infinite M200PRO) for up to 72 h at 28°C under orbital agitation (160 rpm). The absorbance was measured at 570nm every 15 minutes.

Results and conclusions: Genomic libraries from different yeast on multi-copy plasmids can reveal potential genes [4] for the improvement of xylose transport in S. cerevisiae. We obtained 90 S. cerevisiae transformants from the S. stipitis genomic library, and 8 clones were selected based on their capacity to grow on xylose synthetic medium, although the final absorbance in xylose was lower than when glucose was the carbon source (data not shown). Two transformants, BBY-D1Ss6 and BBY-D1Ss90, grow higher than the other transformants in all the sugars tested, with a similar and marked growth in mannose. In contrast, BBY-D1Ss41 and BBY-D1Ss53 transformant strains did not grow on this sugar. The growth strain BBY-D1Ss80 was also expressiv in all sugars (mostly in glucose and fructose). In general, the eight transformants were able to grow on different sugars, not been specific to one carbohydrate neither specific for glucose only. We are analyzing the inserts present in the pPGK plasmid to verify which genes from the S. stipitis genome have been isolated in our work.

Acknowledgements: This work was funded by the Brazilian agencies CNPq, FAPESC and FINEP, and by the Japanese International Cooperation Agency (JICA).

References


P123

Glycine betaine biosynthesis genes differentially expressed in sugarcane under water stress/Glycine betaine biosynthesis genes differentially expressed in sugarcane under water stress

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BMC Proceedings 2014, 8(Suppl 4):P123

Background: Plants have developed a wide range of strategies which allow them to cope with a variety of abiotic stresses [1]. One of those mechanisms is the accumulation of compatible solutes, which protect cell structure against damage induced by dehydration and oxidation [2]. The accumulated compatible solutes may include betaines and related compounds such as sugars and amino acids. Understanding the action mechanism of glycine betaine (GB) and its effects on drought tolerance mechanisms may lead to
the development of cultivars adapted to different hydric conditions. In medicinal plants, betaine is synthesized by two enzymes: choline mono-oxygenase (CMO), and betaine aldehyde dehydrogenase (BADH) [3]. The present study evaluated gene expression profiles of CMO and BADH genes in sugarcane leaves (Saccharum SP) submitted to three levels of water stress (mild, moderate and severe), using DNA microarrays.

Methods: Sugarcane plantlets of cultivars SP83-2847 and SP90-1638, considered moderately tolerant and sensitive to water stress, respectively, were cultivated in greenhouses for 60 days in sterilized soil at 26°C with 56% of humidity. After 2 months, water stress was induced by irrigation suppression. All leaves were collected when water content in the soil was 10%, 30% and 75% respectively, and designated as mild, moderate and severe water stress.

Total RNA was extracted using Trizol Reagent (Invitrogen, USA), according to the manufacturer’s instructions and used to synthesize a cDNA probe which was used to hybridize nylon membranes containing CMO and BADH genes. Membranes were hybridized, for 18h at 58°C, probe was washed out and filters were exposed to imaging plates (FujiFilm, Japan) for 96 hours. Images were scanned using Phosphorimag FLA3000-G (FujiFilm, Japan), and spots quantified by Array Vision (Imaging Research, Canada). Variation in gene expression levels was obtained by log2 of the ratio of values found for control plants and plants under water stress during different trial periods. Northern Blot was used for validation of the results obtained by the microarray.

Results and conclusions: Our analyses showed an increase in the gene expression level for BADH enzyme only under moderate and severe stress for the sensitive cultivar, where differential expression of the tolerant variety started at the beginning stress. No differential expression was observed for CMO at significant levels for both varieties. These results confirm BADH gene modulation under water stress and its relationship with water stress tolerance.

Acknowledgements: CNPq and FAPESP.

References

P124
Molecular analysis and validation of primitive races peach palm (Bactris gasipaes) by means of markers RAPD
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BMC Proceedings 2014, 8(Suppl 4):P124

Molecular markers were used to examine the genetic variability of eight landraces of peach palm (Bactris gasipaes var. gasipaes) and two wild populations (Bactris gasipaes var. chichagui), their relationships and genetic structures. Two hundred plants of these 8 races were used and 18 plants of the two wild populations, one from the Magdalena River, Colombia, and the other from the Xingu River, Pará, Brazil. Eight primers were used to generate RAPD markers, of which 124 markers with 101 polymorphic. The observed heterozygosity was 0.38, with 93% polymorphism, both slightly greater than in earlier studies. The “gene flow medium” was 1.12 among the races of peach palm (Saccharum), which is explained by the distance between most breeds analyzed. These values are significantly larger than those of [1], because it is a less accurate marker for this type of analysis. The Amazonian landraces had greater heterozygosity and % polymorphism than the Central American race. The structure of the dendrogram with the four previously studied landraces, essentially validating it and confirming two landrace groups - one Occidental and one Oriental. When the Juruá landrace was added to the analysis, it joined the other Occidental races, as expected by its geographic position. When the other landraces were added to the analysis, a consistency and some problems were observed. The consistency was that all the landraces joined the Occidental group, expected by their geographic positions. The problems were the grouping of the Vaupés landrace with the Juruá landrace, which are geographically distant and have different fruit sizes and shapes. The relationship between the Cauca landrace and the Inirida landrace was also problematic, since they are geographically separated. The two wild populations joined the landraces at a great distance, suggesting that they did not participate in the domestication of the cultivated landraces and indirectly reinforcing the hypothesis of a single origin in southwestern Amazonia.

References

P125
Specific detection of three Torradovirus species with digoxigen-labeled probes
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Background: Torradoviruses are an emerging group of picorna-like plant virus from the family Secoviridae that infect tomato and other Solanaceae species. The genus Torradovirus include four species: Tomato tomatovirus (ToTV), first found in Europe, and afterward in Central America and Australia; Tomato apex necrosis virus (ToANV), present in Mexico; Tomato chocolate spot virus (ToChSV) and Tomato chocolate virus (ToCHV), both found in Guatemala. The symptoms caused by these viruses include chlorotic regions on the leaves that may develop to necrotic spots and holes, while the fruits show necrotic lines and frequently cracks on the surface, reducing yield and quality [1]. Even though these viruses have not yet been found in Brazil, the sudden spread of ToTV from Europe to Australia and Central America emphasize the necessity of effective methods to detect them. For that reason, we have developed digoxigenin-labelled RNA probes for the detection of three torradoviruses, ToANV, ToChSV and ToTV, through hybridization.

Methods: In order to produce those riboprobes, total RNA was extracted from infected Nicotiana benthamiana plants using TRIzol Reagent and used for RT-PCR with the generic primers pair described by Verbeek and colleagues [2], modified with the addition of the T7 promoter to the 5’ end of the reverse primers. The RT-PCR products were purified and transcribed in vitro using MEGAScript T7 Kit (Invitrogen) and digoxigenin-labeled nucleotides. The RNA probes produced were purified and hybridized to total RNA extracted from plants infected with which one of the viruses and non-infected plants blotted onto nylon membranes. Anti-digoxigenin Fab fragments conjugated to alkaline phosphatase were bound to the hybridized digoxigenin-labeled probes and the chemiluminescent substrate CDP-Star was added in order to produce light. X-ray films were exposed to the membranes and developed.

Results and conclusions: The X-ray films showed that each probe was able to hybridize only to the target virus, while no hybridization was observed for the RNA extractions from non-infected plants or plants infected with other viruses. In conclusion, these results show that the digoxigenin-labeled RNA probes can be used for the effective and accurate detection of the three Torradovirus species.

Acknowledgements: We thank to Science Without Borders CAPES Scholarship - Proc. Nº 5582/12/1, and CDFA Specialty Crop Grant.

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Avaliação da metodologia de detecção e quantificação de membros do gênero Torradovirus. BMC Proceedings 2014, 8(Suppl 4):P126

P126 Overexpression of a truncated form of the MSN2 gene enhances the initial rate of ethanol production in an industrial fuel-ethanol Saccharomyces cerevisiae strain
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BMC Proceedings 2014, 8(Suppl 4):P126

Background: The yeast strain CAT-1 isolated from a Brazilian fuel-ethanol plant (Babrzdadeh et al. 2009) is one of the most common strain used nowadays due to its very efficient fermentation capacity, especially at high sugar concentrations and under the stressful industrial conditions. Since the transcription factor genes MSN4, MSN2, YAP1 and HSFI of tolerant yeast strains are highly expressed under ethanol stress [1], we generated a CAT-1 derived strain named ATT-6 that overexpresses a truncated form of the transcription activator Msn2 through genomic engineering and analyzed the ethanol stress tolerance and fermentation capacity of this modified strain.

Methods: Following the procedure described by Petracek and Longtine [2], for the construction of a yeast strains overexpressing a truncated form of the MSN2 gene, a DNA fragment containing the Km gene which confers resistance to G418 in a yeast transformant was flanked by LoxP regions and the constitutive PADH1 promoter, was integrated into the genomic locus of the MSN2 gene of CAT-1, deleting the N-terminal region (first 48 amino acids) of the protein. The effect of 12-16% (v/v) ethanol addition on cell growth of the strain was evaluated in 96-well plates using a Tecan GENios microplate reader at 30°C and 110 rpm. Fermentation performance was determined using high sucrose concentration (200 g/L).

Results and conclusions: Under microaerobic conditions, the industrial ATT-6 strain overexpressing a truncated MSN2 gene showed increased growth rates and increased tolerance to up to 16% (v/v) ethanol stress, while the industrial control CAT-1 strain did not show cell growth before 60 hours. These results were consistent with those obtained by Hong et al. [3], indicating that overexpression of a truncated MSN2 can increase ethanol tolerance in a laboratory S. cerevisiae strain. Next, we investigated whether this improvement in cellular viability under high ethanol conditions leads to higher ethanol productivities during ethanol fermentation. We used high sucrose concentration (200 g/L) to expose yeast cells to high ethanol concentrations, not only by the initially added ethanol (12-16%), but also by the ethanol produced during fermentation. Our results show that the genetically modified strain ATT-6 grew slightly better than the control strain in the presence of supplemented ethanol. Ethanol production of the ATT-6 strain also showed slight initial higher ethanol productivity during the first 8 hours, when compared to the parental strain. Furthermore, the ATT-6 strain also presents higher invertase activity needed for the sucrose consumption, a result in accordance with Geng and Laurent [4] that indicates that Msn2/MSN4 act specifically in the early phase of Suc2 induction. Since there is limited information available for the function of the truncated form of MSN2, further studies on its regulatory roles for ethanol tolerance are needed. In conclusion, our results show that the industrial ATT-6 strain that overexpresses the truncated MSN2 allele is more tolerant to ethanol and produced more ethanol than the control unmodified strain.

Acknowledgements: This work was supported in part by grants from the Brazilian agencies CAPES, CNPq and FINEP.

References

Metarhizium anisopliae

P127 Detection of GM residues in foods derived from soy in Überlandia - MG
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Soy is a product of great economic importance, since it covers a variety of marketing fields that permeate the human and animal consumption for the processed food industry. Thus, to maximize production and improve product quality, the legume is a frequent target for genetic modification. The concern with the long-term effect of the transgenic product, beyond the consumer’s right to know about the product you eat demanded a law regulating the presence of these products in the market. Existing legislation in Brazil indicates that rates of GM products, with more than 1% should be informed on food labels. To ensure compliance with the law are necessary protocols able to identify and quantify the waste. Therefore, this project aims to evaluate the protocols of DNA extraction by CTAB method proposed by Ferreira and Grattapaglia and the method proposed by FERRARI et al [2], as well as detect PCR transgenic residues in processed foods derived from soybeans. After extraction, DNA was quantified by spectrophotometry and absorbance ratio 260/280 nm was analyzed to determine the quality of the samples. Furthermore, the technique was used to electrofuge agarose gel to compare the quality of DNA samples of soybeans with fresh samples processed soybeans. The protocol for DNA extraction from FERREIRA & GRATTAPLAGIA was more efficient compared to the yield of DNA extracted from different samples, but in relation to quality of DNA both protocols showed similar results. The detection of residues in transgenic samples showed that the PCR amplified only the lectin gene (endogenous), confirming that the samples contained soy, but did not amplify the exogenous gene, suggesting that the samples did not contain residues of transgenic.

References

P128 Viability of Metarhizium anisopliae conidia (metisch.) Sorok preserved in packages containing silica gel
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Background: The entomopathogenic fungi are important microbial agents for their efficient performance as microbial biopesticides in pest control. The development of commercial products formulated with entomopathogenic fungi is in a promising market for biopesticides. However, the fungi can lose their viability when exposed to unfavorable conditions of temperature,
humidity and ultraviolet radiation, affecting the life of conidia. Among these constraints, there is the relative humidity through the presence of free water in formulated as one of the main factors that affect the viability of the organism in the form and function of storage time. The use of desiccant agents, such as silica gel, may be a viable alternative to favor the retention of moisture in the air, reducing the adsorption of water molecules by the system. Thus, the aim of this study was to determine the efficiency of silica gel in maintaining the viability of dry conidia of *Metarhizium anisopliae*. 

**Methods:** The treatments consisted of 0.02 g of dry conidia of *M. anisopliae* with initial viability of 95.5%. The fungi were stored in microcentrifuge tubes with three replicates, containing different amounts of silica gel as drying agent (0.15 g, 0.30 g and 1 g) and a control without the presence of conidia. The treatments were stored in chambers of BOD controlled temperature of 26°C ± 1°C. The viability of conidia was assessed in plates Rodac once a month for 180 days, according to the methodology described by [1].

**Results:** It was observed that the treatments containing silica, independent of the amount (0.15 g, 0.30 g and 1 g) with high spore viability when compared to the control without the presence of silica gel. Since the increase of the silica also maintained a higher viability, since silica is a product which retains moisture in the air by physical adsorption, or a process in which water molecules are trapped in the surface pore desiccants. 

**Conclusion:** The presence of silica gel throughout the analysis period considerably aided in maintaining the viability compared to treatment without the silica gel since it decreases the moisture present in the system increasing the shelf.

**Reference**


**P130**

Structure of the fungal community in the agricultural system in upland and native area Cerrado

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**Background:** Plants are able to survive in a distinctive environment from its usual one, especially due to morphological or physiological changes, thus a good ability of acclimatization and phenotypic plasticity is present [1]. Light and water availability are decisive factors in a plant’s survival, the occurrence of those is irregular, because luminosity is broadly available on the edges and large clearings according to the criteria proposed by Souza, et al. [3] and scarce inside woods [2] and water availability depends on local pluviosity. Considering this, studies relating photosynthesis and water-use efficiency to phenotypic plasticity are major to the discovery of species capable of tolerating degraded environments with diverse luminous and pluvious intensity, thus, contributing to the enhancement of them. Based on the given information, this study aims to look into the behavior of these variables in *Piper aduncum* through gas exchange in distinctive spots of a gully.

**Methods:** The study was performed at a rural property in Alfenas-MG between the months of January-April 2013. The local vegetation found on the region is semi-deciduous with little native species.

*The gas exchange parameter was measured through a portable photosynthesis gas exchange system (IRGA, Model LI-6400XT, Li-Cor, Lincoln, Nebraska, USA). All measures were made through the morning between 8AM and 11AM, on a fully expanded leaf area and with due phytosanitary measures. The evaluated parameters were: foliar photosynthetic rate (A) and the water-use efficiency (A/gs). The measures were taken on a foliar area of 6cm², the chamber’s air flow had a CO₂ concentration of 380 μmol mol⁻¹. The air was collected outside of the gully and then transported inside a protected container and then pumped to the chamber. A photosynthetic photon flux density (PPFD) of 800 μmol.m⁻².s⁻¹ was used from an artificial light source (LI-6400-02B RedBlue LED, Li-Cor). The temperature was kept at 28°C. The experimental designs were entirely randomized with 3 treatments (area of the edge, slope and the gully’s bottom) with twelve repetitions. Averages and standard errors were done for data analysis.*

**Results and conclusions:** In this context, the study has shown that the photosynthetic rate was bigger on the edges of the gully (11.37 μmol.m⁻².s⁻¹ ± 1.06) and the water-use efficiency on the edges and the slope were equal. The difference between the treatments results from the slope and the gully’s bottom can be explained, due to the fact that when the data was collected, sunlight rays passed through the canopy and despite that usually happening in a short amount of time, it can contribute actively to the photosynthetic photon flux, and thus, increasing photosynthetic activity [4]. The gully bottom showed lower water-use efficiency, therefore photosynthesis was small in relation to stomatal conductance. Results lead to conclude that *Piper aduncum* demonstrates behavior prone to acclimatization and consequently of phenotypic plasticity when exposed to diverse luminous and water intensity from different spots of a degraded area.

**References**


The breaking of seed dormancy induced by stress [1] is extremely important with regard to the acceleration of germination process [1,2]. Abiotic stress processes, just like high hydrostatic pressure (HHP), are applied in several biotechnological studies, mainly because HHP induces changes in physiological and biochemical processes [3]. Pretreatment with a stress induction tool can be lethal or induce the synthesis of protective factors. When the stress does not kill the organism, it is called sublethal stress, and it can even increase the resistance to other stresses. High hydrostatic pressure is an important model in stress response study, for it has the ability to change the plasma membrane fluidity so as to keep the organism alive, which allows studies on the metabolic changes. Water is used as the medium of fluid pressure transmission since it has lower compressibility and greater compatibility, resulting in less risk of contamination. In our experiment, Carica Papaya seeds were put into a polyethylene tube containing water, the system is then closed in a capsule of high resistance steel, and suffers pressures between 10 and 400 MPa. The seeds were set to germinate in vitro, while the pressure transfer fluid present in the capsule was used to analyze abscisic acid per HPLC. The HHP generated positive effects due the seed hydration, which reflected in the percentage and speed of germination [4]. The begin of the seeds germination subjected to HHP was anticipated to 70 hours due to greater water retention, characterized by the increasing of weight (about 2.8 times greater when compared to dry weight), followed by germination values between 70-90%, while the seeds subjected to ambient pressure (control group) showed hydration of about 2.4 times and germination around 55-65%. The ambient pressure (0.1 MPa) showed lower water retention, resulting in lower mean percentage and germination speed when compared to HHP treatments. The abscisic acid (ABA) is closely related to tolerance to stress conditions (HHP) and its relation to gibberellin (GA) is crucial in the process of germination. The pressure transfer fluid has proven useful for the detection of abscisic acid, characterized by the presence of chromatograph peak with retention time equals to analyses of samples fortified with industrial ABA. The analyses of the HPLC chromatograms demonstrate changes in the composition of the water used as medium of pressure transfer. The seeds undergoing HHP pressure showed through gas chromatogram, differences between the concentrations of protein, peptides and phenol, when compared to the seeds subjected to hydrostatic control group. The balance between the concentrations of abscisic acid and gibberelin is related to germination. The presence of ABA in fluid of pressure transfer indicates a factor of germination capacity of seeds subjected to HHP stress. HHP stress is an effective biotechnological tool in the search for new mechanisms of stimulation of Carica Papaya seeds in order to provide improved germination and breaking of dormancy. Some data obtained in this experiment were omitted because due to patent submission.

References

P131
Effect of high hydrostatic pressure on seeds Carica papaya
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BMC Proceedings 2014, 8(Suppl 4):P131

P132
Production of polyhydroxyalkanoate biopolymer from vinasse using Ralstonia eutropha
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BMC Proceedings 2014, 8(Suppl 4):P132

Background: Brazil is the world’s leading ethanol producer and exporter. Due to increasing fuel demands, ethanol production has escalated in recent years. Vinasse is generated as a byproduct of the ethanol industry and is mainly used as fertilizer. The excessive usage of vinasse in soil leads to groundwater contamination [1].

The objective of this project is to develop a fermentation process with the bacterium Ralstonia eutropha for the production of polyhydroxyalkanoates (PHAs) using vinasse as the main nutrient and carbon source. This process will enable the utilization of vinasse for production of bio-based, biodegradable P(3HB) biopolymer. These biopolymers are intracellularly accumulated by R. eutropha as carbon and energy reserves. The potential applications of P(3HB) as alternatives to petroleum-based plastics are abundant. In this work, pure vinasse was successfully used as substrate for the production of P(3HB) with the achieved accumulation reaching 50% of cell mass as polymer.

Methods: The microorganism used was Ralstonia eutropha (DSM545). Vinasse was collected at Usina Iracema (Iracemapolis, São Paulo, Brazil). A 50L sample was taken directly after the distillation process, sterilized by filtration and stored at -20°C.

In order to analyze the P(3HB) production, two pre-cultures were prepared, one with NB and a second with vinasse with addition of urea [2]. Batch fermentation was done using a New Brunswick Scientific* reactor with a working volume of 4.0L, with no nitrogen feed. pH was corrected using 2.5 M NaOH and 2.7 M HCl solutions. Culture was followed during 22 hours.

Determination of biomass, nitrogen, and protein content were done according to [2]. The composition of the vinasse was determined by HPLC, as well as the final concentration of P(3HB), according to [3] with modifications.

Results and conclusions: Previous results from our group suggested no inhibition effect of vinasse on R. eutropha growth and the strain can grow with a maximum growth rate of 0.27 h⁻¹. The analysis of the vinasse revealed that its main components are glycerol, fructose, saccharose and mannitol. These substrates can be utilized by the bacteria. According to the experimental analysis, the microorganism barely used fructose to grow and accumulate biopolymer. Saccharose and mannitol, on the other hand, were consumed entirely. 25% of the glycerol was consumed.

The nitrogen concentration when the culture started was 0.063 g L⁻¹, which indicates nitrogen limitation since the beginning of the culture. As a consequence, P(3HB) was accumulated during all culture to a concentration of around 50% during the culture. This is low compared to 80% (gPHB/g biomass) in defined media [4] but promising from a waste product. According to the carbon balance, 79% of the calculated biomass and...
biopolymer production was reached from vinasse, indicating the feasibility of the process.

Acknowledgements: We thank Capes and CNPq for the financial aid to this project through the AJT fellowship awarded to C.S.Gal. We also thank our collaborators Prof A.J. Sinskey and his team from MIT.

References

P133

Ormossia excelsa Bentheed seed aspesis for the initialization of an in vitro

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BMC Proceedings 2014, 8(Suppl 4):P133

Ormossia excelsa Bentheed is an Amazon medicinal plant popularly known as Tento amarelo which belongs to the Fabaceae family and is used in the treatment and prevention of diseases, once its seed extract is being tested to fight tooth decays[1]. It is also used in the timber industry to make cudgels, banks and canoes. Moreover, the seeds are often used in popular craftsworks [2-4]. The aim of this study was to develop an aseptic protocol to determine the best type and concentration of bactericidal and fungicidal agents for disinfection of seeds. The study was conducted at the College of Technology/University of Amazonas Plant Tissue Culture Lab, Manaus/AM where 90 Tento amarelo seeds were obtained from the Native Seed Amazon Center. They were washed with neutral ODD® detergent and rinsed with running water for a minute. Then, they were immersed in 0.2% (w/v) Derosal solution for an hour under 100 rpm constant orbital stirring, followed by a bath in a 70% ethanol solution for 1 minute and finally immersed in a hypochlorite sodium solution at 0.25%, 0.50% and 1.0% (w/v) concentrations respectively, for 30 minutes under the same stirring. Subsequently, the explants were washed three times with sterile distilled water and inoculated on MS sterile basal culture medium. After 30 days, the explants were evaluated for the presence or absence of fungi and bacteria, as well as their survival rate. The experimental design was completely randomized and statistical analysis used simple percentages. There was no statistically significant difference between treatments of hypochlorite sodium at 0.25% and 1.0% (w/v) concentrations, respectively since both provided the same level of alive and axenic explants (100%). It was observed that even with long period of exposure of the seeds to hypochlorite sodium and Derosal, these antimicrobial agents have been effective, since they are not toxic to the seeds of this kind. Even when exposed to high concentrations of these antimicrobial agents, the seeds remained alive and vigorous after germination. Treatments that present high and efficient decontamination effects are indicated for Tento amarelo seed aspesis as this species has a good performance for in vitro germination. We can come to the conclusion that it is possible to start an Ormossia excelsa Bentheed in vitro culture, from this aspesis.

References
make the HHP a valuable tool in biotechnology research, as in the ethanol production [1]. Amino acids play a key role in central metabolism besides being the building blocks of proteins, and they are important to the HHP stress response. In this study, the Saccharomyces cerevisiae BT0510 was exposed to 50 MPa for 30 min at room temperature, followed by incubation at room pressure with aeration for 15 min. Samples of total RNA were collected every 5 min for transcriptional analysis by DNA microarray technique. Bioinformatics analysis demonstrated the upregulation (≥ 2 fold) by HHP treatment of genes related to the sulfur amino acids metabolism, methionine and cysteine. The HHP treatment induced the genes MET3, MET10, MET14 and MET16, which are correlated with the conversion of intracellular sulfur in sulfide, MET2, related to the conversion of homoserine to O-acetylhomoserine, was also induced by HHP, as well as the gene that codes for Met17p, responsible for the incorporation of sulfide in O-acetylhomoserine to form homocysteine, that will be directed to methionine or cysteine synthesis. These amino acids are directly correlated with sulfur assimilation in yeast cells. Methionine is the S-adenosylmethionine precursor, which participates in the biosynthesis of lipids and polyamides, and is also involved in methylation reactions, being a methyl group donor [2,3]. Cysteine is part of iron-sulfur proteins and is the glutathione biosynthesis precursor. Glutathione maintain the redox state in cytoplasm, therefore, playing an important role in cell response to oxidative stress [2,3]. The key gene related to the biosynthesis of methionine (MET6) was upregulated by HHP, while the gene related to the biosynthesis of cysteine (CY54) was unaffected. Five minutes after pressure release MET6 was repressed. The genes related to the conversion of methionine to S-adenosylmethionine, SAM1 and SAM2, were downregulated. Methionine residues are important against reactive oxygen species (ROS) [4], and genes associated with the reduction of methionine sulfoxide (MWO1 and MWO2) were induced by HHP treatment, suggesting that methionine plays an important role in the reduction of ROS resulting from stress caused by HHP [5]. Concerning the regulation of sulfur amino acids metabolism, MET28 was strongly induced during the entire HHP and post treatment. Other factors, such as the transcription factor encoded by MET4 were not affected by HHP, and also MET30 that negatively regulates Met4p. Met28p appears to play an important role in the biosynthesis of sulfur amino acids in response to HHP. It seems that this protein participates in the Met4 complex-DNA stabilization. Methionine biosynthesis upregulation is not related to other stresses, such as heat and osmotic stresses, and appears to be specific to HHP, which reinforces the use of this treatment to study the stress response in microorganisms.

References

Background: The success of a breeding program depends on the existence of genetic variability [1]. Therefore, breeders have recommended to the formation of the population-based study, the interbreeding between superior cultivars and divergent, resulting in hybrid combinations of higher genetic diversity, hence to achieve higher crop yield and quality. The objective of this study was to evaluate the genetic diversity of 35 soybean genotypes determined to their potential as parents for breeding programs. Methods: Using agronomic traits and microsatellite markers to evaluate the genetic diversity of 35 soybean genotypes to determine their potential as parents for breeding programs. Phenotypic analysis was carried out in the field at Fazenda Capim Branco and molecular analysis was conducted at the Instituto de Genética do Instituto de Biologia (IBI) at the Universidade Federal de Uberlândia (UFU-Federal University of Uberlândia). We evaluated 35 soybean genotypes using seven agronomic traits and nine microsatellite markers. These genotypes were then grouped by UPGMA and a cluster analysis, and the relative importance of the agronomic trait was obtained. Molecular analysis was used to calculate polymorphic data for each microsatellite marker. Genetic variability was found for all agronomic traits except for first pod height. UPGMA was used to form ten groups phenotypic analysis and seven groups for molecular analysis. Additionally, the Tocher method was used to form cluster groups for both of these analyses.

Results and conclusions: The nine microsatellite markers amplified 26 alleles, ranging from 2 to 4 and averaging 2.88 per marker. Polymorph data varied between 0.29 and 0.66 and averaged 0.44. Seven potential parents G11, G12, G16, G21, G22, G26 and G33 were identified from the UPGMA and Tocher clusters. These genotypes had average grain yields greater than 5000 kg ha⁻¹. Agronomic traits and microsatellite markers are effective tools for the study of genetic diversity in soybeans. Thus, we concluded that using agronomic traits and molecular microsatellite markers together makes it possible to detect potential parents for the soybean breeding program at UFU. Hybrids of the G11, G12, G16, G22, G26 and G33 genotypes promise segregating populations with superior genetic variability.

Acknowledgements: Brazilian Ministry of Science and Technology (CNPq) and Foundation to Support the Research of the State of Minas Gerais (FAPEMIG).

References

P137
Studies of stability and characterization this enzyme bromelain in pineapple (Ananas comosus)

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BMC Proceedings 2014, 8(Suppl 4):P137

Background: Bromelain is the generic name given to the set of derived endopeptidases belongs to members of the Bromeliaceae family, which belongs to the pineapple (Ananas comosus), being able to break the peptide bond, separating proteins and amino acids [1]. Bromelain possesses a wide range of therapeutic benefit as property of facilitating digestion of proteins, meat softening, ability to facilitate blood clotting [2] and economic importance related to the food industry and textiles and production of
The enzyme extract was obtained from the peel, stem and leaves of Ananas comosus. The plant tissue derived from the peel, stem and leaves of pineapple were processed extractor and then centrifuged at 10,000 g for 20 minutes at 4 °C to remove insoluble material. For the assay of enzymatic activity was used azocasein method, wherein azocasein 1,0% (w/v) (Sigma) was solubilized in ethanol 4% (v/v) and 0.1 M phosphate buffer, pH 7.0, and was used as a substrate. The assay mixture, containing 125 μL of substrate and 125 μL of enzymatic extract was incubated for 10 minutes at 37 °C and the reaction stopped by adding 750 μL of trichloroacetic acid 5% (w/v). The samples were centrifuged at 4000 rpm for 10 minutes and at a temperature of 5 °C. The stability of bromelain was evaluated against various pHs (5.0, 6.0, 7.0, 8.0, and 9.0 and 10.0) and at different temperatures (5, 25, 35, 40, 45, 50, 55 and 60 °C). And readout was taken in a spectrophotometer at 440 nm, and a unit of activity was defined as the amount of enzyme required to produce an increase in optical density by one unit at an interval of one hour.

Results and conclusion: The enzyme activity increased with increasing temperature until it reaches 50 °C, where it began to decline rapidly. Front pH changes, the activity increased to pH 7.0, where declined with the change in the activity. Analysis and extracting and exhibiting a second peak of activity at pH 10.0. The optimum temperature and pH for activity was 50 °C and pH 7.0, in which one observes the greatest activity of the enzyme bromelain. Thus, it was concluded that for this study the enzyme stability is interesting to work with these conditions.

References
0.24 ± 0.11 for the negative pool and the cut-off was calculated as OD = 0.47. 100% of the negative sera were negative and 70% of the characterized positive were identified as positive under these conditions. The average OD based on rLipL32 was 0.2 ± 0.08 for the negative pool, equivalent to a cut-off of OD = 0.36. 100% of negative sera were negative and 50% positive sera were identified as positive. The anti-dog-IgG conjugate exhibited the best specificity and sensitivity. The recombinant protein rLigBrep demonstrated potential as a diagnostic tool for canine leptospirosis, 70.0% sensitivity and 100% specificity. The native protein, LigB, is highly conserved in pathogenic Leptospira spp. and therefore represents an ideal candidate for further studies.

References

P140
Evaluation and comparison of Loop-mediated Isothermal Amplification (LAMP), PCR and nested PCR for detection of Ehrlichia canis in naturally infected dogs
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BMC Proceedings 2014, 8(Suppl 4):P140

Background: The detection of parasites in blood samples by DNA based assays is mostly performed by using the PCR technique, but due to the time of the reactions and costs involved, they still cannot be used in large-scale in routine laboratories. Recently, an alternative are assays based on the technique of Loop-mediated Isothermal Amplification (LAMP), which requires a shorter reaction time, higher specificity and has a lower cost [1]. This study aimed to develop a LAMP assay for the detection of Ehrlichia canis, a Gram-negative endobacteria and the etiologic agent of canine monocytic ehrlichiosis (CME), a major disease transmitted by ticks to dogs [2]. DNA extracted from blood samples of dogs presented to veterinary clinics and laboratories in the city of Ribeirão Preto, showing clinical signs indicative of EMC, were used in the assays. The samples were also previously diagnosed by PCR for presence of E.canis. Two sets of LAMP primers were used for the amplification of a fragment of the genes p30 (P30) and Heat-shock operon groESL (GRO).

Methods: The LAMP reactions were performed according to published protocols [3]. Briefly, each LAMP reaction mixture contained 1 μl extracted DNA, 20 pmol of each FIP and BIP primer, 5 pmol of each F3 and B3 primer, 0.5 mM of each dNTP, and 1X ThermoPol reaction buffer (20 mM Tris-HCl, 10 mM KCl, 10 mM (NH4)2SO4, 2 mM MgSO4, 0.1% Triton X-100, pH 8.8). The final volume was adjusted to 19 μl with autoclaved ultrapure water and the mixture was incubated at 95°C for 2 min, followed by incubation at ice. After the addition of 8 units Bst DNA polymerase large fragment (New England Biolabs, UK), the reactions were incubated at 60°C for 60 min and subsequently at 80°C for 10 min to terminate the reaction. A 10-μl aliquot of each reaction was used for electrophoresis on 2.5% agarose gel in Tris-acetic acid-EDTA (TAE) buffer and visualized under UV light after staining with ethidium bromide.

Results and conclusion: In this preliminary tests of a LAMP assay for detection of E. canis in canine blood samples, the primers directed to the p30 gene presented a better performance than the ones directed to the Heat-shock operon groESL (GRO). The p30 primer set was able to amplify the target gene from samples obtained from different regions of Brazil.

Acknowledgements: This study was supported by grants from Fundação de Amparo a Pesquisa do Estado de São Paulo (2012/02920-7 and 2009/12419-0) and Capes.

References

P141
Heterologous expression and purification of Leptospira spp recombinant proteins to leptospirosis vaccine development
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BMC Proceedings 2014, 8(Suppl 4):P141

Background: Leptospirosis is an infectious disease of humans and other mammals and an important public health problem worldwide, mainly in developing countries. The disease is among the most common zoonosis worldwide and is caused by infection with pathogenic spirochetes of the genus Leptospira. [1]. Rodents and others wild or domestic animals are asymptomatic hosts that can carry Leptospira spp in the kidneys and shed high number of them in urine. The infection usually occurs through direct contact with reservoirs contaminated urine [1]. Leptospires have a double membrane structure, where the LPS are the main constituting antigen in its outer membrane. Those proteins are potential targets to development of vaccines due to its capability of being recognized by the hosts immune system [2-4]. There is no vaccine evaluable worldwide to protect humans against leptospirosis. In the last decades a number of studies evaluated recombinant proteins as vaccine antigens, with limited or no success. We randomly chose three conserved lipoproteins to evaluate as recombinant proteins: those expressed by lct10260, lct10365 and lct11360 ORFs in the L. interrogans L1-130 genome. Lipoproteins are associated with membranes and may be exposed in the cell surface, allowing recognition by immune responses. In this study we cloned, expressed and purified these proteins.

Methods: Primers were designed to add restriction enzyme sites to each gene that were amplified from Leptospora borgpeterseni sorovar Ballum strain 4E genomic DNA. The sequences were cloned into E. coli pAE expression vector. The recombinant vectors were utilized to express recombinant Leptospira proteins in E. coli BL21 (DE3) Star cells. The cultures were grown to log phase and the recombinant proteins expression induced by adding IPTG. The cells were harvested, lysed and the proteins were purified in denaturing conditions using Ni2+ affinity chromatography. Purified proteins were analyzed by SDS-PAGE and Western Blot with anti-6His antibody. The final concentration of each protein was determined using the commercial kit BCA Protein Assay.

Results and conclusions: The cloning was successful and resulted in the pAE/lct10260, pAE/lct10365 and pAE/lct11360 recombinant vectors. The proteins were expressed in E. coli BL21 (DE3) Star and purified, yielding high amount each with satisfactory purity. The WB with anti-6His antibody marked recombinant proteins in the expected size: 12 kDa for lct10260; 37 kDa for lct10365 e 24 kDa for lct11360. These proteins are now target antigens to develop a recombinant vaccine against leptospirosis. Currently we are evaluating these proteins in an established hamster model of leptospirosis using homologous and heterologous challenges.

References

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Evaluation of effectiveness of protein expression of DNA vaccine in CHO Cells
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The caseous lymphadenitis (CLA) is a common disease that affects ruminants around the world. The CLA is caused by gram-positive bacteria known as Corynebacterium pseudotuberculosis, which is an intracellular facultative pathogen. In Brazil, the high prevalence occurs principally in northeast and southwest, where has the major herd of sheep and goat causing important economic losses. The actual commercial vaccine confers some level of protection and as based in attenuated or inactive bacteria. The third generation of vaccine includes the DNA vaccine, which can give some advantages as does not cause infection and the stability [1-3]. Thus the aim of this study was to develop and evaluate DNA vaccines based on genes that encode for secreted proteins of C. pseudotuberculosis. For this, we cloned on eukaryotic expression pTARGET vector, gene fragments of C. pseudotuberculosis namely Cp1002_1957 and Cp1002_1802, which encoded for the LipY protein and transferase B protein, respectively. After PCR using specific primers to each gene, the encode sequence were ligated on pTARGET vector (Promega) and the ligation reaction was used to transform Escherichia coli Top10 by electroporation. The transformation was plated in LB with ampicillin and X-gal substrate. After incubation, the white colonies were select for a screening by digestion with the enzyme EcoRI to confirm gene insertion on vector. Five clones of each gene are selected to transfaction of the CHO cells in order to confirm the expression level of proteins by indirect immunofluorescence using sera of mice immunized with the recombinant proteins (CP1957 and CP1802). The immunofluorescence analysis show high levels of protein expression (CP1957 and CP1802) in CHO cells in all clones tested. Both DNA vaccines (pTARGET/1802 and pTARGET/1957) are able to express the proteins in CHO cells in vitro. On the next step, we will perform the animal immunization to confirm the protection level (challenge test) and the immunomodulatory response.

References

P143 Potential immune of recombinant serine protease of Corynebacterium pseudotuberculosis
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BMC Proceedings 2014, 8(Suppl 4):P143

Background: Caseous lymphadenitis (CLA) has high relevance. It is a chronic disease that affects sheep and goats. The causative agent is Corynebacterium pseudotuberculosis, facultative intracellular bacteria. The prevalence of caseous lymphadenitis is high in many regions of the world, including South America. Brazil has 78% of seroprevalence in goats [1,2]. Therefore, prophylaxis becomes the best strategy as an effective vaccine can eradicate disease, but also to formulate a serologic test able to detect visceral cases. One of the first steps for developing of a serological test and an effective vaccine is the choice of a target, which must present appropriate antigenic characteristics. The CP40 protein (corynebacterial protease 40) have been studied in several studies. This protease is considered one of the virulence factors of C. pseudotuberculosis. Encoded by the gene CP40 and characterized as a serine protease, proteins responsible for several functions, such as providing the activation of pro-inflammatory cytokines which will help in the activation of the immune response. However, the aim of this study was to evaluate the potential immune serine protease recombinant in BALB/c mice.

Methods: To this end, CP40 protein was expressed in a heterologous genes in a prokaryotic system and purified by nickel affinity chromatography. The antigenicity of rCP40 was determined through positive and negative sera from animals for LC by Western Blotting. To determine immunogenicity, BALB/c mice were inoculated with a solution of rCP40, saponin and Freund’s adjuvants in three doses on days 0, 15 and 30. The serum samples of these animals were collected to check the production of specific antibodies rCP40. Statistical analysis was performed using GraphPad Prism version 6.0 for Windows (GraphPad Software, San Diego, CA).

Results and conclusions: Positive sera reacted with the recombinant protein, demonstrating that rCP40 maintained the antigenic properties, which is important because it demonstrates that antibodies generated against the native protein present in the bacteria C. pseudotuberculosis were able to recognize the recombinant protein. The results of the ELISA showed that the development of humoral immune response was generated by the experimental groups were inoculated with rCP40 associated with both adjuvants. The activation of helper T cells express ligands. Cytokines induce isotype switching of immunoglobulins [3] which are fixed more firmly to the antigens and plays diverse functions, forming the most effective mediators of humoral response by involving the cellular immune system, including phagocytosis and complement proteins. In mice the role of CD4 + T-cells helper can provide production class antibody IgG2a and IgG2b that are capable of promoting directly or indirectly a response from Th1 and lead to opsonization and toxicity mediated by cells [4].

References

P144 Development of a recombinant protein based Dot-Blot for the diagnosis of canine leptospirosis
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BMC Proceedings 2014, 8(Suppl 4):P144

Background: Leptospirosis is a globally disseminated zoonosis, recognized as a re-emergent neglected disease [1] and the causative pathogens are spirochetes from genus Leptospira [2]. In Brazil, more than 100.00 leptospirosis cases are reported annually, with outbreaks emerging especially during floods [3]. The microscopic agglutination test (MAT) is considered the standard serological test for the diagnosis of leptospirosis, but it requires paired serum samples, is demanding, difficult to analyse and the results can be variable when compared between laboratories [2]. The absence of an adequate laboratory diagnosis is the main barrier for the implementation of disease surveillance for the control of both human and animal leptospirosis. Hence, there is an urgent need for the development of an adequate laboratory test that is both quick and reliable. Apart from the
danger connected to rodents, which are leptospires main vectors, occurrence of the disease in dogs can generate a higher risk of infection for humans [4]. In the current study, selected recombinant proteins from L. interrogans were tested for their diagnostic potential using a Dot-Blot technique against a canine serum panel previously characterized by the MAT and a whole-cell Leptospira indirect ELISA.

Methods: Nine recombinant proteins were tested for their ability to recognize antibodies in Leptospiriosis positive and negative serum samples. The proteins were based on LigB, LigA, OmpL37, LemA, Flaa1, Flab1, and Lipl32. Three different concentrations were evaluated per antigen: 300, 500, and 1000 ng in each dot-blot. Two pooled serum dilutions were tested: 1:200 and 1:400. Furthermore, two isotypes of conjugated antibodies were evaluated: anti-dog IgG and anti-dog IgM. Selected recombinant proteins were screened using a panel of individual canine serum samples: 19 positive (11 vaccinated and 8 non-vaccinated) and 19 negative (11 vaccinated and 8 non-vaccinated). The degree of reaction was classified in four levels of colour (+, +1, +2 and +3), where +2 and +3 were considered positives and + and -1 negatives.

Results and conclusions: The majority of the tested antigens showed weak, non-specific reactions with the positive sera, except for rLigBrep and rLipl32. These recombinant proteins distinguished between the positive and negative pooled sera. Using anti-IgG, rLipl32 recognised all antigen concentrations at a 1:500 sera dilution. For rLigBrep, the best performance was achieved with anti-IgM and 500 ng and 1000 ng of antigen, regardless of the sera dilution. In an analysis of the individual serum samples, the optimal concentration of rLigBrep was 150 ng, and the sensitivity was 81.8% (9/11) and specificity was 63.6% (7/11). Using serum samples from vaccinated animals, the rLigBrep had a sensitivity of 87.5% (7/8) and specificity of 25% (2/8). rLipl32 failed to discriminate between the positive and negative sera and was not included in further evaluations. From these data, we can conclude that the dot-blot technique showed promising results in discriminating between positive and negative, vaccinated and non-vaccinated canine serum samples. However, further refinement of the technique is required before it can be used for the diagnosis of canine leptospirosis.

References

P145 Preliminary analysis of the transcriptome of salivary glands of Ornithodoros brasiliensis (Acari: Argasidae)

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Ornithodoros brasiliensis is an endemic tick to Brazil, restricted to highlands of the state of Rio Grande do Sul [1]. It is a very aggressive species with human activity and the environment. Dis Clin Microbiol Infect 2013, 20(2):239-44.

BMC Proceedings 2014, 8(Suppl 4):P145

Ornithodoros brasiliensis Aragão is an endemic tick to Brazil, restricted to highlands of the state of Rio Grande do Sul [1]. It is a very aggressive species with human activity and the environment. Dis Clin Microbiol Infect 2013, 20(2):239-44.

BMC Proceedings 2014, 8(Suppl 4):P145

P146 Cloning and expression of DNA encoding growth hormone tambaqui (Colossoma macropomum) in the yeast Pichia pastoris


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BMC Proceedings 2014, 8(Suppl 4):P146

Genetic studies involving the search, cloning and expression of genes encoding proteins involved in important physiological processes and advantageous features of the economic standpoint have become the biotechnology research increasingly promising. Due to its zootechnical importance, the encoding gene of growth hormone (GH) of various fish species have been isolated, cloned and expressed in heterologous expression systems. The tambaqui (Colossoma macropomum), Amazonian fish species considered promising for fish farming, has been the subject of extensive genetic research with biotechnology focus. This study aimed to express the CDNA encoding GH tambaqui (tGH) in the methyleotrophic yeast Pichia pastoris. The nucleotide sequence of CDNA tGH, previously isolated by Sousa [2009] [1], was optimized for expression in P. pastoris and obtained by chemical synthesis. It was then cloned into the cloning vector pUC19 followed by subcloning into expression and secretion vector pHPI9 with the endonuclease Eco RI and Not I. The recombinant plasmid named pPIC-tGH was linearized and inserted into host by electroporation. Recombinant clones were selected in medium auxotrophic for histidine. The expression of recombinant protein was induced by methanol addition during 96 hours occurred in shake flasks. The culture supernatant was analyzed on SDS-PAGE gel and expression of tGH was confirmed by Western blotting. The analysis of the supernatant revealed in both phenotype Mut* and Mut' the presence of a protein band of approximately 23 kDa, which was confirmed to be the tGH recombinant by Western blotting using monoclonal antibody against histidine tail C-terminal. The expression of tGH started already first 24 hours and was sustained throughout the induction period, lasted up to 120 h. These results are similar to those of Li et al. [2], who expressed GH carp (Cyprinus carpio) in P. pastoris and observed that from the first 24 hours of induction the recombinant protein was already present in the medium, and time 72 and 96 hours corresponded to the peak of expression. These results demonstrate that GH tambaqui can be successfully expressed in P. pastoris. The tambaqui, for their economic importance, so far was the first Amazonian fish species to have GH expressed in heterologous system, the first step in the production of GHs of other important species for aquaculture regional and national. In the future, tambaqui recombinant GH
should be analyzed for their efficiency in accelerating the growth of tambaqui towards the possible use in aquaculture production, which represent progress and innovation in technologies of cultivation of fish Amazonian.

References

P147 Hematological adjustments of the bony tongue Arapaima gigas under influence of amazonian waters
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Arapaima gigas, population known as pirarucu is largest freshwater fish in Amazon river, with great, economic and ecological, interest. Despite being an air breather its gill structure is quite close to water breathers, especially in early stages of development [1]. However, in animals above 100 g the pillar cell system are embedded into the filament being the gill, an ion regulation active site. The effects of Amazonian rivers waters is well notices in fish [2], mainly by black water (BW), and white water (WW) where the ion fluxes can be measured [3]. However information on the ionic regulation patters on this species are scarce as well implication by hematological adjustments. Such data could provide inferences on management conditions as well corroborate with what has been suggested by literature, which suggest this species as a panmic population [4] able to deal with the hydrographic barrier formed by the BW in the Amazon basin [5]. The present study aims to be analyzed A. gigas hematological parameters when exposed to BW and WW providing suitable hematological data concerning physiological responses in different types of water. Fish were acclimated seven days in three separated ponds containing BW, WW and well water (C), respectively. Control (C) fish were placed in the latter pond. Blood samples were taken from the caudal vessel at the end of acclimation period in order to perform measurement assays on levels of hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, as well glucose, cholesterol and protein levels. Our findings corroborate the hypothesis stating that BW does interfere on fish adaptation specially in small fish (~100g). These lack of adaptation should be due the gill morphology of small fish which is close to other water breather fish. However in large fish (~1000g) the findings clearly showed that neither WW or BW can interfere on plasma profile of analysed fish. The compromise between gas exchange and ion regulation has been demonstrated previously. As hematological adjustment plays a role on such osmoregulatory compromise, the hematological parameters data presented by this study clearly demonstrate that changes in the hematological features play a crucial role in the water to air breathing transition behavior as well in the A. gigas gill ontogenetic changes. Despite black water systems being an obstacle the species has kept its ion-regulatory mechanisms even when immerse in black waters.

References

P148 Study of antiapoptotic effect of a protein isolated from Megalopyge albicolis (Lepidoptera: Megalopygidae) hemolymph
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BMC Proceedings 2014, 8(Suppl 4):P148

Apoptosis has a central role in many cellular processes and development of some diseases like cancer and Alzheimer’s. Molecules that interfere with the apoptotic process may be used in the biotechnology industry, the control of cell death occurring in high density cultures performed in bioreactors is an important factor in production processes. Has been shown that hemolymph of the Lononimia obliqua (Lepodoptera:Saturniidae) is capable of inhibiting cell death in different models [1-3]. The objective of this study, is to identify the potential anti-apoptotic of a protein isolated from hemolymph of larvae of Megalopyge albicolis (Lepidoptera: Megalopygidae). Methods: The hemolymph was collected and the citotoxicity was evaluated in culture (up to 5%). The anti-apoptotic protein responsible for this activity was isolated and purified by gel filtration chromatography using a gel filtration column system (Superdex 75). The fractions obtained were tested for anti-apoptotic activity in VERO and SF-9 cells. Apoptosis was induced with 25 to 250 μM of Tert-butyl or 800ng/ml of Actinomycin D in cells treated and not treated with hemolymph. After 18 hours, the cells were stained with acridine orange and ethidium bromide and observed in confocal microscope. To study the cytoskeleton, the cells were incubated with filoldina-FITC after 4 hours of apoptosis induction. Results and Conclusions: Cytotoxicity of the isolated Megalopyge albicolis hemolymph was evaluated and no adverse effect was observed. This protein was capable to protect cells against death induced and was able to avoid the lost of cytoskeleton structure. The hemolymph of M. albicolis contains components able to inhibit death by apoptosis induced by chemical agents. Was observed that this component can act in cytoskeleton structure, increasing the cell viability acting to maintain the physiological and functional conditions of the cells. The activities exhibited by this protein are of great interest for the development of products to be employed in cell and tissue culture procedures, where the increase in cell viability is important to maintain the physiological and functional conditions of the cells.

Acknowledgements: FAPESP 10/52434-6

References
Resveratrol as a protective agent of goat sperm submitted to sex sorting in discontinuous Percoll gradient - preliminary results

Effect of antioxidants on sperm motility and progressive motility of goat sperm submitted to sex sorting in discontinuous Percoll gradient. However, all sex semen methodologies can promote irreversible damages to sperm cell, which compromise their fertility [1]. Among the main causes of injuries to the sperm are the reactive oxygen species, which have their production intensified during semen manipulation [2]. Therefore, antioxidants therapies can be an important alternative to protect the sperm during laboratory manipulation [3]. Thus, resveratrol is a powerful antioxidant with an important role on sperm protection [3], the aim of this study was to evaluate the protective effect of resveratrol on goat sperm submitted to sex sorting in discontinuous Percoll gradient.

Methods: To perform this study were used three semen pools from four mature goats, collected with artificial vagina. The fresh semen pools (G1) were analyzed and diluted in 10X DSEM to 800 × 10⁶ sperm/mL. Aliquots of 500 μL of the diluted semen were added on Percoll discontinuous gradients, and prepared according Resende et al. [4], without (G2=sexed semen) or with resveratrol (G3=sexed semen + 75 μM resveratrol). After that, the gradients added of the semen samples were centrifuged (500 × g per 20 min) and the pellet of cell recovered. The spermatic cells recovered from each gradient, with or without resveratrol, were evaluated too. The Sperm parameters accessed were plasma membrane integrity (PMI) by CFDA and PI [5], mitochondrial membrane potential (MMP) by JC-1 [5], total motility (TM) and progressive motility (PM) by CASA. Statistical analysis was done with ANOVA and Teste-t at 5% significance.

Results and conclusions: The percentage of spermatic cells with PMI was higher to G1 (P < 0.05) than to G2 (G1 = 83.00 ± 5.70; G2 = 67.83 ± 0.76); G3 = 76.50 ± 4.82%). No statistics differences (P > 0.05) were observed among all the experimental groups for +MMP (G1 = 87.33 ± 8.31; G2 = 87.50 ± 17.32; G3 = 87.17 ± 9.00), TM (G1 = 84.73 ± 7.12; G2 = 71.50 ± 18.70; G3 = 78.43 ± 7.98) and PM (G1 = 37.35 ± 6.67; G2 = 39.99 ± 12.41; G3 = 48.61 ± 10.12). Nevertheless, sexed semen with resveratrol showed numerically, higher values to the parameters evaluated than without it, fact that consolidates the Sarlos et al. [3] observations about the protective effect of resveratrol. In conclusion, resveratrol does not represent protection to goat sperm submitted to sex sorting in discontinuous Percoll gradient. However, new studies should be realized to reduce the standard variances and determine the real role of resveratrol on sperm protection.

Acknowledgements: The authors are grateful to FACEPE, CAPES, RENORIBIO and CNPq.

References:

Activity of crude extract of the sexual renal segment from Crotalus durissus on spermatic kinematics of thawed dog semen

Effect of a 2010, Espécies reativas de oxigênio e nitrogênio: Acta Vet Crotalus durissus e Sarlós et al. [3] observations about ily on reproductive cycle, once when Squamata 50 Rev Bras Saúde Prod Na has no influence on spermatic kinematics of the Crotalus durissus on spermatic kinematics of 77 Avanços metodológicos na seleção do sexo de dog semen samples (n = 3) cryopreserved in tris-yolk-glycerol 36 R Bras Zootec 8(Suppl 4): to perform this study were used three semen pools from four mature goats, collected with artificial vagina. The fresh semen pools (G1) were analyzed and diluted in 10X DSEM to 800 × 10⁶ sperm/mL. Aliquots of 500 μL of the diluted semen were added on Percoll discontinuous gradients, and prepared according Resende et al. [4], without (G2=sexed semen) or with resveratrol (G3=sexed semen + 75 μM resveratrol). After that, the gradients added of the semen samples were centrifuged (500 × g per 20 min) and the pellet of cell recovered. The spermatic cells recovered from each gradient, with or without resveratrol, were evaluated too. The Sperm parameters accessed were plasma membrane integrity (PMI) by CFDA and PI [5], mitochondrial membrane potential (MMP) by JC-1 [5], total motility (TM) and progressive motility (PM) by CASA. Statistical analysis was done with ANOVA and Teste-t at 5% significance.

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Acknowledgements: The authors are grateful to FACEPE, CAPES, RENORIBIO and CNPq.

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Use of phytopathogenic virus for peptide expression in plant system
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BMC Proceedings 2014, 8(Suppl 4):P151

Background: Some plant viruses have been used to express and/or car peptides from human and animal pathogens for diagnostic purposes or vaccine. This system has presented many advantages over traditional methods, especially with regard to cost and production of peptides free of pathogens [1]. In this context, the possibility of using these viruses in an effort to produce vaccine peptide candidates in veterinary medicine is presented as a very promising idea. Among the relevant diseases in veterinary medicine there is caprine arthritis encephalitis (CAEV) virus infection. Caprine arthritis encephalitis (CAEV). This virus infects goats worldwide, causing arthritis, encephalitis, mastitis, progressive weight loss and mainly fall in production, being consequently a major economic loss to producers [2]. This disease is silent and yet there is still no treatment nor vaccine, and the control accomplished by early diagnosis. Therefore, this study aimed to produce peptides CAEV using a plant system.

Methods: Primers were designed for a segment of p28 protein from the gag gene of CAEV. The insert obtained was cloned into pGEM, inserted into plasmid non-commercial (PNC) and inoculated on susceptible plants. The plants were kept in a greenhouse until the appearance of symptoms. The extraction of proteins was carried out [3] and the sample was submitted to SDS-PAGE and Western Blotting assay.

Results and conclusions: The electrophoretic profile of approximately 55 kDa for the produced protein was similar to the positive control (commercial p28 protein). The Western Blotting confirmed the specific reactivity to this antigen. Thus, it was possible to determine the production of CAEV vaccine candidate peptides in plant systems.

Acknowledgements: The authors are thankful to the Rede Nordeste de Biotecnologia (RENORBIO).

References

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Sequencing of the whole mitochondrial genome of the Brazilian stingless bee, Melipona scutellaris
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Background: In the Americas, from Mexico to Argentina, the genus Melipona has a great diversity of species, mainly in the Amazonian region, and a crucial pollination role. It is already well-known the importance of Brazilian stingless bees as pollinators, as well as producers of good honey, pollen, wax and propolis [1]. The present study is about the characterization of the mitochondrial genome of Melipona scutellaris, a Brazilian stingless bee, commonly found in the Northeast Region [2].

At first, the characterization of bees was based mostly on morphological and behavioral criteria. With advances in molecular biology, such visual criteria proved to be not so robust in all cases. Since the mitochondrial genome is well conserved among all animal species, it has been effectively used as a phylogenetic and evolutionary tool. Furthermore, the interaction between mitochondrial and nuclear genomes has important functions in the gene regulation of both [3].

Basically, the mitochondrial genome is about 16 kb long and it is composed approximately of 37 genes, of which there are the two ribosomal subunits (125 and 165), 22 tRNAs and 13 proteins (three subunits of cytochrome oxidase, cytochrome B, subunits 6 and 8 of ATP synthase and seven subunits of NADH dehydrogenase) and still an A/T repetitive region [1]. In this study, our reference organism is the Melipona bicolor, a stingless bee also found in Brazil.

Methods: Expressed Sequence Tags (ESTs): Previously, transcripts were obtained from the fat body of Melipona scutellaris workers bees. A cDNA library was generated and ESTs were sequenced by means of Sanger sequencing.

DNA Amplification: DNA amplification was performed using “universal” [4] and specific mitochondrial primers of Atta laevigata, a leafcutter ant. Of 15 set of primers 13 amplified, resulting in some unspecific bands. As we succeed to obtain specific bands, the PCR products were cloned using pGEM®-T Easy Vector (PROMEGA, US). The samples were sequenced using the MEGA-BACE 1000 DNA Sequence System (GE Healthcare, UK).

Bioinformatics: The sequences identifications were made using Blast2Go and the NCBI database. The contigs were obtained using CAP3. Finally, they were mapped with our reference mitochondrial genome.

Results and discussion: The sequences of PCR products and ESTs, together covered 28% of the reference mitochondrial genome. Since the mitochondrial genome is relatively short, it has been proved that long PCR products, obtained from high fidelity enzymes, are effective to cover the whole mitochondrial genome in Metazoan species [5]. Therefore, we propose next, using specific primers of M. scutellaris, designed based on the sequences already generated. Still, through SOLID™ sequencing, we obtained reads of mitochondrial sequences. Analyzing these data, we expect to obtain a considerable part of the transcriptome, which will be completed by amplification of DNA with specific primers. The complete mitochondrial genome from M. scutellaris can provide new information about evolution in stingless bees and show new information with biotechnological potential.

References

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Xanthan gum enhances humoral immune response elicited by a DNA vaccine against leptospirosis in mice
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BMC Proceedings 2014, 8(Suppl 4):P153

Background: Traditional vaccines (killed or inactivated) played vital roles in controlling and eradicating infectious diseases for a long time. Antigen-specific T cell response can be induced when mice are intramuscularly
inoculated with naked plasmid DNA. Therefore, DNA vaccines were evaluated in many studies, demonstrating its safety, stability and easy production. Furthermore, it has been reported that the gene expression lasted for one year after intramuscular injection of the plasmid DNA. However, some disadvantages such as the low transfection rate and low immunogenicity make the use of multiple doses necessary [1]. In light of this context, several studies have been performed to improve the immune response induced by DNA vaccines. The xanthan gum is an extracellular polysaccharide produced during fermentation of bacteria of the genus Xanthomonas and has been studied as a new vaccine adjuvant [2]. However, it has not yet been evaluated as an adjuvant for DNA vaccines. The aim of this study was to evaluate the capacity of the xanthan gum to increase the humoral immune response of mice inoculated with a DNA vaccine against a fragment of the leptostrongyloidea antigen LigAni cloned in the mammalian expression plasmid pTARGET.

**Material and methods:** Four to six month-old female BALB/c mice were segregated into 2 groups of 12 animals each, where both groups were inoculated with the DNA vaccine. Animals belonging to group 2 (G2) also received 0.5% xanthan gum as adjuvant. The immunization protocol consisted of 3 doses (days 0, 14 and 21) of 100 μg of the pTARGET/LigAni plasmid injected intramuscularly 45 minutes after 100 μg of a 25% (w/v) sucrose solution was injected. Blood samples were taken 0, 13, 20 and 27 days post-first immunization for the humoral immune response evaluation after each dose through indirect ELISA using recombinant LigAni (5 μg.mL-1) as antigen. Sera were diluted 1:30 in PBS-T. ELISA units were calculated by dividing the mean absorbance of each group by the mean absorbance of the same group at day 0. ANOVA followed by the Tukey’s test was performed to detect significant differences (p < 0.05) between immunizations.

**Results:** ELISA results showed that antibody titers in sera from G2 was statistically higher than titers in sera from G1 after the second and third immunization (p < 0.05). There was no significant difference between the second and third immunizations of G2 though. This way, the results indicate that the xanthan gum had an adjuvant.

**Conclusions:** We conclude that xanthan gum had an adjuvant effect increasing the humoral immune response elicited by a DNA vaccine against leptostrongyloidea in mice.

**Acknowledgements:** We would like to thanks CAPES, FAPERGS and CNPq for financial support and scholarships.

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**Partial results of the construction of cDNA library of Megalopyge albicolis**

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**BMC Proceedings 2014, 8(Suppl 4):P154**

**Introduction:** For more than 350 million years, insects live and survive in almost every ecosystem on the planet, what have resulted in the development of protection and defense mechanisms against adverse situation. This feature has stimulated research into new agents with pharmacological and biotechnology potential in the class of arthropods. Recently, we have identified and isolated proteins of pharmacological and biotechnological interest in the hemolymph of caterpillars from family Saturniidae (Lonomia obliqua). Two proteins have been further characterized: one with antipapoptotic activity and other with an antiviral action.

**Objectives:** The main objective of this project is to build, characterize and compare the transcripts generated by the construction of a cDNA library of the integument of caterpillars Megalopyge albicolis (Megalopygidae family).

**Methods:** The mRNA was isolated using the Dynabeads mRNA Direct kit (Invitrogen) and quantified with the RiboGreen RNA Reagent (Invitrogen). Subsequently, a cDNA library was produced and sequenced with a 454 GS-Junior machine (Roche). These procedures were repeated for two different tissue samples, originated from two different animals.

**Results and discussion:** As a result, we obtained 38,2456 reads for the first tissue cDNA sequencing and 138,177 for the other one, with an average size of 240,66 and 439,10 bases, respectively. After the elimination of small and low quality reads, the poly A tails were trimmed off, resulting in 165,187 reads with an average size of 398,87 bases. These sequences were grouped according to their similarity using the CLC-Genomics program, resulting in 2,519 Contigs (using 117,777 reads), while the remaining 46,410 reads were classified as singlets or singletons. The more abundant transcripts are mainly related to proteins involved in catalysis, transport and binding. We also detected a high expression of the gene encoding for the protein arthroporetin, with is involved in storage and in developmental and metamorphosis processes. Other frequent transcripts are also related to storage (vitellogenin) and to reactive oxygen species detoxification (catalase). These proteins need be further studied to better characterize their biological role, as well as to determinate the biotechnological potential of molecules produced by M. albicolis.

**References**


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Antimicrobial activity from ticks eggs waxes

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BMC Proceedings 2014, 8(Suppl 4)P156

Introduction: Ticks lay their eggs in the environment and cover the eggs in a waxy layer to protect them from desiccation and microbial attack. This wax is produced by an organ known as Gene’s Organ. Bio prospection has shown the presence of active principles in the hemolymph of arthropods as well as in the salivary glands of ticks. Some of these are of interest for the development of new pharmacological drugs. In this study, different tick species were used to test the antimicrobial effect of the extract obtained from the wax enveloping the eggs.

Objectives: The objective of this study is to evaluate the antimicrobial effect of the waxy secretion from the eggs of the following tick species: Amblyomma cajennense, Amblyomma aureolatum, Rhipicephalus (Boophilus) microplus and Rhipicephalus sanguineus.

Methods: The egg masses were treated with iced cold phosphate buffer (pH 6.8) to test against influenza virus (H1N1) to determine the antiviral activity of the ticks’ wax. MDCK cells were infected with influenza viruses, culture of MDCK cells, performed in 96 wells microplate, were treated with 2600, 1300, 650, 325, 162.5, 82, 41 and 20.5 mg/mL of the eggs wax extract 1 h before infection. After 72 h post infection cytopathic effect induced by the virus was observed, the culture medium was removed and the cells in the plate were stained with crystal violet (0.2% in 20% methanol). The egg wax was maintained in culture during the time of infection. The eggs were also treated with chloroform to obtain an extract suitable for the disc diffusion standard methodology stablished by Kirby and Bauer. The microorganisms used to test the activity were: Candida albicans, Micrococcus luteus, Escherichia coli, and Staphilococcus aureus. After incubation the plates were observed for the presence of absence of growth inhibition.

Results: Amounts as small as 325 mg/mL of the extract were able to inhibit the replication of the virus. Besides, the sample presented very low citotoxicity on Vero cells. On the other hand, the organic extract from A. aureolatum and R. sanguineus showed an inhibition zone for the strains of C. albicans, M. luteus, E. coli and S. aureus. This result is in accordance with the antimicrobial activity reported for the wax extracted from other ticks.

References

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Orthologous surface proteins from Mycoplasma hyopneumoniae and Mycoplasma flocculare: in silico comparison and heterologous expression of differential extracellular domains

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BMC Proceedings 2014, 8(Suppl 4)P157

Background: Mycoplasma hyopneumoniae and Mycoplasma flocculare are two closely related mycoplasma species often found in the porcine respiratory tract [1]. However, M. hyopneumoniae is pathogenic, being the causative agent of enzootic pneumonia, while M. flocculare is a commensal bacterium. Enzootic pneumonia is a contagious respiratory disease characterized by chronic cough, growth retardation, low mortality, and a high morbidity. It causes significant economic losses in pig industry worldwide. Some of main interactions between the host and the bacteria are mediated by surface proteins, and a comparison between the two mycoplasma species between these two mycoplasma species can lead to identification of determinants of pathogenicity or commensalism.

Methods: In this work the deduced amino acid sequences of M. hyopneumoniae and M. flocculare surface proteins [1,2] were aligned and comparatively analyzed. Comparative analysis was made by sequencing alignments using ClustalW algorithm available in the MEGA 5.03 software package. Orthologous pairs of interest were selected based on the differential presence of amino acid stretches ≥ 5 residues or regions of significantly reduced homology in comparison to flanking sequences. Topology analyses including predictions of extracellular and transmembrane domains were performed using the TopPred, phobius and TMHMM programs. The differential domains of the selected orthologous pairs were amplified by PCR from genomic DNA of the respective species and these amplicons were cloned into the pGEX4T-3 plasmid by in vivo homologous recombination in Escherichia coli KCB [3]. The recombinant plasmids were expressed in E. coli strains adequate to heterologous expression, and the expressed recombinant polypeptides were purified by affinity chromatography and thrombin cleavage.

Results and conclusions: A total of 170 putative surface protein sequences with identified orthologs in both M. hyopneumoniae and M. flocculare (MHP and MF, respectively) were analyzed. From this survey, three pairs of orthologs were selected for functional analysis, based on the presence of differential amino acid stretches in predicted extracellular domains. In the first pair (MHP7448_0556 and MF_00306), the M. flocculare protein presents an exclusive 53 aa-long stretch in its N-terminal extracellular domain. In the second pair (MHP7448_0094 and MF_00500) less conserved N-terminal sequences (40.5% identical) are present, in comparison to the rest of the proteins (80% identical). And, finally, the third pair (MHP7448_0612 and MF_00357) showed an 114 aa-long stretch with 35% identity between orthologs, flanked by regions with 70%-80% identity. This third pair of orthologs had the differential domains (with 306 bp and 393 bp, respectively) cloned and expressed in E. coli. Star and pLySE strains. GST-tagged recombinant polypeptides of 37 kDa and 40 kDa were expressed and, after purification and cleavage with thrombin, 11.4 kDa and 14.7 kDa polypeptides were recovered for MHP7448_0612 and MF_00357, respectively. The differential domains of the other two pairs of selected orthologs will be also cloned and expressed in E. coli and all the recombinant polypeptides from M. hyopneumoniae and M. flocculare will be produced for immunization of mice, in order to get evidence of potential differences between the immune responses induced by each ortholog.

References
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**Evaluation RNAi silencing in the DH82 canine histiocytic sarcoma cell line**

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**Background:** Cancer is a leading cause of death among dogs worldwide [1]. The disruption of balance in expression of genes that control the cell cycle, including proliferation, differentiation and programmed cell death [2]. The interest in understanding the molecular aspects of cancer in humans and dogs is driven by the possibilities of identification of novel targets for the development of new anticancer compounds [3]. A molecular tool that has been used for this purpose is the RNA interference (RNAi) technique which exploits the mechanism of post-transcriptional silencing, mediated by molecules of double-stranded RNA that trigger degradation of complementary mRNAs [4]. However, for the application of this technique, important aspects must be investigated, since not all cell strains are susceptible to silencing by RNAi, and often the intracellular release of the interfering RNAs has low efficiency [5]. In this work we evaluated the efficiency of RNAi in silencing of topoisomerase IIα, a target for many anticancer compounds, in the canine histiocytic sarcoma DH82 cell line.

**Materials and methods:** DH82 cells at a concentration of 2.4 × 10⁵ cells/well were cultured in 6 and 24 well plates at 37°C and 5% CO₂. Next, siRNAs (small interfering RNA) designed to target topoisomerase IIα and glyceraldehyde-3-phosphate dehydrogenase (positive control) mRNA were introduced into cells with the kit N-TERT Nanoparticle siRNA Transfection System (Sigma Aldrich®). The cells were incubated for 24 hours with a solution of nanoparticles, comprising siRNA (20-30 nM) complexed to peptides N-TERT. After transfection, the silencing was confirmed by real time PCR using TaqMan Gene Expression Assays (Applied Biosystems®). The ΔΔct method was used for calculating differences in gene expression levels between the untransfected and transfected cells. The level of expression is shown as a fold change positive (induction) or negative (repression).

**Results and discussions:** Silencing of topoisomerase IIα (top IIA) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was confirmed by obtaining negative fold change. Following transfection in 6-well plates, it was obtained a fold change of -2.56 and -1.35 for the top IIA and GAPDH genes, respectively. The level of silencing of type IIα decreases after incubation of the transfected cells in the absence of siRNA, as demonstrated by reduced values of the fold change for -0.95 and -0.82 after 24 and 48 hours of incubation, respectively. The transfection in 6 and 24 well plates resulted in silencing of the top IIA of -2.56 and -0.73. The use of well plate for transfection, possibly facilitates the internalization of siRNA/peptide complex by cells. The increased concentration of siRNA from 20 to 30 nM did not result in increased silencing of the top IIA.

**Conclusions:** The lineage DH82 demonstrated susceptibility to silencing mediated by RNAi. The use of RNAi technique in this cell lineage when performed under ideal conditions of transfection was efficient, allowing the use of this model as a tool to assist in the identification of new targets for the development of new anticancer drugs.

**Financial support:** This work was supported for CAPES and FAPESP.

**References**


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**P159**

**Cellular and molecular aspects of adult brain development in honeybee castes (Apis mellifera L.)**

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**Background:** The adult honey bee brain exhibits a complex architecture composed by millions of neurons, glial cells and their respective tracts which form structures known as neuropils. They are organized to produce the optic lobe, antennal lobe, central complex and mushroom bodies. Learning and memory-related skills that honeybee workers use for navigation, foraging, nestmate recognition, and other activities are believed to be associated with these mushroom bodies, which are more developed in the adult members of the worker caste. During larval period, however, the differential feeding offered to prospective queens promotes faster brain development and higher expression of several neurogenic genes (ataxin-2, cryptocephal, dachshund, Eph Receptor, fax, shot, krüppel homolog-1 and tetraspanin SD) [1]. It seems that in some point during pupation, there happens a shift in this trend. In fact, queen's brain experiences extensive cell death events, but worker's brain is favored by higher rates of cell proliferation, resulting in caste specific brains [2].

**Methods:** *Apis mellifera* pupae were collected from colonies (africanized hybrids) at the Experimental Apiary of the Federal Institute of Muzambinho (IFSULDEMINAS) at Muzambinho, Minas Gerais State, Brazil. The developmental stages were classified according to the criteria proposed by Michelettie and Soares [3] and Rembold et al [4]. To investigate whether differences could be detected in brain development in the context of caste differentiation we performed neuroanatomical studies of whole mount preparations of queens and workers pupal brains using actin phalloidin/rhodamin to visualize axons, and DAPI to visualize nuclei. In addition, we used genome-wide expression analyses and normalized transcript expression using RT-qPCR for monitoring specific genes during pupal stages.

**Results and conclusions:** Our morphological results showed that during early pupal stages there are no obvious differences between castes brain development. However, late worker pupae exhibit larger calyces, fundamental parts of mushroom bodies, when compared with queens. Gene expression analyses revealed that the transcription profiles in brains of queens and workers (except for kr-h1) show an opposite pattern to that observed during larval development, with workers' brain with higher levels of gene transcription (e.g., mesencephalic astroycte derived neurotrophic factor, minibrain, signal peptide peptideptide, and tumbledew, all associated to neurogenic events or cell death prevention). This indicates that the respective protein products are responsible for the differential development of adult brains between honeybee castes. Particularly, the expression patterns of the neurogenic genes atx-2 and fax and the *anti-cell death* gene *signal peptide peptideidase* help explain the observed inversion of the neuroblasts area in queens and workers' brain, which, during larval period favors queens (very likely due to the differential feeding), and during pupal development and in adults, favors workers.

**Acknowledgements:** We thank Sávio Dutra and Rubens Castro for technical assistance in the experimental apiary in Muzambinho.

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*Saccharomyces boulardii* ingestion increases the humoral response of a DNA vaccine against leptospirosis in mice

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**BMC Proceedings** 2014, 8(Suppl 4):P160

**Background:** DNA vaccines are a good option to generate a desired antigen using the cellular machinery of the vaccinated subject. This kind of vaccine can induce both the humoral and cellular immune response, and shows high stability and easy working, as well as offer a low-cost production, and safety for immune compromised patients [1]. However, some disadvantages, such as the low transfection rate and low immunogenicity, makes necessary the use of multiple doses [2,3]. Therefore, several studies have been conducted trying to increase the immune response generated by the DNA vaccines. *Saccharomyces boulardii*, a probiotic yeast that is capable to increase the host immune response [4], was not previously evaluated as an adjuvant for DNA vaccines. Thereby, in the present study we evaluated the *S. boulardii* capacity to increase the humoral response of mice using DNA vaccines against a fragment of the leptospiral antigen LigA cloned in the mammalian expression plasmid pTARGET [4,5].

**Material and methods:** Four to six month-old Female BALB/c mice were separated into 2 groups of 12 animals each. Group 1 (G1), control) were fed with antimicrobial-free ration; group 2 (G2) with the same ration containing 10^5 cfu g^-1 of *S. boulardii*, 14 days before the first immunization, for adaptation, and during all the experiment. The immunization protocol consisted in 3 doses (days 0, 14 and 21) of 100 μg of the pTARGET/ligAni plasmid injected intramuscularly. Blood samples were collected from the retro eye venous plexus of the animals on days 0, 13, 20 and 27 for the humoral immune response evaluation of each immunization through indirect ELISA, using recombinant LigAni (5 μg/mL) as antigen and serum dilutions 1:30 in PBS-TELSA units were calculated by dividing the mean absorbance of each group by the mean absorbance of the day 0. The student’s t-test was performed to determine significant differences (p < 0.05) between the groups.

**Results and conclusions:** Sera from G2 has shown ELISA units statistically higher than the sera from G1 after the second (p < 0.0001) and third immunization (p = 0.0264). In addition, there was no significant difference between the second and third immunizations of G2. This way, the results indicate that using *S. boulardii* as adjuvant, only two immunizations are sufficient to obtain an adequate immune response. Therefore, the *S. boulardii* was able to enhance the humoral immune response to a DNA vaccine against leptospirosis in mice.

**Acknowledgements:** We would like to thank CAPES, CNPq and FAPERGS for the financial support and scholarships.

**References**


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Evaluation of immunogenicity of rSeM and use of PAMPs as possible enhancers of the immune response

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**BMC Proceedings** 2014, 8(Suppl 4):P161

The immune system is responsible for the first immune response to infection. This response is based, in part, by Toll-Like Receptors (TLR) that detect pathogens and induce an appropriate immune response[1]. These receptors bind to structures called Pathogen Associated Molecular Patterns (PAMPs), including lipopolysaccharide, flagellin, lipoproteins, nucleic acids, and other molecules 2. The Equine Strangles is a bacterial disease caused by Streptococcus equi subspecies equi (*S. equi*). This bacterium synthesizes several virulence factors, among these M protein (*SeM*) that stands out for its high antifagocit potential, having an important role in the pathogenesis, making it a promising antigen vaccine candidate[4]. The purpose of this study was to evaluate the immunogenicity of rSeM and the use of PAMPs as possible potentiators of the immune response.

Forty Balb/c female mice were immunized intramuscularly on day 0 and 21 of the experiment. Blood samples were collected on days 0, 14 and 28, and processed in order to obtain serum. The mice were divided equally into four groups. The animals belong in groups 1-2 were immunized with the recombinant BL21 (DE3) strain of *E.coli*, which was previously cloned to express the protein of interest recombinant M protein (rSeM)[1]. In group 1, the strain of *E.coli* was inactivated using 0.1% formaldehyde and increased by 10% aluminum hydroxide, and group 2 received the same strain, but was not inactivated and adjuvant was not added in the vaccine composition. Group 3 was immunized only with the recombinant M protein of *S. equi* Purified associated with 10% aluminum hydroxide. Each vaccine dose contains 20 μg of rSeM. Animals of to group 4 were used as control and were inoculated with only PBS1X. The immune response of the animals was evaluated by indirect ELISA. It is noteworthy that the animals were kept and handled in accordance with the legal requirements provided for in the Brazilian National Law for Protection of Experimental Animals. Vaccines containing bacterins, used in group 1 and 2 showed the highest antibody seroconversions compared to the group immunized only with the rSeM recombinant vaccine. These results suggest that group 1 and 2 showed higher antibodies absorbance than group 3 due to the fact that these two groups were immunized with the recombinant BL21 (DE3) strain of *E.coli*, which presents the PAMPs in t.

This work was funded by "Centro de Desenvolvimento Tecnológico (CDTec)" from "Universidade Federal de Pelotas (UFPEl)."

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Detection of polyandric behavior in giant Amazonian river turtle (*Podocnemis expansa*) using microsatellites markers

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**BMC Proceedings** 2014, 8(Suppl 4):P162

**Background:** In some species, presence of multiple paternity, due to polyandric behavior have important consequences in the effective size of a population when compared to unique paternity, mainly when it is about endangered species[1]. Because the exacerbated exploitation of meat, guts and eggs as food by local communities, giant Amazonian river turtle (*Podocnemis expansa*) is at low risk/dependant of conservation, according IUCN. Facing this, studies related to reproductive behavior of this species have a great importance for contributing with programs about its conservation. Studies related to polyandric behavior, already evidenced in
P. expansa, describe genetic benefits for the specie, because together with multiple paternity occurrence, it rises the genetic variability from offsprings and decreases the occurrence of endogamy between individuals [2,3]. The present study aims to verify the existence of polyandric behavior in females of Amazon turtles from Mamirauá Reserve of Sustainable Development (RDSM), using microsatellite markers.

Materials and methods: 120 just-hatched individuals from four nests were analyzed, previously collected from RDSM. Samples of until 500µL of blood were collected by femoral vein puncture by using 1mL syringes and stored in microtubes with 500µL of absolute ethanol at 4°C. After blood sampling, offsprings were released in the site of origin. DNA extraction was performed by CTAB method suitable for nucleated blood cells. After extraction, DNA was submitted to Polymerase Chain Reaction (PCR) following the economic protocol described by Schuelke [4]. Four microsatellites loci were used (Puni1D12, Pe344, Pe519 and Puni1E1) developed for the specie. PCR products were subjected to genotyping according DeWoody protocol [5], performed by an automatic DNA sequencer ABI 3130xl. Observed alleles analysis for each locus was performed by using GeneMaker v2.2 program, in order to identify the genotype of each locus from sampled individuals. Analysis for multiple paternity was done using the minimum method of Alleles Counting.

Results and conclusion: When separately analyzed, four loci in all nests indicated polyandry occurrence, with at least three fathers contributing in each nest. In three nests the most polymorphic locus showed fourteen alleles and one nest had eighteen alleles. The least polymorphic locus, otherwise, showed eight alleles in two nests, seven in one nest and five in the last nest. Such results corroborates with previous studies which support as prevalent the polyandric behavior for P. expansa. RDSM population was considered ecologically extinct, by the high levels of predation in the past centuries and the low number of females spawning nowadays. From late 90s, nesting areas began to be protected in different areas from RDSM by local population. Presence of multiple paternity in similar conditions to most abundant populations can be considered a recovery signal of RDSM population. Results showed are part of the "Conservação de Vertebrados Aquáticos Amazônicos" project (Conservation of Amazon Aquatic Vertebrates), developed by Mamirauá Institute of Sustainable Development and sponsored by Petrobras, through “Programa Petrosbras Ambiental” (Ambiental Petrobras Program).

Acknowledgements: Foundation for Research Support of the State of Rio Grande do Sul (FAPERSG), through project No. 11/1894-0, and CNPq.

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OmpL37 from *L. interrogans* serovar Copenhageni strain Fiocruz L1-130 in hamsters, using prime-boost, DNA, and protein-based immunizations.

**Methods:** The ompL37 gene was cloned into pAE and pTARGET vectors, to obtain a subunit and a DNA vaccine, respectively. The recombinant protein OmpL37 (pOmpL37) was characterized by Western blot (WB) and pTARGET-ompL37 was evaluated by transfection of CHO-K1 cells and analyzed by immunofluorescence. Groups of 6 hamsters were immunized twice with an interval of 21 days as follows: OmpL37-Alcohol (2x 100 μg), pTARGET-ompL37 (2x 100 μg), prime-boost pTARGET-ompL37 (100 μg) plus rOmpL37 (100 μg), pTARGET (2x 100 μg) and PBS-Alcohol. Two independent experiments were conducted. Pooled blood samples, collected at days 21 and 42, were used for RNA extraction using the RiboPure-Blood Kit (Ambion). cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems). Expression profiles of INF-γ, TNF-α, IL-1α, IL-1β and TGF-β were assessed by quantitative real-time PCR using SYBR Green PCR Master Mix (Applied Biosystems). The relative Ct (ΔΔCt) method was used to quantify cytokine gene expression. The CT of each test gene was evaluated in pooled hamster whole-blood samples, the CTs were normalized against the β-actin gene CT (ΔCT) and then compared to the same normalized gene in the respective control groups (calibrator) [4].

**Results and conclusion:** Considering that target genes are up or down-regulated when a 2-fold change in mRNA levels is observed [5], TNF-α was induced at day 42 (ratio > 2.84), and by pTARGET-ompL37 at days 21 and 42 (ratio > 5). In contrast, INF-γ was down regulated in the prime-boost group at day 42 (ratio = 0.41). Similarly, down-regulation of IL-1α was observed at day 42 in the pTARGET-ompL37 (ratio = 0.28) and prime-boost (ratio = 0.19) groups. TGF-β was expressed at basal levels in all groups. Both rOmpL37 and pTARGET-ompL37 were able to induce a pro-inflammatory response, characterized by increased TNF-α expression.

However, the Th1 and pro-inflammatory cytokine levels decreased in the prime-boost group.

**Acknowledgements:** CNPq, CAPES and FAPERGS.

**References**

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Expression and purification of Lipl32 recombinant protein of *Leptospira interrogans* in different *E. coli* strains

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**Background:** Leptospirosis is a zoonosis caused by the infection of pathogenic bacteria of the genera *Leptospira*, often transmitted by direct or indirect contact with urine of infected animal hosts [1]. In national territory, leptospirosis is considered an endemic disease, with high incidence in rainy seasons, but occurring during the whole year [2]. From all the cases, 15% evolve to the icteric form, with serious clinic manifestations, as severe icteric, hemorrhages and kidney disease, including lethal onset in 50% of the cases [3].

From all membrane proteins Lipl32 is the most abundant and found in all pathogenic species of *Leptospira*. Saprophytic strains do not express Lipl32, making the detection of the protein or the detection of specific antibody a potential toll for the development of a laboratory diagnosis [4].

**Methods:** DNA of pAE-Lipl32 was utilized in the transformation of *E. coli* strains BL21-S1, with expression induced by NaCl, and BL21 Star(DE3)pLysS, with expression induced by IPTG. Eight transformed colonies of each strain were inoculated in culture medium, and after the induced expression and analysis, by SDS-PAGE, the results showed a high level of expression in BL21-S1 strains. One of the BL21-SI clone was chosen to express the Lipl32 protein in a higher volume, for purification in a anionic chromatographic column, using a crescent concentration of ampic elution buffer (0,05 to 1,0 M). The purification resulted in 259 fractions combined in 15 protein pools according with the protein spikes observed by spectrophotometry. The 15 proteic pool were analyzed in SDS-PAGE.

**Results and conclusions:** The SDS-PAGE showed only one band corresponding to the Lipl32 protein, from pools six to 15, although, pool six has a visible higher expression, and pools 14 and 15 showed the presence of contaminant proteins, probably due to the high concentration of the eluent. More purifications steps are needed, but the expression of Lipl32 by *E. coli* strain BL21-SI, induced by NaCl was successfully achieved.

**References**

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Cryopreservation of ram epididymis spermatozoa post-slaughter - A feasible biotechnique?

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**Background:** Gametes for breeding high-value livestock is commonly preserved in gene banks for later use in assisted reproduction programs. Such material can be used in artificial insemination or embryos transfer in the field as well as embryos in vitro production [1]. However, when these animals die, genetic material may be lost if it is not possible to recover and preserve spermatozoa from epididymis. Thus, our aim is to establish a recovery technique of ram sperm epididymal post-slaughter and to test its post-cryopreservation viability.

**Methods:** Ten rams were slaughtered and testis-epididymis of each animal was placed in individual bags and sent to the Andrology Laboratory (UFPR). Epididymis were separated, cleaned (alcohol 70%) underwent a slicing technique and immersed in 5.0mL of Tris buffer (375mM Tris, 124mM citric acid, 41.6mM fructose, pH 7.0) for sperm migration for 10 minutes [2]. The suspension buffer-spermatozoa from each animal was evaluated for motility (0-100%) and vigor (0-5) and when approved (60%/2%), diluted in Tris-yolk (20% egg-yolk, 5% glycerol, pH 7.4), packed in straws (0.25mL), cryopreserved in an automated system (TX3000®) and stored (-196°C). After thawing (37°C/30s), semen samples were evaluated for kinematic (SCA*) and structural integrity (plasma membrane-PICD; mitochondrial membrane potential-JC1; acrosome-FITC/PNA).

**Results and conclusions:** Data were expressed as mean ± sd. From 10 samples, 70% were initially approved for cryopreservation. After thawing, the following values were obtained: total motility (64.73 ± 7.10), progressive motility (19.97 ± 4.26), plasma membrane integrity (39.39 ± 4.71), mitochondrial membrane potential (88.81 ± 3.44) and acrosome integrity (49.16 ± 0.76). These results are consistent with other results of post-thaw ram semen obtained by ejaculation [3,4] as well as are indicative of function and structure preservation of sperm cell [5]. Thus, we concluded that cryopreservation of ram epididymal spermatozoa is a feasible biotechnique for germplasm conservation of animals after slaughter.

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Acknowledgments: The authors are grateful to the FACEPE, CAPES, RENORBIO and CNPq.

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P167 Culture optimization of Escherichia coli for expression of rGE from bovine herpesvirus 1 and 5
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Background: The use of Escherichia coli for the production of recombinant proteins is an established strategy to obtain biotechnological tools. However, the recombinant protein expression is dependent on temperature, bacterial culture time, induction period, nutrients, plasmid characteristics, and insert itself. Thus, the aim of this study was to optimize the expression of recombinant rGE protein (rGE) from Bovine Herpesvirus types 1 and 5.

Methods: The expression at various post-induction time-points and during growth at two temperatures was performed in order to standardize the conditions that E. coli maximizes rGE expression. A recombinant vector pAE/gE containing a consensus sequence between BovH-1 and BovH-5 was used to heat-shock transform E. coli strain BL21 Star (DE3). The transformation product was seeded on solid Luria Bertani (LB) medium containing ampicillin (100 μg/mL). After, the colonies selected were grown in LB medium (1 ml) and incubated at 37 °C for 16 h. Then, 0.5 ml of this culture was transferred to 10 ml of LB medium and incubated at 37 °C again to reach the exponential phase of bacterial growth (OD600 0.6 - 0.8). The bacterial culture was induced with 0.6 mM IPTG for periods of 4, 6 and 12 h at 25 °C or 37 °C. Aliquots from each culture condition tested were collected and analyzed by SDS-PAGE and Western Blot (WB).

Results and conclusions: The finding of this study indicated that rGE protein was successfully expressed after induction for 12 h at 25 °C. These conditions will be used to obtain rGE lots for development of immunodiagnostic assays of bovine Herpesvirus type 1 and 5.

Acknowledgments: We thank to Universidade Federal de Pelotas, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) and CNpq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

References

Background: The improvement of in vitro maturation (IVM) protocols has become an alternative to increase the culture medium efficiency. Tretinoin (TTN, all-trans retinoic acid, ATRA), is an active metabolite of vitamin A [1], that mediates cell proliferation, cell differentiation, and embryonic development process. In in vitro production embryos systems, TTN acts improving cytoplasmic maturation process in oocytes, developmental competence in early embryos, and quality in blastocysts [2]. Studies have been demonstrated the presence of α, β and γ subtypes of retinoic acid receptors (RARα, RARβ, RARγ) for TTN in oocytes, hatched blastocysts, and cumulus cells [3]. This molecule can also be used for treatment of skin disorders and for anti-tumor treatment, so researchers have been associated TTN with polymeric nanoparticles to protect it from degradation and to improve its chemical stability and efficacy [4]. The aim of present study was to test the concentration-dependent effect of supplementation of free tretinoin (TTN) and tretinoin-loaded lipid-core nanocapsules (TTN-LNC) in bovine in vitro maturation media, and its influence in reactive oxygen species (ROS) production in two-to four-cell stage embryos. In conclusion, tretinoin-loaded lipid-core nanocapsules added in in vitro maturation media highly protects embryos at early stage of development against oxidative stress.

Methods: The experimental groups were established, and cumulus-oocyte complexes (COCs) were matured in oocyte in vitro maturation medium supplemented with 0.25, 0.5 and 1 μM of TTN-LNC or TTN. Control groups of COCs matured without treatment and treated only with blank lipid-core-nanocapsules (LNC) were also examined. The oocytes were in vitro fertilized in order to evaluate the ROS levels in embryos produced by the different treatments. The ROS formation was evaluated in two-to-four-cell stage embryos as previously described [5] with some modifications.

Results and conclusions: ROS production was lower in embryos derived from oocytes matured in the presence of TTN or TTN-LNC. Both treatments TTN and TTN-LNC protect the cell more effectively against oxidative damage reducing the ROS production. A significant reduction (p <0.05) in ROS production was detected in the presence of TTN-LNC compared with controls. There was no difference between the concentrations in TTN and TTN-LNC groups. Tretinoin-loaded lipid-core nanocapsules offers an increased protection against oxidative stress in embryos produced in vitro. The studies at the molecular level using oocyte competence markers are alternatives to clarify the function of lipid-core nanocapsules in in vitro maturation and in embryonic development.

Acknowledgments: CAPES, CNPq and FAPERGS.

References
Expression of recombinant *Mycobacterium bovis* antigen 85B by *Mycobacterium smegmatis* mc²

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**Background:** Bovine tuberculosis (TB) is a disease which constitutes an important economic and zoonotic problem in Latin America. The etiological agent, *Mycobacterium bovis*, has several antigens described and well characterized. The *M. bovis* antigen 85B (Ag85B), encoded by *fbpB* gene, is the bacillus immunodominant antigen and promising target for vaccine and diagnosis development [1]. Ag85B combined with other immunogens is considered as a potential diagnostic tool for TB ELSA assay [2]. Anti-Ag85B antibodies have high sensitivity and specificity for mycobacteria detection in commercial tests [3]. Here we describe the recombinant Ag85B production by *Mycobacterium smegmatis*, a fast growing mycobacterial specie.

**Methods:** We pairs of synthetic oligonucleotide primers were designed using the Vector NTI software, based on the *M. bovis* AF2122/97 complete genome sequence. The histidine tail codons were included in the reverse primers in order to facilitate recombinant proteins purification by nickel affinity columns. These primers were used for PCR amplification of *fbpB* gene, including its own promoter (1500 bp) and one immunodominant portion (873 bp) of the same gene. The PCR products were digested with *KpnI* and *Xhol* and *HindIII*, respectively. The digestion products were then cloned into the respective mycobacterial expression vectors: *pU*P410 and *pUS2000*, which were previously digested with the same restriction enzymes. The ligations were transformed by electroporation into *E. coli* TOP 10 competent cells and transformed cells were selected on LB agar medium containing kanamycin. The recombinant plasmids were extracted using the QIAprep® Spin Miniprep Kit (Qiagen). To confirm the expression was performed by real-time PCR (qPCR) during a cultivation expression was performed by real-time PCR (qPCR) during a cultivation period between 15 to 60 days. **Results and conclusions:** At the experimental interval, rapid fungus colonization on the lignocellulosic substrate was observed. The RT-qPCR analysis showed that *Lip1* gene expression started before the 15th day of cultivation. On the 15th day, this gene was highly expressed and its expression decline was observed from the 45th day. Comparing *Lip2*, its highest expression was observed in the range between 30 and 45 days of cultivation, while the decrease in the *Lip2* expression occurred from the 45th day. Comparing both genes, an abrupt decrease in the *Lip1* and *Lip2* expression was observed on the 60th day of culture, inferring the possibility that these genes act simultaneously during the degradation of the substrate evaluated. Overall, the results indicate the *Ceriporiopsis subvermispora* potential to degrade coconut fibers, and the gene expression analysis may provide data to understand the fungus extracellular system used to decompose lignin [3,4]. Also, insights on the role of regulating elements in the expression of these ligninolytic enzymes are necessary for efficient biotechnological applications. **Acknowledgements:** Laboratório de Biotecnologia e Biologia Molecular, Embrapa - Tabuleiros Costeiros, SE, Brazil for technical support.

**References**


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Study of lipase production by a filamentous fungus isolated from soil contaminated with lipid residues

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BMC Proceedings 2014, 8(Suppl 4):P171

Lipases are the special kind of esterases belong to subclass 1 of hydrolytic enzyme class 3 and have been assigned sub-sub-class 3.1.1 due to their specificity for carboxylic acid ester bonds. The biological function of lipase is to catalyze the hydrolysis of triacylglycerols to give free fatty acid, diacylglycerols, mono-acylglycerols and glycerol. They constitute the most important group of biocatalysts for biotechnological applications. Furthermore, novel biotechnological applications have been successfully established using lipases for synthesis of biopolymers and biodiesel, production of enantiopure pharmaceuticals, agro-chemicals and flavour compounds. The aim of the present study was to evaluate the production of lipases by filamentous fungi isolated from soil in different growing conditions. The lipase production was evaluated by both solid-state with soybean meal and submerged fermentation with different carbon and nitrogen sources. In addition, different concentrations of olive oil and ammonium nitrate and temperature were evaluated through a full factorial design (34) with three replications at the center point. All submerged fermentations were shaken (120rpm). Lipase activity in the culture filtrate was determined spectrophotometrically at 37°C using p-nitrophenol palmitate (pNPP) as substrate. One unit of enzyme activity (1U) was defined as that amount of enzyme that liberated 1μmol of pNPP per minute under the test conditions. Except for the fermentation at different temperatures, all experiments were carried out at 28°C. Olive oil and ammonium nitrate were, respectively, the best carbon and nitrogen sources. Low lipase activity was found in the solid-state cultures, which is interesting, since soybean meal was used without inducing lipid supplementation. In the factorial design, analysis of variance (ANOVA) showed significant effects when temperature and oil concentration varied. The maximum level of lipase production (206.68U/ml) was reached with 0.5%(W/V) of olive oil, 1.5%(W/V) of ammonium nitrate and at the temperature of 24°C. Lipase production is generally stimulated by lipids and is usually coordinated with the availability of triglycerides. Furthermore, nitrogen sources in the medium have to be carefully considered for growth and optimization of production. Response surface methodology has been widely used to bring insights of the interaction effects of several process parameters, generally resulting in higher production yields. In this work, with the evaluated levels of each tested parameter, we did not achieve an optimized condition. Further experiments are needed in order to get an optimized process of lipase production. Nevertheless, the soil-isolated filamentous fungi demonstrated an interesting ability to produce lipase in both solid-state and submerged fermentation.

References


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Biomass production from Bacillus sp. RAB9 using several carbon sources

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BMC Proceedings 2014, 8(Suppl 4):P172

Background: The NYD medium (glucose, meat extract, yeast extract and peptone) is used for biomass production of some bacteria species that promote plant species growth [1-3]. The main carbon source present in the medium is the glucose that contributes a percentage of 25% to the medium price. An alternative to obtain a more accessible price for the medium is to replace the glucose by sources of lower cost. Several byproducts and feedstock from food industry and agro industry have been applied on microorganisms growth due your high availability and low cost. Among these, the molasses stands out as culture medium in fermentative processes because of its high contents of sugars, nitrogen and vitamins [4]. This study evaluated the replacement of glucose by comercial sucrose, soy molasses and sugar cane molasses seeking better conditions for biomass production from Bacillus sp. RAB9.

Methods: In this study was used the strain of Bacillus sp. RAB9. The assays were performed in 250 ml flasks with 100 ml of medium and incubated at 30°C on an orbital shaker at 150 rpm. The biomass was quantified between 4 and 24 hours. The sugar content in each molasses was determined spectrophotometrically by the DNS method. The biomass was quantified from the absorbance (600 nm) of the medium after centrifugation at 3500 rpm for 15 min using pre defined standard curves. A volume of 0.01 g.L -1 of inoculum, calculated according the standard curves, was inoculated in the fermentation medium. The NYD medium has the following composition (g.L -1 ): glucose 10.0; yeast extract 5.0; meat extract 3.0; peptone 5.0; pH 6.5. Tested: sucrose 10.0, soy molasses 10.0; sugar cane molasses 10.0.

Results and conclusions: In NYD medium the production biomass was 0.74 g.L -1 in 24 hours of fermentation. The results found showed an increase on lineage growth of 39.2% for sucrose, 95.9% for soy molasses and 310.8% for sugar cane molasses. The replacement by cane molasses contributed to a 76.7% reduction in biomass cost. Selected the sugar cane molasses to replace the glucose in NYD medium was evaluated the concentrations of 10 to 20 g.L -1 for choose the best concentration for replacement and subsequently the best temperature of 30 to 45°C. The increase on cane molasses concentration did not contribute for the gain on biomass. Was observed that the best alternative to substitute glucose is the sugar cane molasses on concentration of 10 g.L -1 at 40°C.

References


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Evaluation of the use of raw glycerol in biomass production by Trichoderma reesei QM9414

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BMC Proceedings 2014, 8(Suppl 4):P173

Background: Biodiesel has emerged as a energy, environmental and social solution proposal. However, there are some technical challenges that need
to be solved, for example, use of the by-product of biodiesel production,
crude glycerol (CG). To avoid future problems caused by the accumulation
of CG and simultaneously make biodiesel production more competitive,
the search for the most viable alternatives to the use of this waste has
been proposed. The CG can be the raw material for the synthesis of products
with high added value, obtained through biochemical conversion (fermentation
routes) [1-3]. The biomass of Trichoderma is considered an excellent biological
control agent for pest and disease pathogens in plants and is promising
alternative to conventional chemical control [4,5]. Thus, the aim of this study
was to preliminarily evaluate the use of CG in biomass production of
Trichoderma QM9414 by submerged fermentation.

Methods: The fermentations were conducted in flasks of 250 ml capacity
(100 ml of culture broth). The inoculum represented about 1% (v/v) from
the fermentation medium. The incubation time was 72 hours at 30°C under
agitation at 175 rpm. The initial pH of the tests was 5.5. The basic medium
of fermentation (test T1) presented in g/L: 15.0 of CG, 1.4% of (NH₄)₂SO₄, 2.0
of KH₂PO₄, 0.3 of CaCl₂·2H₂O, 0.6 of MgSO₄·7H₂O, 0.005 of FeSO₄·7H₂O, 0.002
of CoCl₂·6H₂O, 0.0016 of MnSO₄·H₂O; 0.0014 of ZnSO₄·7H₂O and 6.0 yeast
extract (YE). It was also tested modifications of the basic medium: YE
suppression (test T2); YE suppression and increased (NH₄)₂SO₄ (2.8 g L⁻¹
(test T3); decreased YE (0.6 g L⁻¹) (test T4); decreased YE (0.6 g L⁻¹) and
increased (NH₄)₂SO₄ (2.6 g L⁻¹) (test T5). After 72 hours, was analyzed the
consumption of glycerol (high performance liquid chromatography) and the
produced biomass (dry weight).

Results and conclusions: The CG used in the study came from the
production of biodiesel from soybeans (purity of 80.9%). It was found
biomass of T. reesei QM9414 in all fermentation tests using CG. However, the
presence of YE (6.0 g L⁻¹) in higher concentration in the fermentation medium
(T1) provided the best results for the production of biomass (4.49 g L⁻¹).
The lower production of biomass, 1.32 and 1.52 g L⁻¹, were detected, respectively,
in tests T2 and T3. It was noticed that the isolated presence of inorganic
nutrient source ((NH₄)₂SO₄), even at a concentration of 2.8 g L⁻¹, did not
ensure an increase in biomass production. Tests T4 and T5 were respectively
2.33 and 2.48 g L⁻¹ of biomass. Regarding the consumption of glycerol, tests
conducted with YE (T1, T4 and T5) were related to higher percentage (over
73.0%). The lowest percentages of glycerol consumption occurred in the tests
that employed exclusively (NH₄)₂SO₄ (T2 and T3). The results showed that
the presence of yeast extract favored the use of crude glycerol and allowed the
formation of biomass. It was verified the viability of using crude glycerol as
carbon source for the production of biomass T. reesei QM9414.

Acknowledgements: Coordination for the Improvement of Higher
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Volume 8 Suppl 4:8(Suppl 4):

Background: Xanthan gum, a commercial microbial polysaccharide, has
many industrial applications, including the tertiary recovery of oil, due to its
unique rheological behavior (e.g. high viscosity at low concentrations,
pseudoplasticity, solubility, stability over a wide range of pH values and
temperatures, compatibility with many salts) [1]. Its production employs
sucrose or sucrose, which raises the price of xanthan production. One way to
reduce the cost is to use cheaper alternative substrates, like residues [2].
Biodiesel is included in this context because its chemical composition is
susceptible to oxidation, which decreases its capacity as a fuel, resulting in
the possibility of organic residue accumulation [3]. Thus, biodiesel conversion
into xanthan gum by a fermentation process is one alternative for reducing
costs, since the substrate is a critical aspect in its commercial production,
and also for minimizing possible environmental impacts. Accordingly, the goal
this study was to evaluate the effect of soybean biodiesel as an alternative
substrate for non-food grade xanthan gum biosynthesis.

Methods: Experiments were carried out in a medium containing 2% soybean
biodiesel as a carbon source, supplemented with 0.01% urea and
0.1% KH₂PO₄, using sucrose as control carbon source under the same
operational conditions. In order to obtain the polysaccharide, Xanthomonas
campestris pv. campestris 629 was inoculated in these culture media and
incubated at 28°C, 200 min⁻¹ for 96 h. All assays were performed in triplicate.
The recovered biopolymer was dried for analysis. The apparent viscosities of
the gum solutions from biodiesel and sucrose were measured in a
centrifugal rheometer coupled to a wash bath for temperature
control (25, 45, 65 and 85°C), with a shear rate of 25 to 100 s⁻¹. Rheological
data were measured according to the Ostwald-de-Waele model
(μ = K (γⁿ)⁻¹), using a regression analysis to ascertain the apparent viscosity
(K, n, R²) [4]. The xanthan FTIR analysis was obtained in the range 4.000-4.000 cm⁻¹
in KBr pellets at room temperature.

Results and conclusions: The results showed that soybean biodiesel as an
alternative carbon source supported xanthan production with a yield of
937.3 ± 0.2 mPas and flow rate (n) of 0.61 ± 0.01, and R² = 0.99. These values
were similar to those determined for sucrose, the usual carbon source
employed for xanthan gum production. The apparent viscosity of xanthan
gum from biodiesel presented a similar profile when compared to gum from
sucrose. Regarding the FTIR analysis, both gums showed a similar spectral
behavior, probably owing to the polymer structural proximities. The results
indicated that biodiesel is a potential and promising option as an alternative
substrate for the production of non-food grade xanthan, due to the
observed rheological properties indicated through to K and in values [1,5].
However, fermentation conditions need to be optimized in order to improve
yield in this polymer production.

Acknowledgements: CNPq and FAPITEC for financial support and
fellowship.

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Production of xanthan gum from soybean biodiesel: a preliminary
study
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BMC Proceedings 2014, 8(Suppl 4):P174

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Antimicrobial potential of Actinomycetes by NRP5 and PKS-1 pathways
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Background: Actinomycetes may account for 10 to 30% of the total soil rhizosphere microorganisms. The attention given to the actinomycetes in biotechnological applications is a result of their metabolic versatility that is accompanied by the production of primary and secondary metabolites of economic importance, which are a promising source of products (e.g., antibiotics, enzyme inhibitors, antiparasitic and anticancer agents) (1,2). Included in this range of compounds are secondary metabolites synthesized by polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) pathways. An effective method for assessing the presence of these biosynthetic pathways is the detection of PKS and NRPS genes by PCR (3,4). Thus, this study was based on targeted analyses of 31 soil isolate actinomycetes aiming to evaluate their antimicrobial potential through the NRPS and PKS-I pathways.

Methods: The antimicrobial activity was evaluated by the antagonism test against two economically important phytopathogens, the bacterium Xanthomonas campestris and the fungus Thielaviopsis paradoxa, using the technique of double layer. The X. campestris and T. paradoxa were propagated at 28°C in YM (yeast malt) pH 6.0 and PDB (potato dextrose broth) respectively. The results were statistically analyzed using the Bonferroni test.

The presence of genes PKS and NRPS was evaluated by PCR, using degenerate primers for highly conserved regions encoding enzymes associated with biosynthesis of polyketides and peptides.

Results and conclusions: According to the experimental results, 52% of the isolates showed antimicrobial activity against at least one of the target bacterial pathogens tested. Among these active isolates, some belong to rare families. Thus, this finding can be a source of novel biomolecules with antimicrobial activity. From those isolates that presented one of the NRPS and PKS-I genes, 75% of them showed antagonistic activity against one of the phytopathogens evaluated. Preliminary data on this screening demonstrate the importance of the biotechnological potential of these actinomycetes due to the antagonistic activity against plant pathogens of economic interest and the possibility of be used as biocontrol, besides offering a strong area for metabolic research (2,5).

Acknowledgements: CNPq and FAPITEC for financial support and fellowship, and Embrapa- Tabuleiros Costeiro, SE, Brazil (Laboratório de Biotecnologia e Biologia Molecular de Microorganismos do Solo for technical support.

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Background: Proteases are enzymes used in diversified process and industrial products, where the most important is the food sector and the detergent fabrication. The microbial obtention way is the most relevant, and the filamentous fungi are the main synthesis agents in industrial scale. [1]. The genre Coniothyrium, Verticillium, Penicillium e Aspergillus are reported as proteases producers. The species Aspergillus oryzae is considerate not toxicogenic and it is a huge proteases producer, so it has an elevated industrial interest [2,3]. The literature about this theme is vast, there are countless articles using the Aspergillus in semi-solid fermentative processes to proteases of obtention. Nevertheless, the importance of this work was demonstrated by the use of an alternative substrate to protease synthesis, the canola meal. The canola meal it is a byproduct of the extraction process of canola oil, showing high protein content. Nowadays, the industrial sector has encouraged researches that can contribute and adding value to this residue [3]. So, in this context, this work proposed to evaluate preliminarily the performance of five lines of Aspergillus oryzae in relation to the production of proteases in semi-solid fermentation using the canola meal as a substrate inductor.

Methods: Five NRRL strains of Aspergillus oryzae (designated 2220, 1911, 5590, 694 e 2217) were used in these experiments. For the solid state fermentation was used a medium of canola meal at proportion of 100g of bran to 40mL of water. The experiments were done in Erlenmeyers of 500 mL, each flask contained 40g of medium, previously autoclaved. The initial inoculation was of 10⁶ spores.g⁻¹ of fermentation medium. The fermentations were performed in statics conditions under temperature of 20°C during 96 hours. The proteolytic activity was determined each 24 hours using azocasein as substrate and trichloroacetic acid as precipitating agent. A unit of proteolytic activity was defined as a quantity of enzyme that produces a difference of 0.01 of absorbance per minute of reaction between the reactional blank and the samples in the experimental conditions. The results were expressed in U.g⁻¹ of fermentation medium [5]. The statistical analysis, through the Scoot-Knott test was performed.

Results and conclusions: Within 48 hours of fermentation, all extracts showed proteolytic activity, indicating that all strains were able to synthetise proteases. The highest levels of activity were reach at 72 hours of fermentation and no difference was observed after this period. It was observed that A. oryzae NRRL 2217 and NRRL 2220 were protease best producers. The proteases production profile of the canola bran was similar to that demonstrated by Thanapimmetha [4], with Jatropha curcas residue medium. This study shows the viability of using the canola meal to the synthesis of proteases by different strains of A. oryzae.

Acknowledgements: IFISULDEMINAS and UNAERP

References

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Influence of linolenic acid in the production of jasmonate
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Background: Research shows that jasmonic acid (JA) and Methyl Jasmonate (MJ), compounds that participate in the metabolism’s control, development and protection of plant processes [1], also possess anticancer activities [2]. In studies, researchers found that Jasmonate were able to inhibit the growth of human cancer cells, and MJ induced death in lung carcinoma cells [3], breast, prostate, melanoma, lymphoma and leukemic cells [4], without reaching normal lymphocytes. The predominant route of obtaining these compounds is the vegetal extraction, however it takes about 800 kg of flowers of Jasminum grandiflorum to produce 1 kg of jasmonate containing only 0.25% of AJ. However, the jasmonatos can also be produced by the microorganisms [5]. A microorganism which shows promising results in the production of Jasmonate is the fungus Botryosphaeria rhodina, making it possible to produce systems which can control the parameters involved in the fermentation process, including the use of precursors in this process.

Methods: Inventories of line Botryosphaeria rhodina Kfn 3.1 were inoculated in petri dishes containing PDA culture medium, serving later to fermentation. The homogenate was inoculated in a 250 ml Erlenmeyer flask with 50 ml of media M1, M2. The experiment was conducted using the methodology of 2² full factorial design, evaluating time ffluencia (7 and 14 days) and the addition of the precursor linolenic acid (0 and 1 μM). All experiments were performed in triplicate. After the period of each fermentation, the material was vacuum filtered and then subjected to extraction and analysis of the production of jasmonic Acid. The extraction was by liquid-liquid partition using as extracting agent as ethyl acetate. The detection of the AJ extract was made by TLC (thin-layer chromatography comparative) Samples AJ standards (Sigma) were used as reference. Quantifying jasmonates in extracts was performed by HPLC (High-performance liquid chromatography). The calculations of the factors influencing the time and addition of precursor and it's interaction were statistically analyzed by t-test at the 5% level of probability.

Results and conclusions: Through the analysis of the CCD fermentations with culture medium supplemented with linolenic acid was possible to verify that all samples had jasmonates. The addition of linoleic acid (1 μM) induced inhibitory effect on the production of jasmonatos, however, the increase in time from 7 to 14 days to produce positively influences AJ. Thus it was concluded that the conditions evaluated is not necessary to supplement the culture medium with linoleic acid and increased time of fermentation favors the production of AJ by fungus Botryosphaeriaphantherodina.

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Endo and exoglucanases produced by Penicillium citrinum isolated from Amazon
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Background: Cellulolytic enzymes (glucohydrolases - EC 3.2.1.1-) are biocatalysts highly specific. They act in synergy to hydrolyze β-1,4 bonds between monosaccharide units of D-glucose in the cellulose chain releasing its constituents. Cellulases are categorized according to the place they act in the cellulose fibril. Endoglucanases start hydrolysis, exoglucanases act in the reduced terminal produced by endoglucanases followed by β-glicosidase
which act in the product of exoglucanases catalysis releasing glucose monomers.

Fungi are considered the best cellulolytic enzyme producers due to its natural cellulases that complete saccharification of lignocellulose. Species of Penicillium have been reported as excellent producers of cellulolytic enzymes when compared to commercial species and strains [1]. Aiming to contribute to biocatalytic processes and obtaining of new sources for cellulolytic enzyme, this work has as objective the production of endoglucanases and exoglucanases from Penicillium citrinum isolated from an agro industrial residue in Amazon.

Methods: Sample was isolated from sugar-cane bagasse in agro industry of Amazon by the municipality of Presidente Figueiredo-AM-Brazil. 110 km from Manaus. Molecular identification was made by ITS-18S gene amplification, followed sequence analysis of using software Bioedit Sequence Alignment Editor® and BLAST from NCBI (BLASTn). For the production of cellulolytic enzymes fungi was cultivated during 192h in submerse fermentation, having carboxymethylcellulose (CMC) as carbon source [2]. Enzymatic assays were done in triplicate [3,4] incubating the culture supernatant in presence of substrates CMC for endoglucanases and avicel (microcrystalline cellulose) for exoglucanases. Total secreted protein was quantified by using BCA kit (Thermo Scientific®). Enzymatic activity was calculated according to international units IU (1 IU is equal to 1 μmol of released product) and, the values were presented in U/mg. As experimental control all the assays were also done with a commercial strain of Trichoderma reesei (QM 9414).

Results and conclusions: Fungi specie was confirmed as Penicillium citrinum, having 100% identity and similarity in BLASTn alignment. Regarding to endoglucanase production was observed two peaks, one with 72 h and with 144 h of cultivation, and enzyme activity of 9.67 U/mg and 8.32 U/mg, respectively. This results were comparable to those obtained for T. reesei (QM 9414), which was observed activity equal to 12.50 and 14.37 U/mg respectively. Exoglucanase activity was higher in P. citrinum (0.64 U/mg, 96h) than that in T. reesei (0.43 U/mg, 168h), as indicated by Singh and co-authors [5]. Production profile of endoglucanases and exoglucanases of P. citrinum showed to be highly synergic since the exo activity increases gradually and its peak is related to endo activity decay. Therefore P. citrinum presents a good potential as alternative to cellobiolytic enzyme production.

Acknowledgements: This study was funded by CNPq, CAPES, FAPEAM and Biotech Amazônia LTDA-ME

References

P180
Increase in bioethanol production from used office paper by Saccharomyces cerevisiae UFPEDA 1238
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BMC Proceedings 2014, 8(Suppl 4):P180

Background: Used paper is particularly attractive as feedstock for bioethanol production because it is readily available [1]. The aim this work was to hydrolyze used office paper with dilute sulfuric acid for bioethanol production by three industrial strains of Saccharomyces cerevisiae. Acid concentration, time of the hydrolyze and ratio solid:liquid were varying according to factorial design. Reaction time was not significant. Maximum total reducing sugar content was obtained with 5 % V/V acid concentration and 1:10 ratio solid:liquid. Higher ethanol production was obtained by UFPEDA 1238 in 24 h. Increase 7 to 47 % was obtained in ethanol production, when S. cerevisiae UFPEDA 1238 was used in relation to other two strains.

Methodology: Used office paper of Department of Antibiotics from Federal University of Pernambuco, Brazil, was hydrolyzed with dilute sulfuric acid at 120 °C and 1 atm (autoclave). The acid hydrolyzate was filtered through qualitative paper and used in the preparation of fermentation medium, after detoxification at room temperature by mixing with NaOH (4.5 pH). Acid concentration (1, 3 and 5 % V/V), time of the hydrolyze (60, 90 and 120 minutes) and ratio solid:liquid (1:50, 3:50 and 1:10) were varying according to factorial design. Three industrial strains of Saccharomyces cerevisiae (UFPEDA 1238, UFPEDA 1326 e UFPEDA 1337), were used in fermentations carried out at 34 °C and 80 rpm. Samples were used for the determination of sugars, organic acids, furanic compounds and ethanol by high performance liquid chromatography on an Aminex HPX-87H™ column at 60 °C, 5 mM H₂SO₄, 0.6 mL/min and RI-detector [2]. The total reducing sugars (TRS) content of the acid hydrolyzed were measured using the 3, 5-dinitrosalicyclic acid reagent method [3].

Results and conclusions: Increase in acid concentration and mass of paper increased the TRS. However, the reaction time was not significant. Maximum TRS content (28.40 g/L) was obtained with 5 % V/V acid concentration and 1:10 ratio solid-liquid. Starting this result hydrolyses at 10 % V/V acid sulfuric, with 10 g and 1 h in was carried out. Lower content of furfural and 5-hydroxymethyl furfural (HMF) were observed with 5 % V/V (A hydrolysate) than to 10 % V/V (B hydrolysate). In the acid hydrolyze, cellulose is hydrolyzed to glucose and hemicellulose is degraded to pentose and hexose. At high temperature and pressure, xylose and glucose are further degraded to furfural and HMF, respectively [4], which are inhibitors of the fermentation. Fermentations with A hydrolysate (5 % V/V, 0.9 M) were performed, using three strains of S. cerevisiae. Glucose was completely consumed in 24 h. Strain UFPEDA 1326 presented higher growth. On the other hand, higher ethanol production (1600 mg/L) was obtained by UFPEDA 1238 in 24 h. This study showed that ethanol production from used office paper is possible without the addition of cellulase enzyme.

Acknowledgements: The authors acknowledge the financial support from Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brasilia DF, Brazil (CNPq).

References

P181
Efficient maltofritose fermentation through hydrolysis mediated by the intracellular invertase of Saccharomyces cerevisiae
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BMC Proceedings 2014, 8(Suppl 4):P181

Background: It is well known that in the yeast S. cerevisiae the sugars sucrose and maltose/maltotriose are metabolized by different pathways: sucrose is hydrolyzed by extracellular invertase (encoded by SUC genes), while maltose and maltotriose are actively transported into the cell and hydrolyzed by intracellular α-glucosidases (both proteins encoded by the MAL genes). Nevertheless, several reports have shown that some SUC genes can be located proximal to MAL genes at the telomers of different chromosomes. Furthermore, the SUC genes also allow the synthesis of an intracellular form of invertase, an enzyme with no obvious function in yeasts [1]. We have already shown that sucrose can be metabolized by yeast cells through MAL-encoded transporters and α-glucosidases.

Methods, results and conclusions: Now, our results will show that maltotriose can be efficiently fermented by S. cerevisiae cells through its active transport mediated by the AGT1 permease, a MAL transporter required
for maltotriose utilization [2,3], and its intracellular hydrolysis mediated by the cytoplasmic invertase. The Brazilian industrial fuel-ethanol strain CAT-1 cannot ferment maltotriose efficiently due to a defective promoter of the AGT1 gene [4]. To increase maltotriose fermentation by this strain, we placed a strong promoter ($P_{AGT1}$) in the AGT1 gene of strain CAT-1, generating strain GMY05. While the AGT1 gene was indeed over-expressed in this strain (measured by real-time PCR and a specific transport assay), maltotriose was still not fermented efficiently. However, when we over-expressed the intracellular form of invertase, by replacing the signal sequence of the $SUC2$ gene with the strong $P_{AGT1}$ promoter, the resulting ISU2C strain GMY08 fermented maltotriose efficiently. Using conditions were the MAL-encoded α-glucosidase. To be expressed, we could show that the intracellular form of invertase hydrolyzes maltotriose efficiently (but not maltose or p-nitrophenyl-α-glucoside), specially at the cytoplasmic pH of 7.0. Under the same conditions we purified the intracellular invertase by ion-exchange chromatography, and the identity of the enzyme confirmed by mass spectrophotometry. With the purified enzyme we performed enzymatic tests that corroborated our previous analysis, showing that intracellular invertase hydrolyzes maltotriose. Thus, our results indicate an unexpected overlap in sucrose-maltotriose metabolism by yeast cells, showing that the intracellular invertase allows efficient maltotriose hydrolysis, and offers new approaches that can be applied to optimize several industrial fermentation processes that use starch hydrolysates, including production of distilled beverages, brewing and baking.

Acknowledgements: This work was funded by the Brazilian agencies CAPES, CNPq, and FINEP.

References


P182

Comparative study of microbial community from mining wastes - focus on future recovery of copper

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BMC Proceedings 2014, 8(Suppl 4):P182

Background: The interaction between microorganisms and heavy metals has been occurring since the beginning of life on the planet, 4 billion years ago, which allowed the biological evolution of resistance in systems and the survival of these microorganisms in environments containing high metals concentrations of metals [1]. Recently, a bioremediation review presented by Perpetuo et al. [2] considers the bioremediation technique as a feasible alternative for treatment and recovery of sites contaminated by heavy metals. However, a previous study of the microbial community living in these areas is necessary to isolate organisms that are able to tolerate and metabolize heavy metals (copper, zinc, cadmium and chromium). Like in this study, Castro-Silva [4] also related strains of Bacillus resistant to heavy metals, but in a copper mine located in Santa Catarina, Brazil; and Burkholderia sp. has been described in the literature as symbions of plants for the bioaccumulation of heavy metals [5]. Once we have verified cellular growth in the presence of significant metal concentrations and high capacity for metal biosorption under aerobic conditions, these bacteria can potentially be applied to in situ bioremediation of aqueous systems contaminated by heavy metals, also allowing for the recovery of these metals.

Conclusion: This work has great importance due to the low cost of systems treatment compared to conventional ones, allowing a better use of copper wastes and consequently better mining economic return. Moreover, the main advantage is further reduction of environmental impact caused by the mining activity.

Acknowledgements: Fapesp.

References


P183

Periplasmic α-carboxylic anhydrase plays an essential role in Ralstonia eutropha CO2 metabolism

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BMC Proceedings 2014, 8(Suppl 4):P183

Background: Carbonic anhydrase (CA) enzymes catalyze the interconversion of CO2 and bicarbonate. These enzymes play important roles in cellular
metabolism such as CO₂ transport, ion transport, and internal pH regulation. Understanding the roles of CAs in the chemolithotrophic betaproteobacteria *Ralstonia eutropha* is important for the development of fermentation processes based on the bacterium's capacity for carbon fixation using the Calvin-Benson-Bassham cycle. Of the five classes of CA, the alpha-CA is the best-characterized thus far. The gene encoding a periplasmic alpha-CA (caa, H16 82403) has been identified in the *R. eutropha* H16 genome, along with three others CA from different classes. In this study, we evaluated the importance of Caa in the metabolism of *R. eutropha* by examination of CA activity and growth in caa gene deletion, complementation, and overexpression strains. Localization of Caa in the cell was accessed by fluorescent microscopy.

**Methods:** *Ralstonia eutropha* (H16) strains were propagated in TSB or mineral medium [1]. Standard protocols were employed for plasmid and strain construction [1,2]. Purification of tagged-proteins was performed following manufacturer's instructions.

The CA activity assays were based on protocols from Sundaram et al. [3] and Fasseas et al. [4] with some modifications. Cells were observed under 100X magnification using a Nikon Labophot-2 microscope with phase-contrast and fluorescence attachments.

**Results and conclusions:** Purified Caa was found capable of performing the interconversion of CO₂ and HCO₃⁻ with equilibrium lying towards HCO₃⁻ formation. The activities detected were 83.6 ± 14.4 EU/mg protein and 422.2 ± 97.1 EU/mg protein using bicarbonate and CO₂ as substrates respectively. Deletion of caa (strain Re2428 (H16 acad)) resulted in poor growth in all conditions tested, even with addition of CO₂ to culture. In an attempt to recover *R. eutropha* growth and to determine the effect of Caa localization on cell growth the annotated gene with (caa) and without (caab) the nucleotide sequence encoding the 23-aa N-terminal predicted signal-peptide were amplified, cloned separately into expression vectors and reintroduced in *trans* into Re2428. Overall, reintroduction of caa or caab in Re2428 was able to recover only partial growth when compared to the wild type. However, the strain Re2428/ pCaa presented better growth when compared to Re2428/CAab in mineral media. This could be an effect of the different localization of Caa in the cell. Caa deletion and mislocalization adversely affected cell growth by causing damage to metabolism. The periplasmic localization of Caa was confirmed by microscopy using the red fluorescent protein (RFP)-tagged enzyme.

We demonstrated the importance of Caa for the transport of CO₂ and the supply of bicarbonate to *R. eutropha* cells, as its correct localization and balanced expression in the cell are essential for *R. eutropha* during growth. The localization of Caa in the periplasm of the cell is crucial for the conversion of CO₂ to bicarbonate since Caa is probably responsible for rescuing the CO₂ which diffuses into the cell and converting it to bicarbonate then transporting it across the cell membrane back to central metabolism.

**Acknowledgements:** This work is funded by the US Department of Energy (ARPA-E).

**References:**

**P184**

**Synthesis, purification, characterization, and antinociceptive activity of opioids peptides**

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**BMC Proceedings 2014, 8(Suppl 4):P184**

**Background:** Bioactive peptides prospection is important for biotechnology field as well as a starting point in many research areas, such as: new drugs development and production of genetically modified plants [1]. In general, bioactive peptides have been identified as candidates for the new drugs development because their intrinsic properties concerning some potential activities, like high specificity, potency, less toxicity, and also chemistry and biological diversity [2]. Peptides can present diverse activities such as antimicrobial, opioids, hypotensive, antithrombotic among others. In 2010, around 60 synthetic peptides with therapeutic potential have been available to pharmaceutical marketing and can be used in many pathologies like, allergies, asthma, arthritis, cardiovascular diseases, diabetes, gastrointestinal dysfunction, growth problems, inflammation, obesity, infectious diseases, cancer, osteoporosis, pain, vaccines and others. [3,4]. For pharmaceutical industry, in therapeutic area, the most promising projects are those that focus on cancer, pain, diabetes, Alzheimer’s disease and depression [5]. This study aims to design sequences of synthetic peptides that may have dual activity: antinociceptive and hypotensive. These peptides act on different targets, opioids receptors and angiotensin converting enzyme (ACE), respectively.

**Methods:** Peptides primary structure prediction was based on previous work of our group, which focused on prospection and bioactive peptides characterization. These studies were based on the knowledge available on the literature about the biological properties of specific protein domains, molecular targets and drug actions. The peptides were synthesized by solid-phase chemical synthesis using Fmoc strategy followed by purification on high performance liquid chromatography. Purity and confirmation of the primary structure were determined by mass spectrometry, MALDI and ESI. The peptides were tested in mice (6-8 per group) intraperitoneally, equimolar to morphine (positive control) in order to assess its possible antinociceptive activity through hot plate and tail flick assays.

**Results and conclusions:** It was possible to evaluate the antinociceptive activity of these two designed peptides, which present unpublished sequences. Both exhibited pharmacological activity in vivo, which may be denominated opioid receptor agonists, because they promoted antinociception in mice when exposed to thermal stimulation. In naloxone presence, there was no activity, so it can be concluded that the peptides interact with opioid receptors. In vivo tests showed that synthetic peptides have delayed response, longer activity compared to morphine and may have cross the blood-brain barrier. Thus, it is interesting continue studying therapeutic potential of these peptides to contribute with development for new drug candidates.

**References:**
Anaerobic fermentation processes are economically attractive for industry, as costs for aeration and stirring are greatly reduced. An example of such a process is (bio)ethanol production by Saccharomyces cerevisiae, in which a low but positive ATP gain leads to elevated product yields. However, for other fermentative pathways, such as in an engineered homolactic strain of S. cerevisiae, the net ATP gain for the fermentation of glucose to lactate is null due to the requirement of ATP for product export. Therefore, increasing the conservation of ATP is of major importance for such ‘zero-ATP pathways’. One opportunity to increase ATP conservation arises when disaccharides are used as a substrate. In many industrial microorganisms, disaccharides are cleaved by hydrolysis, which results in the dissipation of energy that is available in this cleaving reaction. However, phosphorolytic cleavage could be used to replace the hydrolysis of disaccharides, thereby increasing the ATP yield.

In this study, growth of S. cerevisiae on maltose was used as a model. All known native maltose metabolism genes were removed and replaced by a maltose phosphorylase (Lactobacillus sanfranciscensis) and a single overexpressed copy of the native MAL11 maltose transporter. Because maltose phosphorylase cleaves maltose into glucose and β-glucose-1-phosphate, additionally a β-phosphoglucomutase (Lactococcus lactis) was co-expressed in this strain.

Anaerobic maltose-limited chemostat cultures showed that replacement of maltose hydrolysis by phosphorylase increased the biomass yield of the mutant strain by 26% over the wild type strain, demonstrating the potential of phosphorylase to improve ATP conservation of disaccharide metabolism in industrial microorganisms [1].

Reference

P186 Production of recombinant 503 antigen of Leishmania infantum chagasi using cultivation in batch and fed-batch
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Background: Visceral leishmaniasis is an infecto-parasitaria disease caused by obligatory intracellular protozoa belonging to the genus Leishmania, and might be lethal since there is no precocious diagnosis and proper treatment. Despite the considerable effort, there is no effective and safe vaccine for human use [1]. Genetically modified micro-organisms are used industrially in general for production of hormone, antibiotics and proteins. In the production of heterologous molecules, both the expression and the production step are important [2]. Therefore, the study of cultivation conditions in bioreactor plays an important role for the process viability of batch and fed-batch [3]. Thus, the aim of this work was to study strategies for production of the 503 antigen of Leishmania infantum chagasi using cultivation in batch and fed-batch.

Methods: The strain of E. coli expressing 503 antigen of Leishmania infantum chagasi was kindly provided by Dr. Mary Wilson (University of Iowa, USA) [1]. The clone was cultured in 2xYT medium supplemented with ampicillin and kanamycin. The cultivation were carried out using a bench bioreactor with a working volume of 1.5 L at frequency of agitation of 200 rpm and constant output aeration of 1vvm. The expression of the recombinant protein was induced by the addition of lactose to the cultivation medium at the final concentration of 10 g/L [4]. Optimization of cultivation conditions for 503 antigen was performed in batch and fed-batch. Then, assays of the fractions were performed by Lowry method and electrophoresis. Biomass concentration was monitored by the dry weight and the acetic acid concentration was assayed by high-performance liquid chromatography.

Results and conclusions: Fed-batch culture is one of the most performed strategies to reach high cell densities of E.coli and consequently high recombinant protein productivities. It was observed that to the cultivation in fed-batch, the agitation of 400 rpm resulted increased the biomass of 2.5 g/L to 11 g/L. It was observed the same behavior for the protein using both batch and fed-batch (0.11 g/L). In the present study in the both processes it was not observed the inhibitory effect in the cellular growth as well as on the 503 protein expression. The highest acetic acid concentration was obtained at the agitation speed of 400 rpm (0.2 g/L) that occurred during the first two hours of cultivation. However, this concentration was inferior to 0.9 g/L that according to [5] have no inhibitory effect in the growth.

Acknowledgements: The authors thank the CNPq (Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico) for the financial support and Dr. Mary E. Wilson (University of Iowa, USA) for providing the microorganism strain.

References

P187 Biosynthesis of xanthan gum from residual glycerin from biodiesel production for drilling fluids
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Background: Several reports have focused on the value and biological transformation of industrial wastes as an alternative substrate for the production of high value-added components. In this context, it can cite the crude residual glycerin obtained as the primary byproduct of biodiesel production, which has increased exponentially during the last years. As an abundant residue, its disposal into the environment entails several drawbacks and health risks [1]. The bioconversion of this by-product into added-value products by fermentation processes is an important alternative to overcoming this environmental issue. Glycerin is an attractive feedstock for the production of useful chemicals [2], since the cost of the fermentation medium represents, for example, a critical aspect of the commercial production of xanthan, which is the most important microbial polysaccharide with widespread commercial applications (e.g. foodstuffs, pharmaceutical, agricultural products, petrochemical industries) [3], due to its rheological properties and its capacity to produce viscous solutions at low concentrations, together with other characteristics like its pseudoplastic property. This study investigates the effect of glycerin as alternative substrate for xanthan production by Xanthomonas campestris, by evaluating its operational production conditions as well as their physicochemical properties, aiming at its application as drilling fluid.

Methods: Assays were carried out in 250 mL shake flask cultures with 80 mL of medium containing 2% crude glycerin as an alternative substrate, supplemented with 0.01% urea and 0.1% KH2PO4, compared with sucrose as control under the same operational conditions. In order to obtain the xanthan, the X. campestris mangiferaeindicae 2103 was incubated at 28°C in a rotary shaker at 250 min for 120h. Samples were withdrawn at regular
intervals and analyzed for concentrations of biomass, xanthan, residual substrate. The molecular weight of xanthan was estimated by size-exclusion chromatography. Its viscosity for drilling fluid was determined according to the Petrobrás N-2604 standard [4]. Rheological data were fitted to the Ostwald-de-Waele model: \( \mu = K \cdot n^\lambda \), using a regression analysis to ascertain the apparent viscosity (\( K \) and \( n \)).

**Results and conclusions:** The experimental results showed that glycercin supported xanthan production with a yield of 7.23 g/L, approximately 72% higher than that obtained using sucrose as carbon source. This biopolymer exhibited a consistency index (\( K \)) of 6342.6 ± 0.08 mPa.s and flow rate (\( n \)) of 0.2066 ± 0.01, according with the parameters established by the Petrobrás N-2604 standard [4], with a minimum \( K \) of 1500 mPa.s\(^2\) and maximum \( n \) of 0.5. These values were 70% and 30% higher, respectively, compared to gum from sucrose fermentation. Its molecular weight varied from 28.2 to 36.2 \( \times 10^5 \) Da, analogous value to those obtained from sucrose. Therefore, the results indicated that crude glycercin has the potential to be a cost-effective and promising alternative source of carbon for the production of non-food grade xanthan, whose rheological properties show a promising alternative use as a drilling fluid to enhance oil recovery [2,5], due to \( K \) and \( n \) satisfactory values.

**Acknowledgements:** FAPESB and CNPq for financial support and fellowship, and Laboratory of Drilling Fluid, Carboflex Company for drilling fluids rheology tests.

**References**


**P188**

**Bioremediation potential of Pseudomonas aeruginosa and Enterobacter cloacae isolated from a copper-contaminated area**

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**Background:** The Sossego mine, located in Canaã dos Carajás, Pará, Brazil, has a pond of wastes with low copper concentrations economically unfeasible for extraction. In this place, we can improve environmental conditions and, at the same time, recover part of the ore diluted in these wastes, through evaluation and use of the local biodiversity, in bioremediation processes, once this the technology, will allow the decontamination as well the recovery of these metals with high value. Once there are some restrictions on the microorganisms introduction in the environment, it is important to establish the bioremediation potential of native species from a particular location. Therefore, it is necessary to study the biodegradation processes or biotransformation of compounds in the microbial biodiversity already adapted that are responsible for these processes in the environment, first in a bench scale[1].

In this work, among the 22 strains isolated from environmental samples from a copper mine, two of them presented great potential for bioremediation. Strains were identified and both were subjected to comparative study of their bioremediation potential and showed good results in concentrations up to 320ppm of copper.

**Methods:** Strains were identified by mass spectrometry (MALDI-TOF- Biotyper). Both strains were grown in 150mL DYP medium supplemented with 8g/L of casamino acids and in parallel with the same medium, added of 160 and 320ppm of copper in flasks of 500mL incubated in an orbital shaker at 28 °C and 200 rpm. Samples were taken along 24h (one in one hour) and monitored for cellular growth and copper biosorption by spectrophotometry UV-Vi (600 nm) and atomic absorption spectroscopy (AA), respectively. As a control of copper adsorbed, samples were taken at 0h and 24h and analyzed also by AA. At the end of exponential phase of cellular growth, the biomass was utilized to construct a correlation curve between absorbance and dry mass of the cells.

**Results and conclusion:** Metals removal using micro-organisms selected has become very promising, since they can exhibit high selectivity and removal rate, and also has advantage of having the potential to regenerate biomass, allowing reuse in the further steps, after the metal recovery [2-4]. In this work, most efficient strains for bioremediation of effluents contaminated by copper were identified by mass spectrometry as Pseudomonas aeruginosa and Enterobacter cloacae presenting a high score for species identification (2,43 and 2,24 respectively). P. aeruginosa showed high tolerance to concentrations up to 160ppm of copper and E. cloacae up to 320ppm of copper. Considering the current conditions of the Sossego mine, P. aeruginosa and E. cloacae could be very efficient in the reusing process of copper available in the pond. Moreover, the strains isolated in this study showed better results than those described in the literature[1]. P. aeruginosa was able to remove 30% of copper from medium containing 160ppm of copper compared with 23% of removal described by Sethuraman & Kumar[S], and E. cloacae adsorbed 50% of 320 ppm of copper, compared to 20% in the same study[S].

**Acknowledgements:** CEPEMA-POLI-USP, University of São Paulo, Chemical Engineer Department, PQI-POLI-USP.

**References**


**P189**

**Starting study of microbial genetics diversity in soils of mangrove preserved in sergipe**

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**Background:** Tropical mangroves are considered one of the most productive ecosystems in the world, characterized by high rate of cycling of organic matter and nutrients that occurs between the oceans and terrestrial environments. This paper proposes the characterization and evaluation of structures and diversity of microbial communities in the soil of mangroves in three conservation areas located in Sergipe. The samples were analyzed by the extraction, purification and analysis molecular. Soil samples were collected in PVC tubes 50 cm long and 50 mm in diameter, placed vertically in the sediment of three different regions of the state of Sergipe, in triplicate. For the extraction of total DNA from soil was used Power Soil DNA Kit, an aliquot of 5 μL of the extracted DNA was subjected to electrophoresis on agarose gel 1 % (w/v) stained with ethidium bromide (0.5 mg/ml gel) in TAE buffer. Was used as standard molecular 2 μL of Low Mass DNA Ladder. The gel was subjected to an electric field of 80 V for approximately 30 min and then photo-documented. The extracted DNA was also quantified by spectrophotometry. For purification of PCR products was used Kit GFXTM PCR DNA and Gel Band Purification by following the manufacturer’s directions and visualized on agarose gels and subjected to an electric field of 80 V for approximately 30 min. With tags generated by using a primer
specific 16S constructed a data matrix and calculated the diversity of the samples, using the Jaccard coefficient and UPGMA. The results were generated from the software FreeTree by which a dendrogram was obtained. The genotypes were grouped into three groups among the samples, group I: genotype accesses of the region 1, group II: genotype accesses of region 2, and group III: visualize genotype accesses of the region 3, checking the genetic distance between accesses collected from the regions. The high degree of polymorphism possessed among samples represents heterozygosity for this marker, which can contribute to infer genetic relationships. Such diversity will in comparison studies of the diversity present in genomic databases. Thus, we conclude that there is diversity in the microorganisms of the studied samples. The 16S rRNA gene has been used extensively in phylogenetic inference of microorganisms, however, because it is an initial study is still in the process of identification the total sequences of mangroves to continue with studies analyzing sequences isolated. In an attempt to attribute function to these genes and then use the potential of lineages. Observing this, we realize that the genotypes showed diversity among the samples collected. Such diversity will in comparison studies of the diversity present in genomic databases.

Thus, we conclude that there is diversity among the genotypes of the studied samples. These results allow to conclude that the mangrove has exclusive characteristics, besides contributing to initial information on the study, to select genotypes that may be possible potential in bioremediation and other biotechnological products and industrial to be explored from the knowledge that diversity.

References
loading was studied in the range of 0.0406-0.65 mg HRP / g alginate. Alginic microphases were obtained by dripping in calcium chloride solution (1M) according to literature [1]. The HRTP immobilization in the presence of ISs was conducted using the enzyme loading more appropriate, incorporating in the immobilization medium 1% (w/v) of IL. The ILs studied as additives were: [C4mim]TF$_2$N; [C4mim]BF$_4$; [C4mim]HSO$_4$; [C4mim]Ac e [C4mim]PF$_6$. The activity of free and immobilized enzyme was verified by colorimetric method based on the change of absorbance at 470 nm due to the formation of product in guaiacol oxidation for three minutes. The results obtained for biocatalysts immobilized by encapsulation suggest that the use of IL as an additive in the immobilization process causes a significant increase in the total activity recovery yield (RA) HRP (increase from 5.2 to 23.5 %). The results for the HRTP immobilization showed that RA was 23.5% and 20.40% for the LI$s$ [C4mim]HSO$_4$ and [C4mim]TF$_2$N, respectively.

[C4mim]Ac provided RA of 13.3 %, while the biocatalyst with [C4mim]PF$_6$ showed RA of 17.3 %. The [C4mim]BF$_4$ showed the lowest performance as an additive among the ILs studied. According to Diego et al.(2009) [2] there is not a rule to predict the behavior of immobilized enzymes in the presence of the. The results obtained for biocatalysts immobilized by encapsulation in alginate microphases show that the use of ionic liquids in this process has a positive effect on the activity of the immobilized biocatalyst. Additional studies in the structural characteristics of the immobilized biocatalyst are being conducted to evaluate the influence of the ILs in the supporting structure.

References

#### P192

**Microbial biodegradation of landfill leachates located in São Paulo state, Brazil**

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**Background:** Leachate is one of the most problems present in landfills, mainly due to large production and the ability to cause serious environmental damages next to these areas. An activated sludge process is commonly used for biodegrading organic contaminants in wastewater using a mixed population of microorganisms. This technology is consolidated for wastewater treatment, and recently has been used as the first step in leachate treatment process. In this work, initially, physical-chemical and biological parameters of the leachate from different landfills (Areats and Bandeirantes, both in SP), were evaluated. Also the microbial consortium present in the sludge from a wastewater plant of the Capuava Petrobras Refinery, (SP) was characterized. Purpose of this work was to study the kinetic properties of the activated sludge process by removal of the pollutant organic matter, represented by the decrease of Total Organic Carbon (TOC). In order to verify the bioremediation efficiency, experiments were developed comparing the leachates before and after biological treatments. Results show that combination of leachate and sludge can decrease the organic load significantly, demonstrating bioremediation efficiency.

**Methods:** Bacterial selection was performed by their potential to degrade the leachate and were isolated and identified by growth on selective medium at 37°C. Cellular growth evaluation was performed after 24 hours of incubation and after this time were submitted to identification by mass spectrometry. Analyzed parameters of leachates were: quantification of metals, assessing an inductively coupled plasma optical emission spectrometry (ICP-OES), ammonical nitrogen, phenol, BOD and COD. Biodegradation experiments were performed in flasks incubated at 37°C in a orbital shaker (200 rpm) following the conditions: 100% (containing 120 ml of leachate and 30 ml of sludge), 50% (containing 75 ml of leachate, 30 ml of sludge and 45 ml of minimal mineral medium) and control containing 120 ml of minimal mineral medium and 30 ml of sludge.

#### P193

**Acid stress response in Saccharomyces cerevisiae involves ion homeostasis and calcium signaling pathway**

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**Background:** Leachates present in landfills are a major environmental concern. Microbial biodegradation is an alternative for a primary treatment, but for this is important to keep the test conditions favorable for the growth of microorganisms [3].

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**Methods:**

**Results and conclusions:** The identification of isolated bacterial strains was performed by mass spectrometry (MALDI-TOF). This technique produces a spectrum of bacterial proteins which are analyzed by a software, comparing the profile of each database, confirming usual molecular identification. Isolated strains were identified as *Pseudomonas aeruginosa*, *Micrococcus luteus* and *Enterobacter cloacae*, being all them common microorganisms present in the sludge process[1]. The leachate characterization from Areats landfill showed lower concentrations of contaminants than the Bandeirantes landfill. Main parameters analyzed were: the difference in color, quantity of phenol, Chemical Oxygen Demand, Biological Oxygen Demand and presence of metals. Explanation for this may be due to its location; time of operation; composition of received wastes, among others. However, biodegradation assays showed that the isolated strains were able to degrade significantly both leachates, being an effective alternative for a primary treatment, but for this is important to keep the test conditions favorable for the growth of microorganisms [3].

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**Acknowledgement:** This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Petrobras Petrochemicals. Portions of this content are taken from the following publications: Diego T, Lozano P, Abad MA, Steffansky K, Vautierb M, Ibora JL. On the nature of ionic liquids and their effects on lipases that catalyze ester synthesis. Journal of Biotechnology 2009, 140:234-241.
Production, recovery, and purification of recombinant 503 antigen of Leishmania infantum chagasi using expanded bed adsorption chromatography

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BMC Proceedings 2014, 8(Suppl 4):P194

Background: Visceral leishmaniasis, a disease caused by Leishmania infantum chagasi, represents a major public health problem in many areas of the world. Despite the considerable effort, there is no effective and safe vaccine for human use [1]. Some authors have reported that as much as 50% of overall costs in the biotechnology industries are related to downstream processing. Thus, the development of new and economically advantageous purification methods is a challenge [2]. Expanded bed adsorption (EBA) is an innovative chromatography technology that allows the adsorption of target proteins directly from unclarified feedstock. EBA technology combines solid-liquid separation with an adsorption step in a single-unit operation, aiming at increased overall yield, reduced operational time, and less capital investment and consumables [3,4]. Thus, the aim of this work was to purify the 503 antigen of Leishmania infantum chagasi directly from crude feedstock using EBA chromatography.

Methods: The strain of E. coli expressing 503 antigen of Leishmania infantum chagasi was kindly provided by Dr. Mary Wilson (University of Iowa, USA) [1]. The clone was cultured in 2xTY medium supplemented with antibiotics [5]. The cultivations were carried out using a bench bioreactor with a working volume of 1.5 L, at frequency of agitation of 400 rpm and constant output aeration of 1vvm. The expression of the recombinant protein was induced by the addition of lactose 10 g/L. Optimization of adsorption and elution conditions of 503 antigen was performed in batch mode according to two central composite designs. Then, EBA using Streamline Chelating was employed to purify 503 antigen from unclarified bacterial homogenate with a glass column (30.0 cm × 2.6 cm ID) and an adjustable piston, in order to minimize headspace over the fluidized bed. Analysis of the fractions was performed by Lowry method and electrophoresis on 15% polyacrylamide gels under denaturing conditions. The gels were photographed to estimate protein production, using the software ImageJ.

Results and conclusions: The batch adsorption experiment with Streamline Chelating showed that the optimal binding condition of 503 antigen was pH 8.0 in the presence of 1.625M NaCl. The optimal elution condition for the elution of protein of interest from the adsorbent was in the presence of 600mM imidazole. The adsorption isothermal data of 503 antigen onto Streamline Chelating showed that the data obeyed the Langmuir adsorption isotherm. The EBA assays showed that bed height increased linearly with the linear flow velocity. The fraction recovered after the elution contained 25% of the initial amount of 503 antigen. In conclusion, EBA has been applied successfully to purify the 503 antigen from an E. coli homogenate. The EBA mode combined clarification, capture, and purification of the interesting protein in a single step process, thereby giving rise to a good product recovery.

Acknowledgements: The authors thank the CNPq and CAPES for the financial support.

References

Biomediation is the process in which are employed organisms, plants or microorganisms for removal or reduction of pollutants into the environment, either on the ground or water. The biological process of biomediation is ecologically more suitable and effective for treatment of contaminated environments contaminated with organic and recalcitrant molecules and toxic metals. Recalcitrant molecules which pollute the environment are generally called xenobiotic are the result of anthropic action, as they are synthetic chemical compounds produced industrially by humans and include plastics, solvents, lubricants, detergents, pesticides explosive, comprising thus a large number of molecules of different applications, potential toxicity, and remain in the environment. When these elements are in the natural ecosystem in large quantities, exhibit toxic effects or other undesirable effects by organisms [1,2]. Atrazine pertecente the class of s-triazines is considered a xenobiotic being widely used in agriculture as a herbicide and thus widely detected in groundwater as far surface [3]. The key enzyme for the initial biodegradation of this compound is the atrazine chlorohydrolase (AtzA), performs an action of hydrolytic removal of the ring of the compound [3]. Currently, through the use of bioinformatics tools can be studied in depth the composition and organization of microorganisms and their genetic variations. The NCBI database provides many information about the organisms, such as sequences of specific genes or entire genomes of organisms [1]. This study aimed to evaluate the variations in the gene that codes for the enzyme atrazine chlorohydrolase in 10 bacterial species through the construction of a phylogenetic tree. Through observing the results could be verified that among analyzed bacteria only Rhodococcus corallinus emerges from a different ancestral in the phylogenetic tree and the remainder strains analyzed constitute a group rate descendant of a single common ancestral, with slight variation in the gene encoding the enzyme atrazine chlorohydrolase.
Introduction: Medicinal plants have been used since ancient times as medicines for the treatment of a wide range of diseases. Morinda citrifolia L (Noni) has been used in folk remedies by Polynesians for over 2000 years, and is composed of a broad range of bioactive compounds, including effects against headache, fever, arthritis, gingivitis, respiratory disorders, infections, tuberculosis and diabetes, it is commonly used as herbal medicines and cosmetics.

Objectives: This study aimed to map the patent applications, analyzing the potential and the evolution of technological capabilities through the translated patent applications with regard to the Morinda citrifolia.

Methods: Prospecting was carried out in the databases of the European Patent Office (Espacenet/EP), World Intellectual Property Organization (WIPO), United States Patent and Trademark Office (USPTO) and the Institut National de Propriedade Intelectual (INPI) of the Brazil. The keywords used was Morinda citrifolia, in addition to the substantives skin, cosmetic, antioxidant and dermatological. The research fields filled with the keywords were "title" and "abstract", and subsequently the research was restricted application the field "international patent classification (IPC)" with the codes A61K (regarding preparations for medical, dental or hygiene purposes) and A61Q (regarding the specific use of cosmetics or similar preparations for personal hygiene).

Results: Using the keyword Morinda citrifolia, 391 patent applications were found, 9 (2.3%) in the PTO, 310 (79.28%) in the Espacenet, 23 (5.88%) in the USPTO and 49 (12.53 %) in the WIPO. The selection of patents was restricted combining the words skin (total of 28 applications, 24 of them found in the Espacenet, 01 in the INPI, and 03 in the WIPO); cosmetic (15 applications in the Espacenet), antioxidant (total of 05 applications, 05 in the INPI, 01 in the Espacenet and 02 in the WIPO); and dermatological (01 application in the Espacenet). Japan and United States were the main depositors countries. The highest number of patents obtained with the combination of the keywords Morinda, citrifolia and IPC A61K presented the subcode A61K36, representing pharmaceutical preparations characterized by active organic. Furthermore, the highest number of patents among codes A61Q laid in the subcode A61Q1, representing preparations for skin cleansing. The largest number of applications occurred between 2002 and 2009.

Conclusions: The data showed that the area related to technological research with Morinda citrifolia is promising, and that since 2000 there has been a growing increase in the number of patents, particularly in the years 2005 and 2007. The United States and Japan lead the ranking of depositor countries. The largest technology is involved in the development of cosmetics for skin care, and most exploited in the makeup production and in medicinal preparations.

References


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Evaluation of antimicrobial potential of leaf extract of plants collected in the UFAM campus’ reserve

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BMC Proceedings 2014, 8(Suppl 4):P199

Background: The search for substances with antimicrobial properties is intensifying every day, given the increasing number of microorganisms resistant to the usual antibiotics. The use of plant components in the pharmaceutical field has gradually increased in Brazil. According to the World Health Organization, medicinal plants should be the best sources to obtain new varieties of drugs [1]. Due to the above, the need to assess the antimicrobial potential in some species of Myrtaceae, Anacardiaceae and Oxalidaceae families.

Methods: The leaves were collected in domestic and native plants in the reserve of the university campus. The collected material was triturated in distilled water, centrifuged 8000g and the supernatant filtered through 0.22-μm membrane and stored in 15mL Falcon tube [2]. The leaves were dried at 50 °C for 24h. The antimicrobial activity tests were the agar diffusion (Kirby-Bauer), and when confirmed the presence of antagonism one bioautography was performed to determine the Rf of antibiotic molecules. Test microorganisms were spiked in Plate Count Agar medium (PCA) and incubated at 37°C for 24 hours before testing [2]. For inoculum preparation, the youth cultures of each microorganism were standardized in sterile saline according to the 0.5-MacFarland Scale. The tested extracts included the filtrate liquid and the hydrated powder 10% (w/v). The seeding was carpet-type using “Swab”, 100L of extracts were placed in “Cup plate” incubated for 24h at 37 °C. The positive extracts in the biological assays were weighed and serially diluted to twice with distilled water (200 mg/2 mL) and transferring one mL of this dilution to the successive tube to 10² in In each tube containing the respective dilution by adding 19.0 mL of Mueller-Hinton agar, which was poured into Petri dishes. Thus, the concentration range from 5 to 0.04 mg/mL was obtained. The seeding will be carpet type using “Swab”. The MIC will be considered the lowest concentration that inhibited microbial growth [3].

Results and discussion: Species of Myrtaceae, Anacardiaceae and Oxalidaceae families showed positive results against the test microorganisms Candida albicans, Escherichia coli, Mycobacterium smegmatis and Staphylococcus aureus. In the study concerning the minimum inhibitory concentration (MIC), it was notorious that better results were obtained when using more concentrated extract. The mean inhibitory concentration for samples was 10. The results show that the species of the three families has great antimicrobial potential. Noting the ability of Amazon to research and enhanced absorption of drugs, particularly of antibiotics [2]. Although Brazil is a major producer of pineapple, occupying the first position in 2010 worldwide with a production of 1.5 million tons of fruit, the salt stress, deleterious alterations observed in plants grown in saline conditions, which occur due to intoxication by ions and decline of supply of water and nutrients to the plant [3] is main factor limiting growth and productivity of plants since it causes metabolic responses in plants, affecting and compromising all important processes such as photosynthesis, changes in levels and protein synthesis and activity enzymes, as well as in the synthesis of lipid metabolism and energy [4]. Thus, the objective of this research was to evaluate the influence of salt stress on the activity of bromelain in pineapple plants (Ananas comosus L. Merrill) cv. Pêrola grown in vitro, at sea.

Methods: The bromelain activity was available from crude extracts of leaves and stems from plants of pineapple, Ananas comosus var. comosus, cv. Pêrola, cultivated in vitro in culture medium MS [5] with growth regulator (200 μg mL-1 BAP and 0.5 μg mL-1 ANA) and transferred one mL of this dilution to the subsequent tube to 10² in In each tube containing the respective dilution by adding 19.0 mL of Bovine Serum Albumin was used as a substrate to be hydrolysed by the enzyme bromelain.

Results and conclusion: According to the results, the bromelain activity, total protein concentration and specific activity obtained in pineapple plants cv. Pêrola showed variation in relation to salinity levels and plant tissue analysis (stem or leaf). In quantitative terms, the more significant levels of proteolytic activity of bromelain were obtained in tissues from pineapple stems of the treatment corresponding to a concentration of 100 mM NaCl. Our findings corroborate results obtained in other vegetables and can be used in optimizing crop when in coastal regions or in saline soils.

Acknowledgements: The authors thank the EMBRAPA, FAPITEC/SE and CAPES.

References
Introduction: Acetobacter pasteurianus is among the major bacteria responsible for acetic fermentation and gives the characteristic taste of vinegar, can be found as a contaminant of wine production industry. In industrial fermentation is frequent contamination by lactic acid bacteria such as Lactobacillus fermentum that competes with the yeast for nutrients and inhibiting their growth, slow the fermentation and impair the production of ethanol [1] (AIM). The objective of this study is the molecular identification of bacteria isolated as contaminants of an ethanol industry in the state of Pernambuco, by sequencing the 16S ribosomal gene (16S rDNA).

Methods: The samples were cultured in a sucrose syrup at 35°C for 24 hours and subjected to DNA isolation using the protocol of Sambrook et al (1989) [2] by extraction with phenol / chloroform and precipitation with isopropanol. The PCR reaction was performed using 16S rDNA primers then analyzed by agarose gel electrophoresis observed in UV transilluminator and revealed. The amplified product of the 16S rDNA gene was purified, sequenced and the data were aligned with the tool BLASTn (Basic Length Alignment Search Tool) algorithm enabling the identification by comparison to other sequences deposited in the GenBank database from NCBI (National Center for Biotechnology Information).

Results and conclusions: The PCR products of the gene 16S rDNA of the bacteria had a molecular weight of approximately 1500 base pairs [3]. This drew attention by quantity and rheological properties of a polymer produced molasses medium. The molecular technique used revealed a high degree of conservation of the 16S rDNA gene and comparison of the sequence obtained in this study with sequences deposited in GenBank database resulted in strains of known species showing similarity with the sequences under study.

Acknowledgements: The Fundação de Amparo a Ciência e Tecnologia de Pernambuco (FACEPE) for financial support.

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Molecular identification of Acetobacter pasteurianus and Lactobacillus fermentum, contaminating an ethanol industry in the state of Pernambuco

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BMC Proceedings 2014, 8(Suppl 4):P200

P201
Bioproduction and extraction of jasmonates

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BMC Proceedings 2014, 8(Suppl 4):P201

Introduction: Jasmonates are a growing class of compounds of vegetable origin which present phytoregulator activity and toxicity against some types of cancer cells. Additionally the jasmonates have also shown activity against some types of nematodes [1]. Jasmonates available on the market are of plant origin or synthetic, present costs are still high. Like plants, some microorganisms have the potential to produce jasmonates. Some microorganisms have the potential to produce this class of compounds, including filamentous fungus Botryosphaeria rhodina has shown to be the most promising. The choice of the strain and optimization of process steps are fundamental premises for increasing the scale of production [2]. Furthermore, the optimization of the steps of extraction and purification of the product in the fermentation broth are essential to ensure the viability of the process. Currently, studies of recovery of jasmonates produced by Botryosphaeria rhodina are based on organic solvents. In this work, an alternative extraction route is proposed, based on adsorption, and the determination of the adsorption kinetics of jasmonates present in fermented using ion exchange resins Amberlite 1* is presented.

Methodology: Fermentations were conducted in Erlenmeyer of 250 mL using 50 mL of culture medium M2 and 5 mL of inoculum. After a period of fermentation, the mycelium was removed by vacuum filtration and subjected to fermentation extraction tests. To do this the filtrate collected was adjusted to pH 3.0. Then it was mixed in an Erlenmeyer of 100 mL of fermented and 1 g of resins Amberlite 1* XAD-2, XAD-4 or XAD-7 separately, put under stirring at 130 rpm for 60 minutes [3]. Samples (5 mL) were collected after 2, 4, 6, 8, 10, 20, 40 and 60 minutes of contact and subsequently subjected to extraction in order to determine the content of jasmonates remaining in each sample. The extractions were performed by liquid-liquid partition using ethyl acetate as solvent extractor. The monitoring was performed by CCDC (Thin Layer Chromatography Comparative) using silica chromatoplates with UV indicator. The quantification of jasmonates was performed by HPLC (High Performance Liquid Chromatography) using a chromatograph SHIMADZU (LC-10AD VP) coupled to a detector of diode array. It was used a m) and a solvent system Supelcosil C18 column (25 cm × 4.6 mm id, 5 comprised of MeOH: acetic acid 0.1% (60:40). The solvent flow was 0.85 mL.min-1 with analysis monitored at 210 nm. For quantification it was used the external standard method, by plotting a calibration curve with standard solutions of pure AJ at concentrations ranging from 0.1 - 1.0 mg.mL-1.

Results and conclusions: The results showed that the efficiency of XAD-2 resin was 84.02%, XAD-4 was 96.3% and XAD-7 95.15%. With the data analyzed, it was concluded that even though the Amberlite XAD-4 has a slightly higher efficiency in the time of equilibrium, the resin XAD-7 after 40 minutes showed higher adsorption (93.01%) over XAD-4 (85.19%). Therefore, for the continuation of studies, the Amberlite 1* XAD-7 will be used.

Acknowledgements: FAPESP, CNPq, UNAERP

References:

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Xylose and cellulose fermentation by yeasts isolated from the Brazilian biodiversity

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BMC Proceedings 2014, 8(Suppl 4):P202

Background: The production of fuel ethanol has become important in recent years due not only to the future depletion of fossil fuels, but also environmental concerns. An attractive source of raw material for ethanol production is the lignocellulosic biomass, composed of lignin, cellulose and hemicellulose. In the case of Brazil, the sugarcane bagasse is an interesting source of cellulose and hemicellulose, polymers that can be used in the fermentative process for fuel alcohol production [1]. Although the industrial yeast Saccharomyces cerevisiae efficiently ferments hexoses, this yeast is unable to ferment pentoses such as xylose (present in hemicellulose hydrolysates) or the disaccharide cellobiose (present in cellulose hydrolysates). Thus, we have characterized the enzymes and transport
systems involved in xylose and cellobiose fermentation by yeasts species isolated in rotten wood from several Brazilian ecosystems [2-4].

Methods: The xylose fermenting yeasts Spataplasora arborariae, S. passalidarum and Candida queiroziae were grown on rich YP (2% peptone and 1% yeast extract) medium with 2% of glucose, xylose or cellobiose as carbon source. The xylose reductase activity was measured by monitoring the oxidation of NADPH or NADH, while the xyitol dehydrogenase activity was measured by monitoring the reduction NADP+ or NADPH in the presence of xyitol as substrate [5]. The intracellular β-glucosidase was assayed using pemereabilized yeast cells using cellobiose or p-nitrophenyl-β-glucopyranoside (pNPG) as substrate. The active proton co-transport with xylose or cellobiose was determined using a pH-meter as previously described for other yeast sugar-H+ symporters.

Results and conclusion: Our results showed that the fermentation of xylose, cellobiose and glucose is a variable trait in the yeasts isolated from rotten wood. S. arborariae and S. passalidarum ferment xylose better than glucose probably due to a xylose reductase with significant activity (Km of 10-18 μM and Vmax of 0.38-0.50 U mg-1) not only with NADPH, but also with NADH as cofactor, while the xyitol dehydrogenase was totally dependent on NADP+ (Km of 100 μM and Vmax of 0.25 μM mg-1). Our results also show that S. arborariae has a H+-xylose cotransport system with low affinity and high capacity (km of 25 mM and Vmax of 35 mmol mg-1 min-1) for the sugar. While this last yeast ferment cellobiose, only half of the S. passalidarum strains could ferment this sugar due to the presence of an intracellular β-glucosidase as already described for C.queiroziae [3], a yeast specie which has an high affinity H+-cellobiose cotransport system (Km of 1.5 mM and Vmax of 19 mmol mg-1 min-1). Thus, the xylose and cellobiose fermenting yeasts characterized in this work may constitute an interesting source of enzymes and/or transporters (and their corresponding genes) with more appropriate characteristics for the fermentation of these sugars, that may be used in the industrial yeasts aims to optimizing bioethanol production in Brazil.

Acknowledgements: This work was funded by the Brazilian agencies CNPq, FAPESC and FINEP.

References:

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Effects of different nitrogen sources on the production of Hyaluronic acid by Streptococcus
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BMC Proceedings 2014, 8(Suppl 4):P204

Hyaluronic acid (HA) is a linear polysaccharide with high molecular weight composed of disaccharide units of D-glucuronic acid (GlcUA) and N-acetylglucosamine (GlcNAc). HA is naturally present in vertebrate organisms, as well as in bacteria. HA can be obtained commercially through three routes: human umbilical cords, rooster combs, and strains of group C Streptococcus. This is a natural polysaccharide with extensive range of applications in the medical, pharmaceutical and cosmetics. Due to the viscous, cohesive and hydrophilic, the HA products and derivatives present high aggregated value ranging from US$ 2000 to 60000 Kg^-1, depending on their applications. The most frequently used bacteria in the industrial production of HA are Lancefield group A and C streptococci. These bacteria are nutritionally fastidious microorganisms which require complex nutrients due to their limited ability to synthesize specific aminoacids and B-vitamins. Additionally, there is the nutritional requirement with respect to organic
nitrogen, which also supplies a large portion of carbon for their cellular biosynthesis. Recent studies seek alternatives to allow the cost of production of HA using agricultural derivatives and industrial waste. In this context, the objective of this study was to evaluate the effect of nitrogen sources yeast extract, soy protein, whey protein and corn steep liquor in the production of HA. Fermentation was carried out in 125 mL erlenmeyer flasks containing 25 mL culture medium. The culture medium comprised in (g·L⁻¹): glucose, 30; nitrogen source (yeast extract, soy protein, whey protein or corn steep liquor), 30; K₂HPO₄, 2.5, NaCl, 2.0 and MgSO₄.7H₂O, 1.5. The inoculum was 10% (v/v) and the fermentations occurred at 100 rpm, 37°C and pH 8.0 for 24 hours. Inoculated cultures were centrifuged and the cell free supernatant was treated with ethanol for the precipitation of HA which quantified using a colorimetric reagent Carbazole. The concentration of lactic acid, acetic acid and formic acid were also analyzed in a system of high performance liquid chromatography with IR detector, column Aminex HPX-87H at 60°C and a solution of H₂SO₄ 0.005 mol L⁻¹ as the mobile phase a flow rate of 0.7 mL min⁻¹. The results performed in triplicate were compared by the Tukey test at 5% probability level (p < 0.05). The highest production of HA, 0.534 g L⁻¹ was obtained when using yeast extract as nitrogen source. Subsequently, experiments which resulted in a better yield of the polymer are those containing soybean protein (0.192 g·L⁻¹) and whey protein (0.063 g·L⁻¹). In medium containing corn steep liquor, no microbial growth or production of HA occurred. The decreased production of HA was directly followed by a reduction of the production of lactic acid and acetic acid.

References

P205
Monitoring expression of yeast cell wall protein-encoding genes in response to high hydrostatic pressure
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BMC Proceedings 2014, 8(Suppl 4):P205

Background: The cell wall (CW) is one of the most important structures of the yeast cell, accounting for up to 30% of its dry weight. This organelle determines cellular morphology, affords mechanical protection and provides osmotic support. The yeast CW is a dynamic structure susceptible to many modifications, adjusting its composition and thickness to environmental changes. These responses usually involve changes in gene expression, increasing levels of proteins that have protective functions. High hydrostatic pressure (HHP) is a useful model of stress, which causes CW compression [1]. Exploring this process using the model organism Saccharomyces cerevisiae may allow us to understand the mechanisms of yeast stress tolerance in biotechnological processes and it may also help in searching for effective antifungal drugs, since the CW is a desirable target of action.

Methods: In this work S. cerevisiae strain BT0510 was subjected to a non-lethal HHP of 50 MPa for 30 min, followed by recovery at atmospheric pressure for up to 15 min. RNA samples were collected to perform a time series microarray expression analysis.

Results and conclusions: Through bioinformatics, changes in the expression pattern (>2 fold) of several CW organization and biogenesis genes were identified. HHP induced the expression of HSP12, which protein is present in CW and acts by increasing its flexibility [2], promoting survival under various stress conditions. The CW stress adaptive response is mainly mediated via Cell Wall Integrity (CWI) pathway, and its genes were affected by HHP. Rho1p is the master regulator of CW signaling, and is stimulated by Rom1p [3]. HHP induced the expression of ROM1, Mtl1p and Wsc3p are related in detecting and signaling CW status to Rho1p; their genes were upregulated by HHP. Related to the same pathway, HHP activates PKH1 and PKH2, paralogs genes of which proteins activate components of a signaling cascade required for CW maintenance. The genes related to β-1,3-glucan, β-1,6-glucan and CW chitin biosynthesis were not strongly affected by HHP. Furthermore, MNN1, MNN9 and MNN10, correlated with protein mannosylation were downregulated by HHP. The products of these genes are subunits of mannose polymerase complexes, what suggest a possible change in the outer layer of the CW. Moreover, HHP induced the coding genes of Pir3p Hsp150p, members of proteins with internal repeats family (PIR), correlated with CW reinforcement by interconnecting two or more β-1,3-glucan molecules providing defense against β-1,3-glucanases, common stress in the wild since these enzymes abound in plant tissues [4]. DSE2, DSE4, EGT2, CTS1, SCW11 and SUN4, related with CW degradation and separation of daughter cell from the mother cell, were downregulated by HHP, suggesting that pressure can affect cell division. Many genes involved in CW biosynthesis and organization had their expression changed after HHP treatment, evidencing the importance of the CW to ensure cell survival against this stress. Knowing the key cell-survival proteins is critical to improve biotechnological processes, and the results presented here may help in development of new drugs or in develop stress tolerant cell wall yeast cells.

Acknowledgements: CNPq, CAPES, MCTI, FINEP and FAPES.

References

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Development of active packaging for the preservation of lyophilized pulp fruit
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BMC Proceedings 2014, 8(Suppl 4):P206

Introduction: Innovative technologies are needed to maintain food freshness, quality and safety, while reducing concentrations of chemical additives. This work aimed the development of an active packaging material to preserve lyophilized tropical fruits pulp powder and to study the stability provided by the active packaging.

Methodology: Active packaging material was low density polyethylene (LDPE) film incorporated with Tinuvin 326(UV absorber) and iron powder in different concentrations (0-0.5% and 0-1.5% w/w, respectively). Eleven treatments were performed according to the central composite design (CCD) in order to evaluate changes in chemical, physical and barrier properties of films. Moreover, the influence of selected optimal active packaging on the stability of lyophilized tropical fruits pulp was assessed.

Results: The incorporation of Tinuvin 326 in LDPE did not alter the physical and mechanical properties of the film. Tinuvin 326 promoted barrier activity to UV light with increasing concentrations while maintaining film transparency. Thus, incorporation of 0.5% Tinuvin 326 and 7.5% iron was considered the most suitable conditions according to the Response Surface Methodology, for the physicochemical and microbiological characteristics of lyophilized pulp. Moreover, the lyophilized tropical fruits pulp powder was stable for most of the analyzed parameters during storage of the products.

Conclusions: The developed packaging maintained the chemical integrity, physical properties and resistance to microbial growth of the packaged food, during the storage period. Due to their nature, this package may be produced on industrial scale for lyophilized products preservation, replacing laminated packaging. Therefore, developed active packaging has potential for the preservation of lyophilized fruits pulp powder.
High hydrostatic pressure upregulate central carbon metabolism genes in a distillery yeast strain

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BACKGROUND: High hydrostatic pressure (HHP) is applied on a variety of biotechnological processes, including food preservation, modulation of enzymatic activity, disaggregation of proteins, vaccines development and recently it was reported its use for ethanol yield increase [1,2].

METHODS: In this study we performed a microarray analysis in a distillery Saccharomyces cerevisiae strain (BY05010) submitted to sublethal pressure treatment of 50 MPa for 30 min at room temperature, followed by incubation for 5, 10 and 15 min at room pressure (0.1 MPa). The transcription of the genes involved in central carbon metabolism in response to HHP was investigated for bioinformatics tools.

RESULTS: HHP induced genes related to the phosphorylation of intracellular glucose, HXK1 and GLK1. Glucose-6-phosphate is a glycolysis and pentose phosphate pathway (PPP) intermediary. Glycolysis and gluconeogenesis were slightly induced by pressure, but GDH1 and GUT2, associated with the glycol-3-phosphate shuttle, were upregulated by HHP. This mechanism transfer reducing equivalents from the cytosol to the mitochondria for the reduction of ubiquinone in ubiquinol in the electron transport chain that leads to oxidative stress. Interesting enough, HHP also induced ZWF1 and GND2 genes that encode two enzymes related to NADPH production in PPP. The PPP plays an important role in NADPH generation, which is required in oxidative stress response, in order to maintain the intracellular levels of reduced glutathione [3,4]. Pyruvate, product of glycolysis in cytosol, is converted to different metabolites that are transported to the mitochondria and integrate the tricarboxylic acid cycle (TCA cycle). The HHP upregulated several genes associated to this pathway, such as PDC1, PDC6, ADH1 and the analysis monitored at 210 nm.

CONCLUSIONS: Data showed that several genes related to the central carbon metabolism were induced by HHP, suggesting that yeast accelerates glucose consumption and, consequently, its metabolism. This data analysis allows the design of new cells for ethanol yield increase using HHP.

Acknowledgements: CAPES, CNPq, FINEP, FAPES and MCT.

References:

Optimization of jasmonates bioproduction

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INTRODUCTION: Several studies have shown that jasmonates (jasmonic acid, (+)-7-isojasmonic acid and methyl jasmonate) exhibit potential anti-cancer activity, with the advantage of displaying selective cytotoxicity to cancer cells that spares normal lymphocytes to this function [1]. This class of compounds is present in several families of plants, being also produced by certain microorganisms, including Gibberela fujikuroi and Botryosphaeria rhodina. Bioprocess studies based on the use of strains of B. rhodina have shown the potential of this microorganism to produce jasmonates under controlled conditions [2]. There are several factors that affect the productivity of a fermentative process, including the strain of the microorganism, the inoculum, and the morphological and rheological properties of the broth [3]. Once selected the most producing strains, it becomes necessary to define the conditions of the fermentation process, and the standardization of the inoculum is of fundamental importance when seeking increased productivity. In view of the morphology of filamentous fungus B. rhodina and the difficulty to induce sporulation, the standardization of the inoculum is a major challenge. In this context, the aim of this work was to conduct the standardization of B. rhodina inoculum preparation for jasmonate bioproduction.

METHODOLOGY: For the inoculum preparation, the fungus was grown in culture medium BD for 7 days, then the medium was drained and added to autoclaved distilled water. The mycelium was disintegrated and homogenized using a Turrax homogenizer (Marconi) and subsequently the DO was adjusted to 0.5 (l = 700 nm). Fermentations were conducted in 250 mL Erlenmeyer flask containing 50 mL culture medium inoculated with 5 mL of fungal homogenate M2 or 1/8 of the mycelial plate (control). Fermentations were conducted in the dark at 30°C under static conditions for 14 days. For quantification of jasmonates produced at the end of the fermentation period, the fermented broth was recovered by vacuum filtration, adjusted to pH 3.0 with 4 M HCl and then subjected to extraction with ethyl acetate. The quantification of jasmonates was performed by HPLC (High Performance Liquid Chromatography) using a chromatograph Shimadzu (LC-10AD VP) coupled to a diode array detector. A Supelcosil C18 column (25 cm x 4.6 mm id, 5 mm) was used and the solvent system was MeOH:Acetic Acid at 0.1% (60:40). The solvent flow was 0.85 mL min⁻¹ and the analysis monitored at 210 nm. For quantification, the external standard method was used, by plotting a calibration curve with standard AJ solutions.

RESULTS and CONCLUSIONS: From the obtained data it was observed that the flasks inoculated with fungal homogenate had a higher concentration of jasmonic acid when compared to the control, in which 1/8 of mycelial plate was used. The average AJ concentration in the media inoculated with homogenate was 352.4 mg·L⁻¹, whereas the control was 274.0 mg·L⁻¹, representing a 28.6% increase in the amount of produced AJ.

Acknowledgments: FAPESP - Sao Paulo Research Foundation UNAERP - University of Ribeirao Preto

References:
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Influence of individual HXT transporters in xylose fermentation by recombinant Saccharomyces cerevisiae strains

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BMC Proceedings 2014, 8(Suppl 4):P209

Background: Lignocellulosic biomass is an attractive raw material for bioethanol production since it is an abundant and renewable feedstock that does not compete with food and feed production [1]. Xylose is the most abundant pentose present on these feedstocks, and although S. cerevisiae cannot readily ferment this sugar, the overexpression of the genes for xylose reductase (XR) and xylitol dehydrogenase (XDH) from P. stipitis and xylulokinase (XX) from S. cerevisiae allows the utilization of xylose [2]. However S. cerevisiae also lacks specific transporters for this sugar and thus the uptake of xylose is carried out by native hexose transporters encoded by the HXT1-HXT7 genes [3]. In the present report we analyzed the impact of individual HXT transporters on xylose fermentation by recombinant S. cerevisiae yeast strains overexpressing the genes for XR, XDH and X [4].

Methods: Cultivations were performed in rich (YP) or synthetic complete (SC) medium containing the required sugars and when necessary, 2% Bacto agar, 0.5 mg/l aureobasidin A and 200 mg/l Geneticin were added to the medium. The chromosome-integrative plasmid pAUR-XKXDHXR [4] containing PGK promoters for overexpression of XR, XDH and X was digested with BsiWI and then chromosomally integrated into the AUR1 locus of the yeast strains. HXT1, HXT2, HXT5 and HXT7 genes were obtained by PCR from S288c S. cerevisiae genomic DNA and cloned individually into a pPGK multi-copy plasmid [5], and these plasmids were transformed into the strains lacking all HXT genes or individual HXT genes, respectively. Anaerobic batch fermentations were performed at 30°C in closed 50-ml bottles with a magnetic stir bar and 100 rpm. Assays with 2-6% of glucose, xylose or both sugars were performed. During fermentation cell growth was monitored and samples were removed for further analysis. Glucose, xylose, ethanol, xylitol, glycerol, and acetic acid were determined by HPLC as previously described [4].

Results and conclusion: The deletion of individual HXT genes had no detectable effect on glucose fermentations, but these knockout strains ferment xylose poorly, even under glucose plus xylose conditions. The low-affinity HXT7 permease allowed the maximal consumption of sugars and ethanol production rates during xylose plus glucose co-fermentation, but was incapable to allow xylose consumption when this sugar was the only carbon source. The high-affinity HXT7 permease allowed efficient xylose fermentation, but during xylose plus glucose co-fermentation this permease showed a clear preference for glucose. While the HXT5 permease performed bad with glucose and did not allow xylose utilization, the moderately high-affinity HXT2 permease was a transporter that allowed both xylose and xylose-glucose co-fermentation with the same rates as glucose, even under co-fermentation conditions, but had the drawback of producing stuck fermentations. Thus, our results indicate that new approaches to engineer selected HXT transporters to increase their affinity towards pentoses, or to avoid their sugar-induced degradation, are promising strategies to improve second generation bioethanol production by xylose-fermenting yeasts.

Acknowledgements: This work was funded by the Brazilian agencies CNPq, FAPESC and FINEP, and by the Japanese International Cooperation Agency (JICA).

References

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Evaluation of storage stability of soybean biodiesel using a flame ionization detector coupled to a gas chromatography system

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BMC Proceedings 2014, 8(Suppl 4):P210

Background: Biodiesel, an alternative energy source, has been attracting increasing worldwide interest in recent decades. It is obtained by base-catalyzed transesterification, resulting in a final mixture consisting of mono-alkyl esters and other by-products (e.g. glycerol, alcohol, free fatty acids). This composition provides a level of operational quality control that enables biodiesel to be marketed efficiently, because its viability depends on several factors, such as stability during the storage period. Unlike fossil fuels, which are relatively inert and therefore undergo few alterations to their properties during storage, biodiesel degrades through oxidation and hydrolysis, with consequent alterations to its properties, due to exposure to the environment. Thus, the purpose of this study was to monitor soybean biodiesel quality using a flame ionization detector (FID) coupled to a gas chromatography (GC) system.

Methods: In the alkali catalytic methanol transesterification method, the catalyst (KOH) is dissolved in methanol by vigorous stirring in a small reactor. The oil is transferred into the biodiesel reactor, and then, the catalyst/alcohol mixture is pumped into the oil. A successful transesterification reaction produces two liquid phases: ester and crude glycerin. Crude glycerin, the heavier liquid, will collect at the bottom after several hours of settling. Phase separation can be observed within 10 min and can be complete within 2 h of settling. Soybean biodiesel samples were stored for 7, 15 and 30 days at two different temperatures (room temperature and 8°C). GC-FID was used as single methodology to evaluate the ester content and storage stability of the samples in the following manner: a DB-Waxetr capillary column (30m×0.25mm, 0.25μm), injection volume of 1μL, and N2 as carrier gas. The column temperature was maintained at 170°C for 1min, and then ramped up at 10°Cmin-1 to 210°C, kept at 210°C for 1min, then ramped up at 5°Cmin-1 to 230°C, and kept at 230°C for 6min. These samples were then compared to standard compounds. The FID temperature was 230°C.

Results and conclusion: The GC-FID analysis showed results that allowed biodiesel esters to be identified and quantified, indicating biodiesel degradation during storage periods, a decrease in ester content of 14.40% and 16.0% at 8°C and room temperature, respectively. The GC-FID method is especially suitable as a rapid tool for control purposes in order to determine the methyl ester content quickly, simply and inexpensively, in order to meet Brazil’s National Agency of Petroleum, Natural Gas and Biofuels (ANP) requirements for biodiesel commercialization.

Acknowledgements: Authors would like to express their gratitude to CNPq, UNIT, CAPES and FAPITEC for the financial support and scholarships.

References


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Evaluation of production of xanthan gum utilizing the corn cob liquor as a carbon source in different strains of Xanthomonas campesstris
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BMC Proceedings 2014, 8(Suppl 4):P211

Background: The xanthan gum production media and are commonly employed as glucose or sucrose, which accounts for about 50% of the cost of production. Therefore the use of agro-industrial residues such as corn cob for the production of xanthan gum becomes suggestive, as such residues are abundant. Waste when processed becomes a rich source of carbon, nutrients and salts. Thus this study aims to use hemicellulose fractions, derived from agro-industrial residues.

Methods: The cobs were selected, dried at room temperature and submitted to grinding in knives mill to 16 mesh and then alkaline extraction was performed utilizing 0.75 mol NaOH for 120 minutes, and then filtered and stored on cooling (~20°C) until used [1]. The strain was acquired bank Xanthomonas Institute of Technology and Research, where four strains of Xanthomonas campesstris (629, S6, 254 and 1078) are stored in YM medium consisting of (gL-1): yeast extract 3.0, malt extract 3.0, peptone 5.0, sucrose 1.0.0, and agar 20.0, the microorganism being incubated at 28 ° C for 24 hours. The fermentation process was performed in two steps, the inoculum was prepared by adding 1 ml of bacterial suspension 108 in 14 ml of YM medium incubated on an orbital shaker at 150 rpm, 28 ° C for 24 hours. During the second stage, the inoculum was added to 86 mL of fermentation media. The media were evaluated for carbon sources, and the presence of salts. The nutritive media were: M1 (liquor corn cob), M2 (liquor corn cob + salts), M3 (sucrose), M4 (sucrose + salts). After inoculation, was incubated at 180 rpm for 96 hours at 28 ° C [2]. For each experimental condition was performed four replicates, which was analyzed productivity and apparent viscosity.

Results: The results show that the strains analyzed showed similar behavior as the production of the polymer in the four conditions of fermentation, which emphasized the S6 strain, which exhibited the highest performance in its production in all conditions including in the absence of salts, which the sample M1 obtained 11,7038gL-1 Jh-1 and M2 obtained 6,486475 g.L-1 Jh-1 biopolymer. It can be concluded that the results achieved demonstrated the feasibility of obtaining gum in media with the hemicellulose fractions, enabling greater feasibility of process within the context of a biorefinery. The apparent viscosity of aqueous solutions has shown that the use of the corn cob liquor has improved the viscosity of the polymer obtained.

References

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Evidence of a new intermediate compound of the chitin biosynthesis found in the marine-derived Penicillium roqueforti fungus
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BMC Proceedings 2014, 8(Suppl 4):P212

Chitin derivatives, chitosan and substituted chito-oligosaccharides from fungi present a wide spectrum of applications and they have been studied in many fields such as medicine, cosmetics, agriculture, aquaculture, and food as dietary supplements [1]. Chitin is a copolymer with N-acetyl-D-glucosamine units linked with β-(1-4)-glucosidic bonds which provide rigidity to the cell wall in chitinous fungi. There is evidence that the chitin synthesis is catalyzed by the chitin synthase (CS; EC 2.4.1.16), an enzyme that transfers β-(1-4)-linked anhydro-2-acetamido-2-deoxy-D-glucose (GlcNAc) from uridine diphosphate N-acetylgalactosamine (UDP-GlcNAc) to the nonreducing end of growing chitin chains [2]. However, chitin is synthesized by the regulation of distinct isoenzymes whose number ranges in some hypomycetes [3]. Nevertheless, there is relatively little information on the genes responsible for chitin biosynthesis in filamentous fungi, analyses of DNA fragments from taxonomically diverse fungal species have shown that most fungi have three to six chitin synthase genes [4]. Therefore, this diversity of chitin synthases makes difficult to find a unique model of regulation of the chitin pathway. The chemical screening of the biomass of a new marine-derived strain of Penicillium roqueforti, produced by liquid-state fermentation, led to the identification of several volatile and non-volatile compounds [3]. As a result of this previous study, we have isolated and characterized a new molecule. The chemical structure of the 2-deoxy-2-phosphamino-α-D-glucopyranose isolated was elucidated on the basis of 1D and 2D NMR studies as well as other instrumental techniques. In consequence of this discovery, it has been proposed a biogenetic route aiming to explain its formation as an intermediary component of the chitin biosynthesis.

References
Surfactants are amphiphilic molecules constituted by hydrophobic and hydrophilic regions that usually get distributed on interfaces between fluid phases of different polarities (water/oil and oil/water) [1]. Several compounds with tensioactive properties are synthesized by living organisms (animals, plants and microorganisms), being considered natural surfactants or biosurfactants, which reduce superficial tension and present a significant emulsifying capacity. Endophytic fungi are microorganisms associated to plants, which inhabit, at least during a certain period of time, the interior of vegetal tissues without causing any damage or producing external structures [2]. Plants present a great potential for the obtaining of biologically active compounds. However, there are only a few reports regarding endophytic microorganisms isolated from tropical plants. Among the interesting host species, the genus *Myrcia* has brought the scientific interest due to the presence of a considerable amount of biologically active compounds for the production of antibiotics and antioxidants [3]. Therefore, this work aimed to verify the production of biosurfactants by endophytic fungi isolated from roots and stems of *Myrcia guianensis*. The endophytes were isolated previously [4] and maintained on BDA tubes, being activated on this media at 28°C during 5 to 10 days. It was produced a spore suspension (1.0 × 10^8 spores/mL), which was inoculated in Erlenmeyer flasks containing the liquid media: MgSO4 (0.5 g/L), Na2HPO4 (3.0 g/L), KH2PO4 (1.0 g/L) and yeast extract (1.3 g/L). After autoclaving the media, it was added 0.5 g/L of soybean oil in order to induce the biosurfactant production [5]. The fungi were cultivated in duplicate during 7 days in a shaker at 28°C and 170 rpm. After the experiment, the cultivated media was filtered and the supernatant was used in a quantitative test to determine its emulsifying capacity (Eₚₜ). Eight endophytic fungi were evaluated for the production of biosurfactants (Mgc 3.1.1, MgC 3.3.2, MgRe 1.3.3, MgRe 2.3.1, MgRe 2.1.1, MgRe 1.3.1, and MgC 2.1.2). The results showed that four culture media formed emulsions with kerosene, indicating the production of tensioactive molecules by the isolated fungi. The emulsifying index obtained was 64.93% for MgRe 2.3.1, 69.52% for MgC 3.3.2. 70.0% for Mgc 3.1.1 and 75.75% for MgRe 1.3.1. It is worthy to mention that the synthetic surfactant sodium dodecyl sulfate, used as positive control, presented a emulsifying index of 78.45% at a 1% solution. Hence, it is possible to conclude that the endophytic fungi isolated from *Myrcia guianensis* were able to produce biosurfactants and that they present a great potential as a source of new products.

References

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**Screening of immobilization method in aerogel matrix in the presence of protic ionic liquid**

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Aerogel and xerogel are formed via hydrolysis and polycondensation reactions of silica precursors, such as tetraethoxysilicate (TEOS), always careful not to cause collapse, reduction in surface area and pore size. Several studies showed the use sol-gel entrapment possesses a number of desirable attributes, as the enzyme is physically entrapped in a rigid glass framework that permitted stabilization of the enzymes, tertiary structure owing to the tight gel network. In this study, have focused on the screening of immobilization method in aerogel matrix in the presence of protic ionic liquid (PIL) with evaluation catalytic efficiency and operational stability. The novel mesoporous silica supports (aerogels) obtained by the sol-gel technique was used to immobilize commercial *Burkholderia cepacia* (BC) lipase by physical adsorption, covalent binding and encapsulation in the absence and presence of protic ionic liquid (N-methylmonooethanolamine pentanoate - C₅H₃). Catalytic efficiency was determined in the analyses of hydrolytic activities were carried out on the base loading solution and immobilized enzyme to determine the total activity recovery yield, Ya (%). For physical adsorption the recovery of activity with PIL was 82.95% (ADSLI) and in the absence 70.31% (ADS). Operational stability of the enzyme has also been examined and obtained similar values a half-life of 0.73 h to ADSLI and 0.88 h for ADS occurs with 2 batches. And other immobilization technique, covalent binding the results showed that in aerogels supports in the presence of C₅H₃ obtained 69.13% (CBLI) of total activity recovery yield and 39.11% (CB). Under such conditions, the operational stability tests indicated that a small enzyme deactivation occurs after 15 batches, revealing a biocatalyst half-life of 7.5 h for CBLI. For encapsulation, the recovery of activity with PIL (ENLI) was 45% and 37% in the absence PIL (EN), ENLI exhibited a half life of more than 23 batches with 70% of its activity remaining, with around 11.5 h. And EN the half-life was 6.5 hours, totaling 13 batches. Therefore, ADSLI presented good efficiency with total activity recovery yield but for operational stability ENLI showed the best between immobilization methods studied. Hence, it is rather appropriate for application in hydrolytic processes.

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**Biotechnological route for obtaining methyl esters from crambe oil (Crambe abyssinica)**

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**Introduction:** The fatty acid esters synthesis by transesterification of oils to produce biodiesel commonly involves methanol or ethanol as acyl acceptor. The transesterification of vegetable oils catalyzed by lipases is an alternative process for obtaining biodiesel. These biocatalysts working under mild conditions of temperature, allow for easy recovery of glycerol, synthesis of specific alkyl esters and transesterification of triglycerides with high concentrations of free fatty acids. The seeds of crambe (Crambe abyssinica) have a high oil content and great potential for biodiesel production [1]. In this way, the present work aims at crambe oil transesterification with methanol catalyzed by immobilized lipase Novozyme 435 (Candida antarctica).

**Experimental:** Crambe seeds were powdered and lyophilized for extraction of crude oil by pressing for 72h. For transesterification first was evaluated the effect of molar ratio (MR) methanol/oil in the range of 3:1 to 12:1 with reaction time of 6h and 3.36% (w/w) of enzyme. In the best MR evaluated the effect of reaction time (0.375-24h). The reactions were conducted in thermostatted bath in reactor containing oil (1.5g±1.62 mmols), methanol, immobilized lipase and tert-butanol at 20% (w/w) with respect to the mass of oil. The quantification of fatty acid methyl esters (FAME) formed was performed by gas chromatography according to European standard EN14103, with minor modifications. The conversion was calculated based on a standard curve constructed with biodiesel 100% purity.

**Results and discussion:** Factors such as presence and the molar ratio of organic solvent affecting the transesterification of vegetable oils. The lipase *C. antarctica* tolerates reaction media containing organic solvents, in particular hydrophobic as hexane, [2] but in transesterification reactions, the substrates used (alcohol) and reaction product (glycerol) this type are immiscible solvent, which might result adsorption of these
polar molecules on the hydrophilic support of the immobilized enzyme and low reaction rates. To solve this problem tert-butanol at a concentration of 20% was used as co-solvent because it is moderately hydrophilic (log P = 0.80) being able to solubilize oil, methanol and glycerol forming by eliminating the negative effects of methanol and glycerol on enzyme activity [3]. The high values of MR negatively affect the reaction rate by cause enzyme denaturation see the large concentration of highly hydrophilic compound (methanol) in the reaction medium [4]. At low conversions were obtained with the FAME with MR 9:1 to 12:1 (24 and 17%), while conversions of 64 and 54% were obtained in the MR 3:1 and 6:1, respectively. The profile of the kinetics of formation of FAME showed reactional system in conditions of initial velocity reaction with 3h. From 12h the reaction system tends to equilibrium, reaching maximum conversion of 82% after 24h of reaction. The results show promising potential to obtain biodiesel from crude crambie by enzymatic route.

References
2. Soumanou MM, Bornscheuer UT: Bacterial adherence to polystyrene: a replica method of multivariate analysis results showed that all variables were important (P < 0.0001 X1, + 0.0002 X2, + 0.004 X3). The method chooses for their resistance to microbial attack and cost effectiveness. Although the phenotypic diversity and lower generation time of bacteria make this group the most studied, yeast are also able to adhere and grow on inert surfaces. While free yeast cells have been studied for use in bioremediation, only in the past two decades have studies of immobilized yeast cells been reported [2]. Among the organic environmental pollutants, phenol stands out for its recalcitrance and toxicity. Therefore, the aims of this work were: (i) to evaluate the potential for immobilization of a yeast strain from a petrochemical wastewater in Brazil, (ii) to test flexible polyurethane foam as immobilizing agent for this strain and (iii) to compare the effect of phenol on immobilized and free cells.

Methods: The Candida rugosa yeast strain used in this work was isolated from oil refinery wastewater polluted by phenol. The cell surface hydrophobicity was determined by the replica method [3]. Adhesion to of xylene was evaluated according to the hidrophobicity method [4]. Qualitative production of biofilm was tested by cultivation on Congo red agar (CRA) and microbial adhesion in glass tubes (MAG) [5]. The cell suspension of C. rugosa was standardized to optical density of 0.5 at 600 nm, corresponding to an inoculum of 10^9-10^10 CFU mL^−1. The strain was cultivated on sterilized polyurethane foam matrices. The number of colony-forming units per square centimeter (CFU cm^−2) of polyurethane was determined using the spread plate method. Free and immobilized cells were cultured in a synthetic mineral medium containing different phenol concentrations. Tolerance to phenol was determined by daily measurements of CFU cm^−2.

Results and conclusions: The index of partition and translucent violet areas classified the strain as moderately hydrophobic. Dark red colonies on CRA plates and wall glass tubes stained red indicated that the C. rugosa strain is a biofilm producer. The growth curve of C. rugosa showed three phases: (1) lag phase, from 0 to 2 h, during which the number of adhered cells was 4.3 Log CFU cm^−2; (2) logarithmic phase, which lasted from 2 to 10 h, when the cell count increased from 4.3 Log CFU cm^−2 to 6.4 Log CFU cm^−2; and (3) stationary phase, which lasted from 10 to 24 h, when the cell concentration was 6.4 Log CFU cm^−2. The concentration achieved after 24 h, around 2.5 X 10^7 CFU cm^−2, is suggestive of biofilm formation. Concentrations of 62.5 mg l^−1, 125 mg l^−1 and 250 mg l^−1 of phenol had a lethal effect on the cells in suspension. The increase of the number of cells at 62.5 mg l^−1 and 125 mg l^−1 of phenol indicated the protective effect of the cell immobilization. C. rugosa from petrochemical wastewater presented hydrophobicity and biofilm production, indicating the ability to adhere on solid surfaces. Immobilization of this strain on polyurethane foam might be a useful strategy to improve bioremediation and for treatment of phenol-polluted industrial and petrochemical wastewater.
where, Y, X1, X2 and X3 denoted absorbance at 405 nm, pH, time and temperature, respectively. The high operational stability of PVA/Chitosan/Trypsin film was a characteristic that indicates in this sense, showing stable for about three weeks at different storage conditions, leading to the believe that the immobilization process gives a better stability for the enzyme reaction when compared to the free enzyme.

Acknowledgements: This work was supported by the Brazilian Ministry of Science and Technology through CNPq.

References

P218
Development, characterization and antimicrobial activity and wound healing nanocomposite membranes xanthan: silver using porcine model
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BMC Proceedings 2014, 8(Suppl 4):P218

Background: In recent years, advances in biotechnology has allowed the development of synthetic membranes associated with nanocomposites, which has shown promising results as dermal burns dressings. In this sense, silver nanoparticles (NPAg) has been the focus of interest because of their biological properties such as antimicrobial and anti-inflammatory effect. The incorporation of NPAg in biological membranes of different nature is considered to be an alternative to traditional treatments, with chitosan, polyester, polymethacrylate methyl and cellulose, has been successfully tested in several biological models. The association between NPAg and polymers produced by the micro-organism presents important advantages, such as water solubility and lack of toxicity. Recently we developed a technique for producing NPAg associated with xanthan (GX), a biopolymer with potential application in several sectors of the petrochemical industry, food and pharmaceutical, through fermentation by Xanthomonas sp performed in the presence of silver nitrate.

Methods: Therefore, this study aimed to develop, characterize and evaluate the potential antimicrobial and healing membranes nanocomposite xanthan: silver using porcine model. The incorporation of NPAg in biological membranes of different nature is considered to be an alternative to traditional treatments, with chitosan, polyester, polymethacrylate methyl and cellulose, has been successfully tested in several biological models. The association between NPAg and polymers produced by the micro-organism presents important advantages, such as water solubility and lack of toxicity. Recently we developed a technique for producing NPAg associated with xanthan (GX), a biopolymer with potential application in several sectors of the petrochemical industry, food and pharmaceutical, through fermentation by Xanthomonas sp performed in the presence of silver nitrate.

Results and conclusions: After eight, 18 and 30 days the wounds were examined macroscopically for each lesion area, and the anatomic and histological microscopic study of the scar area observed that XNPAg membranes showed a significant increase in the values of thickness (P < 0.05), density (p = 0.01) and Young's modulus (p < 0.001) and reduced strength strain (p < 0.05) when compared to membranes of xanthan. Were revealed changes in the thermal profile of the two membranes suggesting the incorporation of silver nanoparticles in the polymer xanthan. XNPAg The membrane induced the formation of inhibition zones 9, 7 mm and 9.6 mm and death rate of 89% and 100% for Staphylococcus aureus and Escherichia coli respectively. Histological analysis showed quantitative and qualitative increase in the reaction granulation and best architectural arrangement of collagen fibers along the the healing process of wounds covered with membranes XNPAg. Could be concluded that the membranes nanocomposite xanthan:silver showed satisfactory mechanical properties for its handling, transportation and storage, as well as important antimicrobial activity and pro-healing in dermal burns using porcine model.

Acknowledgements: Authors would like to express their gratitude the UNIF, CNPq, CAFES and FAPITEC for the financial support and scholarships. References

P219
Study of the synergistic activity between industrial yeast strains resistant to high temperature and ethanol concentrations and high fermentative capacity to produce ethanol
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BMC Proceedings 2014, 8(Suppl 4):P219

Fuel-ethanol fermentation process includes a reutilization of the yeast biomass, in which yeast cells are exposed to stress conditions as high temperature and increasing alcohol concentration. This environment may cause a significant delay in fermentation and drop in cell viability. The Bioen program (UNESP) conducted a study to select highly producing yeast strains in a Brazilian distillery. Two of these were selected when comparable to other well established commercial yeast strains: ZFC4 (ethanol best producer) and ZFD4 (most ethanol and temperature tolerant). Based on these data the aim of this work is to study genetic features associated with cell resistance to sustained stress of selected yeast strains in synergy with standards (PE-2, CAT-1 and SA-I) used in Brazilian distillery. The yeast strain was confirmed as Saccharomyces cerevisiae using PCR. The strains were screened together for growth of 25 at 44°C on plates containing 10% and 12% (v/v) ethanol and for fermentation assays. All of these demonstrated optimal responses to the high temperature and alcohol concentration and fermentative capacity when compared to the same yeast strain singly. To gain insight of the cellular mechanisms of resistance to these stresses, global gene expression analysis of the selected strains will be performed.

P220
Respirometric analysis of Penicillium simplicissimum growth in solid-state fermentation using Jatropha cake as culture medium
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BMC Proceedings 2014, 8(Suppl 4):P220

Jatropha cake (JC), a toxic residue derived from the extraction of the oil from Jatropha curcas seeds, can be used as a culture medium for solid-state fermentation (SSF). Through this process, it is possible to detoxify
and add value to this solid residue through the production of enzymes of biotechnological interest. However, one of the major technological bottlenecks in SSF is the microbial growth control. This study aims to monitor the growth of the fungus *P. simplicissimum* in JC by respirometry and compare this process of lipase production in tray-type bioreactor with the one in Raimbaut columns.

First, the experiments were carried out and optimized in tray-type bioreactors. After two sequential experimental designs - Plackett-Burman and Central Composite Rotatable Design - a production of 1400 U/g was reached, representing a 9-fold increase of the initially obtained activity. Based on the conditions indicated by the experimental design, the fermentation process was carried out on Raimbaut columns [1]. In these experiments, it was possible to follow the profile of CO₂ production by *P. simplicissimum* through respirometric analysis. It was observed a high and fast production of CO₂ over the first 24 h of growth. After this time, there is a reduction of the CO₂ release and the fungus achieves its basal metabolism around 96 h. In spite of the intense fungal growth, a low lipase production was reached (500 U/g), in contrast with the one obtained in tray-type bioreactors. This behavior is probably due to the strong compression of the solid culture medium and, consequently, the difficulty of heat and mass transfer, even under forced aeration. In order to reduce the compaction of the medium, a mixture of JC with sugarcane bagasse was prepared. The sugarcane bagasse (SCB) is widely used in SSF with other agroindustrial residue for better structure the culture medium, avoiding compaction problems [2]. After some assays, a medium composed of 15% (m/m) of SCB and 85% (m/m) of JC was used. After sugarcane bagasse addition, the lipase production was 1600 U/g, near to the one obtained in tray-type bioreactor.

The production profile of CO₂ is similar to those obtained by other fungi cultivated in SSF and in other culture mediums [3]. However, in both curves obtained, an unusual small change in the stage of metabolism in the first 24 h was observed in all experiments, probably due to the waste toxicity.

The phlorof ester content (the major toxic component) was 70% reduced reaching a final concentration of 676 μg/g. From these experiments, it is observed that (i) for SSF in columns bioreactors with JC, the use of sugarcane bagasse (or other inert raw material) to structure the culture medium is essential, avoiding compaction, (ii) the fungus *P. simplicissimum* reached a high lipase production when grown in Raimbaut columns with JC and SCB, reaching a production of 1600 U/g, (iii) through the respirometric system to monitoring the fungal growth, it is possible to see the stages of metabolism change of the fungus, allowing further studies and (iv) the fungus was able to reduce 70% of the phlorof ester content after 26 h of fermentation.

References

**P221**

**Purification of endoglucanase produced by *Penicillium citrinum* isolated from Amazon**

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**Background:** The cellulolytic enzyme complex has many important biotechnological applications such as beverage, textile, food, paper and cellulose industries, as well as in degradation of lignocellulosic for ethanol biofuel production [1]. According to the enzymatic activity, cellulolytic complex is subdivided in three classes: endoglucanases, exoglucanases and β-D-glycosidases.

Endoglucanases (endo-β-1,4-glucanase, EC 3.2.1.4) are responsible to initiate cleavage, hydrolyzing randomly internal regions from the amorphous cellulose fiber structures, releasing oligosaccharides from different grades of polymerization. These will be hydrolyzed by exoglucanases releasing cellobiose followed by β-D-glycosidases being hydrolyzed to glucose [1]. *Penicillium citrinum* has worldwide occurrence. Commonly in the soil, this species is described as good xylanase and cellulase producer [2,3]. This work describes liquid chromatography to separate endoglucanases of *Penicillium citrinum* supernatant from submerged fermentation process.

**Methods:** Suspension of 1 mL *P. citrinum* spores (6.4 × 10⁶) was inoculated in 200 mL submerged fermentation medium [4] containing CMC as carbon source for 10 days/28°C/150 rpm. Every 24 hours was sampled 6 mL aliquots, centrifuged and right after the supernatant storage at 4°C. A pool was made of collected points from 72 to 240 hours. It was concentrated by SpeedVac (Thermo). The final volume of 1.5 mL, filtrate (0.45 μm) and injected in chromatograph column Superdex 75 10/300 GL (GE Healthcare) previously equilibrated with sodium citrate buffer at 50 mM pH 4.8. Fractions of 1.5 mL was collected in a flux of 0.6 mL/min in 1.5 column volumes (CV) using Akta Purifier (GE Healthcare). Fractions were analyzed by CMCase activity [5], protein dosage with BCA kit (Thermo Scientific) and SDS-PAGE 12%.

**Results and conclusions:** It was observed a elution profile with three peaks from purification of 12 mL (pool) from culture supernatant, corresponding to the fractions 7, 8, 11 and 16, containing 60, 60, 250 and 30 μg/mL of proteins, respectively. By analysis in SDS-PAGE a band with 33 kDa in fractions 7 and 8 was detected, matching for endoglucanases of genera *Penicillium*, which can vary from 26 to 50 kDa. Only fraction 7 and 8 had CMCase activity (0.4 and 0.3 U/mL, respectively), in which 1 unit is the necessary quantity to release 1 μmol/min of hydrolyzed product. The specific activity CMCase was 6.6 and 4.4 U/mg for 7 and 8 fractions, respectively. Chromatographic profile obtained for each induction time (72 to 240 hours) was comparable to the obtained for the pool, being the analysis referring to the protein content and enzymatic activity in progress.

**Acknowledgements:** Biotech Amazonia LTDA-ME, FAPEAM, CNPq and CAPES.

**References**

**P222**

**Expression of a bacterial xylose isomerase in an industrial strain of *Saccharomyces cerevisiae***

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**Background:** The use of lignocellulosic biomass rather than fossil fuel is an environmental sustainable alternative for bioethanol production. However, fermentation of lignocellulosic hydrolysates by *Saccharomyces cerevisiae* is not viable since this yeast cannot ferment xylose naturally. Current, several studies are being developed to introduce a pathway that allows pentose fermentation by *S. cerevisiae* [1]. The bacterium *Propionibacterium acidipropionici*, employed in many industrial processes, is able to efficiently ferment xylose using the enzyme xylose isomerase. Xylose isomerase, codified by the XI gene, converts xylose to xylulose [2]. This study aims to develop a yeast capable of fermenting xylose through the expression of the *P. acidipropionici* XI gene in *S. cerevisiae*. Furthermore, the effect of the over
expression of a gene that encodes a xyulokinase (XKS1) and the deletion of the gene that codifies an aldose reductase (AR) together with the expression of the XI gene were evaluated. These enzymes are crucial for xylose fermentation since the former converts xylose to xyulose-5-P by the pentose phosphate pathway (PPP) and the last converts xylose into xylitol, which can alter the xylose isomerase activity [3].

Methods: The sequence of the XI gene from P. acidipropionici was obtained from its genome recently published [4]. An industrial strain of S. cerevisiae derived from PE-2, A1 (haploid; URA3Δ), was used in this work. The URA3Δ was used as an auxotrophic mark to select the transformants. The strategy chosen for heterologous expression of the XI gene by S. cerevisiae was its introduction in a high copy number plasmid. This plasmid contains the URA3 gene and the XI gene was cloned with a constitutive promoter and terminator. Three strategies were used to evaluate the heterologous expression of the XI gene: (1) expression of the XI gene alone; (2) expression of the XI and overexpression of the XKS1 gene; (3) expression of the XI gene, overexpression of XKS1 and deletion of the AR gene.

Results and conclusion: Tests of growth performed in a culture medium supplemented with xylose proved the great ability of P. acidipropionici to grow in this carbon source. Considering that the codon usage of S. cerevisiae is substantially different from the P. acidipropionici and given that this bacterium has a high GC content, an optimization of the codons from the XI gene was performed. By this way, the codon adaptation index (CAI), initially 0.49, raised to 0.93 after the optimization. The optimized gene was synthesized and the yeast was transformed with the XI cassette. Preliminary fermentation tests in medium containing xylose as carbon source showed that these yeasts were still not able to ferment xylose. Analysis of RNA samples from all lineages confirmed that the XI is expressed. Since the majority of the XI sequences are protected by patents and the gene from this bacterium is not protected, many efforts are being done to understand the reason why this bacterial gene is not functional in S. cerevisiae.

Acknowledgements: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Laboratório de Genômica e Expressão (UNICAMP).

References:

Background: Microalgae are becoming a viable model for the expression of recombinant proteins. However, suitable levels of expression for commercial production have not yet been obtained. One way to increase the heterologous protein production is the codon optimization of the target gene so that it presents the preferential codons used by the host organism according to its own pool of transfer RNA (tRNA). For codon optimization the Codon Usage Database (CUD; http://www.kazusa.or.jp/codon) has been used. The database provides codon frequency tables based on gene transcripts, regardless of their level of expression. Therefore, the aim of this study was to determine if there are changes in the frequency of codons used when taking into account the level of expression of genes, considering the 50 most expressed genes and 50 least expressed genes from the microalgae Chlamydomonas reinhardtii.

Methods: The transcriptome data, published by Lv et al. (2013), was used to select the genes according to their expression level. The 50 most expressed transcripts (RPKM>2900) were selected from the four stages of growth of C. reinhardtii: Log (LP), stationary (SP), lipid accumulation (LAP), and cellular decline (CDP). As a negative control, we selected the 50 least expressed transcripts (RPKM<15) from the LP growth phase. The sequences of the selected genes were obtained from the website http://genome.jgi-psf.org/ Chire4/ and the codon frequency tables were created using the tool Gene to Cdon Usage (http://www.entelecom.ch/2008/10/gene-to-codon-usage/).

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Gene expression level: is it an important factor in codon optimization for overexpression of recombinant proteins?
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Results and conclusion: The tables created with the 50 most expressed genes in the four stages of growth of the microalgae were not different by analysis of UPGMA. Thus, the LP stage table was used for all other comparisons. The UPGMA method distinguished the three tables. The main difference among the tables was observed in the AAG codon, with a frequency of 98.6 per thousand in the 50 most expressed genes table, compared with 42.3 per thousand from the CUD, and 28.0 from the negative control. Moreover, the codons with zero frequency were only identified in table from 50 most expressed genes: AUC, AU, GGA, and AGA. The chi-square test shows that there is enough evidence to support the hypothesis that the frequencies of the codons are not homogeneous among the three tables. Therefore, we conclude that there is a change in the codon frequency dependent on the level of gene expression. The next step is the production of three genetic constructs with the green fluorescent protein (GFP) gene.
optimized by each of the codon frequency tables in order to observe, in vivo, whether there is an improvement in the production of recombinant proteins.

Acknowledgements: CNPq - Brazil Science without Borders Program (COBIBI).

References

P225
Production and characterization of films from nanocomposite coating of sunflower seeds
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BMC Proceedings 2014, 8(Suppl 4)P225

Background: The culture of sunflower plants presents a great economic relevance. Sunflower’s seed stand out for your high high concentration of oil, which is used in human and animal feeding, and currently, as source of raw material to obtain biodiesel. However, about 12% of worldwide sunflower production is lost per year due to diseases occurrence, being this, the most limiting factor to the culture in most producing regions. The main diseases is Alternaria stain, which affects sunflower culture, are transmitted through the seeds. The coating technique has been researched by food and agricultural industry, once the coating of seeds with biodegradable films enable an uniform application of chemical and biological agents against the attack of pathogens and pathophathogens. This action reduces the impact over the environment and farmer’s health. Additives incorporated in biodegradable films production used in the coaters have as function to protect the seeds against the attack of microorganisms. The aim of this work was to develop a nanobiocomposite of metal-polymer to coat the sunflower seeds.

Methods: Tests were performed according to the experimental delineation: the chosen polymer (pectin, hydrolyzed collagen and xanthan gum); plasticizers (propylene glycol and glycerol) and the addictive (propolis and metal-polyaccharide nanocomposite). The films were prepared by casting technique. This method consists of a method used for food products, that require a uniform coating on an uneven surface. After the immersion, the excess coating material is drained from the product and then it is dried or left to stand to solidify. The films were evaluated against their mechanics properties and activity against Alternaria sp.. The coating in the sunflower seeds was observed by scanning electron microscopy. The propylene glycol-plasticized films were developed experimentally, and the best-performing formulation (2% pectin and 0.6% propylene glycol) was selected for HPE incubation. The film with 5% HPE had antifungal activity against the phytopathogen and was used to coat the sunflower seeds.

Results and conclusion: The incorporation of HPE improved the mechanical properties of the film and did not change other film characteristics. Photomicrographs were obtained of the films and coated seeds, this procedure showed their morphological characteristics and total seed coating when the film is incorporated. The films containing metal-polymer presented a similar behave as the pectin coated films, having a superior biological activity comparing with films containing red propolis, showing, therefore, an excellent biotechnological potential to seeds coating technology.

Acknowledgements: Authors would like to express their gratitude the UNT, CNPq, CAPES and FAPITEC for the financial support and scholarships.

P226
Isolation, purification and partial physicochemical characterization of a lectin in Andira pisonis Mart seed
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BMC Proceedings 2014, 8(Suppl 4)P226

Lectins are ubiquitous proteins in nature, with non-immune origin, which have at least one non-catalytic domain that binds carbohydrates specifically and reversibly. They can be found in vegetables leaves, stems and seeds. The Dalbergieae tribe has lectins which have specificity for different carboxydrates and also have several biological activities such as induction of rat paw edema, release of chemotactic mediators by macrophages, vasorelaxant effect in rat aortas, among others. This study aimed to isolate, purify and physicochemically characterize a lectin found in seeds of Andira pisonis Mart (Dalbergieae). Andira pisonis Mart seeds were ground into a fine powder and subjected to total protein extraction in 1 M ammonium sulfate. Soluble proteins were subjected to hemagglutination activity quantification by the Bradford method and essays of hemagglutination inibition activity by sugar. The lectin from Andira pisonis Mart (APL) was purified by affinity chromatography on Sepharose-Mannose matrix eluted in 0.1 M glycine buffer pH 2.6 with 0.15 M NaCl. The eluted fraction was dialyzed against distilled water, lyophilized and subjected to ion exchange chromatography on HiTrap SP XL 01. APL was eluted on 20 mM sodium acetate buffer pH 4.5 gradient of 0-1M NaCl. APL hemagglutinated rabbit erythrocytes (enzymatically treated) and other lectins from the tribe Dalbergieae and showed specificity for mannose (25 mM). SDS-PAGE analysis showed that APL is composed of a major 34 kDa double band and a minor 8 and 9 kDa double band. APL showed thermostability at 60°C. Further studies are still needed in order to better physicochemically characterize this protein and study its biotechnological potential on the referred conditions of vasorelaxant effect and chemotatic mediator.

P227
Physico-chemical characterization and partial sequence of a lectin from Canavalia bonariensis Lindl seeds
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BMC Proceedings 2014, 8(Suppl 4)P227

Lectins are (glycol) proteins that bind specifically and reversibly to carbohydrates. These proteins, in particular those from plant, are important tools in glycochemistry and glycochemistry. Canavalia bonariensis Lindl is a species of Leguminosae family, Papilionoideae subfamily, tribe Phaseoleae, subtribe Dioicleinae, native of the southern region of the country. The objective of this work was to purify a lectin from C. bonariensis (CaBo) seeds through affinity chromatographic. The process of purification of CaBo (Canavalia bonariensis Lecitin) was monitored by SDS-PAGE and hemagglutinating activity and showed that the purified lectin is characterized by an electrophoretic profile consists of a higher band with approximately 26 kDa, and two bottom bands with apparent molecular mass of 14 and 12 kDa. The analysis by mass spectrometry indicated that CaBo has a chain with molecular mass of 25,512 kDa and and two subunits (B and y chains) with molecular mass of 12,999 Da and 12,537 Da, respectively. CaBo also had its primary sequence partially determined by tandem mass spectrometry, obtaining 61% of the total sequence of the protein. CaBo was tested for the thermostability of their hemagglutinating activity after incubation for one hour at different temperatures (40° to 80° C), losing activity only at 80° C after one hour. Regarding its stability at different pH (4.0 to 10.0), CaBo was stable in a pH range between 7.0 and 9.0. The CaBo activity was also affected after serial dilution in the presence of the chelating agent EDTA and it was recovered significantly after addition of CaCl2 and MnCl2 0,005 mol/L, proving to be dependent of divalent metal cations.
P228
The role of junior enterprises in the development of human resources in biotechnology: a case report in Rio de Janeiro state
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BMC Proceedings 2014, 8(Suppl 4):P228

Introduction: Junior Enterprises are nonprofit entities run by undergraduate students. The Junior Enterprise Antonio Paes de Carvalho (EJ-APC) was established in 2010 with the major goal of stimulate the entrepreneurial skills of students in the life sciences area by the development of biotechnological products and services. Since human resources trained in basic science at the university are poorly incorporated into the private sector, we aim to demonstrate that new technologies can be developed or new markets can be achieved by the students in order to stimulate their professional career out of the academic locus.

Methods: Consists in the creation, establishment and management of the EJ-APC itself. The students incorporated as director can experience the daily work of an enterprise at all stages of its operation, from production to the presidency. Besides, students enrolled in products development can enhance their skills at the bench, using specific technologies.

Results: Since its inception, it has had four directories, yearly elected, and for selection processes for new members. In 2010, there was one single project line (polyclonals antibodies), whereas in 2013 we already run 4 different lines: polyclonals antibodies, academic histologic slides, plated materials and genotyping. A series of changes in the structure and organization of the company were driven by directors: i) the creation of a complex selection process; ii) greater participation in MEJ (Junior Enterprise Movement); iii) the establishment of strategic planning; and, iv) implementation of SOPs (Standard Operating Protocols).

Conclusion: The EJ-APC has an increasing number of students who engage in the management and implementation of projects, diversifying their professional perspective. Despite these advances sounds like an expected progression, we consider it an achievement in view of such strongly academic niche in which the EJ operates. At EJ-APC, students can learn and experience subject different from those in the traditional university teaching.

P229
Teaching biotechnology through practical cases
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BMC Proceedings 2014, 8(Suppl 4):P229

Background: A good way to teach biotechnology is showing practical cases after explaining the theory.

Methods: Three cases of study and application of biomedical signals are analyzed:
a) Development of a multisensorial prosthesis of upper limb commanded by myoelectric signals (sEMG)
b) Development of a robotic wheelchair commanded by eye blinks (myoelectric signals - sEMG) and by brain signals (EEG)
c) Use of myoelectric signals (sEMG) from disabled children to interact with mobile robots in order to carry out actions in the environment

In the first one, students are motivated to understand how a myoelectric signal is produced and then this knowledge is applied in upper limb prosthesis design.

In the second case, brain signals are also explained. Then EDR/ERS (Event Related Desynchronization /Event Related Synchronization) in alpha rhythm and Steady State Visual Evoked Potential (SSVEP) are used to command a robotic wheelchair. Myoelectric signals are also used to command the wheelchair; in this case, these signals are captured from the user face due to eye blinks.

Finally, for the third case, myoelectric signals are captured from disabled children to allow them to interact with a mobile robot, in order to the mobile robot carrying out movements of grasping objects using its tweezers, paint, etc.

Results: Several videos of experiments about the three cases are shown to students and discussions about the strategy of using the methodology of "hands on" when teaching biotechnology are exposed, based on these practical cases.

Acknowledgements: To CNPq, CAPES, UFES, PPGBioteck.

P230
Influence of recombinant S2 cell population enrichment on rabies virus glycoprotein expression and specific RNA and DNA quantities
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BMC Proceedings 2014, 8(Suppl 4):P230

Background: Different strategies have been evaluated for increasing the productivity of Drosophila melanogaster (S2) cells expressing recombinant rabies virus glycoprotein (RVGP). The maintenance of selective pressure during all the cultivation time and new methods for population enrichment with high level expressing cells are two methods which can increase the productivity through increased expression of RVGP gene. To understand how these methods can improve glycoprotein expression, analysis of the quantities of heterologous DNA and RNA were performed [1,2].

Methods: From S2MRVGPPhy cell population (untreated control) three populations were generated: S2MRVGPPhy+Hy was obtained after selective pressure using hygromycin for 2 weeks; S2MRVGPPhy-M2 and S2MRVGPPhy-M3 were obtained after immunomagnetic enrichment of RVGP expressing cells (MACS, Miltenyl Biotec), using rabbit polyclonal antibody and mouse monoclonal antibody, respectively [3]. Cell populations were induced with CuSO₄ for RVGP expression and submitted to cycloheximide (CHX) treatment before sampling in different periods. Samples were analyzed by ELISA, qPCR and qRT-PCR.

Results: All cell populations presented no significative differences in RVGPDNA content as analysed by qPCR. Before treating the cultures with CHX, cell populations showed very similar amounts of RVGPMRNA and were expressing RVGP at concentrations of 23.1, 31.9, 65.3 and 45.7 ng / 10⁵ cells for S2MRVGPPhy, S2MRVGPPhy-M2, S2MRVGPPhy-M3 and S2MRVGPPhy+Hy, respectively, showing that both population enrichment strategies were successful on improving RVGP expression. When CHX was added to cultures, the amounts of RVGP decreased probably due to degradation and translation interruption [4]. As expected, RVGPMRNA levels increased.

Conclusion: Immunomagnetic enrichment of RVGP expressing cells and hygromycin treatment showed to be efficient on increasing RVGP productivity. These methods are easy to perform and may be used for additional selection of recombinant S2 cell populations that are unable to clonal selection because of absence of growth in low cell concentrations. As these methods not statistically changed the amount of RVGPDNA copies / cell between the cell populations in study, the differences in RVGP expression could be attributed to different transcription and translation rates. For studying expression profiles, an inhibitor of translation in eukaryotes (CHX) was added to cultures for translation blockage. The amounts of accumulated RVGPMRNA showed that cell populations exhibited different profiles of transcription and translation for glycoprotein expression. While S2MRVGPPhy-M3 produced the highest level of RVGP (65.3 ng/10⁵ cells), it showed the smallest level of RVGPMRNA accumulation (R = 5.7) among all populations. As S2MRVGPPhy+Hy produced the second highest level of RVGP (45.7 ng/10⁵ cells), and showed the highest RVGPMRNA accumulation level (R = 499.4), probably cells present different rates of processing due mainly to metabolic differences. Future experiments undergoing more broadly kinetic evaluations of RVGP and RVGPMRNA may contribute do better understand these results.

Acknowledgements: Fundação para o Desenvolvimento Administrativo (FUNDAF) and Instituto Butantan.

References
Background: Spider dragline silk is considered to be the toughest biopolymer on Earth due to an extraordinary combination of strength and elasticity. With synthetic biology it is possible to express recombinant spider silk proteins, which are characterized by a highly repetitive rich glycine and alanine sequence [1]. However, production of high molecular weight spider silk protein can be difficult due to DNA instability, transcription and translation errors. Here we show, for the first time, Masp2 (105 kDa) spidroin silk protein production from the Brazilian spider Parawixia bistrata in different metabolically engineered E.coli strains.

Methods: A Masp2 monomer gene from P. bistrata was designed (DNA2.0), and a 32 mer plasmid was constructed [2]. To increase the glycyl-tRNA pool, tRNA4Gly[7] and glycyl-tRNA synthetase genes were cloned in pACYC184 plasmid [3]. All vectors were confirmed by DNA sequencing. The bacterium BL21(DE3) was co-transformed with the 32 mer and one metabolic plasmid. pACYC184 and pET19b vectors was used as controls. Cells were grown in a 2L flask culture with 1L of LB medium with antibiotics, at 37°C and 200 rpm. Silk proteins were induced with 1 mM IPTG, for four hours, and growth curves were established. Protein histag N-terminal extraction was performed under native conditions and purified by IMAC. All samples were analyzed by SDS-PAGE gels, staining with Colloid Blue and by Western blot. Dialysis was at 4°C against 10 mM Tris-HCl pH 8.0 for the first 24 hours, and water for an additional 24 hours. Samples were lyophilized and weighed. Statistical analyses were determined by ANOVA and unpaired Tukey test (ASSISTAT 7.7)

Results and conclusions: All E. coli that received Masp2 grew, expressing spider protein, and data yields are shown in table 1 (N = 3). There were no expression differences between BL21(DE3) and pACYC184, nor in the strain containing one gene copy to increase tRNA4Gly pool. This shows no interference from the initial plasmid used for bacterial metabolic stress. When the tRNA4Gly and glycine pool were overexpressed by pETgly2, pETglyWY-glyA and pETgly2-glyA, an improvement was noted in the production of 105 kDa Mass2 (P < 0.01). In similar studies, the same synergistic effect was reported for high molecular weight protein from Nephila clavipes [3]. The same authors also reported that protein sizes are directly associated with fiber quality properties. The 105 kDa Masp2 produced was able to be spun in fibers, which will be characterized in future analyses. Currently, there is no ideal heterologous organism to produce spider silk proteins, and metabolic engineering together with synthetic biology can optimize spider silk protein production. Different spider silk proteins may result in new types of protein-based biomaterials with wide applications in medicine and industry.

Acknowledgements: Embrapa Genetics Resources and Biotechnology, University of Brasilia, CNPq, CAPES, FAPDF, the NIH P41 Resource Center (EB002520), Tufts University.

References

Table 1 (abstract P231) Spider silk Massp2 protein yield in different engineered E.coli

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Means reference: mg protein/g pellet. P < 0.01; CV = 13.72%
Studies showed that the presence of extracellular IBT causes quinone depletion, reducing activity for enzymes that utilize it for their electron-carrier capability [5]. In E. coli, deletion of AcnAB-ToIC units increased IBT tolerance by reducing this quinone depletion [3]. Our study has shown that this mechanism might be similar in R. eutropha response to IBT stress.

Acknowledgements: This project is funded by the Advanced Research Projects Agency-Energy (ARPA-E). My thanks to Conseil National de Desenvolvimento Cientifico e Tecnologico (CNPq) for financing my stay in the United States through the Science Without Borders program.

References

P234
Evaluation of antioxidant activity and chemical prospecting of metabolites produced by Streptomyces hygroscopicus S75-5
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BMC Proceedings 2014, 8(Suppl 4):P234

Background: Streptomyces hygroscopicus is a species that produces a variety of antibiotics belonging to different classes. It has a distinct structure and possesses certain biological properties, such as antimicrobial and antitumor [1]. According to a study by Rong and Huang (2012) [2] more than 650 kinds of bioactive substances have been produced by Streptomyces hygroscopicus and species related to it. Members of this clade are still one of the main means of screening new substances. The aim of this study was to investigate the antioxidant activity and chemical profile of metabolites of Streptomyces hygroscopicus S75-5 obtained by fermentation in liquid and solid media. The antioxidant activity evaluation was conducted using the DPPH method [3] and chemical prospecting following the methodology described by Harborne [4]. The results showed that the extracts of fermentation in the liquid medium presented the highest percentage of antioxidant activity. Chemical prospecting also made it possible to determine the presence of phenolic compounds in the ethyl acetate extract of the metabolic liquid by fermentation in a liquid medium.

Methods: Streptomyces hygroscopicus S75-5 was grown in an ISP-2 medium liquid for 48 hours (30 °C / 120 rpm) and inoculated into a liquid medium (soybean meal) for 96 hours (30 °C / 120 rpm) and a solid medium (parboiled rice) for 21 days (30°C). After cultured in a liquid medium, the biomass was separated from the liquid metabolic and extracted with methanol. The metabolic liquid was extracted with ethyl acetate. Metanol was also added to the solid medium. The antioxidant activity evaluation was conducted using of the methodology of sequestration free radical 2,2-diphenyl-1-picrylhydrazyl [3], where 80 \% of the extracts and the standard solution, quercetin, were added to 500 \mu L of a DPPH solution (44 \mu g/mL). After 30 minutes, the absorbance was read in a UV-Vis spectrophotometer at 517 nm. Each sample was tested in triplicate and the activity expressed in percentages according to the equation: AA% = 100 - ([(Abs extract - Abs blank) / 100]) / (Abs control).

Results and conclusions: In the antioxidant activity, the methanolic biomass extracts, ethyl acetate of the metabolic liquid and methanolic of the solid fermentation showed, respectively, 62.2%; 76.9% and 49.1% capability to sequester the free radical, respectively. The ethyl acetate extracts of the oil liquid from fermentation in a liquid medium was responsible for the highest percentage of antioxidant activity, making it possible to detect the presence of alkaloids, reducing sugars, phenolics compounds, flavonoids, tannins, triterpenes and steroids, using revealing specific [4].

Acknowledgements: The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amaro a Ciência e Tecnologia de Pernambuco (FACEPE) for financial support.

References


**P235**

Perception of 3rd year high school students of two schools in Dourados-MS about biotechnology

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BMC Proceedings 2014, 8(Suppl 4):P235

**Background:** It is known that biotechnology is present in all sectors of contemporary life. Still, it is quite common the divulgation of mis-conceptions about it, which leads to insecurity and prejudice by the part of society. Thus, to demystify and disseminate biotechnology as science, have been developed by students of Biotechnology from Federal University of Grande Dourados (UFGD), the extension project “Biotechnology for All”. Through expository lectures the academics seek to elucidate, clarify doubts and stimulate the interest of students of the 3rd year of high school for this science. So, This study was conducted to evaluate the perception of students of the 3rd year of high school in two schools of Dourados-M5, on topics related to Biotechnology. As a result, it was possible to assess the similarities and differences between the data collected in both institutions.

**Methods:** We developed a questionnaire with ten questions addressing general knowledge on topics related to biotechnology, that were applied on students of the 3rd year of high school at a private school and in a public school in Dourados-M5. The evaluation was conducted based on questionnaires completed by 48 students, being 22 private schools (45.8%) and 26 of public (54.2%).

**Results and conclusions:** All public school students said they had had contact with biotechnology for a vehicle of communication, and television was identified as the main source of information on the subject for these students. Among students of private education, 20% reported never having had contact with biotechnology by any means of communication. The internet was identified as the main source of information about biotechnology by these students. When asked about the importance of biotechnology to society, 76.5% of respondents said that they considered important but could not explain why, and less than half of the respondents were able to correctly define the meaning of “biotechnology”. Respondents were asked to mark among several options the topics that were believed to be related to biotechnology: 86% of public school students and 80% of private school students indicated the correct alternatives. People often mistakenly relate areas such as robotics and computing to biotechnology, probably due to the suffix “technology”. At the same time, concepts like transgenics, stem cells and cloning were correctly marked by the most students probably by the large diffusion of these issues on the media. It was found that 75% of students said they knew the meaning of transgenics, although only 39% cited examples such as soybean, oil, and “biscuits”. Regarding the acronym GMO (genetically modified organism) 90% of private school students reported not knowing their significance, and 65% of public school students said they knew the meaning of GMOs, but they were not able to cite examples. Most students reported owning a detached position in relation to GMOs and 25% could not answer because they have not enough knowledge about it. In general, respondents showed no basic knowledge about biotechnology, so the project “Biotechnology for All” is an important source of dissemination of this science to society.

**Acknowledgements:** To Pro-Rector of Extension and Culture (PROEX) of UFGD.

**P236**

Molecular characterization of strains of *Zymomonas mobilis* by sequencing the 16S ribosomal

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BMC Proceedings 2014, 8(Suppl 4):P236

**Background:** *Zymomonas mobilis* has attracted great interest in the scientific, industrial and biotechnological due to its high potential fermentation. From the viewpoint of taxonomic *Z. mobilis* is the only species of the genus *Zymomonas*, and is subdivided into three subspecies: *Z. mobilis* subsp. *mobilis*, *Z. mobilis* subsp. *pomaceae* and *Z. mobilis* subs. *franciensis*. Differentiation between these three species is based on physiological tests. These tests are time consuming and often unreliable. Therefore, molecular techniques are proposed as a quick and reliable way to characterize the genetic variability of these bacteria. This study aimed to perform molecular characterization of 6 strains of *Zymomonas mobilis* deposited in the Collection of Microorganisms of Department of Antibiotics, Federal University of Pernambuco (UFPEDA).

**Methods:** The strains were grown in SSDL for 24 hours at 30 °C, followed by centrifugation and extraction of chromosomal DNA. PCR reactions were performed using specific primers and conditions for amplification of the 16S rDNA. The products of the amplified 16S rDNA were purified and sequenced using a ABI PRISM® 3500 Genetic Analyzer (Applied Biosystems) at the Sequencing Platform LABCEN/CCB in the Universidade Federal de Pernambuco (Recife, Brazil). The data obtained by sequencing the 16S rDNA were analyzed and compared by BLASTn programs and aligned by MultiAlin.

**Results and conclusion:** The sequences obtained were different degrees of similarity to strains from institutional collections. Multiple alignment of the sequences of the 16S rDNA of strains of *Z. mobilis* UFPEDA gene regions revealed a high degree of conservation. Sequence analysis of the 16S rDNA confirmed that all the strains belong to the species *Zymomonas mobilis*. However, it was not possible to differentiate the level of subspecies. From the results obtained by multiple alignment of the sequences was possible to prove the stability and degree of conservation of all lineages. Based on these results, other phylogenetic markers should be used to better characterize the genetic variability of strains of *Z. mobilis* deposited at the collection UFPEDA.

**Acknowledgements:** The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support and the Sequencing Platform LABCEN/CCB in the Universidade Federal de Pernambuco (Recife, Brazil).

**P237**

In vitro antioxidant activity of alkaloids from southern Brazilian Psychotria: a comparative analysis

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BMC Proceedings 2014, 8(Suppl 4):P237

**Background:** Monoterpenoid alkaloids (MIAs) comprise a class of secondary metabolites of dual biosynthetic origin, characterized by the presence of an indole and a terpene portion. MIAs are widely studied because of their bioactive properties and pharmacological potential. In this study, major MIAs from native species of *Psychotria* from southern Brazil were used: *P. brachyceras*, *P. umbellata* and *P. leiocarpaa*. The same habitat and accumulate the alkaloids brachycerase, psychollatine and N-D-glucopyranosyl vincosamide, respectively. The three MIAs are related and have structural similarities. Previous work identified antioxidant properties of these alkaloids and induction of their accumulation by oxidative stress, suggesting that they play roles as antioxidant compounds, something regarded as unusual for this metabolite class [1]. MIAs may have a role on the control of oxidative burst generated by stress, aiming at reducing the reactive oxygen species' harmful effects and promoting the maintenance of plant fitness. Herein, the antioxidant potential of the three alkaloids against superoxide anions and singlet oxygen is comparatively evaluated, and putative structure-activity relations are considered.

**Methods:** The antioxidant capacities were evaluated according to previously established protocols. Quenching activity against superoxide anions was measured by Nitro Blue Tetrazolium (NBT) reduction (solution coloration turns blue) by spectrophotometry at 560nm [2]. The singlet oxygen quenching was assessed by rubrene color decay monitored by spectrophotometry at 440nm [3]. The alkaloids were tested in equimolar concentrations (5mM for superoxide and 2mM for singlet oxygen). The positive control was Trolox® (vitamin E synthetic analogue) and the negative control was performed by addition of the solvent solution, only.

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comparison was also done with the flavonoid rutin, a well known natural antioxidant phenolic, at the same concentration. Antioxidant capacity is presented in percentage of reduced NBT, in IC50 of antioxidant activity (alkaloid concentration able to mitigate 50% of ROS) and by rubrene color decay.

Results: Superoxide anions: The alkaloids GPV and brachycerine decreased NBT reduction rate under to 10%, result comparable to the positive control (Trolox®), which decreased to 15%. Psychollatine brought reduction down to 27%. The reaction without the quenchers achieved 90% of NBT reduction. The alkaloids quencher activity of superoxide was comparable to that of the flavonoid rutin. IC50 activity against superoxide yielded the following results: GPV was able to mitigate 50% of ROS at the lowest concentration, followed by brachycerine and psychollatine (0.0475mM, 0.0624mM and 0.0637mM, respectively). These values were higher than but comparable to those of rutin (0.0224mM). Singlet oxygen: quenching activity was similar for GPV, psychollatine and Trolox®; observable by the lower decay of rubrene color. Brachycerine was not so efficient, but still better than the negative control.

Conclusions: Structural groups such as rings, double bonds, amines and glycosylations affect the efficiency of antioxidant activity, GPV appeared to have the best antioxidant activity in both assays, probably as a result of the contribution of a tertiary amine and additional glycosylation for combating ROS. Despite the significant structure similarity between brachycerine and psychollatine, these metabolites did exhibit specificity in ROS mitigation: the first was more efficient against superoxide anions and the second, against singlet oxygen.

Acknowledgements: Research was supported by grants from National Council for Scientific and Technological Development (CNPq-Brazil) and Propesq-UFRGS.

References

P238 Isolation and characterization of a lectin from Andira anthelmia seeds Cleane Moreira 1, Mayara Silva 1, Camila Nobre 2, Thaiz Miguel 2, Antonia Nascimento 2, Celso Nagana 2, Kyria Nascimento 2, Benildo Cavada 2, Ivancie Silva 1
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BMC Proceedings 2014, 8(Suppl 4):P238

Andira anthelmia seeds, a species belonging to the Leguminosae family, Papilionoideae subfamily, Dalbergieae tribe, have a glucose/mannose specific lectin that agglutinates rabbit erythrocytes treated with trypsin. The Dalbergieae tribe has lectins which have specificity for different carbohydrates including mannose and also have several biological activities such as induction of rat paw edema, release of chemotactic mediators by macrophages, vasorelaxant effect in rat aortas, termitecidic action, potential fungicidal action, among others. This study aimed to isolate, purify and physicochemically characterize a lectin found in seeds of Andira anthelmia. The lectin from Andira anthelmia seeds was purified by affinity chromatography on Mannose-Sepharose matrix followed by ion exchange chromatography on DEAE-Sephacel matrix. This procedure resulted in a purified lectin, named AAL. AAL purification process was monitored by specific hemaggulinating activity and SDS-PAGE, in which it was observed that this lectin has a molecular weight of approximately 20 kDa and four others subunits of approximately 15 and 14 kDa. This lectin is a glycoprotein with approximately 1.89% of carbohydrates on its composition and shows high stability, being able to maintain their hemaggulinating activity in a wide pH range and after exposure to temperatures of 70 °C for one hour. After dialysis against the chelating agent EDTA, AAL lost its hemaggulinating activity, but recovered its action after the addition of metals, being, therefore, dependent on divalent metal cations. In this study a new lectin from Dalbergieae was purified and characterized. Further analyzes are needed in order to best evaluate their biotechnological applications.

P239 Cloning and expression of lipase gene from Ralstonia eutropha in Escherichia coli
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BMC Proceedings 2014, 8(Suppl 4):P239

Background: Lipases are enzymes belonging to the class of hydrolases, acting in the aqueous-organic interface demonstrating considerable levels of activity and stability in aqueous environments and non-aqueous [1]. Its biological function is to catalyze the hydrolysis of long chain triglycerides to form fatty acids and glycerol, in addition to performing esterification [2]. They have wide industrial applicability and can be used in the composition of detergents, in food industry, in wastewater treatment and fine chemistry [3]. The goal this work was clone and express lipase gene from Ralstonia eutropha in Escherichia coli BL21DE3.

Methods: The lipase gene was isolated by polymerase chain reaction from a metagenomic library of Terra Preta de Indio of Amazon soil previously constructed in our laboratory. The Lip5 fosmid containing lipase gene Ralstonia eutropha was used as template and PCR was performed using Lip5 primers forward and reverse. The amplicons were inserted into cloning vector pCR4 TOPO, multiplied in E. coli. The fragment was released by cleavage with restriction enzyme EcoRI / NotI and subcloned into intracellular expression pGSM vector. E. coli BL21DE3 was transformed with recombinant vector and positive transformants were selected by halos formation around colonies in LB solid medium containing 2% Olive Oil as lipase substrate. Recombinant clones are inoculated in LB liquid medium, the culture was incubated with shaking at 37°C until the OD600 reached 0.5. IPTG was then added to a final concentration of 1 mM. Cells were collected 8 h after IPTG addition for protein detection by SDS-PAGE.

Results: A fragment 1200 bp was amplified of Lip5 fosmid and subcloned into intracellular expression pGSM vector. The recombinant plasmid was transformed into E. coli BL21DE3. Expression of the lipase gene was induced by IPTG. Translucence degradations halos were observed in solid medium indicating the production of lipase. SDS-PAGE analysis revealed the presence of protein in the supernatant obtained after sonication treatment. The protein had an approximate molecular weight of 39 kDa. We are performing the purification and characterization of recombinant lipase expressed in bacteria and proceeding the cloning in Pichia pastoris yeast.

Acknowledgements: This study was supported by the Conselho Nacional de Pesquisa and Desenvolvimento Tecnológico (CNPq-scholarship), Centro de Apoio Multidisciplinar da Universidade Federal do Amazonas (CAM/UFAM).

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P240 Identification and proteome analysis of petroleum degrading bacteria Júlio Nino Souza-Neto 1, Solange Araújo 1, Ieda Batista 2, José Pereira 1, Edmar Andrade 1
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BMC Proceedings 2014, 8(Suppl 4):P240

Background: The natural environment has suffered high accumulations of pollutants which affect not only the soil, groundwater, rivers, lakes, rainwater routes and air, but also human health due to the formation of unhealthy conditions. Excessive use of pesticides, crude oil spills, discharges of industrial waste, among other xenobiotic compounds accumulate in the
environment and become worrisome for its persistence and its harmful effects to life. Therefore, bioremediation becomes an option for solving this problem, which is a tool that uses microorganisms to biochemical pollutants degradation compounds, transforming them into less or non-toxic substances. In this context, this work presents the results of identification of bacteria that degrade crude oil, as well as some results of a proteomic analysis of isolates grown in the presence of that pollutant to evaluate the profile of protein expression.

Methods: Bacterial strains previously isolated from macrophyte Eichhornia sp., Cyperus sp. and Hymenachne sp., the outputs of Petrobras refinery influent / Nanuau-AM (REMAN), were identified by phenotypic analysis and sequencing and comparison of the 16S gene region to identify the species level using the BLAST. Qualitative assays of degradation crude oil were performed for all strains identified using minimum medium and petroleum (1.3%) as carbon source, followed by sample preservation. The sample which had the most degrading activity was selected to proteomic investigation. Growth curve, extraction and quantification of protein content, followed by two-dimensional electrophoresis analysis, were determined for bacteria cultured in presence of 1.3% crude oil or 0.1% yeast extract as carbon source. All assays were performed using proteins extracted at the end of the exponential phase (24h for growth crude oil and 12h for growth yeast extract). The analysis of the spots was made using Image Master Platinum version 6.0 (GE Healthcare) according to the saliency and smooth parameters, which were equal to 100 and 2, respectively. Proteomic profiles of bacteria cultured in presence of petroleum and yeast extract were compared each other to identify proteins related to crude oil degradation.

Results and conclusions: Seven samples with the potential for bacterial degradation of crude oil were identified, which are Pseudomonas aeruginosa (SB41), Lysinibacillus fusiformes (SB63 and SB121), Acinetobacter junii (SB102 and SB132) and Bacillus pumilus (SB123 and SB139). The species identified showed redundancy and the molecular results were corroborated by phenotype ones. The sample identified as A. junii SB132 was used in proteomic analysis which preliminary profiles had been obtained. The number spots identified was 178 and 124 for bacteria cultured in presence of petroleum or yeast extract, respectively. Compared each other protein content for both conditions presented 36% similarity. These data show that the protein profile is different and there is differential expression between the two conditions. Investigation of proteomic profile of other bacterium sample and identification of proteins involved in crude oil degradation are in progress.

Acknowledgements: This study was supported by CNPq, CAPES and FAPEAM.

P242
Cloning of oxidosqualene cyclases from Maytenus ilicifolia for synthetic biology
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BMC Proceedings 2014, 8(Suppl 4):P242

Introduction: Pentacyclic triterpenes are secondary metabolites which are promising molecules in the pharmaceutical field and food and material industries. These compounds are formed by the cyclization of oxidosqualene catalyzed by oxidosqualene cyclases (OSC) [1]. The aim of this study is to clone the OSC genes from the leaves of Maytenus ilicifolia, a medicinal species from Brazil.

Material and methods: For this purpose, we adopted the strategy of RT-PCR using degenerated primers obtained from sequences of known plant OSC genes. Total RNA was extracted from leaves of M. ilicifolia and used in the synthesis of cDNA. The PCR amplification of core fragments of OSC genes was performed with partially degenerated primers designed to anneal to highly conserved regions among OSC genes. The cloned fragments were sequenced and specific primers were designed for the rapid amplification of cDNA end (RACE) of 3' regions and for a first round amplification of 5' extension. After sequencing, another set of specific primers were generated for the RACE of 5' regions and for a first round amplification of the full-length cDNA of the OSCs and confirm the predicted identity of the genes by sequencing [2].

Results and discussion: In silico assembly and sequencing of the full-length cDNA covered the ORF of two main different groups of OSCs with ~2500 bp. Multiple alignment of these genes showed identity of about 50% between those two groups. Comparison of M. ilicifolia OSC genes with those described in the Genebank revealed that one group showed high identity (~90%) with cyclartenol synthase enzymes, while the other one showed high identity (>75%) with triterpene synthases as beta-amyrin and lupeol synthases.

Conclusions: Cloning of OSC genes from the leaves of M. ilicifolia demonstrated two main groups of OSC enzymes present: the cyclartenol synthase, which is part of the plant primary metabolism [3] and one triterpene synthase, which may be part of the secondary metabolism [3]. Future work using functional expression of these cloned genes in Saccharomyces cerevisiae will further characterize the oxidosqualene cyclases from M. ilicifolia leaves.

References

P241
Development of sensitized solar cells by photosynthetic pigments
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BMC Proceedings 2014, 8(Suppl 4):P242

Background: Grätzel solar cell, also known as dye-sensitized solar cell (DSSC), is a dye-sensitized nanocrystalline photovoltaic cell of TiO2 (titanium dioxide). It has a surface of nanoporous TiO2, a semiconductor of a wide bandgap. The basic principle is to transform light energy into electrical energy. When a dye molecule absorbs light, an electron is replaced by an excited state, and this dye can jump to the conduction band of TiO2. In the composite electrode, the electron diffuses from this TiO2 to the conductive glass. From there, the electron is taken to the electrode through a conductor. Similar to this process, the plant cell transforms the light into energy using photosynthetic pigments for absorption and many other mechanisms for the transfer and storage of energy, by a process known as photosynthesis. Observing and analyzing these methods, our objective was to use some photosynthetic pigments found in plants in Grätzel solar cells to evaluate the potential use of these pigments in the production of solar panels.

Methods: The extraction of pigments was performed of 4 algae: Codium tomentosum (CT), Ulva lactuca (UL), Afneltia plicata (AP), Pterosiphonia complanata (PC); 2 fruits: Rubus ulmifolius (AN), Prunus spinosa (AN); and spinach leaves: Spinacia oleracea (CL), using 100% acetone in the case of algae and leaves (extraction of chlorophyll, carotenoids and xanthophylls) and 0.1%-2HCl (pH=2) in ethanol in the case of the fruits (extraction of anthocyanins). A TLC was performed with a filter paper using a mixture of hexane:aceton (7:3), an absorbance reading of the extracts for the separation and a verification of pigments found in each sample. Subsequently, DSSCs were constructed. Was added a TiO2 (10%) layer in the conductive glass, heated at 450°C to attach to the glass layer. Then, submerged in the pigments solutions for 24 hours. After that, another conductive glass scratched with graffiti was arranged and closed up with tweezers. A few drops of solution iodine/iodide were added. The DSSCs were connected in a multimeter and were putted in a solar simulator, with the objective of measuring the voltage when stimulated and unstimulated by light in different time periods.

Results and conclusions: Analyzing the samples voltage in the presence and absence of light, it can be concluded that the voltage produced in the absence of light is insignificant when compared to the voltage at light. Of all samples measured, the ones that showed the best results in relation of the voltage and stability over a certain period of time were those with a higher proportion of chlorophyll: UL and AP. CL also showed elevated
values, though quite ranged over the days, which may be due the degradation of the pigment. PC and AN had high stability but very low voltage. ANM showed irregular and low results. Continuing with the samples for several days, it might be concluded that the cells with pigments of AP and UL remain in operation longer than expected, and UL, moreover, maintains stability. Thus, the use of photosynthetic pigments for the solar cells production from this study is feasible.

Acknowledgements: Thanks to ITMA, UniOvi, CNPq and the program "Science without Borders".

P243
Plant biotechnology: use of tissue culture techniques in species Boerhavia paniculata Rich and Crinum americanum L as alternative for the production of new drugs in vitro
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Background: Crinum americanum L. popularly known as lily is a ornamental plant that belongs to the family Amaryllidaceae alkaloids possessing anti-inflammatory, antimicrobial and inhibition of cholinesterase. The species Boerhavia paniculata is a plant from the family Nyctaginaceae popularly known as "Pega Pinto" that produces isoflavones that have action antiinflamma
tory, urinary tract and with its concentration in the roots. Aimed at the production and accumulation of these substances in vitro, this work aimed to study the induction of roots of the species Boerhavia paniculata Rich and callus induction of the species Crinum americanum L through the techniques of tissue culture protocols developed cell culture for production of these metabolites and evaluate the qualitative HPTLC by these cells [1,2,5].

Methods: For callus induction species Crinum americanum L bulbs explants were cultured on MS medium supplemented with 3% sucrose, 0.8% agar, pH adjusted to 5.8 and 2.26 μM of 2,4-D. The explants were used in a chamber (BOD Eletrolab) for 30 days in the dark at 24°C, after which the explants subcultures were performed in mesams conditions, totaling three subcultures. Boerhavia paniculata Rich seed species were germinated on rich medium containing 1.5% glucose, 0.8% agar in the counter 28°C. The roots of seedlings grown in vitro were inoculated in liquid MS medium containing 3% glucose, 2.46 μM of IBA and pH 5.8. The vials were kept in partial light at a temperature of 28°C ± 2°C. For qualitative analysis of the chemical profile of the samples was evaluated in vitro comparisons by HPTLC with fresh samples to assess the production potential of the metabolites of interest through the visualization of bands in UV irradiation chamber at wavelengths 365-254 nm and after development reagent specific classes of metabolites: alkaloids and Dragendorff to vanillin specificity of the presence of these substances. They applied approximately 5 μL of hexane extracts samples from the chromatoplates Boerhavia paniculata Rich and eluted in 20 mL of Acetate / Hexane (9:1). For samples Crinum americanum L 5 μL was used and eluted with methanolic extract in a system chloroform/ methanol (4:1), all tests were conducted at a temperature of 25°C [2-4].

Results and conclusion: The results showed that roots of Boerhavia paniculata Rich obtained in vitro showed satisfactory results with IBA 2.46 μM for induction of roots. For the species Crinum americanum L callus induction MS medium containing 2.26 μM of 2,4-D showed induction potential in these culture conditions. From a qualitative HPTLC chromatographic profile of the samples in vitroBoerhavia paniculata Rich and Crinum americanum L was possible to assert that the objective was achieved, where the plates containing the spots observed at 365-254 nm and after development, suggest the presence of possible chemical constituents of the class of alkaloids and isoflavonoids.

Acknowledgements: Amazon Foundation for Research Support Para, FAPESP.

References

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Evaluation of process parameters in the production of jasmonic acid
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Introduction: The term jasmonates is used to describe lipid derivatives synthesized via octadecanoid pathway and are mainly represented by jasmonic acid (JA) and its ester methyl jasmonate (MJ), in plants, jasmonates act in the defense mechanism [1], and recent reports have attributed to JA and MJ the ability to inhibit the growth of human cancer cells, yet having cytotoxic selectivity [2]. Due to the diverse biological activities related to jasmonates, studies have been conducted to optimize the bioproduction route of these molecules, and the use of strains of Botrysphaeria rhodina seems to be a viable alternative when compared to the extraction from plants [3]. In order to optimize the production process, some of the important parameters must be standardized and controlled, such as the microorganism strain, the culture medium composition and the operational features of the process [4]. This study aimed to evaluate the influence of the inoculum size, medium supplementation with tryptone, the fermentation time and the interaction among these factors on the jasmonates production by the fungus Botrysphaeria rhodina.

Methodology: The fermentations were performed using M2 culture medium with and without the addition of 5 g/L of tryptone. The volume of inoculum was 5 or 20 mL of homogenate (OD = 0.5 at λ = 700 nm), with average fermentation times of 5 and 10 days. Fermentations were conducted in the dark at 30°C under static conditions. The experiments were performed following a full 24 factorial design and performed in duplicate. For quantification of jasmonates produced at the end of the fermentation period, the fermented samples were recovered by vacuum filtration, the pH was adjusted to 3.0 with 4M HCl and then the liquid subjected to extraction with ethyl acetate. The jasmonates quantification was performed according to [1]. Based on AJ concentrations of each treatment, the variance of effects was calculated and T-test was applied at 5% significance level.

Results and conclusion: From the measurements of JA in the fermented broth, the effects of each factor and their interactions were calculated. It was possible to verify that the JA production was significantly influenced (at 5% level) by the fermentation time, inoculum size, and the interaction between inoculum size and tryptone, so that the elevation of 5 to 20 mL of inoculum resulted in a lower production of JA. The inoculum size factor presented a negative effect, namely, an increase of 5 to 20 mL of inoculum resulted in a lower production during JA fermentation. The factor medium supplementation with tryptone showed no significant effect; however, there was a negative interaction between time and tryptone, ie, the addition of tryptone along time resulted in a lower production of JA. Thus the highest production of JA by the fungus B. rhodina under the tested conditions was 421.5 mg/L using the M2 culture medium without supplementation with tryptone, using 5 mL of homogenate for 10 days of fermentation.

Acknowledgements: FAPESP - São Paulo State Research Foundation, UNAERP - University of Ribeirão Preto.

References
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Effect of carbon source on lipase production by Aeromonas sp. isolated from dairy effluent
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BMC Proceedings 2014, 8(Suppl 4):P245

Background: Lipids are one of the major pollutants in domestic and industrial effluents. The use of lipases in the treatment of these effluents as well as in the bioremediation of contaminated environments represents an environmentally safe alternative to chemical methods [1]. Lipases (EC 3.1.1.3) are carboxylesterases that catalyze synthesis and hydrolysis of long-chain acylglycerols (>10 carbons) and have great potential for industrial and biotechnological applications. Microorganisms are the major source for lipases and have advantages such as ease production and diversified enzymatic properties [2]. Effluents containing high concentrations of lipids represent a good source for the isolation of lipase producing microorganisms and dairy industries are responsible for production of large quantities of this of this kind of effluent [3]. In a previous study two lipase producing microorganisms were isolated from dairy effluents. This report presents the results of lipase production by these microorganisms in different carbon sources.

Methods: Isolates LODO 9 and LODO 10 were cultured overnight in DYG’s media (28°C, 200 rpm) and genomic DNA was extracted using AxyPrep™ Bacterial Genomic DNA MiniPrep Kit (Axygen Biosciences) according to manufacturer recommendation. The 16S rDNA was amplified from chromosomal DNA using primers F1 (AGAGTTTGATCCTGGCTCAG) and R1 (AAGGAGGTGATCCAGCC) for Escherichia coli K-12 [4]. The 16S rDNA gene sequences obtained were compared with sequences of other Aeromonas deposited in the GenBank database by using ClustALW program and a consensus neighbor-joining tree was constructed using Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0 [5]. Isolates were grown (30°C, 200 rpm, 48 h) in minimal medium (30 ml) containing (g/L): NaN3 (4.0), KH2PO4 (1.5) FeCl3 (0.05), MgSO4 (0.2), CaCl2 (0.01), Na2HPO4 (0.5), yeast extract (0.05) and carbon source (10). Each carbon source (glucose, sucrose, lactose, cellobiose, xylose, glycerol, soybean oil, engine oil, diesel and gasoline) was tested separately from the other. The carbon source with best result was tested at concentrations of 0 to 250 g/L. The growth was analyzed by optical density at 600 nm and lipase activity by hydrolysis of pNPB (37°C, 10 min, 410 nm). A lipase unit (U) was defined as 1 mmol of pNP released per minute per ml supernatant under assay conditions.

Results and conclusions: Isolates were identified as Aeromonas sp. (Aeromonas sp. LODO 9 and Aeromonas sp. LODO 10) and phylogenetic analysis grouped both isolates together, closely to Aeromonas punctata ATCC 13468. Lipase activity was detected only in cultures containing sucrose, cellobiose, glycerol, and soybean oil. The latter showed the best results with 95.02 and 87.80 U for Aeromonas sp. LODO 9 and Aeromonas sp. LODO 10, respectively. Both isolates reached the maximum lipase activity with 50 g/L of soybean oil. At this concentration of soybean oil, biomass and lipase activity of Aeromonas sp. LODO 9 were 6.38 mg/mL and 19.15 U, respectively. At this same condition, biomass and lipase activity of Aeromonas sp. LODO 10 were 8.55 mg/mL and 202.22 U, respectively.

Acknowledgments: CAPES/PNPD

References

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Polyhydroxybutyrate production by a sugarcane growth promoter bacterium
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BMC Proceedings 2014, 8(Suppl 4):P246

Background: The plastics derived from petrochemicals have many applications. However, public concern and environmental laws led to conservation policies and establish programs that stimulate the research and use of new products based on renewable resources. Polyhydroxyalkanoates (PHAs) are biopolymers synthesized by microorganisms under conditions of nutrient stress with similar properties to conventional plastics and are completely biodegradable. One of the PHAs is polyhydroxybutyrate (PHB), which accumulated in several bacteria [1]. Gluconacetobacter diazotrophicus is a plant-growth-promoting bacterium, which interacts and lives within sugarcane plants [2]. Genome annotation of this bacterium offers a new scenario to better understand plant-bacteria interactions and reveals novel potential bioproducts. Sugarcane is cultivated mostly for sugar and fuel ethanol production. In this work, we propose the production of biodegradable plastics from sugarcane coupled to its natural partner growth promoter bacterium, G. diazotrophicus.

Methods: An ORF encoding a conserved hypothetical protein sharing approximately 53% identity with the PHB synthase from Acidiphilium multivorans was identified within G. diazotrophicus genomic sequence. This putative G. diazotrophicus PHB synthase gene, consisted of a 987 bp (encoding 328 residues), with a molecular mass of approximately 35 kDa. This PHB synthase was analyzed for structural motifs by scanning against PROSITE patterns and profiles (http://www.expasy.org/prosite) and against Pfam (http://pfam.sanger.ac.uk) to certify about the presence of domains associated with PHB. A multiple alignment analysis was performed using CLUSTALW and the phylogenetic trees were generated using MEGA based on the maximum likelihood (ML) method and the topology of the trees was evaluated by bootstrap analysis on the basis of 1,000 replications.

Results and conclusions: Analysis of the G. diazotrophicus genome sequence for putative PHB polymerase gene resulted in the identification of a protein with a polyR/-hydroxyalkanoic acid (PHA) synthase domain, class III, that represents the Phac subunit of a heterodimeric form of the polyhydroxyalkanoic acid synthase, which links D-(-)-3-hydroxybutyryl-CoA to an existing PHA molecule by the formation of an ester bond. A deeper analysis revealed that G. diazotrophicus PHB polymerase and a putative polyhydroxyalkanoate synthesis repressor (PhaR) are clustered with hypothetical proteins, a hydrolyase and an acetocetyl-CoA reductase, suggesting they are gathered functionally, maybe as operon. The PHA synthase appear to require PhaR for activity in vivo and in vitro [3]. This PhaR exhibits the PHB/PHA accumulation regulatory DNA-binding domain, that binds short chain hydroxyalkanoic acids and PHA granules, and may regulate the expression of itself, of the phasins that coat granules, and enzymes that direct carbon flux into polymers stored in granules [4]. Interestingly, the phylogenetic analysis revealed that G. diazotrophicus PHB forms a cluster with other relevant PHB enzymes with intriguing metabolic activities. Although additional approaches are still needed for reach production levels, these data show that G. diazotrophicus has potential for producing PHB and be employed in the bioplastic production, integrated to the sugarcane industry.

Acknowledgments: FAPERJ and UEZO.

References
P247
Cloning of a synthetic chimeric gene containing recombinant Mycoplasma hyopneumoniae antigens for expression in Pichia pastoris
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BMC Proceedings 2014, 8(Suppl 4):P247

Background: Mycoplasma hyopneumoniae is the primary etiologic agent of Swine Enzootic Pneumonia (EP), one of the most common respiratory disease that affects swine worldwide, causing significant economic losses. Vaccination constitute one of the main practices to control EP. The production of recombinant experimental vaccines against EP has been considered an important approach towards the development of an improved vaccine [1]. This study aimed to clone a synthetic gene composed by the fusion of M. hyopneumoniae antigens R1 (P97), P42 and NrdF to Escherichia coli fragment B of the heat labile enterotoxin (LTB) into the vectors pPICZB (intracellular expression) and pPICZaB (secreted expression), in order to produce this chimeric recombinant protein in Pichia pastoris and to test it as a vaccine candidate. The M. hyopneumoniae antigens were selected due to their capacity to confer partial protection in pigs when evaluated individually [2-4]. These antigens were associated to LTB, a potent mucosal and parenteral adjuvant.

Methods: The DNA fragment coding for the recombinant chimeric protein was designed in silico [5]. The synthetic gene and the vectors pPICZaB and pPICZB were digested with the enzymes BamHI and EcoRI and ligated. The ligation product was transformed into E. coli TOP10 strain, plated on Luria - Bertani (LB) culture medium containing the antibiotic Zeocin™ (Invitrogen). The resulting colonies were selected through a rapid screening method using a protocol with phenol - chloroform. Recombinant clones were expanded in LB liquid medium with antibiotic, the plasmid was extracted and then characterized with restriction enzymes BamHI and EcoRI.

Results and conclusions: The synthetic gene coding for the chimeric protein, composed by the fusion of M. hyopneumoniae antigens R1 (P97), P42, NrdF and to LTB were efficiently cloned into the two expression vectors pPICZB (intracellular expression) and pPICZaB (secreted expression), in order to produce this chimeric recombinant protein in Pichia pastoris and to test it as a vaccine candidate. The M. hyopneumoniae antigens were selected due to their capacity to confer partial protection in pigs when evaluated individually [2-4]. These antigens were associated to LTB, a potent mucosal and parenteral adjuvant.

Acknowledgements: MAPA, CNPq, CAPES and FAPERGS.

References

P248
Cloning of oxidosqualene cyclases from Maytenus ilicifolia for synthetic biology
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BMC Proceedings 2014, 8(Suppl 4):P248

Introduction: Pentacyclic triterpenes are secondary metabolites which are promising molecules in the pharmaceutical field and food and material industries. These compounds are formed by the cyclization of oxidosqualene catalyzed by oxidosqualene cyclases (OSC) [1]. The aim of this study is to clone the OSC genes from the leaves of Maytenus ilicifolia, a medicinal species from Brazil.

Material and methods: For this purpose, we adopted the strategy of RT-PCR using degenerated primers obtained from sequences of known plant OSC genes. Total RNA was extracted from leaves of M. ilicifolia and used in the synthesis of cDNA. The PCR amplification of core fragments of OSC genes was performed with partially degenerated primers designed to anneal to highly conserved regions among OSC genes. The cloned fragments were sequenced and specific primers were designed for the rapid amplification of cDNA and (RACE) of 3' regions and for a first round amplification of 5' extension. After sequencing, another set of specific primers were generated for the RACE of 5' regions. Finally, it was possible to clone the full-length cDNA of the OSCs and confirm the predicted identity of the genes by sequencing [2].

Results and discussion: In silico assembly and sequencing of the full-length cDNA covered the ORF of two main different groups of OSCs with ~2500 bp. Multiple alignment of these genes showed identity of about 50% between these two groups. Comparison of M. ilicifoliaOSC genes with those described in the Genebank revealed that one group showed high identity (~90%) with cycloartenol synthase enzymes, while the other one showed high identity (>75%) with triterpene synthases as beta-amyrin and lupeol synthases.

Conclusions: Cloning of OSC genes from the leaves of M. ilicifolia demonstrated two main groups of OSC enzymes present: the cycloartenol synthase, which is part of the plant primary metabolism and one triterpene synthase, which may be part of the secondary metabolism [3]. Future work using functional expression of these cloned genes in Saccharomyces cerevisiae will further characterize the oxidosqualene cyclases from M. ilicifolia leaves.

Acknowledgements: PADC-UNESP, CNPq and FAPESP.

References

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Gold nanoparticles synthesis to application as nano biosensors
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BMC Proceedings 2014, 8(Suppl 4):P249

Background: The particularities of gold nanoparticles (AuNPs) have stimulated different areas of research in recent years. The exploitation of...
the optical properties, electronic and magnetic materials has allowed these his job in different fields of application, such as the construction of biosensors in gradual release system for drugs, lubricants, solar cells, catalysis and other [1].

The properties of the nanoparticles are dependent on the size and shape of the particles. The same material composition can be determined with different physical and chemical characteristics just modifying characteristics such as size, self-organization, crystalline structure and shape, the point of the material in the nanometer range show distinct physical and chemical properties of materials on the macroscopic scale [2].

The aim of this work was to optimize the synthesis of gold nanoparticles using the reduction method sodium citrate, assessing concentrations of this reducing agent and the synthesis time. The attempt is to develop a route to generate nanoparticles from a precursor and facilitate the use of these nanomaterials in biosensors.

Methods: To check the effect of variables on conversion of the reaction, as well as finding the conditions that maximized the synthesis of nanoparticles, one factorial design (3²) with 3 levels and 2 variables was performed. The variables evaluated were: Concentration of Reducing Agent (1.0, 2.0 and 3.0%) and the time synthesis (5, 10 and 15 min). These intervals were defined to cover most of the studies described in the literature [3].

To prepare the AuNPs, the reducing agent (sodium citrate - Na₃C₆H₅O₇) was added under experimental design to the precursor solution gold (HAuCl₄ 2.5 x 10⁻⁵ M) at 65 °C and was maintained under stirring for the time evaluated.

AuNP’s samples were collected after the synthesis step and had their optical properties assessed by spectrophotometry UV-visible (SHIMADZU). The size and morphology of AuNP’s were examined by transmission electron microscopy (JEM-1400, JEOL Inc, USA).

Results and conclusions: Results were obtained from the spectra of the solutions in electronics and suspension compared with the images of transmission electron microscopy, which showed the difference in particle size.

Surface charts were generated in order to optimize the results and Pareto was introduced to allow better visualization and identification of variables and interactions that affect the synthesis of gold nanoparticles (p < 0.05). The two variables were significant in the process and higher absorbance were found in greater synthesis times. It was concluded that the reducing agent acts as a stabilizer evaluated, since in higher concentrations there is a decrease in absorbance and thus, the smaller the size of the nanoparticles.

Acknowledgements: Brazilian Ministry of Science and Technology (CNPq Grant 483036/2011-0), The Education Ministry (MEC-PROEXT Grant 04/2011) and The Ministry of Science and Technology (MCT/FINEP/CT-INFRA grant PROCINTRA 01/2006).

References:


P250
Study of females of Podocnemis unifilis (Tracajá) (Troschel, 1848) in areas between Xambioá-TO, Antonina-PA and Tucuruí-PA, by molecular tools

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BMC Proceedings 2014, 8(Suppl 4):P250

Background: The turtles are usually associated with low substitution of individuals in the population because they have longer life than any other vertebrates. The order Testudines or Chelonida, the oldest among all vertebrates, comprising the turtles, terrestrial, marine and freshwater. These have as main characteristics, low growth rates and long periods required for mature, causing many turtles are vulnerable to extinction. Like other turtles, P. unifilis, popularly known as tracajá, leaves long-lived animals with late sexual maturity, characterized by a small mortality of adult animals, but high mortality rate of embryos and hatchlings, with natural predation as one of the most important factors of low hatching success of these species. This species is one of the most important representatives of the fauna of the Amazon turtle, but their eggs, meat and offal provide food for local communities, and have their hooves used as adornment and household items. The implementation of large hydropower enterprise cause many environmental damage. The scientific emphasis in EIA/RIMA should be held primarily in the study of special targets, and should take into account the genetic structure of populations. Mitochondrial molecular markers have particular maternal characteristics and high rates variation, which allow us to analyze the behavior of populations. The deployment of hydropower modifies the natural habitat of the turtles, which have difficulty in adapting. This work aimed to study the genetic structure of populations of P. unifilis analyzing the behavior of females in conditions of ambient modified by Tucuruí, and Antonina-PA and Xambioá-TO, where will be built the dam of Santa Isabel.

Methods: We performed the sequencing of fragments of the control region of mitochondrial DNA, generating data of gene diversity and nucleotide, numbers of haplotypes and segregating sites, AMOVA, levels of structuring by pairwise comparison of qST and Mantel test in ARLEQUIN program. Clades were grouped checked by software Treefinder and the haplotype tree was held at hapview software.

Results and conclusions: The study populations were similar to each other and have high diversity within them, but the region of rocks and waterfall in Xambioá associated with hydroelectric Tucuruí, act as barriers to gene flow. Tucuruí has the largest number of groups haplotypes, upriver this number decreases by Antonina and especially Xambioá. The Mantel test showed no correlation between genetic variation and spatial distribution, noting that the species does not present a long-distance migratory pattern. The region Xambioá and Tucuruí should be considered distinct management units due to the influence of anthropogenic activities differently in each place.

References:


P251
Bioprospecting of culturable bacteria with potential polyhydroxalkanoates (PHA) producer, isolated from contaminated sites of Manaus-AM/Brazil

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BMC Proceedings 2014, 8(Suppl 4):P251

Polyethylene and polypropylene, known as conventional plastic, from non-renewable sources, like oil, are successfully replacing by glasses and papers in packaging industry due to their low cost, variety and durability [1]. However, these plastics for being slow degradability and hinder the exchange of gas and the decomposition of other compounds cause health problems when they are not disposed of properly [2]. Nowadays, there are another renewable plastic sources, known as polyhydroxalkanoates (PHA), manufactured by plants, fungi and/or bacteria source. From the ecological view this plastics are important because their degradation are faster and requires less energy in their manufacture processes, reducing significantly the CO₂ emissions [3].

The subject of this work was a bioprospect culturable bacteria potentially producers of PHAs, from water and plant species contaminated from anthropogenic actions.
Bacteria were isolated from two plants (Superfamily Leguminosae) and water collected from two points of Forty affluent of the city of Manaus - Amazonas. For the isolation of the water samples, 50 I were grown in two culture media (Nurient Agar Agar and LB) containing 50 mg / mL of clindamycin at 26 °C for 7 days. The plant samples (leaf, stem and root) were subjected to aseptic procedure described by Souza et al., 2004 [4]. Isolated bacteria were purified by the method of cross-striations and then evaluated by analyzing the morphology of the Gram stain. For detection of potentially producing PHA bacteria it was used the test of Sudan Black plating cell culture with 24 wells containing VM medium and incubated at 26°C for 72 hours according to the protocol described by the National Center for Biotechnology Education [2012 [3]]. Of the four samples collected at two points of Igarapé do Quaraonta, 80 bacteria were isolated, which of 64 were randomly selected and evaluated for potential production of PHA. From all bacteria evaluated, 13 were positive (30%), six (9.37%) of the plant #2, three (4.71%) of the plant #1 and two (3.12%) of each point of collated water. From the 13 positive results, 12 were characterized as Gram negative and Gram negative as streptococci isolated from the root of the plant 1. This research showed a predominantly Gram-negative microbiota, whose endophytic bacteria were the main source, compared with water of potentially producing of PHA bacteria and therefore the bacteria present in polluted waters and adjacent plants of Igarapé do Quaraonta in the city of Manaus may be considered potentially producing PHA.

References

P252 Comparison between the synthesis of gold nanoparticles with sodium citrate and sodium tetraborate
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Background:
With gold nanoparticles (AuNPs) is possible to develop nanoscale devices that can interact with chemical and biological systems. The phenomenon explored in these nanosystems is called Localized Surface Plasmon Resonance (LSPR), which promotes electromagnetic wave oscillation electronics on these small metallic structures. It is interesting to note that this resonance is directly linked to the size of the nanoparticles, the nature of the dielectric material and support environment where the device is being studied [1,2]. This work makes a comparison between results obtained in the synthesis of AuNPs reduction method using a Sodium Citrate (Na2C6H5O7) and Sodium Borohydride (NaBH4). Was expected to demonstrate the viability of these two reducing agents and highlight the potential differences obtained in each mechanism. Since knowledge is the ability of stabilizing citrate ions and the strong reducing action of NaBH4 [3]. The dominance of this knowledge will provide the development of systems with particle size specific for various applications in biosensors.

Methods:
In these experiments consisted initially in the preparation of the following aqueous solutions: 100 mL Tetrachloroauric Acid (HAuCl4) 2.5 x 10-4 M, 5 mL of 1% NaBH4 and 5 mL of Na2C6H5O7 also 1%.

The synthesis of nanoparticles were carried out simultaneously with the reducing agents, for it was placed 50.0 mL of HAuCl4 solution in two Erlenmeyer and the following adding agent reducing in agitation. The system with sodium citrate was put into a heating temperature of 65 °C with NaBH4, has not been heated. The reaction time for both was 25 min, but the use of heating in half with Na2C6H5O7 was off to achieve 10 min of reaction. The procedure used here was based on literature data, with some adaptations [3].

The samples produced AuNPs had their optical properties assessed by UV-Vis spectrophotometry. The size and morphology of AuNPs were examined by Transmission Electron Microscopy (TEM) (JEOL-1400, JEO/ USA Inc.).

Results and conclusion:
The UV-Vis spectrophotometry shows the absorption of the samples obtained in experiments, it is noted that the easy synthesis with NaBH4 did not generate nanoparticles with a reasonable size for the LSPR occur. Phenomenon responsible for the peak at 530 nm in the spectrum of sodium citrate (Na2C6H5O7).

Due to capacity reduction of NaBH4 lot nuclei initiators nanoparticles were generated, but reduced in size. Na2C6H5O7 already has a good ability to stabilize, thus required temperature rise in the synthesis process to increase capacity reduction. Nuclei being formed ions favor the stabilization phase of growth, thus generating nanostructures favorable process RPS to 530 nm.

Acknowledgements: Laboratory of Cellular Ultrastructure Carlos Alberto Redins (LUCCAR)-CCS/UFES. Brazilian Ministry of Science and Technology (CNPq Grant 483036/2011-0) and The Ministry of Science and Technology (MCTI/FINEP/CT-INFRA grant PROINFRA 01/2006).

References

P253 Isolation of entomopathogenic bacteria in the Southwest region of Paraná state in Brazil
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BMC Proceedings 2014, 8(Suppl 4):P253

Background: The use of entomopathogenic bacteria to control harmful insects has increased the interest and researches about spore forming bacteria. Species from genus Bacillus are described as rod-shaped cells, sometimes in chains, and mostly capable of producing endospore. Although hundreds of bacteria species are known to be associated with insects, there is only a few which can be used as biological control for agricultural pests as well as, for vectors of tropical diseases. Several agricultural pests seem to be resistant to both chemical pesticides and known toxins produced by entomopathogenic bacteria. Therefore, the goal of this study was to describe the isolation of an entomopathogenic bacteria contributing with researches for new potentially toxic isolates in biological control of insects.

Methods: Strains of bacteria were isolated from soil samples of eleven locations. These locations were characterized according to the occupation and the use of the land, and allocated as SISLEG map of Universidade Tecnológica Federal do Paraná - UTFPR - Campus Dois Vizinhos. The method of isolation was recommended by the World Health Organization, as follows, 1g of each soil sample was placed into sterile tubes with 10mL of saline solution and stirred by vortex for 2 minutes, then 1.5mL were transferred to sterile microtubes and heated during 12 minutes at 80°C and kept 5 minutes on ice. Samples were then diluted in sterile saline (1000-fold), seeded on Petri dishes with nutrient agar medium and incubated at 30°C for 48 hours. After incubation, colonies of Bacillus spp. were selected [1] by initial identification by differential growth in medium NYSM [2] with penicillin (100mg/L). The isolates from this medium that presented small
crystals of circular and oval size when stained with crystal violet observed with optical microscope (40x) were identified as Bacillus thuringiensis [3,4].

Results and conclusions: Bacteria growth was observed in all eleven soil samples processed in nutrient agar. Heat shock was applied in order to obtain sporulating strains. Colonies were selected according to morphological characteristics standards. Area (1) - Pinus, presented more variability with fourteen morphological types (14), followed by area (2) - Eucalyptus with nine morphological types. Area (9) - Pastures presented the lowest diversity with three types. When a selective entomopathogenic medium was used (penicillin), the area (1) showed extensive growth of strains resistant to this antibiotic, featuring the Bacillus thuringiensis, however area (2) showed little growth of colonies.

References

P254 Genetic transformation to integrate two expression cassettes into the genome of yeast Pichia pastoris
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BMC Proceedings 2014, 8(Suppl 4):P254

Background: Pichia pastoris is a methylotrophic species of yeast, which means that it can grow on methanol as its sole carbon and energy source [1]. The Pichia expression system has several advantages: short length of the oligosaccharide chains added to proteins, correct folding and very high cell densities [2]. P. pastoris vectors are designed for homologous integration into either AOX I locus, one of the two homologous AOX I genes present in this species or his4 locus. Gene insertion events at the AOX I (GS115) loci arise from a single crossover events between the loci and any of the three AOX I regions in the vector, the AOX I promoter, the AOX I transcription termination region (TT), or sequences even further downstream of AOX I (‘AOX II’) [3]. In either GS115 gene insertion events at his4 locus arise from a single crossover event between his4 locus in the chromosome and His4 gene of the vector. This results in the insertion of one or more copies of the vector in his4 locus [3].

Methods: Recombinants Clones P. pastoris GS115 (phenotype His+/Mut-) with pPG Vector (pIC9/ glucomalayse cDNA of Aspergillus awamori) integrated in the AOX I locus were transformed with pPAMy vector (pIC9 vector/α-amylase gene of Bacillus licheniformis), both previously constructed in our laboratory. The pPamy was linearized by digesting with Sac I enzyme to His4 locus integration. Transformation was carried out by electroporation of freshly prepared competent cells. The electroporated cells were recovred in 1 M sorbitol and spread onto MM agar containing starch 1 % (w/v) to identify positive clones. Transformants with larger halos and clear were considered as positive recombinant strains expressing enyzmes in comparion the control strain (Strain previously constructed). Positive strains were then inoculated in 50 ml BMGY at 28°C for 48 h in shaking flasks. The cells were harvested by centrifugation and then grown in 25-m1 BMMY with methanol induction (0,5% [v/v]) at 28°C for 120 h. The supernatants were collected by centrifugation and subjected to enzyme activity assay by Fuwa and DNS methods. Extracellular culture supernatant samples were used to protein detection by SDS-PAGE 10%.

Results and conclusions: A total of 744 transformants were screened on MM plates. Eight positive transformants with the highest halos amylase activities were analyzed by SDS-PAGE. The gel electrophoresis revealed expression expression two protein. One protein band with molecular weight of 116 kDa and another with 58 kDa, corresponding to glucomalayase and α-amylase respectively. When we compared the protein pattern of secreted enzymes is agree with protein pattern observed in control supernatant, previously studied in our laboratory. However, in preliminary analyse, non enzymatic activity was detectad in enzymatic assay by Fuwa and DNS methods. However, new transformations and integrations strategies has been performing by study group.

Acknowledgements: This study was supported by the Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico (CNPq-scholarship), Centro de Apoio Multidisciplinar da Universidade Federal do Amazonas (CAM/UFAM).

References

P255 Production of reactive oxygen species in macrophages treated with essential oil of Croton argyrophyllyth Kunth
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Background: Essential oils are complex systems which consist mainly of volatile compounds of lipophilic source, as terpenes, sesquiterpenes and some non-terpenes [1]. Resulting from secondary metabolism, essential oil from Croton argyrophylyllyth (EOCA) is commonly extracted from its leaves. It is known that the essential oils from Croton species exhibit good antioxidant activity against DPPH free radical and reactive oxygen species (ROS) [2]. Several diseases are attributed to the increase of free radicals that show the importance of endogenous antioxidants like enzyme superoxide dismutase (SOD). Considering the use in folk medicine, it is relevant to find which concentration of EOCA has antioxidant effect against free radicals. To answer this question, the study aimed to evaluate ROS production in peritoneal macrophages of essential oil from C. argyrophylllyth.

Methods: After collection in the field, the essential oil from leaves of C. argyphylyllyth was extracted by hydrodistillation method using apparatus of the type Cleveger having a yield of 0.5%. Male Swiss mice (n = 5) that were 8 weeks old were used. All procedures were approved by and followed the guidelines of the institutional ethics committee on animal research at Federal University of Sergipe. After being sacrificed, were injected 10 mL PBS, then after vigorous massage was obtained peritoneal exudate. The macrophages were prepared in 96-well plate, 10 μL/well NBT (2.0 mg/mL) and EOCA in different concentrations (5, 10, 25, 50 and 100 μg/mL) [3]. NBT reduction was measured in response to zymosan (250 μg/mL in RPMI). Next, the micro plate was incubated at 37°C for 120 minutes, and the reaction was stopped by discarding the supernatant. The sediment was resuspended by addition of 120 μL DMSO/80 μL KOH (2M) in each well. The results were obtained in micro plate ELISA reader using as a blank the NBT in wells with unstimulated cells. The optical density (OD530nm) of formazan produced (insoluble blue deposit) was directly proportional to the ROS generated by phagocyte combined with a lower activity of the enzyme SOD. The data were subjected to variance analysis (ANOVA), with Tukey post hoc. The data variation were tested in triplicate and the differences considered statistically significant when p < 0.05.

Results and conclusions: Some sesquiterpenes found in the composition of the EOCA have proven antioxidant activity such as bicyclogermacren [4]. In the NBT test, the EOCA concentrations from 10 μg/mL to 100 μg/mL showed inhibitory activity of SOD and consequently generated more ROS.
Thus, the concentration 5 µg/mL was the most effective, both in comparison with the blank and the negative control (2µL). It was concluded that the lower concentrations of 10 µg/mL have antioxidant activities and therefore it is suggested cellular protection against free radicals.

Acknowledgements: We would like to thank Drª Ana Paula Prata for the botanical identification of the species.

References

P256
System biology of bacterial cellulose production
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BMC Proceedings 2014, 8(Suppl 4):P256

Background: Reconstruction and applications of genome scale metabolic models have truly influenced the field of systems biology [1,2]. These models have a promising ability to describe cellular phenotypes accurately and to relate the annotated genome sequence to the physiological functions of a cell [3,4]. Gluconacetobacter has been extensively characterized and is a model system for cellulose biosynthesis [5]. Using high throughput sequencing technologies a high quality draft assembly of the genome of this bacteria, Gluconacetobacter hansenii ATCC 23769 (GenBank number: CM000920 and taxonomy ID: 714995), has been completed and can be used to understand how this bacteria is able to produce cellulose. Bacterial cellulose has been used in the medical field as wound dressing and artificial skin material. Computational tools for predicting fluxes in biochemical networks are applied in the fields of integrated systems biology, bioinformatics, and genomics.

Methods: The reconstruction process involves the following steps: (1) creation of a draft model; (2) manual curation; (3) conversion into a mathematical format; (4) identification and filling of gaps; and (5) simulation and correction of the annotated data of the genome sequence of G. hansenii were used as the input for the Pathway Tools software, which can mapping genes to reactions in an automated manner. Moreover, a resource called Model SEED was used by creating a database of the organism. Then, to correct any mistakes and improve the reconstruction, the manual curation must be done using data from many different sources. An essential step is the addition of reactions which are not inferred from genome annotation and will decrease the number of dead-ends. The metabolic network file was loaded into MATLAB® using functions available in the COBRA toolbox. Moreover, a set of computational systems biology tools, written by our group called GENSys (Genomic Engineering System), was applied to the network. The GENSys comprises several modules that allow analysis and simulation of biochemical reaction networks, such as FBA (flux balance analysis) and MFA (metabolic flux analysis).

Results and conclusions: In this work, we present an initial draft of genome-scale metabolic reconstruction and network analysis of G. hansenii. Using the model in conjunction with constraint-based methods, we simulated the metabolic fluxes induced by three different environmental conditions to understand the effects of different carbon sources: glucose, glycerol and mannitol on cellulose production. We decided to maximize cellulose to evaluate the results. Also the maximum yield can be calculated for each carbon source. We summarized the biochemistry of each reaction in the G. hansenii model and we focused in the central pathways and cellulose biosynthesis to choose the reactions and metabolites in the core model. The core is a small scale model that can be used for testing and evaluating new constraint-based analysis methods, but still can be updated. This simplified model is representative and elevated our capability for understanding and predicting the cellular behavior of G. hansenii under any perturbations.

Acknowledgements: CAPES, CNPq and FINEP

References
References

P258
Use of quantum dots of sizes magic in biological systems
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QDs are inorganic semiconductor nanocrystals with quantum confinement and physical and chemical properties different from their counterparts. These properties are controlled depending on the size, shape and structure of nanocrystals, which can absorb and emit a predetermined range of the electromagnetic spectrum. QDs measure 2-10 nm but was recently created the MSNs QDs (nanocrystals sizes magics), measuring 1-2 nm with well-defined structures, high stability during and after the growth, the presence of a few units cells in their composition, show strong quantum confinement and different thermodynamically stable structures. In biomarkers, MSNs QDs have several advantages over organic dyes: exhibit high molar absorption absorption spectrum continuous, high intensity luminescence and high stability.

Therefore QDs may be used in nanomedicine detection of cancers. Once injected into the body dialing UV indicate the location of the tumor devices that capture this fluorescence particle, greatly improving diagnostic procedures. Therefore, the objective is to improve the QDs MSNs for use in biological systems. Laboratory of New Insulating Materials and Semiconductors (LNMS) were synthesized, probably for the first time. These QDs were tested in HeLa cells and Ehrlich. The results showed that are stable after 4 years the synthesis. In vitro assays showed high fluorescence scattered throughout the cytoplasm, demonstrated by double-labeling with DAPI, up to 20 hours after incorporation by the cell. Thus, incorporation of these QDs by tumor cells and their stability allows them to be used in studies of tumor cell migration in vivo.

P259
Adaptation to serum-free culture of HEK 293T and Huh 7.0 cells
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Background: The fetal bovine serum (FBS) is a component of higher value added to the medium, which may cause difficulty in the process of recovery and purification of bioprocduct. Considering all these reasons, it is essential to develop a simple cell adaptation protocol for the culture of cells in serum-free, animal component-free, or protein-free medium conditions [1,2]. The adaptation of cellular lineages to culture FBS or animal protein free medium can allow optimization of cell growth and expression of heterologous genes, facilitating recovery of recombinant proteins expressed in these cells. Mammalian cell growth can be adapted to serum-free media through progressive reductions of serum concentrations [3]. This gradual reduction over time increases the probability of successful adaptation to low-serum or serum-free media by allowing the self-adjustment of the cells to the environment, with the time required depending on the cell line and the composition of the media [4]. Our aim was to establish and to study mammalian cell lines adapted to serum-free medium for the expression of recombinant proteins. Thus, we assessed cell growth, nutrient consumption and metabolite production kinetics in the cell culture media tested.

Methods: In this work, HEK 293T and Huh 7.0 cells were adapted to different serum free media (SFMs) using sequential adaptation approach. HEK 293T cells were adapted to 2 different SFMs: Hybridoma®-SFM and CHO®-S-SFM II; while HUH 7. cells were adapted to 4 SFMs: Hybridoma®-SFM, VP®, SFM, CHO®-S-SFM II; and Pro293a®. Kinetics parameters of adherent cells were analyzed in duplicate for 5 days in 6 well plates with 2 mL of medium. The initial cell concentration in all experiments was 2 x 10³ cells/mL.

Results and discussion: Established protocols to adapt HEK293T and Huh7 to growing cells in SFM. By changing gradually of medium, we obtained two lines adapted HEK 293T to two SFM and Huh 7.0 to four SFM. The Huh 7.0 cells showed morphology and growth better than HEK293T cells. The cells was evaluated and the maximum growth of the line Huh-7.0 in the medium SFM-Hybridoma and CHO-S-SFM II were 120hrs with 1.0x10⁶ cells / ml with viability higher than 90%. The maximum concentration HEK 293T cells and viability were similar to Huh 7.0 cells, but those achieved maximum peak of growth in different times. The SFM CHO-S-SFM II obtained maximum growth in 96 hours and Hybridoma-SFM in 72hrs. Metabolic analysis showed differences in the consumption of nutrients and production of metabolites in different lineages.

References

P260
Effect of ouabain on NFkB and p-38 activation in macrophages: a new biotechnological application
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Background: Inflammation is a response to external challenge or cellular injury that leads to the release of several inflammatory mediators and restoration of tissue structure and function. On the other hand, inflammation deregulation can cause tissue damage and is related to many diseases. During inflammation, a complex program of intracellular signal transduction and transcription events, driven by multiple pro-inflammatory mediators and cytokines, is activated. Ouabain, a potent inhibitor of the Na+-K+-ATPase, was identified as an endogenous substance of human plasma, able to affect various immunological processes, such as lymphocyte proliferation, apoptosis and monocyte function. These effects are particularly attractive for anti-inflammatory molecules bioprospection. We have also demonstrated the ability of ouabain to modulate inflammation, but little is known about the mechanisms involved.

References
Aim: The aim of this work was to evaluate, in vivo, the role of ouabain on NF-κB and p-38 activation. Additionally, we tested ouabain effect (1, 10 and 100 nM) on FITC-dextran endocytosis ability of peritoneal cells. 

Methods: In all experiments, 0.56 mg/kg ouabain or phospho-buffered saline (PBS) was given intraperitoneally (i.p.) for three consecutive days. After 1h of the last day of ouabain treatment, mice were euthanized and macrophages and neutrophils from peritoneal cavity were collected. Cells were seeded in 24-well plates and incubated for 1h. After the incubation period, macrophages were tested using antibodies anti-P-NF-κB e anti-Pp38 by flow cytometry. Besides that, ouabain was tested on FITC-dextran endocytosis ability of peritoneal cells. Peritoneal cells from unstimulated animals were selected for more specific analyzes aimed at quantification and expression compared to the PBS group. Ouabain led to a 65% reduction of potential with biotechnological applications related to the immune system. However, further studies are necessary to elucidate the mechanisms involved.

Results: Ouabain treatment decreased basal levels of P-38 and P-NF-κB expression compared to the PBS group. Ouabain led to a 65% reduction of P-38 activation and 60% reduction of P-NF-κB. Besides that, ouabain was also capable of reducing by 84% the expression of both protein NFκB and p-38 in the double positive cell subpopulation. Finally, after one hour of incubation, approximately 58% of cells endocytosed FITC-dextran particles, and this process was inhibited at 4°C. Moreover, we also observed that ouabain did not affect the endocytosis of dextran particles.

Conclusion: In agreement with the immunosuppressive effects that have been previously observed by us, ouabain pretreatment reduced the basal activation of proteins NFκB and p-38, but did not alter in vitro phagocytosis of dextran particles by peritoneal cells, suggesting a new mode of action of this substance. The present work reveals ouabain anti-inflammatory potential with biotechnological applications related to the immune system. However, further studies are necessary to elucidate the mechanisms involved.

References

P261
Isolation, identification and screening of hydrolytic enzymes producing phylloplane yeasts
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BMC Proceedings 2014, 8(Suppl 4):P261

Background: Microorganisms are common colonizers of superficial (phylloplane) and internal tissues (endophyte) of a variety plant species [1]. Phylloplane yeasts are considered a promising source of new and interesting biotechnological activities, particularly hydrolytic enzymes; however, the biodiversity of yeasts colonizing plant phylloplane and is still poorly known for most plant species. Considering the immense diversity of Brazilian flora, which include many species with medicinal properties, this knowledge is even more limited. Given the above, this study objective to isolate, identify and to evaluate the production of hydrolytic enzymes of yeasts associated with leaves, stems and flowers of alecrim-do-campo (Baccharis dracunculifolia DC), a plant recognized as the main source of exudates used by bees to produce green propolis and also, for its medicinal properties.

Methods: For isolation of yeasts, samples leaves, stems and flowers of B. dracunculifolia were collected; 10 g were macerated in 90 mL of saline solution (0.85%) and dilutions of the suspension were spread in solid medium BDA and TSA. After purification and preservation, the isolates were characterized by colony morphology and grouped. Isolates representative of each group were subsequently identified by molecular techniques based on the amplification and sequence analysis of ITS1-5, 85 ITS2 rDNA. For evaluation and screening of potential enzymes producers, enzymatic activity was performed in solid media [2,3]. Proteolytic and celulolitic activities were tested in TEPG media with 2% casein 0.5% carobomethylcellulose (CMC). Activities of the enzymes pectinase, amilase, lipase and esterase were performed in YNB media containing 1% pectin, 0.2% starch, 1% oil and 1% Tween 80, respectively. After growth for 3-5 days at 28 °C the enzymatic activities of the isolates were evaluated by the production of degradation halo around the colony, which is the visual indication of hydrolysis of their respective substrates. Assays were performed with three replicates and expressed by enzymatic index (IE), which was obtained by ration between diameter of halo and diameter of colony (mm).

Results and conclusions: Were obtained 69 isolates, being 74% of these isolates of yeasts and yeast-like fungi. On the basis of on colony morphology the isolates were grouped into five morphotypes (white, brown, black or salmon colony). The majority of the isolates was originating from samples of flowers and showed black colonies on PDA media. By analysis of ITS1-5,ITS2 rDNA sequences in Genbank database, the isolates were identified as members of the species Starmerella bombicola, Aeurobasidium leucospermi, A. pullulans, Rhodotorula mucilaginosa and Occultifur externus. The isolates were promising for enzyme production, specially for amylopatic and lipolytic activities which were predominant among the isolates. The most promising isolates were belonging to the genus Auraobasidium sp., which showed activities for the five enzymes tested. The isolates identified as promising for the production of enzymes, especially those of the genus Aeurobasidium sp. were selected for more specific analyses aimed at quantification and perfecting of culture conditions that allow greater production and can contribute to the development of alternatives to enzymes already available in market.

Acknowledgements: Luzia D. and Hungria, M. by the material support.

References

P262
Biotechnology: a non-existing word/world for the Brazilian deaf community
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BMC Proceedings 2014, 8(Suppl 4):P262

Background: Biotechnology is a complex theme that is directly or indirectly present in our lives, from food to medicine. Despite that, the understanding about biotechnology is still difficult for most of society due to this complexity. This is worsened for Brazilian deaf community since the word Biotechnology does not exist in Brazilian Sign Language (LIBRAS), the official deaf language. This young language, similar to some foreigner Sign languages, still misses several scientific terms/words [1]. These terms have to be created by the deaf community to help them guarantee the information access, their citizens rights and a better academic formation [1,2]. Therefore, our purpose as a
group formed by deaf and hearing people is to suggest a sign for the word Biotechnology to help on the understanding of the biotechnological area.

**Methods:** Initially six deaf students from a public high school were invited to participate in this work as requested by the deaf community for this type of project. To guarantee their understanding, we created a class about this theme in an inquiry-oriented teaching way, also using materials for contextualization (e.g. bottle of transgenic vegetal oil) [1,3].

**Results and conclusions:** LIBRAS is a language whose signs represent the concept of the words [2,4]. Therefore the word should be previously fully understood by deaf before creating the respective sign. The six deaf students that participated in this work are from a previous stimulated environment under the project Spreadthesign (http://www.spreadthesign.com), an international dictionary of 25 different sign languages from several countries now including LIBRAS from Brazil with our participation. We were also helped by an interpreter that always interrupted the class when one concept seemed to be not fully understood. Thus, cheese production, the snake venom biotechnological potential, the creation of transgenics for food production, and the insulin biotechnological production were fully discussed in this class. In the end, three signs for Biotechnology emerged: a) the combination of the sign of biology - right hand with fingers together on the left shoulder - with another movement; so the final sign is the right hand in the position of letter B (for Bio) rubbed in the palm of the left hand, to remind the process of generating some product using biotechnological methods; b) They used dactylology for the letters B, I and O and the sign of technology (hands in the position of letter T slightly spinning in front of each other). Finally, c) the combination of the sign meaning life - right hand with fingers together on the left shoulder - for Bio, followed by the sign of technology described above. After those sign propositions construction, they decided to send them to the Brazilian Biotechnology Society Event (5th BSBiotec) to ask for the society analysis and opinion. As citizens, deaf community should be allowed to understand the implications of the major changes brought about by biotechnological advances [3-5] and on that matter, the creation of this and other scientific signs may help this community on guarantee the access and understand of current biotechnological information.

**References**


**P263 Production and characterization of metal nanoparticles for chemical reduction in order to application in biological systems**

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BMC Proceedings 2014, 8(Suppl 4):P263

**Background:** Several studies have demonstrated the use of metallic nanomaterials with applications in the biological area with very promising results. Gold nanoparticles (AuNP’s) have attracted great interest from researchers due to its electronic properties, optical, thermal, and its great potential for self-organization in physical, chemical and biological [1].

In general, the AuNP’s are prepared by chemical reduction starting from a precursor (usually HAuCl₄) is a strong reducing agent. The shape and size of the nanoparticles depend on the synthesis process variables such as concentration of the reactants, temperature, pH, reaction time, and others. The same material composition can be determined with different physical and chemical characteristics just modifying characteristics such as size, self-organization, crystalline structure and shape, the point of the material in the nanometer range show distinct physical and chemical properties of materials on the macroscopic scale [2]. The aim of this work was to prepare and characterize gold nanoparticles using the method of chemical reduction with sodium borohydride (NaBH₄) in order to evaluate the influence of the concentration of the reducing agent and the time of synthesis on the properties of NP’s. The proposal is to use the material generated to evaluate its bacterial effect.

**Methods:** In order to evaluate the effect of variables on conversion of the reaction, as well as finding the conditions that maximized the synthesis of nanoparticles, one factorial design (3²) with 3 levels and 2 variables was performed. The variables evaluated were: Concentration of Reducing Agent (1.0, 2.0 and 3.0 %) and the time synthesis (5, 10, 15 min). These intervals were defined to cover most of the studies described in the literature [3,4]. To prepare the AuNP’s, the reducing agent (sodium borohydride - NaBH₄) was added according to experimental design gold precursor solution (HAuCl₄, 2.5 × 10⁻⁵ M) at room temperature and was kept under stirring the pre defined. AuNP’s samples were collected after the synthesis step and their optical properties were evaluated by UV-vis spectrophotometer (Shimadzu). The size and morphology of Au nanoparticles were examined by transmission electron microscopy (JEM-1400, JEOL Inc., USA).

**Results and conclusions:** The preparation of the material was evaluated on the basis of electronic spectra obtained and characterized by transmission electron microscopy was performed to determine the diameter and distribution. The results were analyzed by STATISTICA software 12 and showed that the concentration measured was only significant in study levels. The electron microscopy images showed a non-uniformity of the size of the generated material, probably because the character does not have borohydride stabilizer. The synthesized material will be evaluated for its bacterial effect.

**References**


**P264 Antimicrobial activity of Plectranthus barbatus (Lamiaceae)**

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BMC Proceedings 2014, 8(Suppl 4):P264

**Introduction:** The medicinal potential of plant is due to the presence of active principles, capable of producing several pharmacological effects such as analgesic, anti-inflammatory, diuretics, expectorants, tranquilizers, digestive healing, emollients, anti-diarrhoeal, among others [1]. The deep cultural roots of the Brazilian population facilitated the permanence of the use of herbal medicine to the present day acknowledging its effectiveness and legitimacy. [2] A strong scientific interest in herbal medicine has grown in recent years, which has led to the development of several studies that were based on popular practices with the use of plants for various therapeutic purposes [3]. One of medicinal plants, Plectranthus barbatus, is traditionally used as anti-inflammatory and antifungal agents, and has been recognized for its effects against the bacteria that cause dental caries such as Streptococcus mutans and Streptococcus sobrinus [4].

**Objective:** To evaluate the antimicrobial potential of leaf extracts from Plectranthus barbatus front of species which often causing infection of wounds.
Methodology: The extracts were prepared by maceration in 96% ethanol. The crude ethanol extract was tested against microorganisms: *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Enterobacteraeaeogenes, Salmonella enterica, Streptococcus pneumoniae, Acinetobacter calcoaceticus, Escherichia coli* and *Candida albicans* by agar perforation methods. Subsequently, it was determined the Minimum Inhibitory Concentration (MIC) by the broth micro dilution method using the culture media Brain Heart Infusion (BHI) broth in 96-well plates. The solubilization of the samples was performed with saline solution 0.9% and Tween 80. The positive control used was Ceftriaxone. It was used as a developer 2,3,5-triphenyl tetrazolium chloride (0.5 mg / ml). All experiments were performed in triplicate. Statistical analysis was performed using Kruskal-Wallis test. Data were considered significant when p < 0,05.

Results and conclusion: The extract showed zone of inhibition average of 14.33 (± 0.47) against *Staphylococcus aureus* is considered active according to the methodology AYRES [5]. The MIC had favourable outcomes for *Staphylococcus aureus* (3.12 mg/ml), *Staphylococcus epidermidis* (6.25 mg/ml), *Streptococcus pneumoniae* (6.25 mg/ml) and *Escherichia coli* (6.25 mg/ml). The results allowed to conclude that *Plectranthus barbatus* possesses the ability to inhibit pathogenic bacteria, proving that possesses antimicrobial activity that are prospects for obtaining natural antibiotics. The healing properties, their cytotoxic potential, as well as the development of bioproducts, specifically for infected wound, are underway.

References