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ABSTRACTS

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Aims and Scope

Current Pharmaceutical Biotechnology aims to cover all the latest and outstanding developments in pharmaceutical biotechnology. Each issue of the journal contains a series of timely in-depth reviews, research articles, letters written by leaders in the field covering a range of current topics in both pre-clinical and clinical areas of Pharmaceutical Biotechnology. ***Current Pharmaceutical Biotechnology*** is an essential journal for academic, clinical, government and pharmaceutical scientists who wish to be kept informed and up-to-date with the latest and most important developments.

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1st Biotechnology World Congress

Under the Patronage of

H.E. Sheikh Nahayan Mabarak Al Nahayan

Minister of Higher Education and Scientific Research

Chancellor, Higher Colleges of Technology

WELCOME MESSAGE

It is our pleasure to extend a very warm welcome to the honourable scientists and young researchers participating in the two conferences --- the ***1st Biotechnology World Congress*** and the ***4th International Conference on Drug Discovery & Therapy*** here in Dubai.

This series of conferences has attracted eighteen Nobel Laureates and many other leading scientists to Dubai. The conferences are serving to nurture collaborations with scientists in the region and to establish linkages between scientists in the developing world with those in the advanced Western countries.

Challenges faced by researchers include diseases associated with ageing populations, the spread of transmissible diseases in an interconnected world and the growing threat of resistance to drugs.

We wish to convey our special thanks to **His Excellency Sheikh Nahayan Mabara Al Nahayan**, Minister of Higher Education and Scientific Research, Chancellor, Higher Colleges of Technology for his patronage of these important scientific events. We are also most grateful to all the scientists who have travelled from the four corners of the world to the UAE to participate in these scientific symposia.

We hope that you will find your visit to Dubai intellectually stimulating and socially enjoyable.

PROF. FERID MURAD
(Nobel Laureate)
Co-President

PROF. ATTA-UR-RAHMAN, *FRS*
(UNESCO Science Laureate)
Co-President

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PLENARY LECTURES

PL-64

IRX-2 - A NOVEL IMMUNOMODULATOR FOR CANCER

John W. Hadden II

IRX Therapeutics, Inc., 140 West 57th Street, Suite 9B, New York, NY. 10019, USA;
E-mail: jhadden@irxtherapeutics.com

Progressive cancer is associated with immune dysfunction and many cancers invoke multiple different immune inhibitory pathways as they progress. An effective immune response to cancer is associated in multivariate analyses as an independent positive prognostic factor. IRX-2 is a biologic comprised of multiple cytokines generated from robust stimulation of donor peripheral blood mononuclear cells with Phytohemagglutinin (PHA). A well-defined manufacturing and release process was established and highly reproducible lots of IRX-2 can be generated. IRX-2 has been shown in multiple pre-clinical models to stimulate dendritic cell activation and generation of antigen-specific T cells. IRX-2 protects T cells from activation-induced cell death and augments the function of Natural Killer cells. IRX-2 reverses HPV-induced inhibition of Langerhans cell activation. We have shown that vaccine induced antigen-specific T cell activation is enhanced when various immunogens presented by peptide, protein, cellular or viral platforms are combined with local injections of IRX-2. In early phase clinical trials, we have shown that the IRX-2 immunomodulatory regimen administered into the area of draining lymph nodes of head and neck cancer patients has only mild local toxicities and induces clear increases in lymphocyte infiltrates into the primary tumors. Both radiologic improvements and improvements in survival are associated with patients who had the highest level of IRX-2 mediated lymphocyte infiltrates. Based on these results IRX Therapeutics is organizing multiple trials to establish the clinical impact of the IRX-2 immunomodulatory treatment including a randomized trial in operable head and neck cancer, a Phase II in HPV-related cervical intraepithelial neoplasia, and a Phase I/II trial of IRX-2 and a novel WT1 vaccine in patients with hormone refractory prostate cancer.

PL-2

FROM SUPRAMOLECULAR CHEMISTRY TOWARDS ADAPTIVE CHEMISTRY BIORGANIC AND DRUG DISCOVERY ASPECTS

Jean-Marie LEHN

ISIS, Université de Strasbourg, France; E-mail : lehn@unistra.fr

Supramolecular chemistry is actively exploring systems undergoing *self-organization*, i.e. systems capable of spontaneously generating well-defined functional supramolecular architectures by self-assembly from their components, on the basis of the *molecular information* stored in the covalent framework of the components and read out at the supramolecular level through specific interactional algorithms, thus behaving as *programmed chemical systems*.

Supramolecular chemistry is intrinsically a *dynamic chemistry* in view of the lability of the interactions connecting the molecular components of a supramolecular entity and the resulting ability of supramolecular species to exchange their constituents. The same holds for molecular chemistry when the molecular entity contains covalent bonds that may form and break reversibility, so as to allow a continuous change in constitution by reorganization and exchange of building blocks. These features define a *Constitutional Dynamic Chemistry* (CDC) on both the molecular and supramolecular levels.

CDC introduces a paradigm shift with respect to constitutionally static chemistry. The latter relies on design for the generation of a target entity, whereas CDC takes advantage of dynamic diversity to allow variation and selection. The implementation of selection in chemistry introduces a fundamental change in outlook. Whereas *self-organization by design* strives to achieve full control over the output molecular or supramolecular entity by explicit programming, *self-organization with selection* operates on dynamic constitutional diversity in response to either internal or external factors to achieve *adaptation*.

Applications of this approach in biological systems and to drug discovery will be described.

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PL-1

DISCOVERY OF NITRIC OXIDE AND CYCLIC GMP CELL SIGNALING AND THEIR ROLE IN DRUG DEVELOPMENT

Ferid Murad

GWU Medical Center, Biochemistry & Molecular Biology Department, 2300 Eye Street NW, Suite 530, Washington, DC 20037, USA; E-mail: bcmsaa@gwumc.edu

The role of nitric oxide in cellular signaling in the past three decades has become one of the most rapidly growing areas in biology. Nitric oxide is a gas and a free radical with an unshared electron that can regulate an ever-growing list of biological processes. Nitric oxide is formed from L-arginine by a family of enzymes called nitric oxide synthases. These enzymes have a complex requirement for a number of cofactors and regulators including NADPH, tetrahydrobioterin, flavins, calmodulin and heme. The enzymes are present in most cells and tissues. In many instances, nitric oxide mediates its biological effects by activating the soluble isoform of guanylyl cyclase and increasing cyclic GMP synthesis from GTP. Cyclic GMP, in turn, can activate cyclic GMP-dependent protein kinase (PKG) and can cause smooth muscles and blood vessels to relax, decrease platelet aggregation, alter neuron function, etc. These effects can decrease blood pressure, increase blood flow to tissues, alter memory and behavior, decrease blood clotting etc. The list of effects of nitric oxide that are independent of cyclic GMP formation is also growing at a rapid rate. For example, nitric oxide can interact with transition metals such as iron, thiol groups, other free radicals, oxygen, superoxide anion, unsaturated fatty acids, and other molecules. Some of these reactions result in the oxidation of nitric oxide to nitrite and nitrate to terminate the effect, while other reactions can lead to altered protein structure function and/or catalytic capacity. These effects probably regulate bacterial infections, inflammation of tissues, tumor growth, and other disorders. These diverse effects of nitric oxide that are cyclic GMP dependent or independent can alter and regulate numerous important physiological events in cell regulation and function. Nitric oxide can function as an intracellular messenger, an antacoid, a paracrine substance, a neurotransmitter, or as a hormone that can be carried to distant sites for effects. Thus, it is a unique molecule with an array of signaling functions. However, with any messenger molecule, there can be too little or too much of the substance, resulting in pathological events. Some of the methods to regulate either nitric oxide formulation metabolism, or function have been in clinical use for more than a century, as with the use of organic nitrates and nitroglycerin in angina pectoris that was initiated in the 1870s. Inhalation of low concentrations of nitric oxide can be beneficial in premature infants with pulmonary hypertension and increase survival rates. Ongoing clinical trials with nitric oxide synthase inhibitors and nitric oxide scavengers are examining the effects of these agents in septic shock, hypotension with dialysis, inflammatory disorders, cancer therapy, etc. Recognition of additional molecular targets in the areas of nitric oxide and cyclic GMP research will continue to promote drug discovery and development programs in this field. Current and future research will undoubtedly expand the clinician's therapeutic armamentarium to manage a number of important diseases by perturbing nitric oxide formation and metabolism. Such promise and expectations have obviously fueled the interests in nitric oxide research for a growing list of potential therapeutic applications. There have been and will continue to be many opportunities from nitric oxide and cyclic GMP march to develop novel and important therapeutic agents. There are presently more than 80,000 publications in the area of nitric oxide research. The lecture will discuss our discovery of the first biological effects of nitric oxide and how the field has evolved since our original reports in 1977. The possible utility of this signaling pathway to facilitate novel drug development and the creation of numerous projects in the Pharmaceutical and biotechnology industrials will also be discussed.

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PL-63

PLx PHARMA - DEVELOPING A GI SAFER ASPIRIN

Ron Zimmerman

PLx Pharma Inc., 8285 El Rio Street, Ste. 130, Houston, TX 77054, USA;
E-mail: ron.zimmerman@plxpharma.com

PLx Pharma Inc. is developing a GI safer formulation of aspirin (PL 2200) which achieved a 71% reduction of risk for ulcers in a recent clinical trial comparing it to regular aspirin. PLx's GI protective formulation utilizes a naturally occurring refined soy derivative, phosphatidylcholine (PC), in pre-association with aspirin to maintain aspirin's therapeutic effectiveness while substantially reducing undesirable GI bleeding and ulceration. This product has the potential to positively impact patients and overall healthcare costs by enabling aspirin to be used in a broader population for prevention of the two leading causes of death, cardiovascular disease and cancer. PLx is preparing to file a New Drug Application for PL 2200 in the USA in the first quarter of 2012.

Clinical Results. In a six site endoscopy trial of 200 patients over 50 years of age which compared PLx's lead product, PL 2200 Aspirin 325 mg, to 325 mg immediate release regular aspirin, PL 2200 achieved a 71.0% reduction of risk for ulcers ($p = 0.0069$). PL 2200 has also successfully completed bioequivalence trials which demonstrated bioequivalence to 325 mg immediate release aspirin while maintaining full antiplatelet activity.

PL 2200 Market Opportunity. Studies show that 10% of low dose aspirin users will develop ulcers and 2.5% will develop potentially deadly bleeds, with 30% of NSAID-induced deaths caused by low dose aspirin use. In the US alone, 43 million Americans take aspirin for prevention of heart attack and stroke with most taking 81 mg. For many of these 325 mg aspirin is the appropriate antiplatelet dose but they cannot tolerate a regular 325 mg dose. A similar number of patients with cardiovascular disease do not take any aspirin as they cannot tolerate it. PLx will address these large and growing markets in the US and globally. This will expand the use of a GI safer 325 mg dose aspirin to diabetics and obese whom are at high risk for cardiovascular disease and need a GI safe full antiplatelet dose of aspirin. It will also allow arthritis sufferers using other NSAIDs and aspirin together, which places them at high risk for GI problems, to have a safer aspirin for cardio benefit.

PL2200 Aspirin will provide a safe alternative to anti-secretory drugs, which are limited by drug interactions and a growing risk of potential side effects, including an elevated risk of fractures and infections.

There have been a number of retrospective studies suggesting that chronic aspirin use may significantly reduce the risk of several cancers. A recent prospective study of gastrointestinal cancers provides confirming evidence of aspirin's potential to reduce the risk of GI cancer. While additional prospective studies need to be undertaken, a GI safer aspirin that enables chronic use of 325 mg daily or BID by reducing aspirin's GI toxicity may have a profound impact on cancer rates and patient outcomes.

Additional clinical trials are in preparation that aim to demonstrate that PL 2200 is faster acting than enteric coated aspirin and a more reliable antiplatelet agent.

PLx's NSAID-PC Platform. In addition to PL 2200, PLx's product pipeline includes GI protective formulations of ibuprofen, as well as other currently marketed NSAIDs. NSAIDs induce GI toxicity in part by disrupting the naturally occurring PC barrier to acid that is found in the stomach lining. By non-covalently pre-associating an NSAID with PC, the NSAID becomes more lipophilic allowing it to move through the stomach's barrier to acid with minimal disruption. This NSAID-PC complex more safely delivers an equivalent therapeutic dose of the NSAID into systemic circulation by mitigating the interaction between the NSAID and the naturally occurring PC. As a result, the gastric protective barrier can remain intact and repel acid without altering the acid level in the stomach, thus avoiding the side effects of the current standard of care, chronic acid suppression.

SPECIAL INVITED LECTURES

SIL-5

MEDICINAL PLANT BIOTECHNOLOGY- A ROUTE TO DRUG SUSTAINABILITY

M. Iqbal Choudhary and Atta-ur-Rahman

International Center for Chemical and Biological Sciences, (H. E. J. Research Institute of Chemistry and Dr. Panjwani Center for Molecular Medicine and Drug Research), University of Karachi, Karachi-75270, Pakistan; E-mail: hej@cyber.net.pk

Most traditional medicine systems utilize herbal plants as the main source of therapeutic substances. Globally there is a revival of interest in the use of medicinal plant products for the treatment of various ailments. This is mainly due to an increased awareness of the limited horizon of synthetic pharmaceutical products to control major diseases, high cost of currently available synthetic medicines, reported cases of adverse side-effects of modern medicines and perceived gentleness of natural medicines. A number of products from medicinal plants have emerged successful in recent years, which highlights the continuing importance of medicinal plants in the development of modern medicines.

Major development in medicinal plant research include *ex-situ* cultivation, tissue culture-based propagation of plants, designing of plants which can produce edible vaccines and bioactive secondary metabolites by using biotechnological interventions, and understanding the ecological and genetic niches which lead to the production of certain phytochemicals.

During this presentation, recent developments in the biotechnology and chemistry of the medicinal plants will be reviewed along with the presentation of some of the results of our own work in this field. This includes our efforts to use medicinal plant cell suspension cultures for the structural transformations of chemical compounds, development of rapid dereplication techniques for medicinal plant chemistry, production of secondary metabolites in plant cell suspension cultures, etc. The main objective of our on-going research study is to discover new and effective lead molecules from medicinal plants against various important biological targets.

INVITED LECTURES

IL-86

Track: *Medical Biotechnology*

FIBRINOGEN BASED NANOFIBRES FOR GUIDING THE BEHAVIOR OF ENDOTHELIAL CELLS

George Altankov

ICREA Research Professor, Institute for Bioengineering of Catalonia, Barcelona, Spain;
E-mail: george.altankov@icrea.cat

Regeneration of tissues by providing the right cues to cells is an attractive option for advanced tissue engineering therapies. Electrospinning is a technique capable of producing nanofibers (NFs) with dimensions similar to these of the extracellular matrix (ECM) fibrils thus providing opportunity for mimicking ECM organization and it cells guiding properties. We are recently interested on the endothelial cells response on some spatiotemporal signals from the ECM, therefore we electrospun NFs from pure fibrinogen (FBG) – an important multifunctional protein involved in various physiological and pathological processes, including the provisional ECM formation during regeneration. We showed that FBG NFs are well recognized by endothelial cells and designed as random or aligned may elicit distinct functional responses. The major drawback of these NFs is that they represents poor mechanical properties; they either break at lower densities or tend to fuse at higher - thus adhering cells loss their orientation. Here we report also on the development of a novel type of composite FBG/PLA (poly-L, D-lactic acid) NFs with significantly improved biomechanical properties. The endothelial cells interact better with these fibers, representing higher degree of alignment along their orientation. The well developed focal adhesion complexes, actin cytoskeleton and the improved functional parameters of adhering endothelial cells, confirm their superior biological performance. Collectively, our data show that the composite PLA/FBG NFs combine the good cell recognition properties of FBG with the excellent mechanical properties of PLA, which characterizes them as an advanced biomimetic scaffold for tissue engineering application.

IL-101

Track: *Medical Biotechnology*

NANOSKIN BACTERIAL CELLULOSE: AS ALTERNATIVE ROUTES FOR REGENERATIVE MEDICINE

Pierre Basmaji, Ligia M. Manzine Costa, Gabriel M. de Olyveira and Lauro Xavier Filho

Innovatec's - Biotechnology Research and Development. São Carlos, SP, Zip Code:13566-610, Brazil;
E-mail: nanoexpertise@yahoo.com.br

The present paper describes NANOSKIN production for tissue regeneration applications. Nanoskin is produced from the bionanotechnology process. Nanoskin is a highly hydrated pellicle made up of a random assembly of ribbon shaped fibers less than 100 nm wide. These fibers themselves are composed of a bundle of much finer micro fibrils of nanometric size. These features allow its applications in scaffold for tissue regeneration, and medical applications **.** In the medical field, it's worth highlighting its application as a temporary skin substitute in the treatment of burns and wounds of difficult healing. In addition, Nanoskin membrane is used for: Drug Delivery, Stent covering to avoid re-stenosis and, for replacing of Dura Mater and intervertebral disc, Ophthalmologic prostheses, Scaffolds for organs cultures and Diabetic injury. Another advantage, the Nanoskin dressing reduces the treatment time and thereby reduces the cost of hospitalization of patients with burns or chronic wounds. In the regenerative medicine, we show that the Nanoskin nanocomposite is an osteoinductor or be, stimulates the bone regeneration, enabling bigger migration of the cells for formation of the bone fabric. The use of Nanoskin cellulose bacterial on intact skin to deliver beneficial components has distinct advantages of using water to enhance the delivery by increasing skin permeability. The creation of changes in the pores of highly hydrated intact skin may be a safe and efficient means to conduct transdermal drug delivery of a variety of drugs and pharmaceutical agents, both small (e.g., antibiotics) and large molecules (e.g., insulin). These multiform aspects of Nanoskin bacterial cellulose indicate its potential applications in the pharmaceutical field.

Keywords: Regenerative medicine , wound care, diabetic ulcer, drug delivry, biocellulose.

IL-78

Track: *Other Areas: Nanobiotechnology*

NANOLUX® FIBERS AS NEW NATURAL FOOD APPLICATIONS

Pierre Basmaji, Ligia M. Manzine Costa, Gabriel M. de Olyveira and Lauro Xavier Filho

Innovatec's - Biotechnology Research and Development. São Carlos, SP, Zip Code:13566-610, Brazil; E-mail: nanoexpertise@yahoo.com.br

Food nanotechnology is an area of emerging interest and opens up a whole universe of new possibilities for the food industry. The present paper describes Nanolux® Natural fiber Product based on polysaccharides composed of hemicelluloses protein for wide variety of applied scientific Endeavour's. Nanolux® is produced from the bionanotechnology process. Nanolux® is a highly hydrated pellicle made up of a random assembly of ribbon shaped fibers less than 100 nm wide. These fibers themselves are composed of a bundle of much finer micro fibrils of nanometric size. This nano bio process produces acetic and lactic acid but also small amounts of a potent detoxifying substance, glucuronic acid. Normally this organic acid is produced by the liver in sufficient quantities to neutralize toxins in the body. However, when the liver function becomes overloaded additional glucuronic acid taken in the form of Nanolux® is to be a powerful aid to the body's natural cleansing process, a boost to the immune system and a proven prophylactic. The presence of polyphenols, a naturally occurring antioxidant, and the beverage is to combat harmful free radicals and help keep the body free from in Nanolux® diseases. In this regard, Nanolux® helps maintain the overall well-being of the body.

Nanolux® considered supplement feed based on fibers polysaccharides transformed enigmatically by the fungus Xylan in liquid environment. They increase the activity of the cells NK (Natural Killer Cell), cells T (cells T are a kind of lymphocytes - white cells of the blood) and the cells (cell B is a kind of lymphocytes constituted the immune system). Nanolux plays an important role in immunomodulation (immunostimulation, anti-inflammation, anti-allergy, antioxidation processes). Overall, it is considered to enhance the body's natural healing abilities, alleviate the adverse drug reactions of chemotherapeutic agents, and improve quality of life.

Keywords: Nanofood, Nanotechnology, Natural nanofibers.

IL-85

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

CARBON CAPTURE, SOIL ENRICHMENT AND CROP IMPROVEMENT BY MANIPULATING THE GENETICS OF PLANT: MICROBE INTERACTIONS

Jeffrey L. Bennetzen

Department of Genetics, University of Georgia, Athens, Georgia 30602, USA

Plants actively export a very large percentage of their biosynthetic products into the area around their roots, commonly called the rhizosphere. Only a few of the myriad exported products have known functions, but it is clear that many are involved in the attraction and sustenance of an enormously complex microbial community. These rhizosphere microbes provide resistance to some plant diseases, assist root development, and help the roots in the uptake of essential nutrients. When plants are harvested, the decaying roots and rich microbial community account for the great majority of the carbon captured in the soil. This removal of carbon from the atmosphere also enriches the soil, improving future crop yield and yield stability. Despite the tremendous value of the plant::microbe interface in the soil, very little is known about how it is determined or can be enhanced. We are using the genetics of the host plant as a tool to dissect the general determinants of the rhizosphere plant::microbe interactions, using metagenomics as a phenotypic assay. I will present result showing the segregation and mapping of genes that determine many different species plant: microbe interactions, using whole genome analysis of the model grass Setaria and such biomass target species as switchgrass and sorghum.

Keyword: Biosynthetic products, rhizosphere, biomass, switchgrass and sorghum.

IL-33

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

DATE PALM GENETICS, GENOMICS AND BIOTECHNOLOGY FOR BASIC RESEARCH AND CROP IMPROVEMENT

Jeffrey L. Bennetzen

Department of Genetics, University of Georgia, Athens, Georgia 30602, USA

The date palm, Phoenix dactylifera, is one of the most important agricultural crops in desert regions of the world, and has been since its domestication in the Persian Gulf region about eight thousand years ago. P. dactylifera is also the first palm with a published genome sequence. Description of the P. dactylifera genome provides powerful new tools for the improvement of date palm yield, quality and nutritional characteristics, while lowering production costs and environmental impacts. In addition, the date palm genome sequence provides insights into the genetics of the family Arecaceae, which includes oil palm (Elaeis guineensis), coconut palm (Cocos nucifera) and several other important agricultural species. My lab has initiated collaborations with several labs worldwide to undertake genetic mapping, interspecies comparative genomics, diversity analyses, nutritional characterization and agronomic assessment of date palm germplasm. The initial results of these studies will be presented, including an analysis of genome evolution in the Arecaceae compared to the Poaceae, another important family of monocots that includes maize, rice, sorghum, wheat and barley.

Keywords: Phoenix dactylifera, genetic mapping, interspecies comparative genomics, diversity analyses,

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IL-100

THREE YEAR STUDY OF A PRIVATE CORD BLOOD AND MESENCHYMAL CORD- BANK CRYOBANKS OF IASO MATERNITY PEDIATRICS RESEARCH AND GENERAL HOSPITAL-THE GREEK EXPERIENCE

Eirini Mitrou, Konstantinos Ntallis, Nikos Panagiotopoulos, Emmanouil Bougioukas, Vaso Kalodimou and Ekatherina Charvalos

Director of Central Labs and CRYOBANKS-IASO Maternity, Gynecologic, Pediatrics and Research Hospital, Greece

CRYOBANKS is one of the most reputated stem cell bank of Greece. Historically, Cryobanks International Services of Athens-IASO is the 1st stem cells bank kicked off in 2005 and the 1st accredited bank from AABB (American Association of Blood Banks) in Greece. CRYOBANKS is divided in two units, namely, the laboratory and storage unit (cord blood and cord mesenchymal bank) and the regenerative, research and flow-cytometry unit. Two major projects are under consideration /accomplishment, the adipose tissue development technique for esthetic surgery gynecology-oncology purposes and the development of flow-cytometry for purposes other than for testing of cord blood bank products.

The stored 22.000 cord blood units as well as the 7000 umbilical cord- mesenchymal stem cells units translate into years of experience in the field of cellular therapy products and a frame of continuing effort for excellence. We briefly report here, a study of CRYOBANKS cord blood collection and storage during a period of three year 2008-2010. The whole procedures have been accomplished according to AABB Standards for Cellular Therapy Products (4th Edition) and taking in consideration regional ethical and cultural issues. Most of the samples have been collected at IASO from deliveries or ceasareans. From a total number of 11482 units collected, 11111 have been processed by the AABB approved protocol. Five hundred forty six were with low volume and from those 364 have been discarded for this reason. Twenty seven samples have been discarded because of client's request.

From a number of 4368 of units processed in 2008, 3644 in 2009 and 3099 in 2010, 0.69%, 0.93% and 1.32% cultured specimen respectively were positive for the presence of bacterial /fungal pathogens after inoculation and culture by the BACT T alert system. Ceasarean collections mostly were free of contaminants. By routine culture procedures we identified (descending order), Enterobacteria, *Staph aureus, Enterococci, Bacilli, Diptheroides, Streptococcus spp, Pseudomonas sp and Candida sp.* Antibiotic resistance profiles have been identified by using the Vitek 2 system (Biomérieux, France). The strains in all the cases showed usual antibiotic sensitivities. The above mentioned contamination rates, kind of bacteria and antibiotic resistances are negligible, confirming thus the capacities of our premises.

Cord stem cell banking started in October 2010 using a classical cell extraction protocol, but with major improvements (results presented at the 1st International conference on Stem cell research, Turkey, 28- September- 2 October 2011). Private vs public banks, new products for cellular therapy are among our priorities. We would like to open the dialog with private banks in the Mediterranean and Middle East region to create bridges, exchange ideas and promote our capacities.

IL-76

Track: *Medical Biotechnology*

EFADCHIP® - REVOLUTIONIZING CLINICAL DIAGNOSIS OF 21ST CENTURY THROUGH MULTIFACETED APPLICATIONS OF LOAC

Y.U. Albert Cheung-Hoi

Chairman & CEO, Hai Kang Life Corporation Limited, Hong Kong; E-mail: achy@haikanglife.com

A novel patented field assisted hybridization technology is invented to be used on lab-on-a-chip (LOAC) system, EFADchip®. This technology has the capacity to achieve sensitive and accurate detection of nucleic acid in minutes, enabling diagnostic results to be delivered within the hour from sampling stage. Experiments conducted proved its analytical sensitivity and specificity without generating adverse electrolytic effects unlike those observed in other electricity-based chip systems. The EFADchip® system has tremendous potential application in rapid point of care diagnosis including genotyping that is essential for personalized medicine. The technology behind EFADchip® will be presented and highlighted with its multifaceted applications in revolutionizing diagnosis in the 21st century. Featured in a recent issue of Science magazine (VOL. 332, 24 June 2011), the EFADchip® is being quoted as a prime example highlighting how Hong Kong's recent biotech development is putting the city onto the biotech map.

IL-69

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

PLANT REGENERATION AND AGROBACTERIUM-MEDIATED GENETIC TRANSFORMATION IN VALERIAN (*VALERIANA OFFICINALIS* L.)

Seema Dhir, Kaisa Mûller, Tomeka Howard, Jarred Williams and Alicia Williams

Department of Biology,1005 State University Drive, Fort Valley State University, Fort Valley, GA 31030, USA; E-mail: dhirs@fvsu.edu

Valerian (Valeriana officinalis L.) is a perennial, flowering plant used in her bal medicine. The goal of this work was to establish a simple one step method for plant regeneration and stable gene expression using Agrobacterium tumefaciens into different explants from which the whole transgenic plants can be regenerated. A highly reproducible multiple shoots regeneration system in Valerian using node as an explants and studying the effect of benzyl amino purine (BAP) and kinetin (KN) was developed. Leaves, stems and roots of in vitro plants were used as the explants for indirect organogenesis. The highest callus formation frequency was achieved n Murashige and Skoog"s (MS) media supplemented with (0.1 mg/L KN + 2 mg/L 2, 4-D) for leaf and root explants and (0.1 mg/L KN + 2.5 mg/L 2, 4-D) for stem explants. Furthermore, the highest percentage of shoot regeneration was obtained on a medium containing 0.5 mg/L NAA + 2 mg/L BAP in leaf-derived calli. Similarly, leaf explants cultured on MS media supplemented with 0.1- 3.0 mgl-1 of KN or BAP alone or in conjunction with 0.1 mg/L of NAA and 2, 4-D resulted in induction and maturation of somatic embryos. Leaf explants from one-month-old in-vitro-grown plants were infected by A. tumefaciens carrying a binary vector that harbors β-glucuronidase (GUS) and Neomycin Phosphotransferase (nptII) genes. The infected leaf explants were incubated for three days before they were subjected to GUS histochemical assay. The transformability was determined as the percentage of leaf explants expressing the GUS gene and as the intensity of gene expression (blue color). Several parameters including different concentrations of acetosyringone during co-cultivation, the length of the pre-culture period of explants prior to infection, co-cultivation period, different bacterial density (OD) and duration of immersion periods were tested. The results based on transient GUS gene expression of explants suggested that one-month-old leaf explants inoculated for 60 minutes with 0.4 OD and 150 µm acetosyringone and co-cultivated for 3-4 days in MS medium with 2, 4-D showed 80-90% transformation efficiency. To our knowledge, this is the first report of Valerian

susceptibility to A. tumefaciens-mediated genetic transformation. This procedure will allow us to introduce relevant genes in order to control of the synthesis of secondary metabolite through metabolic engineering.

Keywords: Valeriana officinalis L., Agrobacterium tumefaciens, GUS, nptII gene.

IL-10

Track: *Medical Biotechnology*

INDUCED HUMAN PLURIPOTENT STEM CELLS – THEIR THERAPEUTIC POTENTIAL AND ETHICAL CONCERNS

Norman M. Ford

Lecturer in P/G and U/G Bioethics and Healthcare Ethics, Melbourne College of Divinity - (Catholic Theological College), Melbourne, Australia; E-mail: nmdford@gmail.com

In 2006 Dr. Shinya Yamanaka and Dr Kazutoshi Takahashi showed that it was possible to reprogram mouse somatic cells back to the pluripotency stage by modifying a few key transcription factors under specific culture conditions. When these induced pluripotent stem cells [iPSCs], were injected into mouse blastocysts, they contributed to the formation of the developing embryos. In 2007 Takahashi discovered that human iPS cells could be generated from adult human fibroblasts cells. This led to the creation of directly reprogrammed human iPSCs that were heart muscle cells and eventually to other patients and disease-specific stem cells that could be transplanted for therapeutic purposes without risk of immune rejection. It was hoped that cell and tissue engineering would pave the way for medical treatments for organ failure. Indeed, iPSCs are seen as a likely suitable long-term source of transplantable dopaminergic neurons. At the same time, some iPSCs have been found to undergo premature ageing and this may involve iPSC therapy could lead to tumour formation.

It needs to be said that first more research needs to be done on the safety of using iPSCs for clinical use. Attention needs to be given to the safety of iPSC therapy in the light of its potential risks for cancer formation. It is necessary that no transgene be used to avoid any risks that may be involved. Human trials of this novel means of therapy for diseases raise ethical concerns related to informed consent, patients' recruitment, harm minimization and the unavoidable uncertainty and risks involved in human treatments for the first time. It is also necessary to bear in mind the seriousness of the potential risks, the unreliability of available animal models and the vulnerability of the target patient group.

Before iPSC-based therapeutic strategies can be applied in a clinical setting of neurological diseases they need to be thoroughly evaluated in preclinical animal models. In future years, iPSCl technology may play a major role in regenerative therapy. However, iPSC technology has several problems that remain to be solved, including the present low efficiency of iPSC generation without genetic alterations, the possibility of tumor formation in vivo, and unregulated growth of the remaining cells that are partially reprogrammed and refractory to differentiation. These issues must be solved before iPSC technology can be successfully used in clinical applications.

IL-96

Track: *Medical Biotechnology*

PERSON – THE FOCUS OF HEALTH CARE ETHICS

Norman M. Ford

Don Bosco Youth Centre & Hostel, 715 Sydney Road, Brunswick Vic 3056, Australia; E-mail: nmdford@gmail.com

The provision of pastoral care in hospitals throughout the world is evidence of the need to recognise that patients are always to be regarded as persons. A human person does not exist in the abstract without a name, family ties, a personality, a nationality and a culture. These values are important for everyone: touch them and you touch the person. We identify with our inner self most of all in serious conscientious judgements.

The exercise of free will is highly valued and reveals our personal dignity when we submit to the summons of conscience demanding that we do good to be true to the inner core of ourselves, the centre of our subjectivity. Our desire for good health always engages the power of our mind and free will: we cannot but wish to be happy and good health is

important for everyone. We realise we belong to a human community of equals and recognise others share our pursuit of health and happiness for all.

Reflection on the death of our loved ones brings home to us the limitations of our nature. Modern medicine can put death off for a time but it cannot take it away nor suppress our natural fear of it. Neglect of human nature is neglect of oneself as a person. Notwithstanding individual differences, we recognise all persons are equal in dignity, with the same rational nature, feelings and emotions. The foundation for our typical human experiences and capacities is found in the *rationality* of human nature. Free will is an important power of our rational nature: it is the basis of our autonomy and self-determination which enables us to be responsible for our actions.

There are serious implications that can be drawn from our understanding of the nature of the human person for hospitals, their administrators, doctors, nurses and allied health professionals. Patients are persons who are to be cared for, including the support they need when they are ill and especially as they are passing through their own end of life journey. Patients are not simply clients or customers in need of medical treatment, health care or palliative care. They are to be treated and cared for as persons. The respect due to competent patients requires that an adequate explanation of treatment options be given to them in plain language as a necessary condition for their informed consent.

IL-91

Track: *Medical Biotechnology*

COLLAGEN-ENGINEERED FIBRIN MATRIX TECHNOLOGIES FOR UROLOGICAL TISSUE ENGINEERING

P. Frey, L. Micol, E. Ballet, K. Lorentz and J. Hubbell

Institute of Bioengineering, Swiss Federal Institute of Technology, EPFL, Station 15, 1015 Lausanne, Switzerland; Email: peter.frey@epfl.ch

Diseases or congenital malformations of the human urinary tract might benefit from regenerative efforts by tissue engineering.

The basics of urinary tract cells culturing, and in particular the development of a functional multilayered urothelial construct are discussed.

The development of flat and tubular compressed collagen structures and their physical behavior *in-vitro* are presented. The functionality of tubular urethral structures is demonstrated in a rabbit model.

Further compressed urinary tract cell-loaded collagen-polymer hybrid structures, studied *in-vitro* and *in-vivo* in a rodent model, are demonstrated.

In addition to collagen, fibrin is discussed as a cell-carrier. The development of a novel advantageous engineered fibrin to which TG-aprotinin or KPI can be covalently bound to prevent early degradation is discussed. Also the technology of binding of IGF 1 to fibrin to promote smooth muscle growth is demonstrated and *in-vivo* results in a rat model are discussed.

Keywords: Collagen, plastic compression, engineered fibrin, IGF1, aprotinin, KPI, urethral reconstruction, collagen-e-caprolactone hybrid matrix.

IL-19

PEPTIDE DESIGN FOR PROTEIN-SURFACE RECOGNITION

Ernest Giralt

Institute for Research in Biomedicine (IRB Barcelona), Barcelona Science Park, Baldiri Reixac 10, 08028 Barcelona, Spain; E-mail: ernest.giralt@irbbarcelona.org

Both from a basic science perspective as well as from a drug design point of view there is no doubt that proteins can be considered as privileged targets for binding of small ligands. In this context the design of ligands able to disrupt protein-protein interactions is emerging as an even more relevant issue. The breakthrough concept that proteins function as a contact network rather than as independent

individuals is not only one of the most important advances in our comprehension of living systems, but also translates to a new era in drug discovery. The few reported examples of diseases caused by "impolite" protein social behavior certainly represent only the tip of the iceberg. Therapeutic intervention through molecules designed to selectively modulate the strength and specificity of protein-protein interactions is becoming a reality. This will not only feature molecules with inhibitory capacity: equally or even more interesting are those compounds which can rescue pre-established interactions or structures whose loss results in disease.

Protein-protein interactions are the result of an ensemble of exquisitely regulated molecular recognition events that take place at protein surfaces. This can be referred to as a 'protein recognition code'. In order to understand protein-protein interactions and to achieve the efficient design of molecules with the capacity to modulate these protein-protein interactions, it is necessary to decipher this molecular recognition code, the language that proteins use to communicate. Unfortunately, progress in this field is highly unsatisfactory. Indeed, we are not completely illiterate, in the sense that we know the letters of this alphabet. They are the non-covalent interactions, such as hydrogen bonds, electrostatic interactions, π-cation interactions, Van der Waals forces, and the others. However, we could be compared with a child who is learning to read and attempts Dickens's Oliver Twist.

In this context, a comparison of binding in gas phase and solution can pave the way towards a better understanding of molecular recognition at protein surfaces. Peptide ligands are highly suited for these types of studies. The comparison of the relative affinities of collections of ligands with the same molecular weight facilitates the interpretation of data from MS because it is not necessary to make corrections that are not very reliable with the present state of the technique.

Keywords: Therapeutic peptide, therapeutic target, protein, NMR, drug discovery.

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IL-65

Track: *Medical Biotechnology*

SURFACE-MODIFIED NANOPARTICLES AS DESIGNED BIOMARKERS FOR OPTICAL IMAGING

Sailing He and Jun Qian

Department of Eletromagnetic Engineering, Royal Institute of Technology (KTH), 100 44 Stockholm, Sweden;
E-mail: sailing@kth.se

Optical bio-imaging, which utilizes the optical properties and the spatial variation of a bio-specimen, can overcome many existing problems of traditional bio-imaging modalities (e.g., X-Ray, CT, MRI). Optical biomarkers are especially important in this method, since they can provide lots of rich information of bio-specimen (e.g., luminescence intensity, spectrum, lifetime, polarization, etc). Nano-materials of particle shape, such as gold nanoparticles, silica nanoparticles, graphene, have many special physical and chemical properties, and their biocompatibility can also be achieved through some surface functionalization and bio-conjugation. Due to the above advantages, nanoparticles have been widely applied in various biomedical applications during recent years. In this talk, we will give a brief introduction to the properties and design of some typical nanoparticles such as gold nanorods, organically modified silica (ORMOSIL) nanoparticles, and graphene oxide. We will also show some of our recent results for various applications, such as tumor targeting, deep tissue imaging/diagnosis, imaging guide photodynamic therapy.

IL-56

Track: *Industrial and Manufacturing*

SCALABILITY OF DOWNSTREAM PROCESSING: FROM µL TO M³

Alois Jungbauer

Department of Biotechnology, University of Natural Resources and Life Science Vienna, Austrian Centre of Industrial Biotechnology, Vienna, Austria, Muthgasse 18, 1190 Vienna, Austria; Email: Alois.jungbauer@boku.ac.at

Currently downstream processing of biopharmaceutical proteins consists of a multi-step process with a combination of centrifugation, membrane filtration and chromatography in the most cases. Scale down of unit operations allow a faster optimization, because less material is requested and experiments can be conducted in parallel. The questions remain to which extent do these types of experiments - usually conducted in the 10-250 µl scale - allow a prediction to large scale. Three unit operations will be considered in detail; dissolution of inclusion bodies, refolding and chromatography. These process steps have been scaled down to microliter scale a nd engineering parameters extracted. Then processes with the same feed stock are preformed in large scale and predictability has been assessed. In case of inclusion body dissolution and refolding the maintenance of the redox-potential is the most crucial problem. In chromatography a lot of relevant parameters can be even extracted at 25µl scale, such as equilibrium binding conditions and adsorption kinetics. An example will be presented how prediction of large scale operation can be made with such parameters applying the proper engineering model, in this case a pore diffusion model. An outlook on new development in holistic optimization of downstream processing will be given.

IL-11

Track: *Other Areas: Food; Marine; Bio-safety; Systems Biology; Bioethics*

FERMENTATION AND ENZYMATIC HYDROLYSIS OF MILK PROTEINS: THE CASE FOR THE PRODUCTION OF BIOACTIVE PEPTIDES FOR THEIR USE AS NEUTRACEUTICALS

Ara Kanekanian

Cardiff Metropolitan University (UWIC), Cardiff School of Health Sciences, Centre for Nutrition, Dietetics and Food Science, Western Avenue, Cardiff, CF5 2YB – United Kingdom; E-mail: akanekanian@cardiffmet.ac.uk

Bioactive components in plant and animal products such as polyphenols, caroteinoids and conjugated linoleic acid have been used for their health benefits. Other milk derived bioactive compounds from casein hydrolysis have also been studied and they have been the focus of our research. The bioactive peptides (BAP) from casein hydrolysis using probiotic microorganisms such as *Bifidobacterium animalis* subsp *lactis* were studied together with those produced from peptic and tryptic hydrolysis. It was found that these BAP exhibited several heath promoting activities such as having antioxidant, hypocholesterolemic and hypotensive properties. Their activities as anticarcinogenic and immune modulating have also been investigated. Our research work has shown that most of these bioactivities increased as the incubation with probiotics and enzymatic hydrolysis time increased. The *in vitro* studies of the crude casein tryptic hydrolysates and of the individual fraction isolated using size exclusion chromatography showed clear activities for cholesterol reduction of up to 70% when compared with the control. Similar positive results were observed regarding hypotensive activities and ACE inhibition, with up to 51% reduction compared with the control sample. It was apparent that low molecular weight peptides of <1kDa isolated by ultrafiltration showed better activity than the 10kDa peptide fraction or the crude hydrolysate. The HPLC-MS analysis of these low molecular weight fraction indicated that these peptides to be pentapeptides containing Phe-Trp- Tyr-Arg-Lys or Pro. There was also a clear antioxidant activities with the <1kDa fraction of 24% with radical scavenging activities. The interaction of caseinoglycomacropeptide with tooth enamel to prevent its erosion by acidic drink was studied using scanning electron microscopy. The results were encouraging and showed clear adherence of the peptides onto the surface of the tooth which makes it viable component to add to such drinks. However, the anticarcinogenic activity of the peptides isolated from β-casein hydrolysate on pancreatic and colonic cancer cell lines showed very little effect on the proliferation of the cells which require further investigations.

IL-4

Track: *Pharmaceutical Biotechnology: biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering.*

CREATION OF UNIVERSAL VECTORS FOR PROPHYLACTIC AND/OR THERAPEUTIC RECOMBINANT VIRUS VACCINES

C. Yong Kang

Department of Microbiology and Immunology, Schulich School of Medicine and Dentistry, Siebens-Drake Medical Research Institute, The University of Western Ontario, London, Ontario N6G 2V4, Canada; E-mail: chilyongkang@gmail.com

Vaccination against infectious agents has proven to be the best way to prevent infectious diseases. We have created genetically modified recombinant M gene mutant of the India na serotype of vesicular stomatitis virus (VSV_{Ind}) and M gene mutant of the New Jersey serotype of VSV (VSV_{NJ}) as universal vectors for the development of recombinant virus vaccines. The priming vaccine vector should be antigenically distinct from the boost vaccine vector in order to maximize the boost effects. $rVSV_{Ind}$ with the mutations of G21E/M51R/L111F in the M protein (VSV_{Ind}GML) and $rVSV_{NJ}$ with the mutations of G22E/M48R+M51R in the M protein ($rVSV_{NJ}$GMM) was attenuated to a degree that mice injected with 50 million of these genetically modified infectious viruses directly into the brain showed no neurological signs or any other adverse effects. In contrast, 100 infectious wild-type VSV_{Ind} or wild-type VSV_{NJ} kills mouse within 48 hours. Foreign genes inserted into these VSV vectors elicit strong B cell and T cell immune responses against the inserted gene products when we prime animals with VSV_{Ind}(GML) followed by boost immunization with $rVSV_{NJ}$(GMM) carrying the same genes of interest. Animals can tolerate more than 5 x 10^9 PFU each of recombinant infectious VSV_{Ind}(GML) and recombinant infectious $rVSV_{NJ}$(GMM) and showed high levels of gene expression and immune responses. Our results show clearly that $rVSV_{Ind}$(GML) priming and $rVSV_{NJ}$(GMM) boosting is the best way to induce ultimate humoral and cellular immune responses. We will describe the advantages of these dual serotype VSV vectors for future vaccine development against infectious diseases and cancers.

Keywords: Vaccines, gene mutation, infectious diseases, cancers, vesicular stomatitis virus (VSV_{Ind}).

IL-12

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

SYNTHETIC BIOLOGY - CHALLENGES AND LIMITS FOR THE HETEROLOGOUS BIOSYNTHESIS OF NATURAL PRODUCTS AS DRUGS IN MEDICINAL PLANTS

Oliver Kayser

Technical Biochemistry,Technische Universität Dortmund,44221 Dortmund,Germany;
E-mail: oliver.kayser@bci.tu-dortmund.de

In recent years classic genetic and molecular biology strategies (Bioballistics, Agrobacterium tumefaciens transformation, recombinant enzymes) for production of natural compounds or even breeding of medicinal and aromatic plants have expanded and improved productivity of plant-derived fine chemicals. Among those high value natural products with medicinal and cosmetic purpose (e.g. essential oils, paclitaxel, artemisinine, Vinca-Alkaloids) play a major role. Applying genetic and biotechnological techniques like metabolic engineering, site directed mutagenesis, and pathway optimization for plant optimization to reduce costs and increase productivity are in the main focus of academia and industry. Because of some drawbacks with plant cell cultures and isolated enzymes giving no sufficient high production for commercialization, research strategies shifted more and more to metabolic engineering. From the past, engineering a microorganism is proven as a valuable tool and concepts have been transferred to plant science and opened new promising perspectives for improving plants and cell lines. First, engineering crop plants was conducted, but applying these techniques for medicinal plants is rather new and has not yet been explored so well. Today cloning and expression of multiple genes in polycistronic vectors and genomic integration is of high interest and allows the reconstitution of biosynthetic pathways in heterologous organisms either plants or microorganisms. Combining science and engineering in this research field was claimed as Combinatorial Biosynthesis and later as Synthetic Biology. Synthetic biology includes a large number of subareas, including enzymology, protein assembly and interactions, metabolomics, gene regulation, signal transduction and computational biology and is considered as a future approach for

biotechnological plant optimization. The possibilities show exciting perspectives for the exploitation of medicinal and aromatic plants to increase the level of wanted natural products, gain insight in metabolic pathways even for new biosimilar chemicals, to improve nutrional and health promoting effects of food (nutraceuticals), and to reduce the amount of unwanted by products with potential toxic or allergic activities.

Keywords: Transgenic Plant, Synthetic Biology, Metabolic Engineering, Biotransformation, Plant Biotechnology, Natural Product Chemistry, Cancer.

IL-104

Track: *Business Development*

VERTICAL FARMING FOR URBAN PRODUCTION OF MEDICINAL PLANTS

Oliver Kayser and Gerhard Schembecker

Technical Biochemistry, Technische Universität Dortmund, 44221 Dortmund, Germany;
E-mail: oliver.kayser@bci.tu-dortmund.de

Vertical Farming is an innovative concept to produce economically and environmentally efficient plants for food purpose. Due to reduced available land area and increasing energy costs, vertical farming is getting attractive for cost efficient production in urban areas. The same high demand is also obvious for medicinal plants where 90% of traded and medicinally used phytomedicines are collected from the wild. To meet the demand of pharmaceutical industry for standardised products, high level of desired constitutents and ecologically friendly production, we have initiated a vertical farming project on technical scale. In contrast to already existing urban or vertical farming initiatives, fully integration of cultivation, LED-lightning, online biomass control, extraction, and energy supply is planned in an integrated agricultural production unit. Main goal will be to construct an energy and CO2 neutral production unit reusing heat, energy, water, and CO2 from power stations.

IL-107

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

USING MOLECULAR APPROACHES TO IMPROVE CEREAL CROPS FOR ADAPTATION TO MARGINAL SOILS

Leon Kochian

USDA-ARS Robert Holley Center for Agriculture and Health, 538 Tower Road Ithaca, NY 14853, United States;
E-mail: lvk1@cornell.edu

Plants have evolved a number of different mechanisms for dealing with toxic metals in the environment, and these can involve both avoidance (exclusion of the metal from a plant tissue/organ) or true tolerance, which involves binding and/or sequestration of the metal in an internal compartment. The best characterized mechanism of plant metal tolerance is associated with crop aluminum (Al) tolerance. Al toxicity is a worldwide problem that arises when soil pH values drop to 5 or below; in these acidic soils toxic forms of Al are solubilized into the soil solution, damaging roots and resulting in reduced water and nutrient uptake.

The considerable genetic variability for aluminum (Al) tolerance within many crop plant species has been utilized by plant breeders for a number of years to enhance Al tolerance. But beyond this, genetic variability in Al tolerance has been an excellent experimental resource that is being mined by researchers to elucidate the molecular basis for this trait. Because of the agronomic importance of Al toxicity, research on the identification of crop Al tolerance genes has attracted significant interest from a number of laboratories around the world.

This talk will focus on identifying genes underlying a major Al tolerance mechanism which involves Al exclusion from the root tip mediated by Al activation of specialized transporters that release organic acids into the rhizosphere, where they chelate and prevent Al from entering the root. To date, the Al tolerance genes that have been identified are from two different families of membrane transporters mediating this root organic acid efflux. For both types of transporters, Al-inducible regulation of transporter gene expression plays an important role in differential Al tolerance. It is likely that differences in protein structure and function also play a role in differential tolerance, although to date there is no data supporting this. The identification of genes conferring Al tolerance now provides the necessary molecular tools to use in

both biotechnology and molecular breeding to more effectively address a worldwide agronomic problem that is only exceeded by drought stress with regards to abiotic limitations to crop production.

Keywords: Transgenic crop, molecualr breeding, aluminum tolerance, aluminum toxicity, acid soil, tolerance genes, organic acid transporter.

IL-34

Track: *Plant and Environment*

OPTIMIZATION OF HAIRY ROOT TISSUE GROWTH USING STRUCTURED GROWTH MODEL SIMULATIONS AND A CUSTOMIZED IMAGE RECOGNITION SOLUTION FOR DATA ACQUISITION

F. Lenk, M. Vogel, T. Bley and J. Steingroewer

Institute of Food Technology and Bioprocess Engineering, Dresden University of Technology, Bergstraße 120, 01069 Dresden, Germany; E-mail: felix.lenk@tu-dresden.de

Secondary metabolites produced by plant *in vitro* cultures such as *Betanin* (red-dye in beetroot) or Oleanolic and Ursolic acid found in sage are nowadays a main research focus within the branch of White Biotechnology. Cells genetically altered using Agrobacterium rhizogenes form Hairy roots which can be cultivated in hormone free media in modern bioreactors (Georgiev *et al.,* 2007). To improve the cultivation process (higher yield, shorter cultivation time) and the bioreactor design (bubble column vs. stirred) a structured growth model with consequent simulations and visualization is presented as until now no theoretical description of the growth processes exist (Bley, 2010). The determination of relevant parameters such as the number of branching points or specific segment length is carried out using an automatic image recognition software for pictures taken from the Hairy root tissue networks on Agar plates. With the presented innovative customized solution it is possible to quantitatively track a morphological growth process over the cultivation period. Experimental imaging results of the cultivations of *B. vulgaris* are compared with the results of numerical simulations.

Keywords: *In vitro, Betanin*, Oleanolic and Ursolic acid.

IL-35

Track: *Medical Biotechnology*

ENGINEERED CHO CELLS AND VECTORS FOR FAST AND HIGH-LEVEL ANTIBODY PRODUCTION

Mélanie Grandjean, Valérie Lefourn, Pierre-Alain Girod, Alexandre Regamey and Nic Mermod

University of Lausanne, Lausanne, Switzerland; E-mail: Nicolas.Mermod@unil.ch

Incorporation of epigenetic regulatory DNA elements in expression vectors can be used to prevent gene silencing events and increase transcription rates. This has yielded stable and very high specific transgene expression from CHO or primary stem cells, and increased antibody production in the bioreactor. This approach forms the basis of an antibody-screening platform that allows high throughput expression and assay of hundreds of antibody variants in cultured CHO cells, with quick progression of the best antibody towards pharmaceutical production. Specific productivities obtained from CHO cell lines are so high that new cellular bottlenecks are currently emerging, encompassing transgene genomic integration, protein secretion and cell physiology. Methods to engineer the recipient cells for increased genomic integration of multiple copies of the transgene by homologous recombination will be presented. Proper protein secretion and modifications is another bottleneck, especially for difficult to express immunoglobulin variants. We will show how the faulty steps can be identified at the molecular level and how the expression of secretion/modification pathway proteins may solve processing and secretion limitations. This presentation will illustrate how the use of a systematic and multi-level approach can be used to construct improved gene transfer methods and recipient cells for improved expression of pharmaceutical proteins.

IL-89

ECONOMIC MODELS FOR FUTURE BIOREFINERIES: CHALLENGES AND OPPORTUNITIES

David Mousdale

Beòcarta Ltd, Registered Office 76 Dumbarton Road, Glasgow G81 1UG, UK; E-mail: dmousdale@beocarta.co.uk

Both "narrow" biorefineries - those set up to produce only liquid or gaseous biofuels - and more broadly based future biorefineries able to transform plant biomass feedstocks into a range of fine chemicals, enzymes and other products have been extensively assessed for their sensitivity to raw materials input costs. Especially for fermentation facilities capable of rivalling conventional petrochemical refineries and synthesizing a spectrum of chemical intermediates this analysis is too limited, and a broader range of features must be incorporated into economic models. To access sufficient biomass, for example, it is desirable for biorefinery sites to be capable of handling multiple or sequential plant biomass inputs throughout a production year. Equally, variable market factors could dictate time-dependent commercial outputs throughout all or much of the 21st century as fossil fuels continue to dominate relevant industrial sectors. How will these features be incorporated into scenarios for an evolving upstream and downstream infrastructure? Can truly flexible fermentation sites be viable? Are plant biomass supplies even theoretically capable of meeting an increasing demand for bio-based products? Or are genetically modified (GM) crops the only solution to providing biomass on a global scale without increasing the actual (or perceived) conflict between food and fuel?.

IL-52

Track: *Bionanotechnology*

EMERGING TRENDS - NANOTECHNOLOGY FOR AGRICULTURE AND BIOSYSTEMS

Suresh Neethirajan

Department of Biological Engineering, University of Guelph 50 Stone Road East Guelph, ON N1E0B3, Canada; E-mail: s.neethi@uoguelph.ca

Bionanotechnology is an emerging interdisciplinary field at the interface of biotechnology and nanotechnology. Being in the incipient stage, the topical areas of bionanotechnology research are in a wide spectrum. Nanoscale technology is well suited for understanding the interfaces between organisms in systems biology. The challenges associated in characterizing the diverse organisms involved in the plant-microbe interface and dissecting their molecular-based exchanges are being currently addressed by our developed analytical tools and devices. Microfluidic devices combined with nanoscale features aids in functionally assaying bacteria in investigating the species variation during motility. Imaging studies of colonization and surface adhesion kinetics of bacteria using confocal fluorescence and atomic force microscopy (AFM) reveals the evolution of distinct microbial biofilm morphologies and the ultra structure of the bacterial pili. Non-intrusive investigation of single biomolecules is challenging but useful for screening and diagnostic purposes as there are connections between biomarkers and genetic disorders. An accurate methodology for karyotyping of chromosomes and fabrication of DNA nanowire templates are being discussed. This presentation will highlight the uses of micro and nanotechnologies that have been developed for studying biomacromolecules as well as understanding the various aspects of plant-microbe interface.

IL-95

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology, Clinical Research/clinical trials; bioethics; nanobiotechnology*

THE END OF THE MECHANICAL VENTILATOR? EXTRACORPOREAL MEMBRANE OXYGENATION (ECMO) V2.0

Matthew L. Paden*, James D. Fortenberry, Ajit P. Yoganathan, Kevin O. Maher, Carlee Bishop, Shean Phelps and Courtney L. Crooks

Department of Pediatrics, Division of Critical Care, Emory University, 1405 Clifton Road, Atlanta, GA, 30322, USA; E-mail: matthew.paden@choa.org

Positive pressure mechanical ventilation is the most common form of invasive respiratory support worldwide. While a relatively simple lifesaving lung support technology, it is not physiologic breathing and thus with every breath also directly injures the very organ it is trying to support. Alternately, extracorporeal membrane oxygenation (ECMO) is an established, but complex, technique for providing temporary cardiac and/or pulmonary support for critically ill patients when traditional therapies fail. In ECMO, blood is removed from the large veins of the body, pumped through a membrane oxygenator which performs the function of the lung, and the blood is returned to the patient. ECMO was traditionally used in critically ill neonatal and pediatric patients, however ECMO use is increasing in adults, due to the improved survival with ECMO use seen during the worldwide H1N1 influenza epidemic and by the success of the United States military transporting of critically ill service members from the current theaters of operation to definitive care facilities. The complexity and high risk of complications inherent in the current ECMO implementation, limits use to patients with very high risk of death (~20% predicted survival). Since 1975, the Extracorporeal Life Support Organization (ELSO) registry documents 44,824 patients treated with ECMO, with 62% survival. In 2010, 2,701 patients received ECMO therapy with 56% survival, however they suffered 9,493 complications, highlighting the complexity of the therapy. Leveraging the inherent advantages of ECMO, while working towards reducing complications correlated with increased mortality, represent an opportunity to improve ECMO for use in a broader community of patients. For ECMO to be a replacement for mechanical ventilation, a need exists for a standardized, simplified, compact, less expensive, and portable ECMO device to make this therapy safer, easier to use, and available to a broader group of patients. A multi-disciplinary collaboration of critical care physicians, cardiologists, and engineers from Children's Healthcare of Atlanta, Emory University, Georgia Institute of Technology, and the Georgia Tech Research Institute has been assembled to address these challenges. A review of ECMO technology will be presented, with a detailed focus on limitations of the current implementation, imminent innovations, and challenges for the future.

IL-109

Track: *Other areas: Biosafety*

OSTEOPOROSIS AS A SOURCE OF TISSUES MINERALIZATION. INITIAL RESULTS OF A RESEARCH ON OSTEOPOROSIS THERAPY AND DISSOLUTION OF ARTERIES MINERALIZATION

Maciej Pawlikowski

Lab. Biomineralogy, Cath. Mineralogy, Petrography and Geochemistry, AGH-University of Science and Technology, Al. Mickiewicza 30, 30-059 Cracow, Poland; E-mail: mpawlik@agh.edu.pl

The research conducted at the Laboratory of Biomineralogy of the AGH Unive rsity of Science and Technology in Cracow prove that osteoporosis is a process not only attenuating bones but also a process of transferring elements from bones to soft tissues, including arteries. This phenomenon is one of main causes of mineralization (calcification) of tissues, numerous illnesses, and death in effect. The research on mineralization of arteries prove that it has an inorganic, organic or mixed character, e.g. cholesterol and phosphate. Calcification of arteries results in a growth of blood pressure and is one of main causes of heart failures and haemorrhages.

Retention of osteoporosis or its reduction in cases when it is developed not only strengthens bones but also can decrease a transfer of elements from bones to soft tissues. The Laboratory studies prove that it is possible to launch a natural mechanism of self-repair of bones by an organism of a patient suffering from osteoporosis.

The studies on dissolution of arteries mineralization prove that there is a possibility of their partial cleaning from present concrements. It shall have an influence on improved blood circulation, simultaneously decreasing the risk of heart attacks and embolism of arteries. Initial results of the research on dissolution of arteries mineralization dissolution, conducted *in vitro*, are quite promising but require continuation of studies at a larger scale.

The presented issues, details of them and of existing solutions for these essential problems, shall be presented during the lecture.

IL-88

ATOMIC STRUCTURAL TEMPLATES OF THE EARLIEST LIFE ON EARTH: VIBRATION AND LIGHTNING EXPERIMENTS WITH QUARTZ AND AMINO ACIDS

Maciej Pawlikowski

Lab. Biomineralogy, Cath. Mineralogy, Petrography and Geochemistry, AGH-University of Science and Technology, Al. Mickiewicza 30, 30-059 Cracow, Poland; E-mail: mpawlik@agh.edu.pl

The atomic structure of minerals and organic molecules is remarkably similar. In particular, between minerals and amino acids, which are the building blocks of life on Earth. The hypothesis tested is the possibility of energy transfer from minerals (quartz) to amino acids, in the form of energy as early stage of life creation.

The scenario under investigation is that of lightning reacting with quartz sand grains exposed at the surface. The quartz grains contain water within the pore spaces, which has basic amino acid structures within in. The experiments determined that the vibration of piezoelectric quartz altered the structure of amino acid molecules.

IL-15

Track: *Medical Biotechnology*

V617F MUTATION INDUCES NUCLEAR LOCALIZATION OF JAK2 IN CD34+ CELLS BUT NOT GRANULOCYTIC, MEGAKARYOCYTIC OR ERYTHROID CELLS OF PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASIA

Ciro R. Rinaldi, Paola Rinaldi, Vitalyi Senyuk, Nicola Esposito and Giuseppina Nucifora

Department of Hematology-Oncology, Pilgrim Hospital, Boston, Lincolnshire, United Kingdom;
E-mail: ciro.rinaldi@ulh.nhs.uk

OBJECTIVES: Recently, Dawson *et al.* identified a previously unrecognized nuclear role for JAK2 in the phosphorylation of the tyrosine 41 of the histone H3 in haematopoietic cell lines and in one case on peripheral CD34+ cells from a JAK2V617F mutated PMF patient.

MATERIALS AND METHODS: We stably transfected K562 with pMSCV-Puro-JAK2V617F construct and compare with K562 expressing pMSCV-Puro-wild type-(WT)-JAK2 and performed immunofluorescence and western blot analysis. We searched the nuclear localization of JAK2 in total BM and in 4 sorted cell populations (CD34+, CD15+, CD41+ and CD71+) of 10 MPN patients with JAK2V617F and 5 patients with WT MPN.

RESULTS: Confocal immunofluorescent images and Western blot analysis on nuclear and cytoplasmic fractions confirmed nuclear JAK2 in K562 although with the strongest nuclear signal in JAK2V617F expressing cells. We found a strong nuclear signal within the nuclei of 3-5% of mononucleated cells in 10 of 10 JAK2 mutated patients but not in un-mutated cases. We found nuclear JAK2 in CD34+ cells but not in other cell populations. No nuclear JAK2 was detected in differentiated erythroid, granulocytic or megakaryocytic colonies obtained from all patients.

CONCLUSIONS: We identified a nuclear JAK2 in total BM and only in sorted CD34+ cells of patients affected by all subtypes of JAK2 mutated MPN and not in patients with WT diseases.

IL-92

GATA1 IS OVEREXPRESSED IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND POLYCYTHEMIA VERA BUT NOT IN PATIENTS WITH PRIMARY MYELOFIBROSIS OR CHRONIC MYELOGENOUS LEUKEMIA

Ciro R. Rinaldi

Department of Hematology-Oncology, Pilgrim Hospital, Boston, Lincolnshire, United Kingdom;
E-mail: ciro.rinaldi@ulh.nhs.uk

GATA1, the founding member of the GATA transcription factor family, is essential for cell maturation and differentiation within the erythroid and megakaryocytic lineages. GATA1 regulates many genes whose expression is essential in these lineages; its function depends upon its ability to bind both DNA and protein partners[1]. Disruption of either of these functions causes severe hematopoietic dysfunction and results in blood disorders, such as thrombocytopenia, anemia or even leukemia. Enforced expression of GATA1 in the murine myeloid cell line M1 induces c-Mpl appearance and megakaryocytes (MK) differentiation[2]; the same phenomenon has been showed to occur in hematopoietic stem cells, in which a forced increased level of GATA1 blocks self-renewal and induces the exclusive generation of Meg E lineages [3]. Recently, several studies have suggested a connection between GATA1 and myeloproliferative disorders (MPDs), such as the "GATA1 low" mouse model, which develops a disease closely resembling human primary myelofibrosis (PMF) [4]. This association was confirmed in some patients affected by PMF, who showed decreased GATA1 expression in BM sections after immunostaining[5]. Considering the *in vitro* studies on GATA1 overexpression inducing megakaryocytic commitment, and focusing on a possible role of this gene in PMF, we have hypothesized a pathogenic role of GATA1 in myeloproliferative disorders. We studied the expression of GATA1 in 92 bone marrow (BM) aspirates of patients affected by MPDs such as essential thrombocythemia (ET), polycythemia vera (PV), PMF, or chronic phase of chronic myelogenous leukemia (CML) and in patients with acute myeloid leukaemia (AML); we found that GATA1 is significantly overexpressed in ET and PV ($p < 0.003$) but not in the other MPDs; interestingly, we did not found any correlation between the increased levels of GATA and the presence of JAK2 mutation in ET patients, thus indicating that upregulation of GATA-1 may be a central event in the pathogenesis of this myeloproliferative disorders [6]. Anagrelide (Xagrid®, Shire) has been proven to be active drug in reducing platelet count and thrombotic risk in management of ET and PMF patients [7-10], however, the mechanisms by which this drug induces its effect is still unclear. Recently Erusalimsky and colleges have reported on down-modulation of the expression of GATA1 and its co-factor FOG1 in MK during the *in vitro* differentiation in the presence of Anagrelide in culture media [11]. Taking together these results indicate a central role of GATA-expression for the pathogenesis of ET and PV and indicate that it may be a molecular target of the Anagrelide, therefore, it is worth to explore in detail the effects of Anagrelide on MKs and their precursors both in normal and MPD marrow to define the effect of this drug on GATA1 and FOG1 expression. In addition, it is important now to confirm this preliminary results in a larger cohort of patients and more. We could identify the molecular mechanism of Anagrelide action and possibly stratify the patient population according to the GATA1 levels as a predictor factor for the Anagrelide response.

Keywords: NA

IL-82

Track: *Other Areas: Marine biotechnology*

THE POTENTIAL OF MICROALGA FOR THE PRODUCTION OF BIOACTIVE MOLECULES OF PHARMACEUTICAL INTEREST

Virginie Mimouni, Lionel Ulmann, Virginie Pasquet, Marie Mathieu, Laurent Picot, Jean-Paul Cadoret, Annick Morant-Manceau and Benoît Schoefs

Mer Molécules Santé, EA 2160, L'UNAM Université, Faculté des Sciences et Techniques, Université du Maine, Avenue Olivier Messiaen, 72085 Le Mans cedex 9, France; E-mail: benoit.schoefs@univ-lemans.fr

Through the photosynthetic activity, microalgae process more than 25% of annual inorganic carbon dissolved in oceans into carbohydrates that ultimately serve to feed the other levels of the trophic networks. Beside, microalgae synthesize bioactive molecules such as the carotenoids that exhibit health properties. In addition, abiotic stresses, such as high irradiance, nutrient starvation, UV excess,

trigger metabolic reorientation ending with the production of other bioactive compounds such as omega-3 fatty acids or astaxanthin. Traditionally, these essential fatty acids are acquired through the dietary alimentation. The increasing, and often unsatisfied, demand for compounds from natural sources, combined

with the decrease of the halieutic resources, forces the search for alternative resources for these bioactive components. Microalgae possess this strong potential. For instance, the diatom *Odontella aurita* is already commercialized as dietary complement and compete with fish oil for human nutrition. In this contribution, the type and health effects of the most important bioactive molecules produced by microalgae will be briefly reviewed. Then, the results on the impact of abiotic stresses on the metabolic reorientation and the production of selected bioactive components will be presented. The presentation will end by a presentation of possibilities to improve the production of bioactive compounds by microalgae.

Keyworks: Bioactive compounds, algae, pigment, lipid, health benefit, abiotic stress,metabolic reorientation.

IL-81

Track: *Medical biotechnology*

POROUS TITANIUM COMPOSITE AS A PERSPECTIVE MATERIAL FOR RECONSTRUCTIVE SURGERY

M.A. Shevtsov, M. Pitkin, G. Rayakhtsaum, J. Pilling, N.M. Yudentsova, M.I. Blinova, G.P. Pinaev, I.L. Potokin, M.V. Protasov, O.G. Genbach, D.N. Suslov, V. Moxson, V. Duz and O.V. Galibin

Institute of Cytology RAS, St. Petersburg, Russia, Tikhoretsky ave., 4, 194064, Russia; E-mail: Shevtsov-max@mail.ru

Developing of synthetic material for better osteointegration in reconstruction surgery is a goal of many clinical investigations. We aimed to develop a strong porous pylon for integration of surrounding skin and bone for further attachment of limb prostheses. On mathematical modeling and mechanical tests we developed titanium pylon of sintered titanium particles that approximated the strength of anatomical bone. For further tissue integration *in vitro* we coated pylon with human fibroblasts cells in gel. After 7 days of incubation fibroblast monolayer could be detected by electron scan even inside titanium implant. In *in vivo* experiments on Wistar rats we implanted titanium pylon inside animal femur. Morphological analysis demonstrated clear integration of the porous pylon with the surrounding skin. Electron scan of cross-section of implant in experimental group showed tissue elements in the pores of metal. These data were further confirmed on rabbit experimental model, where implanted titanium rod served as pylon in animal movements. Direct skeletal attachment would improve the outcomes of prosthetic management for people with limb amputations. Developed porous titanium composite material demonstrated the capability to act as scaffold for cells and thus could be used for reconstructive surgery.

IL-31

Track: *Industrial and Manufacturing*

BIOGAS ENERGY AND KERATINASE ENZYME: FROM DISCOVERIES TO COMMERCIALIZATION

Jason C.H. Shih

Professor Emeritus, North Carolina State University, USA; Advisor and Founder, BioResource International, Inc., NC, USA; E-mail: jasonshih.bri@gmail.com

Anaerobic digestion is a microbial process that converts organic waste into biogas, which with 60-70% methane is a combustible gaseous fuel. A thermophilic anaerobic digestion (TAnD) system for poultry manure was systematically studied in the laboratory and on the research farm since 1980. At 50-60^{o}C, TAnD was found to produce biogas at 4-10X higher rates than those operated at mesophilic (30-40^{o}C) and ambient (15-25^{o}C) temperatures. The concept was proven and multiple benefits were demonstrated with a pilot-scale TAnD on farm. Supported by UNDP, a full-scale TAnD processing 5 tons of manure daily from a 50,000 hen farm was designed by the author, constructed and operated in China for nearly 20 years since 1992. In recent years, large-scale digesters processing hundreds tons of manure daily become increasingly popular in

China and Europe. Biogas from large digesters can be a significant source of energy to generate electric power or to fuel transportation. Beyond energy production, the digester is a potential source of valuable bio-products. In 1990, a feather-degrading bacterium was discovered and isolated from a TAnD in my lab. A keratinase enzyme and its gene were subsequently isolated and sequenced and over expression by genetic manipulation, accomplished. Scale-up fermentation made the production of the enzyme in industrial scale. The enzyme was found useful in cooking feather meal, improving feed digestibility and breaking down prion proteins. This series of studies have generated a total of 10 patents and a new biotechnology company (www.briworldwide.com). Thousands of tons of the enzyme product are marketed worldwide.

IL-3

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

A STUDY OF SOME ENZYMES OF BIOTECHNOLOGICAL POTENTIAL FROM THE BLUE-GREEN ALGA SPIRULINA PLATENSIS

S. Sivakami, Mosami Galvankar and Rutwik Thengodkar

Department of Life Sciences, University of Mumbai, Santa Cruz(E), Mumbai, India-400 098;
E-mail: sivakami_s2000@yahoo.com

The cyanobacterium, Spirulina occurs naturally in tropical and subtropical lakes under conditions of high pH and with high content of carbonate and bicarbonate. Spirulina has been found in a variety of environments from brackish waters to freshwater and sea water, thus revealing that the organism is capable of surviving in a variety of habitats and colonizes environments in which other organisms fail to survive. Spirulina formed a part of man's diet, particularly among the Kanembu tribe in Lake Chad in Africa and Mexico.

Spirulina is a photosynthetic, filamentous, spiral-shaped, multicellular, blue-green microalga. The two important species are *Spirulina platensis* and *Spirulina maxima.* Among the cyanobacteria, the heterocystous nitrogen fixing ones like *Nostoc* have been studied in detail in relation to their genome, the enzymic apparatus and their biochemical characterization. However there is scant information on the biochemical characterization of proteins and enzymes from Spirulina. In view of the wide use of this organism as a food supplement, feed and fodder and the commercial and biotechnological uses of its proteins, it was decided to carry out a biochemical study of some of the identifiable activities of *Spirulina platensis.*

Two main approaches were employed for this objective : first, assays of commonly used enzymes of biotechnological potential; second, gene sequences of some of the enzymes.

Primers were designed for several activities and Polymerase Chain reaction (PCR) was carried out to obtain partial or full sequences for Superoxide Dismutase (SOD), Catalase, Thiorexoxin Reductase, Urease (beta-subunit) and amylase. These sequences have been deposited in the NCBI database. The PCR products were sequenced and their identities confirmed by the use of the BLAST search.

Two activities, an amylase and an alkaline phosphatase were purified and characterized from the cell free extracts. It was observed that the purified alkaline phosphatase was a monoesterase that was capable of degrading the pesticide Chloropyrifos.

Spirulina platensis was observed to grow in media containing upto 80ppm of the oragnophosphorous pesticide, Chloropyrifos. The disappearance of the Chloropyrifos peak was marked by appearance of a peak corresponding to its less toxic primary degradation product, 3,5,6 – trichloro-2-pyridinol (TCP) during HPLC. The alkaline phosphatase activity was purified by a combination of ammonium sulphate precipitation and ion exchange chromatography. The purified protein was run on a 2-Dimensionsional Polyacrylamide gel electrophoresis, stained with silver, the protein spot manually excised and subjected to MALDI TOF. It was identified to be an Alklaine phosphatase belonging to the class 3.1.3.1. This activity was capable of releasing inorganic phosphate from p-Nitro Phenyl Phosphate as well as sodium beta-glycerophosphate. The purified enzyme showed an optimum pH of about 40^{o}C. The activity was stable to storage for long periods and retained activity over a wide range of temperatures up to 60^{o}C. Its optimum pH was found to be 10.5. It was observed that the purified enzyme (25ug protein) was capable of reducing the Chloropyrifos concentration to about 50% of the original amount in one hour. Since *S.platensis* grows widely under harsh environmental conditions, this organism is ideal for usage in biodegradaton especially in outdoor conditions. Besides, the alga grown under bioremedial conditions can also be used as feed and fodder.

The amylase activity in cell free extracts was identified using starch as substrate by the Dinitro salicylate Assay (DNSA). This activity was purified by conventional protein purification methods. Its biochemical properties like optimum pH and temperature were studied. Chemical modification using group specific reagents revealed the possible presence of histidine, arginine, carboxylate (aspartate and glutamate), serine and tryptophan at the active site. The amylase activity from *S.platensis* has been cloned and characterised. An overview of the enzymic machinery of *S.platensis* and characterization of two of these enzymes will be presented.

Keywords: *S. platensis,* Dinitro salicylate Assay (DNSA), Chloropyrifos, 2-Dimensionsional Polyacrylamide gel electrophoresis, Catalase, Thiorexoxin Reductase, Urease (beta-subunit).

IL-40

Track: *Medical Biotechnology*

SINGLE USE TECHNOLOGY ENABLING BIOSIMILAR MANUFACTURING

William Whitford

BioProcessing Market, Thermo Scientific Cell Culture & BioProcessing, Thermo Fisher Scientific, 925 West 1800 South, Logan, Utah 84321, USA; Email: bill.whitford@thermofisher.com

Biosimilars differ from small molecule generics in a number of ways, resulting in distinct statutory, regulatory, technical, clinical and business considerations. Recent guidance on their development is clarifying such input as biosimilar entity comparability (US: highly similar), clinical studies and manufacturing process parameters. It yet remains unclear just how closely some of those factors, as well as others involving markets and commercialization, will imitate those of small-molecule drugs. The result is that developers of biosimilars are driven to keep investment and operating costs low; development times and costs reduced; and production formats transportable and flexible. Commercially available single use technologies (SUT) and systems allow, or specifically support, such initiatives. Single-use production technology provides a number of benefits from cost savings to process efficiency to heightened safety. Costs savings begin with a reduction in capital requirements and process footprint through eliminating entire operations with their associated service requirements and validation activity. Efficiency is gained by such features as the greatly reduced process turnaround time, ease and safety in product changeover, and economy of site-to-site transfer or replication. Reviewed here are the specific features afforded by SUT as they bear upon the special biosimilar development requirements mentioned above.

IL-30

Track: *Medical Biotechnology*

CELL PHYSIOLOGICAL, NANOBIOTECHNOLOGICAL AND IMAGE PROCESSING APPROACHES FOR CHARACTERIZATION OF CELLULAR TRAFFICKING AND NANOTUBULAR EXTENSIONS

Peter M. Vassilev

Director, Electrophysiology Laboratory, Division of Endocrinology, Diabetes and Hypertension Department of Medicine, Brigham & Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA; E-mail: pvassilev@rics.bwh.harvard.edu

In these studies new combinations of cell physiological, nanotechnological and image processing approaches were used to characterize the trafficking of subcellular membrane vesicles and their interrelations with networks of nanotubular extensions formed between different types of cells. Some of them can be defined as cytonemes or new special type of tubulovesicular processes. The formation of these unique structures is affected by the phospholipase activities of defined multifunctional membrane ptoteins contributing to ion transport. They are linked to endo/lysosomal trafficking. We used imaging techniques to observe the transport of some of these specifically labeled subcellular vesicles between cells along nanotubular intercellular bridges. The networks of the cellular extensions were disturbed in cells from patients with lysosomal and other disorders. Using fluorescence imaging and nanocarrier-assisting techniques as well as electrophysiological, and other biophysical methodologies we characterized the dependence of these processes on defined cell signaling pathways, Ca2+ transport and other ion homeostatic and lipid metabolic mechanisms.

Keywords: Nanobiotechnology Image Processing Cellular Trafficking Nanotubular Extensions.

IL-6

Track

OXIDATIVE STRESS AND ALTERATIONS IN ACTIN CYTOSKELETON TRIGGER GLUTATHIONE EFFLUX IN *SACCHAROMYCES CEREVISIAE*

S. Versari, L. Barenghi and S. Bradamante

CNR-ISTM Institute of Molecular Science and Technologies, via Golgi 19, 20133 Milan, Italy; E-mail: silvia.versari@istm.cnr.it

Glutathione (GSH) is the most abundant non-protein thiol compound present in living organisms. GSH, used in pharmaceutical, agro-alimentary, and cosmetic industries, can be produced by enzymatic or direct fermentative methods. In an attempt to shed light on GSH homeostasis and extracellular release we carried out the experiment SCORE (*Saccharomyces cerevisiae* oxidative stress response evaluation) during the FOTON-M3 space mission. Microgravity and hyperoxic conditions induced an enormous extracellular release of GSH from *S. cerevisiae* cells (yield ~ 40% w/dw), changed the distribution of the buds, and activated the high osmolarity glycerol and cell wall integrity pathways, as well as protein carbonylation. The results from the single spaceflight experiment were validated by a complete set of experiments in simulated microgravity and indicate that cytoskeletal alterations are mainly responsible for the observed effects. The results of ground experiments in which we induced cytoskeletal modifications by means of treatment with dihydrocytochalasin B, a potent inhibitor of actin polymerisation, or (R)-(+)-trans-4-(1-aminoethyl)-N-(4-pyridyl)cyclohexanecarboxamide dihydrochloride monohydrate, a selective ROCK inhibitor, confirmed the role of actin in GSH efflux. We also found that the GSH release can be inhibited using the potent chloride channel blocker 5-nitro-2-(3-phenylpropylamino) benzoic acid.

Keywords: GSH, oxidation, actin, yeast, chloride channels, microgravity.

IL-84

BIOTECHNOLOGY OPPORTUNITIES IN HONG KONG AND THE PRD REGION, CHINA

Albert Cheung-Hoi Yu

Chairman, Hong Kong Biotechnology Organization; Chairman & CEO, Hai Kang Life Corporation Limited, Hong Kong; Vice Director & Professor, Neuroscience Research Institute, Peking University, P.R. China; Chief, Laboratory of Translational Medicine, Institute of Systems Biomedicine, Peking University, P.R. China; Professor, Department of Neurobiology, Peking University, P.R. China

Hong Kong has experienced accelerated growth in the biotechnology field, especially in recent years, even after the waves of the financial crisis. The Hong Kong Government is determined to develop biotechnology as part of the six new pillar industries, capitalizing on Hong Kong's unique competitive advantages. China's 12th Five-Year Plan has for the first time highlighted Hong Kong and the Pearl River Delta (PRD) region as important regional centers for innovation and technology development. As Hong Kong's biotechnology quickly evolves and become more visualized in the international arena, the Hong Kong Biotechnology Organization (HKBIO) promotes Hong Kong's biotech industry growth, building on the solid foundation which the city already possessed and enhancing collaborations between Hong Kong and the PRD, where numerous new opportunities for biotech development are foreseen. Prof. Albert Yu will introduce the infrastructure of Hong Kong and PRD that accelerates biotech growth, and how international players can tap into the China market utilizing the unique advantages available in Hong Kong and PRD.

SESSION LECTURES

SL-87

Track: *Industrial and Manufacturing*

PRODUCTION AND CHARACTERIZATION OF PEROXIDASES FROM FUNGAL ISOLATE *ASPERGILLUS NIGER* SA1

Safia Ahmed and Shazia Erum

Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan; E-mail: safiamrl@yahoo.com

Aspergillus niger SA1 was selected for the production of Peroxidase enzymes (Laccase, Lignin peroxidase and Manganese peroxidase). Production of Laccase by *Aspergillus niger* SA1 was maximum (1784.38 IU/ml) in production media supplemented with AR 151 dye (10 ppm). Optimum time of incubation was 168 hrs. Characterization of crude lignin peroxidase gave significant rise in the activity of LiP with an elevated concentration of substrate from 2.5 to 10 mm. Optimum pH was 7 for LiP activity. Significant interaction ($p<0.05$) was observed at different temperatures with LiP activity from fungal strain, highest LiP activity (63.92 IU/ml) at 80°C and it reduced (10.06 IU/ml) at low temperature (10°C). Molecular weight of the enzymes was found 66.2, 43 and 38Kda, respectively. Enzyme was purified from the fungal broth via DEAE cellulose column chromatography.

Textile dyes (AR 151, DBK_2RL, Orange II and Sulfur black) were analyzed via decolorization assay with crude and purified enzyme minimal time of 10 sec was found to be sufficient for decreasing in absorbance of initial color for all four textile dyes with crude and purified (lyopholized) enzyme. The stability of crude peroxidase with commercial laundry detergents was positive and showed better effect of stain removal from the cloth.

SL-108

Track: *Other areas: bioethics*

REGULATING NOVEL METHODS OF REPRODUCTION: WOMB TRANSPLANTATION, THE NEXT BREAKTHROUGH?

Amel Alghrani

Centre for Social Ethics and Policy, University of Manchester School of Law, Williamson Building, Oxford Road, Manchester, M13 9PL, UK; E-mail: amel.alghrani@manchester.ac.uk

As scientific progress in the realm of assisted reproduction and biotechnology continues to race ahead, the next revolutionary breakthrough on the horizon is the prospect of womb transplantation. Fertility doctors around the world are researching how to attain the first human pregnancy following a womb transplan t. The world's first human womb transplant has already been attempted in Saudia Arabia in 2000 with some success [1]. The purpose of the procedure would be to restore fertility to patients with an abnormal, damaged, or absent uterus. In the UK alone, it is estimated that approximately 15,000 women per year who seek the help of fertility specialists are found to be incapable of becoming pregnant because of uterine factor infertility [2]. Even though these women may have functioning ovaries, the lack of a functioning womb means they have no chance of gestating their own child to term. The only other option for such woman to achieve genetic motherhood is via surrogacy. For Muslim women surrogacy is regarded as prohibited in Islam and thus not an option. The possibility of womb transplantation would provide a welcome means by which these women could experience gestational motherhood. However this advance raises a host of legal and bioethical questions that have thus far been relatively neglected in legal and ethical discourse. Should this "non-life-saving transplant" be permissible? Is it akin to the transplantation of any other major organ or does it raise separate and unique ethical questions? This presentation outlines the research currently underway into this advance and considers the legal, bio-ethical and regulatory ramifications this advance could raise.

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SL-77

Track: *Industrial and Manufacturing*

A STUDY OF BIODESIEL FROM NON-EDIBLE OIL SEEDS

Elizabeth Funmilayo Aransiola

Department of Chemical Engineering, Cape Peninsula University of Technology, Cape Town 8000, South Africa; E-mail: aransiola4@yahoo.com

This study investigated production of biodiesel from non edible oil seeds of Jatropha curcas and neem obtained in Nigeria with a view to develop a database for the development of biodiesel production, and to encourage the cultivation of these plants in Nigeria. The effects of reaction temperature and oil-to-alcohol molar ratio on the biodiesel production from these oils were investigated with a view to confirm the existing base case. The maximum biodiesel conversion from these two oils was obtained at 333 K and oil-to-alcohol molar ratio of 1:6. A maximum yield of fatty acid methyl esters (FAME) of 87% with a viscosity of 5.64 cSt was obtained for Jatropha biodiesel. This viscosity was used as an index for maximum conversion of biodiesel (BD) from neem oil. The viscosity obtained for neem oil biodiesel was 5.53cSt. Different blends of neem oil biodiesel were tested on an internal combustion engine. The emissions of different blends showed that neem biodiesel has lower emissions of CO and NO than petrol diesel but higher NOX. The physical properties of the BD obtained from Nigerian Jatropha and neem oils met the ASTM standard of D-6751.

Keywords: Biodiesel, transesterification, Jatropha oil, Neem oil, biofuel.

SL-60

Track: *Industrial and Manufacturing*

ACTIVITY AND THERMOSTABILTY IMPROVEMENT OF LACCASE FROM *PLEUROTUS OSTREATUS* IBL-02 BY HYDROPHOBIC GEL ENTRAPMENT IMMOBILIZATION

Muhammad Asgher, Shagufta Kamal and H.M. Nasir Iqbal

Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan;
Email: mabajwapk@yahoo.com

Laccase produced by an indigenously isolated strain *Pleurotus ostreatus IBL-02* during decolorization of reactive textile dye Drimarene brilliant red K-4BL (DBR K-4BL) was purified through ammonium sulphate fractionation, dialysis, gel filtration and ion exchange chromatography. The purified enzyme was immobili zed by hydrophobic gel entrapment in xerogels of different hydrophobicity prepared by using different molar ratios (1:1, 1:5, 1:10, 1:15, 1:20 & 1:25) of trimethoxy silane (TMOS)/proplytetramethoxy silane (PTMS). The free and immobilized laccases were characterized to investigate the effect of immobilization on activity and thermostability of the enzyme. Immobilization of purified laccase was found to enhance its activity and thermostability. Laccase immobilized in the xerogel of 1:5 T:P ratio was the most active and thermostable enzyme having optimum pH 4 and optimum temperature, 55^{o}C with a half life of 3 h and 35min. at optimum temperature. Kinetic study revealed that laccases immobilized in 1:2 and 1:5 T:P ratio gels had higher K_M (83 & 100mM) and Vmax (1000 & 1111mM/mg) as compared to free laccase. The catalytic efficiency for immobilized laccase (85 S^{-1}) was higher than free laccase (42 S^{-1}). The thermostability and kinetic characteristics reflect the suitability of this enzyme for industrial applications

Keywords: Laccase, Xerogels, Immobilization, Characterization, Thermostability.

SL-23

Track: *Plant and Environment Biotechnology*

PHYTOREMEDIATION OF HEAVY METALS CONTAMINATED-SOIL BY *CHROMOLAENA ODORATA* (L) K & R PLANTS TREATED WITH VERMICOMPOST

Harrison I. Atagana

Institute for Science and Technology Education, University of South Africa, P.O. Box 392, UNISA Pretoria 0003, South Africa; E-mail: atagahi@unisa.ac.za

This study investigated the effect of the application of vermicompost on the growth and biomass accumulation of Chromolaena odorata and its uptake of heavy metals from contaminated soil in a greenhouse. The heavy metals, Cd, Cu, Pb, Ni and Zn were used to amend the soil to give final concentrations 0, 10, 50, 80 and 100 mg kg^{-1}. The soil was mixed with vermicompost in a ratio of 3:1 in plastic soil pots and six weeks old Chromolaena odorata propagated from stem cuttings were transplanted into the contaminated soil and kept in the greenhouse for twelve weeks. The growth of Chromolaena odorata was not significantly inhibited by concentrations of the heavy metals below 50 mg kg^{-1}. The application of vermicompost enhanced the growth of Chromolaena odorata and the uptake of heavy metals. Overall, the uptake of metals was increased by the application of vermicompost; however, the effect was least evident in the Cd amended treatments while the Zn treated experiments showed more uptakes.

Keywords: vermicompost, *Chromolaena odorata*, Zn.

SL-55

Track: *Medical Biotechnology*

INDIGENOUS SHEEP BREEDS OF PAKISTAN AS RESERVOIR OF GENETIC DIVERSITY

Masroor Ellahi Babar, Tanveer Hussain, Asif Nadeem, Akhtar Ali, Kamran Abbas and Haleema Sadia

Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore-Pakistan 54000; Email: drbabar@hotmail.com

Sheep are among the fastest growing ruminants of Pakistan. Pakistan is blessed with a wide range of sheep breeds spread throughout the country with distinct features contributing to rich Animal Genetic Resource. The genetic diversity of indigenous breeds was explored using nuclear (Microsatellites markers) and extra nuclear genome (mitochondrial D-loop region and Cytochrome b gene) through genotyping and sequencing respectively. Analysis of sequences revealed 151 variable sites in 2679 bp amplified region in fifty animals of ten different sheep breeds of Pakistan. All Sheep breeds had unique haplotypes. There were 61 A↔G tranitions and 70 C↔T transitions. One hetromorphic transversion which also showed a pattern of transition C→A/T. Transition to transversion ratio was 8.6:1.4. The phylogenetic analysis using bioinformatics softwares of these breeds along with reported sequences throughout the world indicated interesting information about their relationships and origins of sheep in Asia. The paper also embrace comprehensive information of ISAG recommended microsatellite markers in selected sheep breeds like effective number of alleles, observed and expected heterozygosity, Hardy-Weinberg equilibrium, Fis, Fit values. The data demonstrated remarkable dendograms. The data will highly useful for genetic differentiation and forensic applications in future. The findings of this study will facilitate the researchers and breeders to understand the genetic relationships and breed differences for making future conservation policies to preserve this rich reservoir of genetic diversity in the country.

Keywords: Genetic resource, Polymorphisms, Phylogenetic analysis, Sheep breeds, Pakistan.

SL-29

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

BIOREMEDIATION OF TEXTILE MILL EFFLUENT USING MARINE FUNGI

S. Saravana Babu

Department of Botany and Plant Biotechnology, Chikkiah Naicker College, Erode, India;
E-mail: ssbabuflora@yahoo.com

Textile effluents are highly complex and are characterized by high toxicity and intensive colour. Treatment of textile waste water is carried out by physical or chemical or biological or combination of the above methods. The biological method of treating the effluent was proved to be an effective one compared to the physical and chemical method. In the present study an attempt was made on the decolourization and defoxification of textile effluent using marine fungi. Trichoderma harzianum and A.flavus isolated from marine environment. Among these two fungus, the Trichoderma sps proved to be an efficient one in detoxifing the effluent. The decolourisation is about 85%. Mass spectrometric studies revealved the degradation of toxic compounds in the effluent is an effective one. The results are discussed.

Keywords: Decolourization, Defoxification, Trichoderma harzianum, Trichoderma.

SL-94

Track: *Business Development*

PATENTABILITY OF BIOTECHNOLOGICAL AND MEDICAL INVENTIONS IN IRAN

Mohammad Reza Bakhtiari and Masoud Fallahpour

Biotechnology Deptartment, Iranian Research Organization for Science and Technology (IROST), No 27, Forsat St., Tehran 15819, Iran; E-mail: bakhtiari@irost.org

In January 23rd, 2008, the Iranian parliament enacted a new intellectual property law called PITRA2008 which stands for "Patents, Industrial Designs, and Trademarks Registration Act. PITRA 2008 has been drafted mainly based on an IP law model proposed by the World Intellectual Property Organization (WIPO). This law replaced the previous 1931 act and has considerable modifications, especially in the fields of biotech and pharmaceutical inventions.

According to PITRA2008, methods of treatment in humans and animals are excluded from patentability, plainly. While pharmaceutical inventions are patentable in contrast to the 1931 act. Unfortunately, the biotech inventions seem not to be patentable because biological processes for producing genetic material and components have been totally excluded from patentability in article four of the law. However, an inquiry bill has been submitted to the parliament in order to amend the article.

In this paper I will try to review articles of Iran PITRA2008 relevant to patenting pharmaceutical, medical and biotech inventions. General requirements of patentability, the patentable subject matters and exclusions, compulsory licensing, and parallel imports will be discussed in detail.

One of the objectives of presenting such information is to clarify the situation of the legal protection of biotech inventions in Iran for those researchers or companies who intend to have research collaborations with Iranian counterparts or plan to do joint projects on technology transfer.

Keywords: Iran IP law, pharmaceutical, medical, biotech inventions, patents.

SL-25

Track: *Medical Biotechnology*

DEVELOPMENT OF MAMMALIAN EMBRYOS AT THE INTERNATIONAL SPACE STATION

D.M. Barry

Department of Animal Sciences, University of Venda, Private Bag X5050, Thohoyandou 0950, South Africa;
E-mail: Daniel.Barry@univen.ac.za

During the launch of the Soyuz rocket and Space Capsule No. 33 by Energia, the Russian Rocket and Space Corporation, from Baikonur, Kazakhstan, we sent mammalian embryos for a period of 10 d in a portable incubator (Biotherm, Australia) with Mark Shuttleworth, (the first African in space), to the International Space Station (ISS).

One-cell stage embryos were collected from multi-ovulated local Tegeres sheep ewes, and the 1-cell mice embryos (n = 510) were collected from F1-hybrid mice bred in Moscow, Russia. The sheep and mice embryos were collected at Baikonur, close to the Soyuz launching site. The embryos were cultured at 38.5° C at the ISS.

During the flight the culture medium in the test tubes containing the embryos were changed every second to third day by injecting the medium from reservoir tubes into the specially adapted culture test tubes in the portable Biotherm incubator. The culture medium used was TCM-199 with 20% FBS, 1% antibiotics, and 0.5% of both essential and non-essential amino acids. A filter in the culture test tubes prevented the suspended experimental embryos from being flushed out of the culture tubes. The spent medium was collected into a plastic bag by means of a fixed tube. Control embryos samples were cultured under similar conditions on earth for the duration of the space flight.

After their return from outer space, the embryo and the corresponding control samples were frozen immediately (Freeze Control, Cryologic, Australia) in liquid nitrogen, using ethylene glycol as a cryo-preservative. The control and space samples were then transported to a Biotechnology laboratory in South Africa for evaluation and further analysis. It was shown that the sheep and mouse embryos grown under micro-gravity conditions for the 10-d interval at the ISS developed past the hatching stage of the embryo, and an enlargement and outgrowth of the inner cell mass was observed.

Of the sheep embryos cultured at the ISS, 37.5% reached the hatched blastocyst stage, while 25% of the control sheep embryos hatched.

Keywords: Sheep, mice, mammalian embryos, International Space Station, culture.

SL-13

Track: *Plant and Environment Biotechnology*

ISOLATION AND CHARACTERISATION OF PHENOL-RESISTANT BACTERIA FROM INDUSTRIAL EFFLUENT CONTAMINATED SOIL AND STUDY OF THEIR BIODEGRADATION POTENTIAL

Sreela Pal Basak and Priyabrata Sarkar

Department of Polymer Science & Technology, University of Calcutta, 92 A.P.C. Road, Kolkata-700009, India;
E-mail: sreela_basak@yahoo.com

Five phenol-degrading bacteria designated as CUPS-1 to CUPS-5 were isolated from the oil-effluent dumped sites of Haldia Industrial Area of Mednipur district of West Bengal, India (22° 05'N, 88° 03' E) and characterized. Of these five strains, CUPS-3 was found to be a novel strain designated as Stenotrophomonas maltophilia GU358076, while the other four were CUPS-1-Delftia sp.; CUPS-2-Pseudomonas sp.; CUPS-4 –Micrococcus luteus; CUPS-5-Pseudomonas aeruginosa respectively. All of these strains were able to utilize phenol as the sole carbon source to support their growth. Most of these bacterial strains such as CUPS-1, CUPS-2, CUPS-4, and CUPS-5 could tolerate phenol concentrations upto 15mM, while CUPS-3 was tolerant to phenol concentration upto 20mM. The phenol degrading potential of these bacteria are being exploited and it is found that almost (89-92%) of about 1500mg/l of phenol concentration could be degraded within 72 hours.

Keywords: phenol-degrading bacteria, CUPS-1 to CUPS-5.

SL-72

Track: *Industrial and Manufacturing*

UTILIZATION OF AGRICULTURAL WASTES FOR PRODUCTION OF LIPASE BY *PENICILLIUM NOTATUM*

Haq Nawaz Bhatti and Saima Rehman

Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan;
E-mail: hnbhatti2005@yahoo.com

In the present study cultural conditions were optimized for enhanced production of lipase by *Penicillium notatum*. Different agricultural wastes viz. canola oilseed cake, sesame oilseed cake, linseed oil cake, cotton oilseed cake; rice bran and wheat bran were used as substrates for the produc tion of lipase under solid state fermentation conditions. Optimization of different physicochemical factors led to 2 folds enhancement in lipase production. Under optimum conditions an enzyme activity of 5335U/gds was observed. *Penicillium notatum* lipase was purified to homogeneity by four step purification strategy to achieve 28.88 fold purified enzyme with 13.4% recovery and 26779U/mg specific activity. The molecular mass of the homogeneous lipase was 46kDa as determined by SDS-PAGE. It was optimally active at pH 9.5 and 40°C. The Michaelis Menten constants (Km) and Vmax of lipase from *Penicillium notatum* for para nitrophenyl palmitate hydrolysis at optimum temperature were 3.33mM and 232.6 μ mol/mL min-1 respectively. The enzyme was stable in the broad pH range from pH 6.0 to 12, with maximum stability in the range of 8.5-11.0. The enzyme show a high thermo-stability with half lives of 8.25, 3.2, 1.12 and 0.58 h 40, 50, 60 and 70°C. The activation energy for denaturation was 81.1 kJ/mol. Exposure to hydrophobic environment (urea solution and organic solvents), did not affect the enzyme significantly. However when incubated with protease solution, denaturation of enzyme was observed.

Keywords: Lipase, Production, Purification, Thermal stability, Denaturation.

SL-103

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology, Clinical Research/clinical trials; bioethics; nanobiotechnology*

THE CYBORG: A CORE CONCEPT FOR ANSWERS AND QUESTIONS OF NEUROTECHNOLOGY

Paolo Benanti

Pontifical College of Anagni (Fr) part of Pontifical Theological Faculty Teresianum, Istituto Teologico di Assisi (PG) parto of Pontifical University Lateranum, Pontifical Gregorian University, Conv. S. Antonio, Via S. Paolo 2, 06081 Assisi (Pg), Italy; E-mails: fr.paolo@gmail.com; benanti@unigre.it

Neuroethics can – and arguably should - be used to define and ad dress the moral questions concerning the current and future use or misuses of neurotechnology. As defined by Clyne and Kline, the cybernetic organism, or "cyborg" has evolved numerous meanings –from that of Sci-Fi literature, to the reality posed by both our scientific progress, and an increasing reliance upon, and devotion to such technology. Thus, the cyborg stands as a critical example for hopes and fears, as well as answers and enduring questions that arise in, and from neurotechnology. To address these emotions and issues, this lecture will present trans-humanist /post-humanist scenarios, in which the cyborg might be seen as a model of both techno-reductionism (the human as machine) and of enhancing human flourishing through the use of machines. In both scenarios, the cyborg is an emergent state of an evolving, complex biotechnologic-human system. So defined, the cyborg raises numerous moral and ethical questions and problems, that may prompt us to ask not only "…how much human-technologic fusion is too much?", but also "why?" In this light, a draft of neuroethics is proposed that is based upon a commitment to personal morality in the use of technology. It will shown such a system of neuroethics does not attempt to develop a set of absolute rules that answer every kind of problem, but rather affords a set of tools and instruments to enable effective and morally sound governance of neurotechnology.

SL-114

ETHICAL PARADIGMS FOR THE EVALUATION OF NEUROTECHNOLOGIES IN HUMAN ENHANCEMENT

Paolo Benanti

Pontifical College of Anagni (Fr) part of Pontifical Theological Faculty Teresianum, Istituto Teologico di Assisi (PG) parto of Pontifical University Lateranum, Pontifical Gregorian University, Conv. S. Antonio, Via S. Paolo 2, 06081 Assisi (Pg), Italy; E-mails: fr.paolo@gmail.com; benanti@unigre.it

Knowledge's improvement in neurosciences and large number of neurotechnological application, big part of them over human beings, should be well understood and evaluate to void serial ethical implications. These experiments, often characterized by low invasiveness, are aimed to better understand brain functioning and its relationship with the thought and behavior. These trials have given a significant contribution to the debate in the cognitive field, stimulated a philosophical debate about free will-responsibility and attracted interest from the public. In this context we show how ethics and bioethics should try to analyze how neurosciences and neurotechnologies, with their progress, require to be understood and analyzed mainly in relation to their impact on society to answer with an effective governance. In our lecture we would like to draft an outlook of this new frontier and let emerge how and why governance of neurotechnology and enhancement drugs is urgently required. We will summarize ongoing ethical paradigm that are now used to evaluate those phenomena ("Fear for uncertain", "Equality and pursuit for happiness", and "Policy) and we will propose a new paradigm based on recent acquisition of philosophy of technology that we call "empiric turn".

SL-112

Track: *Medical Biotechnology*

EFFECTS OF LEAD AND MERCURY ON THE BLOOD PROTEOME OF CHILDREN: THE FOCUS ON APOLIPOPROTEIN E"

Robert E. Birdsall, Michael P. Kiley, Zaneer M. Segu, Christopher D. Palmer, Milan Madera, Brooks B. Gump, James A. MacKenzie, Patrick J. Parsons, Yehia Mechref, Milos V. Novotny, and Kestutis G. Bendinskas

Department of Chemistry, 226B Snygg, SUNY- Oswego, Oswego, NY, 13126, USA;
E-mail: kestutis.bendinskas@oswego.edu

The proteomics approach was utilized in order to find biomarkers related to lead and mercury exposure at concentrations significantly below CDC guidelines. Blood plasma and serum samples from 34 children were depleted of their most abundant proteins using antibody-based affinity columns and analyzed using two different methods, LC-MS/MS and 2-D electrophoresis coupled with MALDI-TOF/MS and tandem mass spectrometry. Among other 15 potential biomarker targets, apolipoprotein E demonstrated a significant association with lead concentrations (average being one microgram/deciliter) as deduced from LC-MS/MS and 2-D electrophoresis and confirmed by Western blot analysis. Cloned and serum apolipoprotein E exhibited in vitro binding to IDA columns loaded with lead. Serum proteins directly binding to lead and other metals are currently being investigated. The study is the first in the field of proteomics to study toxicology of heavy metals in blood in a general population of children. It is the first to statistically relate a cardiovascular protein Apolipoprotein E with sub-clinical blood Pb levels and to prove apolipoprotein E- Pb binding. These findings also support previous evidence from our group that have associated lead exposure in children with an increase in risk factors related to cardiovascular disease.

SL-62

Track: *Plant and Environment*

TRANSGENIC BARLEY AS BIOREACTOR FOR HUMAN LACTOFERRIN PRODUCTION

A. Yemets, I. Tanasienko, S. Isayenkov, V. Radchuk and Ya. Blume

Institute of Food Biotechnology and Genomics, National Academy of Sciences of Ukraine, Osipovskogo str., 2a, Kiev-123, 04123, Ukraine; E-mail: cellbio@cellbio.freenet.viaduk.net

Because barley seeds are natural protein storage they could be used as efficient bioreactor for producing of recombinant proteins. Here we describe a system for transfer and successful expression of recombinant human lactoferrin gene (*hLF*) in a few Ukrainian barley varieties using *Agrobacterium*–mediated and biolistic transformation. Two vector constructs carrying *hLF* gene driven under glutelin promoter and terminator were created. Binary vector pBiLF with selectable marker gene *hpt* for hygromycin resistance was used for agrobacterial transformation and construction pHLFTubA with mutant α-tubulin gene conferring resistance to dinitroaniline herbicide, trifluralin (selectable gene), was used for biolistic transformation. Isolated mature barley embryos were transformed with *Agrobacterium* and callus cultures were bombarded respectively. Transformed cells were selected on respective media containing selective concentrations of hygromycin or trifluralin and regenerated. Seeds from all selected plants (F1) were used for analysis of lactoferrin expression levels. Respectively, total RNA was extracted from two days germinated seeds. Then purified RNA was used to synthesize first strand cDNA for further PCR with lactoferrin specific primers. The 542 length fragments of the *hLF* gene were amplified during molecular analysis from transgenic seeds that confirm a successful integration and expression of recombinant *hLF* gene in transgenic barley plants.

Keywords: *Agrobacterium*, pBiL, recombinant *hLF* gene, cDNA, biolistic transformation.

SL-90

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

MOLECULAR ANALYSIS OF ENDOSYMBIOTIC BACTERIA NODULATING RETAMA SHRUBS LEGUMES IN NORTHEAST OF ALGERIA

Farida Boulila, Katia Djenadi, Djellali Belhadi, Nacer Ramdani, Gisèle Laguerre and Abdelghani Boulila

Faculté des Sciences de la Nature et de la Vie. Université de Béjaia. Algeria; E-mail: abadifarida@yahoo.fr

Shrubby legumes of the Retama genus are endemic to the Mediterranean Basin and distributed in the various Mediterranean climates from humid to arid, since Retama shrubs are tolerant to extreme drought conditions (Allen and Allen, 1981, Quezel and Santa, 1962). Three species, Retama monosperma, Retama raetam, and Retama sphaerocarpa, are recognized within the Retama genus (Allen, Allen, 1981). These plant species are of ecological interest for dune stabilisation, soil fixation, and revegetation of semiarid ecosystems (Caravaca, *et al.* 2003). Little information has been available about genetic diversity, host specificity, and symbiotic performance of the rhizobia spontaneously associated with Retama shrubs, despite their potential for revegetation projects (Rodrıguez Echeverrıa, Perez Fernandez 2005).

In this study, isolation and molecular characterization of 67 rhizobia-nodulating Retama species growing at seven sites in northeastern Algeria are reported. The sampling sites were chosen along an ecological–climatic gradient en compassing humid zones of coastal dunes to interior zones of the country with a semiarid climate. The genetic diversity of indigenous populations of Retama-nodulating rhizobia was assessed by restriction fragment analysis of PCR-amplified DNA fragments (PCR-RFLP) of four loci, the 16S rRNA gene, the intergenic spacer (IGS) between the 16S and 23 S rRNA genes, and the symbiotic genes nifH and nodC. Genetic relationships of these isolates to other rhizobia were investigated by sequencing the IGS and housekeeping (dnaK,glnII, and recA) genes for representative haplotypes.

Keywords: Shrubby legumes, Retama monosperma, Retama raetam, and Retama sphaerocarpa, PCR-amplified DNA fragments (PCR-RFLP).

SL-54

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

DEVELOPMENT OF THE MOLECULAR DETECTION METHODS FOR FUSARIUM OXYSPORUM F. SP. NIVEUM

Ying-Hong Lin, Kan-Shu Chen, Jenn-Wen Huang and Pi-Fang Linda Chang

Department of Plant Pathology, National Chung Hsing University; No. 250 Kuo-Kuang Rd., South District, Taichung City, Taiwan 40227, ROC; E-mail: pfchang@nchu.edu.tw

The genus *Fusarium* causes devastating plant diseases worldwide, in which *Fusarium oxysporum* is one of the most severe pathogens of crops. The pathogen has over one hundred host-specific forms (known as *forma specialis*, f. sp.) according to the host it specifically infects. For example, *F. oxysporum* f. sp. *niveum* (Fon), the targeted pathogen in this study, is considered as one of the greatest damaging pathogens causing vascular disease of watermelon which in turn is one of the most important restricting factors for watermelon production. A rapid and reliable detection method of pathogen is essential to a sound integrated disease management practice in many crops. This study describes an efficient method for the specific detection of Fon in infected soils and samples of watermelon plantlets and seeds. With optimized PCR assay, the molecular methods could detect Fon in diseased watermelon plants and infected seeds and soil with extreme sensitivity of detecting low numbers of Fon. The PCR detection methods developed for different samples are useful tools that can be beneficial to quarantine purposes, breeding program, as well as other basic researches.

Keywords: *Fusarium oxysporum* f. sp. *niveum* , pathogens, PCR.

SL-118

THREE YEAR STUDY OF A PRIVATE CORD BLOOD AND MESENCHYMAL CORD- BANK CRYOBANKS OF IASO MATERNITY PEDIATRICS RESEARCH AND GENERAL HOSPITAL-THE GREEK EXPERIENCE

Eirini Mitrou, Konstantinos Ntallis, Nikos Panagiotopoulos, Emmanouil Bougioukas, Vaso Kalodimou and Ekatherina Charvalos

Director of Central Labs and CRYOBANKS-IASO Maternity, Gynecologic, Pediatrics and Research Hospital

CRYOBANKS is one of the most reputated stem cell bank of Greece. Historically, Cryobanks International Services of Athens-IASO is the 1st stem cells bank kicked off in 2005 and the 1st accredited bank from AABB **(American Association of Blood Banks) in Greece.** CRYOBANKS is divided in two units, namely, the laboratory and storage unit (cord blood and cord mesenchymal bank) and the regenerative, research and flow-cytometry unit. Two major projects are under consideration /accomplishment, the adipose tissue development technique for esthetic surgery gynecology-oncology purposes and the development of flow-cytometry for purposes other than for testing of cord blood bank products.

The stored 22.000 cord blood units as well as the 7000 umbilical cord- mesenchymal stem cells units translate into years of experience in the field of cellular therapy products and a frame of continuing effort for excellence. We briefly report here, a study of CRYOBANKS cord blood collection and storage during a period of three year 2008-2010. The whole procedures have been accomplished according to AABB Standards for Cellular Therapy Products (4th Edition) and taking in consideration regional ethical and cultural issues. Most of the samples have been collected at IASO from deliveries or ceasareans. From a total number of 11482 units collected, 11111 have been processed by the AABB approved protocol. Five hundred forty six were with low volume and from those 364 have been discarded for this reason. Twenty seven samples have been discarded because of client's request.

From a number of 4368 of units processed in 2008, 3644 in 2009 and 3099 in 2010, 0.69%, 0.93% and 1.32% cultured specimen respectively were positive for the presence of bacterial /fungal pathogens after inoculation and culture by the BACT T alert system. Ceasarean collections mostly were free of contaminants. By routine culture procedures we identified (descending order), Enterobacteria, *Staph aureus, Enterococci, Bacilli, Diptheroides, Streptococcus spp, Pseudomonas sp and Candida sp.* Antibiotic resistance profiles have been identified by using the Vitek 2 system (Biomérieux, France). The strains in all the cases showed usual antibiotic sensitivities. The above mentioned contamination rates, kind of bacteria and antibiotic resistances are negligible, confirming thus the capacities of our premises.

Cord stem cell banking started in October 2010 using a classical cell extraction protocol, but with major improvements (results presented at the 1st International conference on Stem cell research, Turkey, 28- September- 2 October 2011). Private vs public banks, new products for cellular therapy are among our priorities. We would like to open the dialog with private banks in the Mediterranean and Middle East region to create bridges, exchange ideas and promote our capacities.

SL-24

TANGY SCENT IN *TOONA SINENSIS* (MELIACEAE) LEAVES: ISOLATION, FUNCTIONAL CHARACTERIZATION, AND REGULATION OF TWO KEY TERPENE SYNTHASE GENES IN THE BIOSYNTHESIS OF THE SCENT COMPOUND

Chih-Yao Hsu and Shu-Miaw Chaw

Biodiversity Research Center, Academia Sinica, 128 Academy Road, Section 2, Taipei 115, Taiwan; Tel: 886-2-2787115; E-mail: smchaw@sinica.edu.tw

Toona sinensis (Chinese Mahogany; Meliaceae), a subtropical deciduous tree, has a tangy scent resembling a mix of shallots and garlic. *Toona* has long been know for its medicinal *efficacy for treating* enteritis, dysentery, itch and some cancers. fficacious components, volatile scents, and biosynthesis genes remain unknown. In this study, we identified the spectrum of volatile compounds, isolated and functionally characterized two terpenoid synthase genes, *Tstps1* and *Tstps2*, responsible for terpenoid synthesis in *Toona* leaves. TsTPS1 and TsTPS2 have multiple products and mainly regulate the biosynthesis of (+) limonene and β-elemene *in vitro*, respectively. Our *headspace analyses show* that 80% of leaf volatiles were sesquiterpenoids and the developing leaves released a greater diversity and quantity of volatiles

than the mature leaves did, but β-elemene was the dominant in both of them. These data suggested that tangy scent of *Toona* consists a combination of terpenoids and that *Tstps2* was the major gene involved in the terpenoid biosynthesis in *Toona*. *In situ* hybridization revealed that glandular cells of the leaf rachises accumulated abundant *Tstp1* mRNA transcripts. Our GFP-based assay further unprecedentedly demonstrated that the transit peptide of TsTPS1 targets specifically to the mitochondria.

Keywords: *Toona sinensis*; terpene synthase; terpenoid; volatiles; (+) limonene; β-elemene.

SL-43

Track: *Pharmaceutical Biotechnology: biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering.*

FIBRINOLYTIC ENZYME 'ACTINOKINASE' – AN *IN-VITRO* EVALUATION

R.R. Chitte, P.P. Kanekar, L. Krishnan, R.S. Kartha and G.S. Bhuvaneshwar

Microbial Sciences Division, MACS-Agharkar Research Institute, Pune 411 004, India; E-mail: rrc10@rediffmail.com

Cardiovascular diseases, particularly cerebral stroke, myocardial infarction are becoming prevalent all over the world. The therapeutic agents specially fibrinolytic enzymes available in market exert side effects like hemolysis, hemorrhage and immunogenicity thus directing researchers to search for new thrombolytic agents. A novel fibrinolytic enzyme namely 'Actinokinase' isolated from thermophilic *Streptomyces sp.* MCMB 379 is found to have potential as a thrombolytic agent. The process for production of the enzyme and the product is patented.

To study specificity of the enzyme and fibrin degradation products, the crude enzyme obtained from thermophilic *Streptomyces sp*. MCMB -379 was purified using DEAE ion exchange column. The purity of eluant fraction was tested by SDS-PAGE analysis and compared with that of crude extract. *Ex-vivo* fibrin clot lysis was investigated by comparing with clinically applied Urokinase with equal concentration of both the proteins. The *ex-vivo* clot lysis at 37 ^{0}C was monitored at fixed time intervals. The fibrin degradation products (FDP) and D-dimer were analyzed in lysate by immunochemical method using commercially available reagent kits. The activity of Actinokinase was observed to be comparable with that of Urokinase. The enzyme Actinokinase was thus found to be a potent fibrinolytic enzyme in *ex-vivo* clot lysis assay.

The enzyme Actinokinase is fibrin specific, non-hemolytic and non- allergenic in nature.

Keywords: *Streptomyces sp.* MCMB 379, Cardiovascular diseases, lysis assay, fibrinolytic.

SL-42

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology, Clinical Research/clinical trials; bioethics; nanobiotechnology.*

BIOLOGICALLY ACTIVE COMPOUNDS IN SEAWEED EXTRACTS - THE PROSPECTS FOR THE APPLICATION

K. Chojnacka and A. Saeid

Institute of Inorganic Technology and Mineral Fertilizers, Wroclaw University of Technology, Wroclaw, Poland; Email: katarzyna.chojnacka@pwr.wroc.pl

The possibilities of using algal extracts as the components of cosmetics and growth stimulants for plants is discussed. Various methods of the production of ext racts from seaweeds: chemical, physical and biological are presented.

Biologically active substances in the extracts useful in cosmetic products are characterized: phlorotannins, fucoxanthin, polysaccharides (alginate, fucoidan). The mechanism of their advantageous effect on human skin is shown. A particular attention is paid to anti-ageing, anti-oxidant, whitening properties. Polysaccharides – important components of extracts posses the properties of emollients. These compounds are hygroscopic and bind water. This improves the mechanical properties of skin – eg. elasticity and prevents from the wrinkles formation.

Also, the components having stimulatory effect on the growth of plants are characterized: plant growth hormones (auxins, cytokinins, gibberellins), polysaccharides (laminarin, alginate, fucoidan), betain, sterols. The advantageous

activity of these compounds on plant growth is described: increased growth, crop yield, resistance to biotic and abiotic stress. The products available on the market are shown.

The future aspects and new methods of algal extracts production are discussed, e.g. using extraction with supercritical fluids.

SL-105

Track: *Industrial and Manufacturing*

THE COMPLEX BEHAVIOUR OF THE OXYGEN TRANSFER COEFFICIENT IN HYDROCARBON-BASED BIOPROCESSES

Kim G. Clarke, Leslie D.C. Correia, Musaida M. Manyuchi and Peter G. Hollis

Department of Process Engineering, University of Stellenbosch, Private Bag X1, Stellenbosch 7602, South Africa; E-mail: kclarke@sun.ac.za

Successful biotechnology ventures require efficient processes with maximal yields and productivities. Since optimal kinetics are achieved only when oxygen transfer rates are sufficient to meet the metabolic demand, the oxygen transfer coefficient (KLa) remains key to process design, operation and scale up. In hydrocarbon-based bioprocess where KLa is depressed at higher viscosities, this is particularly critical. Here the complexities of KLa behaviour in hydrocarbon-based bioprocesses have been addressed so that the valuable feed stock potential of hydrocarbons can be realised. Additionally, advancements in KLa measurement methodology were made which greatly improved accuracy.

KLa behaviour was quantified under a range of process conditions in alkane-aqueous dispersions with and without solids (inactive yeast). Bubble diameter was determined using high speed photography and image analysis, and with measured gas hold up, defined the interfacial gas-liquid transfer area. KLa behaviour was dependent on the available transfer area and regimes of optimal KLa were characterized according to the bubble diameter and gas hold up. KLa was significantly decreased with increased yeast loading; further, a negative interactive effect of yeast loading and alkane concentration was exhibited. KLa behaviour in these systems will be discussed in terms of the varying fluid properties with changes in process conditions.

Keywords: Oxygen transfer coefficient, interfacial transfer area, multiphase bioprocesses.

SL-27

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

GENETIC TRANSFORMATION OF ARUNDO DONAX: A BIOMASS ENERGY PLANT USING PARTICLE BOMBARDMENT

Sarwan K. Dhir

Center for Biotechnology, Fort Valley State University, USA; E-mail: dhirs0@fvsu.edu

Arundo donax L. (giant reed), an erect, woody perennial plant with high biomass (source for renewable biofuels), is of great relevance to global economic and ecological issues. Currently, strategies are being developed using plant genetic engineering approaches for enhancement of biofuel production. We have evaluated tissue culture parameters for giant reed's ability to produce embryogenic and regenerable cultures that would be useful for genetic transformation. Embryogenic calli were induced using immature inflorescences as explants and axillary buds isolated from plants. Callus induction media supplemented with 2, 4-dichlorophenoxyacetic acid (2, 4-D) and 6-benzyladenine (BA) influenced both callus induction frequency and later plant regeneration on hormone-free media. Media supplemented with 2, 4-D appeared to harmonize callus formation, shoot formation and subsequent whole plants regeneration. Optimized particle bombardment parameters to introduce DNA into embryogenic callus cells were developed. The physical and biological parameters tested for optimal transient expression of ß-glucuronidase (GUS) and green fluorescent protein (GFP) genes were: helium pressure, distance from stopping screen to target tissue and vacuum pressure together with other factors such as gold microparticle size, DNA concentration and the number of bombardments. The highest transient GUS and GFP expression was obtained when cells were bombarded twice at 1100 psi, with 9 cm target distance, 24 mm Hg vacuum pressure, 1 mm gold particle size, 1.5 µg DNA per bombardment,

three days pre-culture prior to bombardment and six days post bombardment culture. Kill curves for both paromomycin and hygromycin have been established to optimize selection criteria. We have used a gene construct consisting of ubiquitin promoter from rice driving expression and have successfully introduced this gene construct into giant reed, producing a total of over 12 stably transformed individual events with transformation efficiencies of about 1.4% per experiment. This is the first report of optimization of particle bombardment parameters for high-efficiency DNA delivery combined with minimum damage to target giant reed tissues.

Keywords: Genetic Transformation, Arundo donax, Particle bombardment.

SL-117

ASSESSING THERMAL INACTIVATION OF *SALMONELLA* ON COOKED BROILER CHICKEN CARCASSES IN TRINIDAD AND TOBAGO

Mark M. Dookeran, Gail S.H. Baccus-Taylor, John O. Akingbala, Berhanu Tameru and Anna M. Lammerding

Food Science & Technology Unit, Department of Chemical Engineering, University of the West Indies, St. Augustine, Trinidad and Tobago;
Emails: Mark.Dookeran@sta.uwi.edu; markdook@tstt.net.tt

Salmonella, zoonotic bacteria normally present in broiler chicken flocks, are a major cause of food-borne illnesses of known aetiology in Trinidad and Tobago and in the wider English speaking Caribbean. Although cooking is regarded as an acceptable method for thermal destruction of these pathogens, consumption of undercooked and re-contaminated cooked broiler meat remains a common mode of transmission to humans. Since the proportion of undercooked chicken is largely unknown, an assessment of various cooking methods would necessitate the intervention strategies that are required to ensure food safety. Cooking time and temperature for fry, boil, bake and grill cooking methods determined from survey and sampling methods and D-values from published data were inputs into a modified model. The model was constructed in a Microsoft Excel^{TM} workbook and simulated using @risk add-in computer software using 100,000 iterations and Latin Hypercube Sampling. Thermal inactivation of *Salmonella* on broiler chicken meat occurred during boiling (0%) and frying (0%) but *Salmonella* survived baking (0.001%) and grilling (0.012%). Differences in the expected value were due to differences in cooking time, temperature, environment and size of chicken cuts. Air, the heat transfer medium for both baking and grilling may be the most important factor linked to inadequately cooked chicken.

Keywords: *Salmonella*, Broiler Chicken, Thermal Inactivation Model, Mark Dookeran.

SL-75

TRANSFORMATION OF AZOTOBACTER VINELANDII FY10 WITH AN ACC DEAMINASE ENCODING GENE FROM PSEUDOMONAS FLUORESCENS FY32

Davoud Farajzadeh

Department of Cellular and Molecular Biology, Biological Science Faculty, Azarbaijan University of Tarbiat Moallem, Tabriz, Iran ; Phone: +98 (412) 4327541; E-mail: farajzadeh@azaruniv.edu

ACC deaminase containing bacteria can cleave the ethylene precursor ACC and thereby lower the level of ethylene in developing or stressed plants and protect plants from the deleterious effects due to high level of ethylene. However, despite considerable amount of experimental data concerning azotobacteria simulation of plant development, evidence to indicate that members of *Azotobacter* genus are able to produce ACC deaminase so far has not been provided. So, in this study, some indigenous azotobacteria isolated from rhizosphere soils of wheat and barley plants cultivated in different areas (drought- and salt- affected soils) in Tabriz, were assayed by biochemical and molecular approaches to find out their ability to produce ACC deaminase. The results revealed that none of the studied isolates contain ACC deaminase activity. Therefore, as part of an effort to obtain an ACC deaminase encoding *Azotobacter*, *acdS* gene was amplified from FY32 strain of *Pseudomonas fluorescens* which had high ACC deaminase activity and then cloned in the broad host range plasmid pBI121 under the control of the 35S-CaMV promoter and transferred into indigenous *Azotobacter vinelandii* FY10 by electroporation. *Azotobacter vinelandii* FY10 transformants showed ACC deaminase activity, lower than that observed in *Pseudomonas fluorescens* FY32. However, the expression of ACC deaminase improved the

existing growth promoting activity of *Azotobacter*. The bacterial inoculation of wheat plants with the transformed bacterium had significantly positive effects on the root and shoot wet weigh in comparison to the wild type and control (non-inoculated), respectively. We also showed that the recombinant plasmid is long lasting in *Azotobacter vinelandii* FY10 and can be stably replicated even in the tenth generation. Therefore, to the best of our knowledge this is the first report of ACC deaminase expression in *Azotobacter* as another PGPR property.

Key words: *Azotobacter vinelandii*, *Pseudomonas fluorescens*, ACC deaminase, Gene transferring.

SL-82

Track: *Industrial and Manufacturing*

HIGH HYDROSTATIC PRESSURE ACTIVATES STRESS TRANSCRIPTION FACTORS WHICH ARE INVOLVED IN *SACCHAROMYCES CEREVISIAE* STRESS TOLERANCE

Fernanda Bravim, Lucas F. da Silva, Diego T. Souza, Soyeon Lippman, James R. Broach, A. Alberto R. Fernandes and Patricia M.B. Fernandes

Núcleo de Biotecnologia, Centro de Ciências da Saúde, Universidade Federal do Espírito Santo, Vitória, ES, 29040-090, Brazil; E-mail: patricia.fernandes@pq.cnpq.br

The transcriptional control elements are activated when *Saccharomyces cerevisiae* cells are submitted to different stressful conditions, among them the high hydrostatic pressure (HHP). HHP exerts a broad effect on yeast cells, similar to the common industrial stresses, as temperature, ethanol and oxidative stresses; and a mild HHP also induces multi-stress resistance. A time series microarray based expression analysis was performed in a wild *S. cerevisiae* strain submitted to 50 MPa for 30 min and 5, 10, 15 min after the treatment. The most frequent genes related to the general yeast stress response were not up-regulated immediately after HHP. However, 5 min after the piezotreatment those genes were up-regulated, increasing over post-pressurization time. It´s worth to note that those genes are regulated by the same transcriptional factors (CIN5, YAP1, HSF1, XBP1, RPN4 and MSN4), which were activated after pressure treatment, being CIN5 the most up-regulated one (~4.3 fold). Moreover, a high induction of genes related to oxidative and heat shock stress response was observed. Therefore, the response of wild yeast to HHP stress has an important application on the biotechnology industries since multiple protection mechanisms, induced by the mild HHP treatment, are required during the fermentative process.

Financial support: FINEP, CAPES, CNPq, FAPES.

SL-67

Track: *Industrial and Manufacturing*

INFLUENCE OF THE PLASMA MEMBRANE FLUIDITY ON THE RESPONSE OF SACCHAROMYCES CEREVISIAE TO HYDROSTATIC PRESSURE STRESS

Jessica M. Freitas, Fernanda Bravim, David S. Buss, A. Alberto Ribeiro Fernandes and Patricia Machado Bueno Fernandes

Núcleo de Biotecnologia, Universidade Federal do Espírito Santo, Vitória, ES, Brasil;
E-mail: patricia.fernandes@pq.cnpq.br

High hydrostatic pressure (HHP) interferes with cellular membrane structure. The orientation of lipid molecules is changed, especially in the vicinity of proteins, leading to decreased membrane fluidity. Adaptation to HHP in prokaryotes requires increased membrane fluidity, often achieved through a rise in the proportion of unsaturated fatty acids. Unlike other fungi, the predominant unsaturated fatty acids in *Saccharomyces cerevisiae* are palmitoleic (C16:1) and oleic (C18:1). In this work a desaturase deficient *S. cerevisiae* mutant strain (*OLE1* gene deletion) was grown in media supplemented with fatty acids differing in size and number of unsaturations, and submitted to pressure up to 220 MPa for 30 min. Experiments were conducted to verify cell survival and lipid peroxidation. Desaturase-deficient yeast supplemented with palmitoleic acid demonstrated increased sensitivity to pressure compared to cells supplemented with oleic acid or a proportionate mixture of both acids. In contrast, yeast cells grown with linoleic (C18:2) and linolenic (C18:3) acids were more piezoresistant than cells treated with oleic acid (C18:1). Growth with palmitoleic acid not only led to piezosensitivity, but also higher levels of lipid peroxidation compared to cells supplemented with the other fatty

acids. Intracellular trehalose during HHP treatment increased cell tolerance to pressure. However, if trehalose was added just before pressurization, so that it remained extracellular, cells were sensitized to pressure. Therefore, fatty acid composition and trehalose content might play a role in the protection of the cell membrane from oxidative damage produced by HHP, confirming that alteration in cell membrane fluidity is correlated with pressure resistance in yeast.

Financial support: CNPq, CAPES, FINEP, FAPES.

SL-58

Track: *Plant and Environment Biotechnology*

SEEDS - POTENTIAL ENERGY SOURCE TOTAL USE POLICY: OIL, BIODIESEL AND CHARCOAL, GAS AND BIO-OIL BY LTC

Mauricio G. Fonseca, Gilberto A. Romeiro, Luciano N. Batista, Viviane F. Silva, Erica C.G. Pissurno, Romeu J. Daroda and Valnei S. Cunha

Fuel and Engines Laboratory – Metrological Chemistry Department / INMETRO – National Institute of Metrology, Quality and Technology, Brazil; E-mail: mgfonseca@inmetro.gov.br

The search of new renewable fuels has been developed by many research groups around the world due to the fossil fuels scarcity and the high polluting degree of its derivatives. Biomass has becoming an attractive alternative energy source cause helps to solve social problems, increasing the rural areas development.

Waste has becoming a large worldwide problem. An important tool to reduce this problem is the use of Low Temperature Conversion [1] methodology. When biodiesel is produced from seed oil are obtained mainly two products: In the oil extraction, are obtained oil and the solid waste, extraction tart. The oil seed is used to produce biodiesel by transesterification [2] reaction and the solid waste can be transformed in pyrolysis oil, charcoal, gas and waste water by LTC [3] process.

The obtained products can be employed as alternative energy sources. Biodiesel and the pyrolysis oil can be mixed and used in engines. Charcoal can be used in heating, industrial furnaces. The obtained gas can be employed in industrial heating and the waste water can be used in agricultural irrigation.

All the obtained products are characterized by GC-FID, IR-FT and physical chemistry parameters: Flash point, water content, cloud point, copper corrosion, oxidative stability and specific mass.

Acknowledgements: CNPq and FINEP

Keywords: Low Temperature Conversion and Biodiesel.

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SL-17

Track: *Plant and Environment Biotechnology*

REGULATED OVEREXPRESSION OF TREHALOSE BIOSYNTHETIC FUSION GENE (TPSP) IN CEREAL PLANTS IMPROVES SALINITY AND DROUGHT STRESS TOLERANCE

Ajay K. Garg, Ju-Kon Kim, Thomas G. Owens, Yang Do Choi, Hye-Ji Kim, William B. Miller, Leon V. Kochian and Ray J. Wu

Department of Molecular Biology and Genetics, Department of Plant Biology, Robert W. Holley Center for Agriculture and Health, USDA-ARS, Cornell University, Ithaca, NY 14853, USA; E-mail: Ajay.garg@uaeu.ac.ae

Rice and maize are the most important cereals in the world and are the primary source of food energy to more than half of humanity. Drought and salinity are the major abiotic stresses reducing agricultural productivity worldwide. High soil

salinity levels and water scarcity affect large terrestrial areas of the world, and the loss of land due to soil salinization and drought is in direct conflict with the needs of a growing world population. Efforts to improve crop performance under environmental stresses have met with limited success because the fundamental mechanisms of stress tolerance in plants remain to be completely understood. Fortunately, it is now possible to use transgenic approaches to improve abiotic stress tolerance in agriculturally important crops with far fewer target traits than had been previously anticipated.

Trehalose is a non-reducing disaccharide of glucose that functions as a compatible solute and in the stabilization of biological structures under abiotic stress in bacteria, fungi and invertebrates. With the notable exception of the desiccation-tolerant "resurrection plants", trehalose does not accumulate to significant levels in the vast majority of plants, in spite of the proliferation of plant trehalose pathway genes. Phylogenetic analyses of protein sequences derived from the corresponding DNA sequences from the completely annotated genomes of a monocot (rice) and a dicot (*Arabidopsis*) plant species showed the presence of one or two trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP) gene families, indicating the genomic complexity of trehalose biosynthetic genes in plants. However, recent studies have shown that trehalose metabolism is of immense importance in numerous plant processes and its manipulation has great potential in crop improvement. Here, we report our results on regulated overexpression of trehalose biosynthetic fusion gene in cereal plants for the purposes of improving abiotic stress tolerance and other agronomic traits. The expression of the transgene was under the control of light-inducible, tissue-specific or stress-dependent promoters. Compared with non-transgenic plants, several independent transgenic lines showed higher levels of trehalose accumulation and exhibited sustained plant growth, less photo-oxidative damage and more favorable mineral balance under salt and drought stress conditions. These results demonstrate the potential of manipulating sugar metabolism in cereal plants to improve abiotic stress tolerance.

Keywords: trehalose biosynthetic fusion gene, Rice and maize.

SL-45

Track: *Medical Biotechnology*

YEAST PROGRAMMED CELL DEATH: A NEW EXPERIMENTAL PLATFORM FOR BIOMEDICAL AND AGRI-FOOD SCIENCES

Nicoletta Guaragnella, Masa Zdralević, Lucia Antonacci, Salvatore Passarella, Ersilia Marra and Sergio Giannattasio

Istituto di Biomembrane e Bioenergetica, Consiglio Nazionale delle Ricerche, Italy; E-mail: s.giannattasio@ibbe.cnr.it

Similarly to animals and plants, yeast cells can commit to programmed cell death (PCD) exhibiting several characteristics of apoptosis, including chromatin condensation, DNA breakage, flipping of phosphatidylserine to the outer membrane, production of reactive oxygen species, and cytochrome c release from the mitochondria. Yeast PCD has been shown to occur in response to a variety of exogenous stimuli, such as oxidative stress, exposure to acetic acid and expression of mammalian pro-apoptotic proteins. This program is also inherent to the yeast life cycle, as aged cells and cells exposed to pheromone also display an apoptotic and necrotic phenotype.

We are interested in understanding of yeast PCD and its regulation through mitochondria-nucleus cross talk. In this presentation, recent achievements in our own work in the mechanisms of yeast PCD induced by acetic acid will be reviewed. The elucidation of how cell adaptation or death occurs in yeast in response to stress is of great relevance for biomedical applications. Indeed, this study allows for identification of new target molecules, involved in a particular metabolic or signalling pathway that is specific to a disease condition, and hence development of new drugs through yeast-based chemical screens. On the other hand, traditional food production technologies, such as brewing and wine making, involves exposure of yeasts to numerous environmental stress sequence. Exploitation of natural yeast biodiversity to improve the efficiency of stress response in yeast strains will lead to innovation in food production processes.

Keywords: Yeast, programmed cell death, mitochondria, drug design, yeast biodiversity.

SL-116

Track: *Medical Biotechnology*

HERE TODAY, GONE TOMMORROW: NEXT DAY COSMETIC OUTCOMES OF PTERYGIUM EYE SURGERY USING HUMAN PLACENTA

Arun C. Gulani

Gulani Vision Institute, 8075 Gate Parkway (W), Suite 102, Jacksonville, Florida 32216, USA;
E-mail: gulanivision@gulani.com

Purpose: "No-Stitch" Amniotic surgery can be used to correct blinding pterygiums and corneal scars raising the bar to cosmetic outcomes next day after surgery.

Methods: 300 eyes of patients with pterygium underwent "Iceberg"concept of pterygium surgery to remove the entire lesion followed by Amniotic graft placement with Tisseel glue.

Results: This technique has resulted in very satisfied patients with ecstatic reactions to their sparkling white eyes as well as early recovery of vision and return to their occupations.Teh recurrence rate has come down from national average of 39% to 0.003%.

Conclusion: Advanced sutureless pterygium surgery using Human placenta can result in pleasing aesthetics, early recovery and gratified patients in raising the bar on ocular surface surgery to cosmetic outcomes. This also lends itself to vision corrective surgery as the next stage in enhancing the recovered vision.

SL-32

Track: *Other Areas: Food*

SAFETY OF RAW VEGETABLES GROWN IN BEKAA VALLEY, LEBANON

Mahmoud. A. Halablab, Imtithal. H. Sheet and Hanafi. M. Holail

College of Science & Information Systems, Hariri Canadian University, P.O. Box: 10, Damour, Chouf 2010, Lebanon; E-mail: halablabma@hcu.edu.lb

Food safety in Lebanon plays a significant role in the national economic and healt h development by safe guarding the health of the nation, enhancing tourism, national and international trade for production, distribution and consumption of safe food, preventing avoidable losses and conserving natural resources. Moreover, Food safety is an increasingly important public health issue, not only in Lebanon but also all over the world. Governments are intensifying their efforts to improve food safety in response to increasing numbers of food safety problems and rising consumer concerns.

A simple definition of food safety is protecting people from illness or injury from handling or consuming food. But, providing safe food can be complex. Efforts must be applied at every step in the process from where foods are produced across the value supply chain to through where they are consumed. Yet food safety practices, legislation and regulatory oversight vary between and even within nations. These differences can affect the integrity of local, regional and global food supply chains. That's why Lebanese society and consumers are working jointly to achieve global food systems that are more transparent, harmonized and science-based in order to enhance public health outcomes and strengthen food security. Fresh fruits and vegetables do not naturally contain pathogenic microorganisms (e.g., bacteria, viruses and parasites) that can cause food-borne illness. All green plants possess a resident microflora which normally subsists on the minute traces of carbohydrates, proteins and inorganic salts which dissolve in the H2O that exudes from the epidermis of the plant or condenses from the atmosphere onto the plant. However, fresh produce can become contaminated in the field through contact with soil, contaminated water, wild or domestic animals, or improperly composted manure. Fresh produce can also come in contact with harmful microorganisms during and after harvest, if it is not properly handled, stored, and transported. In addition, fresh fruits and vegetables can become contaminated through contact with raw food items such as meat, poultry, seafood, and their juices.

Lebanon's position in the heart of the most deprived region of fresh water resources has gained it a relative importance that has started to decline due to increased water demands and improper water management. The major fresh water source in Lebanon is the Litani River, which drains over fifth of Lebanon's total area. Exceeding 170 km and an estimated average annual discharge rate of over 750 million cubic meters, it originates from Al-Olleik spring near Baalbek, in the fertile Bekaa Valley, and flows south then west until reaching the Mediterranean Sea 7 km north of Tyre. The Litani River has multiple tributaries that flow from the slopes of the Lebanon and Anti Lebanon mountain ranges. Two main ones are the Ghozayyel River and the Berdaouni River. The Litani River suffers from potential water pollution problems due to the uncontrolled solid and liquid domestic and industrial waste disposal practices, agrochemical usage and lack of sustainable wastewater treatment system. Most of the fresh vegetables in the Bekaa Region in Lebanon are irrigated from Litani River. This situation causes direct negative effects on the environment, public health, and socio-economic development which urge the need for proper management plans for the surface and ground water resources to eradicate or reduce these impacts and pave the way for environmentally sustainable and socioeconomically viable use of these vital resources.

A microbiological study on raw vegetable samples collected from several regions in Bekaa Valley areas and irrigated from Litani River. These samples included lettuce, parsley and *Malva.* Other control samples taken included those irrigated using ground water wells. All the samples collected were examined for microbial contamination level. The microbiological quality of fresh vegetables ranged from 4.3 to 10.4, 2.0 to 0.71, 1.0 to 8.77 and 1.47 to 8.77 (log10 CFU g-1) aerobic bacteria, coliforms, *E. coli* and *S. aureus,* respectively. Lettuce samples had significantly higher microbial loads including coliforms, *E. coli* and *S. aureus* than parsley samples collected from different locations in Bekaa Valley. Neither *E. coli* nor *S. aureus* had been detected on *Malva* samples. In addition, Barelias had higher microbial loads, coliform, *E. coli* and *S. aureus* than any other location investigated in Bekaa Valley. Moreover, *E. coli* was significantly higher in lettuce samples (42.30%) than in parsley samples (13.8%) and *S. aureus* was significantly more often detected in lettuce samples (51.5%) than in parsley samples (38%). This study demonstrated that lettuce and parsley samples which are usually consumed raw may contain pathogenic microorganisms and represent a risk for human health. Such potential risk increased through irrigation by untreated wastewater or by production practices (manure for fertilizer) and human handling. Therefore, it is urgently required that disposal of untreated sewage to local rivers is prohibited and that the rivers' water is not used until it clear from indicators of sewage contamination.

SL-70

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

DETECTION OF IONIZATION RADIATION EFFECT USING MICROORGANISM (*ESCHERICHIA COLI*)

Maytham Al- Shanawa, A. Nabok, A. Hashim and T. Smith

Material and Engineering Research Institute (MERI), Sheffield Hallam University, Sheffield, City Campus, Howard Street, Sheffield S1 1WB, UK; E-mail: A.Hashim@shu.ac.uk

The environmental pollution and radiation have become the biggest problem threatening the life of people, animals and other living species. Thousands of waste sites around the world contained different pollutants and toxins, particularly heavy metals and radioactive elements. The most dangerous type of pollution is the radioactive contamination which results from the detonation of nuclear devices, the waste products of nuclear power plants, and also from conventional weapons using depleted uranium. The main aim of this study is the development of novel sensing technologies for detection of radiation pollution using microorganisms, such as bacteria. The most common bacteria, Escherichia coli (E. coli for short) was selected for this study. Several experimental techniques, such as fluorescence microscopy, fluorescence spectroscopy, and light scattering (OD_{600}) were utilised for counting living bacteria. The results obtained for freshly prepared bacteria samples were compared to those after exposure to Gamma-radiation (Co-57 source with the dose equivalent to 2000 μSv/h was used) for a different time periods (from 1 to 60 hours). The results showed that the ratio of living/dead bacteria goes down exponentially with the accumulated dose of radiation (Fig. **1**). In addition, the ability of *E. coli* to attenuate Gamma-Radiation was researched, the attenuation factor was found to be of 0.2 Cm^{-1}.

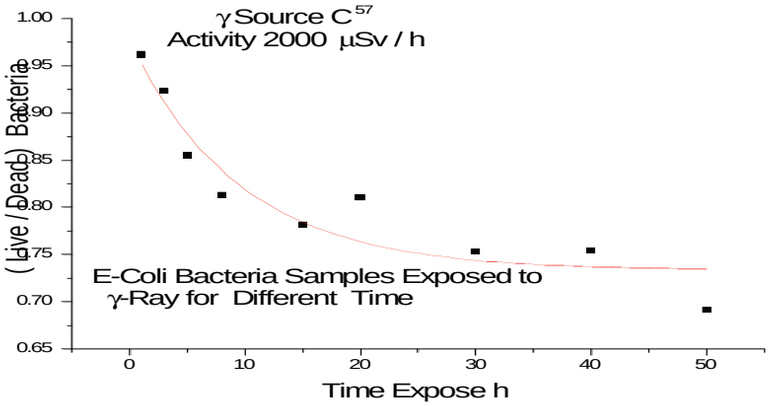


Fig. (1). The dependence of live/dead bacteria ratio on radiation dose obtained form fluorescence microscopy experiments.

Keywords: *Escherichia coli,* fluorescence microscopy, fluorescence spectroscopy, and light scattering (OD_{600})m, Gamma-Radiation.

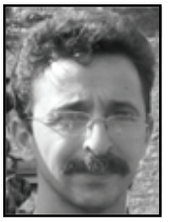
SL-74

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

THE EXTREMOPHILE GRASS *AELUROPUS LITTORALIS*: A SOURCE OF CANDIDATE GENES FOR IMPROVING SALT AND DROUGHT STRESS IN CEREALS

Walid Ben Romdhane, Rania Ben Saad, Adullah Al-Doss, Emmanuel Guiderdoni and Afif Hassairi

University of Sfax, Centre of Biotechnology of Sfax (CBS), LPAP, POBox 1117 3018 Sfax, Tunisia; E-mail: afif.hassairi@gmail.com



Biotic and abiotic stress effects can limit the productivity of plants to great extent. Tomorrow's plants will be required to yield more in spite of less favorable conditions and to limit the environmental impact of cultivation. Drought and salt stresses are the major limiting factor of plant growth and crop yields. To resolve the problem unless the use of traditional plant breeding has achieved significant results it was found that this process is time consuming and expensive. Plant biotechnology appears to be an attractive alternative in respect of the possibility for direct introduction of single or multiple genes into crops.

We have constructed Suppression Subtractive Hybridization (SSH) cDNA libraries from root (RSD45) and leaf (LSD45) tissues of C4 perennial halophyte monocotyledonous plant *Aeluropus littoralis* grown in the presence of 300 mM NaCl. We have also constructed full-length cDNA library from salt stressed (300 mM NaCl) roots (RSTL15) during 15 days. Sequencing revealed 492 independent transcripts from all these libraries. Comparison using BlastX, 335 (68%) ESTs (Expressed Sequence Tag) were classified into putative known functions and unclassified proteins, 59 (12%) have homology only to unidentified homologous sequences. A total of 98 (20%) of the ESTs have no homologies to known sequences in the protein databases which can be considered as novel. We described here an example of a novel gene, *AlSAP*, and its promoter isolated from *A. littoralis*. The *AlSAP* gene has an intron at its 5'UTR. The AlSAP protein is characterized by the presence of two conserved zinc-finger domains A20 and AN1. *AlSAP* is induced not only by various abiotic stresses such as salt, osmotic, heat and cold but also by abscisic acid (ABA) and salicylic acid (SA). We generated tobacco plants expressing the *AlSAP* gene under the control of the duplicated CaMV35S promoter. These plants exhibited an enhanced tolerance to abiotic stresses. We found that the steady state levels of transcripts of eight stress-related genes were higher in *AlSAP* transgenic lines than in wild-type tobacco. To extend these findings to important crops, we generated marker-free transgenic **durum wheat** of the commercial cv. Karim and transgenic **rice** plants expressing the AlSAP gene. AlSAP wheat lines exhibited improved germination rates and biomass production under severe salinity and osmotic stress conditions. Following a long-term salt or drought stress greenhouse trial, AlSAP lines produced normally filled grains whereas wild-type (WT) plants either died at the vegetative stage under salt stress or showed markedly reduced grain filling under drought stress. Measurements of the RWC (relative water content) and endogenous Na^+ and K^+ levels in leaves of AlSAP plants, showed a lower water loss rate and a higher Na^+ accumulation in senescent-basal leaves, respectively, compared to those of WT plants.

The expression of *AlSAP,* in rice cv. Nipponbare, enhances plant tolerance to cold, drought and salt stresses. Under a severe drought stress treatment (Fraction of Transpirable Soil Water down to 0.1), *AlSAP* lines exhibited enhanced *TE* (Transpiration Efficiency) and maintained a high *A* (Assimilation rate) value (22 µmol.m-2s-1) while these values dramatically decreased (*A*= 4 µmol.m-2s-1) in control plants which were subsequently unable to recover from the stress. Noteworthy, *AlSAP* rice plants yielded a similar and a 60% seed set under control and stress conditions respectively with regard to WT plants grown under control conditions. This indicates that *AlSAP* expression imposes no yield penalty and allows seed production even following a severe drought stress at the vegetative stage. Furthermore, *AlSAP* rice was shown to accumulate transcripts of a pilot set of eight stress-related genes at a significantly higher level than WT plants, both under control and stressed conditions. The results suggest that *AlSAP* expression generates stress tolerance in plants through maintenance of the photosynthetic apparatus integrity and by stimulating an endogenous adaptive potential which is not effectively accomplished in WT plants.

Keyword: *Aeluropus littoralis*, *AlSAP* gene, cold, drought, salt stress, transgenic, tobacco, wheat, rice.

SL-41

Track: *Industrial and Manufacturing*

BIOTECHNOLOGICAL PRODUCTION AND APPLICATION OF ENVIRONMENTALLY FRIENDLY INDUSTRIAL ENZYMES IN BANGLADESH

Dr. Md. Mozammel Hoq, Sumaiya Waliullah, Md. Asaduzzaman Shishir, Md. Ilias and Shakila Nargis Khan

Department of Microbiology, University of Dhaka, Dhaka 1000, Bangladesh; E-mail: mmzhoq@gmail.com

Microbial enzymes unlike chemical catalysts are biodegradable, having high specific catalytic activity under mild temperature and pressure conditions rendering it to be environmentally friendly. Biotechnological production of such enzymes (Alkaline protease, keratinase, cellulose-free xylanases) locally could save a considerable amount in foreign exchange besides replacing/reducing the usages of harsh environmental chemicals. Many leather manufacturers still use chemicals (lime-sulphide) for hide processing resulting in inferior leather quality and pollution problems contrary to protease and its other bio-remediation applications. In conjunction, keratinase functions in deharing of hairs from the skins and solubilize feathers to feed. Hence, this investigation aims at biotechnologically producing the enzymes alkaline protease and keratinase for technical applications employing the Bacillus bacteria. Three strains of B. licheniformis (strain # MZK-3 MZK-4, MZK-5) identified following conventional and 16SrDNA gene sequence analysis, have been successfully isolated from effluent of tanneries and poultry firms that demonstrated both proteases and keratinase activities at varying levels on a basal medium containing feather meal as C and N sources at 37°C in bioreactor and shake cultures. They also demonstrated varying properties of pH optimum and stability, temperature tolerances and protease types i.e. serine, metallo- or aspartate. The crude enzymes, viz. protease or keratinase mix and single, from B. licheniformis MZK-5 were found to be very effective in soaking, unhairing and bating processes of hides improving the quality of the leather while decreasing the usage of harsh chemicals by 50 %. Furthermore, the keratinase from B. licheniformis strains solubilized native chicken feathers completely within 8 to 12 hrs. Also the serine protease produce was stabilized with Ca and Mn ions in the complex ingredients of commercial detergents demonstrating its usage as cleansing aid in detergent formulation. The results on the cloning of the kerA gene in E. coli will also be included in the presentation.

SL-106

Track: *Medical Biotechnology*

SINGLE- AND HYBRID-DESIGN TISSUE ENGINEERING SCAFFOLDS: INVESTIGATION ON ACCELERATED IN VITRO DEGRADATION

M. Enamul Hoque, L. Chuan Yong and Ian Pashby

Mechanical, Materials and Manufacturing Engineering, University of Nottingham Malaysia Campus, Malaysia; E-mail: enamul.hoque@nottingham.edu.my

Polymer degradation is mainly governed by molecular weight, crystallinity and susceptibility to hydrolysis. So far, the degradation of polymeric scaffolds has been largely explored. However, little detail information is available in regard to the effect of scaffold architecture/design on degradation kinetics. This current study focuses on the investigation of degradation of single- and hybrid-design scaffolds made of polycaprolactone (PCL). The hybrid-design scaffold integrates two or more lay-down patterns in one scaffold, while the single-design scaffold consists of only one lay-down pattern. The PCL scaffolds were degraded in vitro over five weeks in 5M NaOH solution. The degraded scaffolds were analyzed by means of differential scanning calorimeter (DSC), thermo gravimetric analyzer (TGA), densimeter, scanning electron microscope (SEM) and mechanical microtester (Instron). Both the single- and hybrid-design scaffolds realized homogeneous hydrolytic degradation via surface erosion resulting in a consistent and predictable mass loss. The linear mass loss rendered uniform increase in porosity that led to the decrease in mechanical properties accordingly. Indeed, the design variation did not influence the degradation kinetics significantly. However, the hybrid-design may offer better opportunity to optimize and/or modulate the scaffold properties as required for tissue engineering applications.

Keywords: Degradation, Hybrid-design, Tissue Engineering, Scaffold, PCL, SEM.

SL-65

Track: *Medical Biotechnology*

DETECTION OF β-CATENIN DNA IN THE SERUM OF COLON CANCER PATIENTS – A NONINVASIVE TOOL FOR EARLY DIAGNOSIS OF COLON CANCER

W.L.Wendy Hsiao

Center for Cancer & Inflammation Research, School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, China; Email: bowhsiao@hkbu.edu.hk

Colorectal cancer is one of the most common malignancies in men and women. More than 70% of colorectal cancer develops from sporadic adenomatous polyps. Early detection of polyps can greatly reduce the risk of colorectal cancer formation. Traditional screening tests suffer from the limitation that they are either insensitive or unpleasant and invasive. β-catenin, a key message in the Wnt-signaling pathway, has been implicated in the oncogenesis of colorectal cancer. Our recent observations showed that the level of β-catenin nuclear translocation is highly correlated with the purported stages, lymph node metastasis and mortality rate of colorectal tumor. We hypothesized that β-catenin might be present in the circulating blood of individual with cancer, but not of healthy individuals. To explore this possibility, we examined serum β-catenin from 15 colorectal carcinoma patients, 10 adenoma patients and 10 normal volunteers using PCR. Results show that β-catenin DNA was detected in 15 of 15 (100%) of colorectal carcinoma patients; 9 of 10 (90%) of adenoma patients; and 0 of 10 (0%) of normal objects. Our findings suggest that serum β-catenin DNA is closely associated with colorectal cancer and could be a useful diagnostic tool for early detection and population screening of the disease. [A US patent has been awarded for this current invention]

SL-20

Track: *Medical Biotechnology*

PHARMA-GRADE DEVELOPMENT AND CLINICAL IMPLEMENTATION OF CELL-BASED THERAPIES

Ralf Huss and Christine Günther

Apceth GmbH & Co. KG, Max-Lebsche-Platz-30, 81377 Munich, Germany;
E-mail: r.huss@apceth.com

"Cells as a drug". The implementation of innovative cell- and gene therapies for clinical application is a challenging process with regard to the biology of cells and the regulatory requirements. For manufacturing, quality control and licensing of Cell-based Therapeutics high regulatory requirements have to be met by the pharmaceutical applicant. The regulations and standards are not harmonized within the EU and FDA as well as other authorities worldwide including Asia. There are still many open issues for applicants as well as for authorities.

Apceth aims at the translation of the basic and preclinical research into clinical application, in order to make safe and high-quality cell therapies for different non-malignant and malignant diseases available to patients and clinicians in a shortest time possible. Apceth obtained the manufacturing license for mesenchymal-cell based Somatic Cell Therapeutics and the approval for their application of clinical trials in 2010 by the German authorities and is open to engage joint ventures as well as contract manufacturing for cell therapeutics.

Potential curative effects of cell-based therapeutics and open issues. Of particular interest are therapeutics based on mesenchymal-like cells (MSC). MSC are characterized by their intrinsic migratory capacity to sites of inflammation or tissue injury, their regenerative activity (wound healing and vascularization), immunomodulatory activity (e.g. treatment of autoimmune diseases) and the induction of immunotolerance (e.g. organ transplantation). Their potential as carriers of therapeutic genes for the correction of inherited disorders like rare metabolic diseases or red blood cell disorders is challenging but also an opportunity for cancer therapy. MSC can be isolated from different human organs like bone marrow of fat tissue and propagated by *in vitro* expansion. However, despite their "popularity", the consensus on the therapeutic dose of MSC, the mode and route of their application and the necessary degree of matching between donor cells and recipient in case of allogeneic therapy has yet to be achieved. Similarly, the *in vivo* fate of applied MSC in the patient's body is still a matter of debate.

Manufacturing and quality control. Somatic Cell- and Gene Therapeutics must comply with the EU regulation EC 1394/2007 for advanced therapies. The GMP-compliant manufacturing and quality control of cell-based products follow the pharmaceutical requirements for medicinal products. The procurement, the aseptic processing of cells/ tissues and the *ex vivo* expansion under clean-room conditions are necessarily bound to high investments in technology and require highly-qualified and continuously trained personnel. One of the major obstacles on the way to a successful pharmaceutical cell product is upscaling of the production process. The quality control of the cells and cell products comprises extensive characterization of somatic cells with regard to identity, purity, potency, genetic stability and sterility. Special interest should be paid to the early and concomitant development of potency assays and testing of genetic stability and development of biomarkers. The potency assays should reflect the intended therapeutic activity *in vitro*. The testing of genetic stability of cells after prolonged *in vitro* expansion is of major significance for the safety of the patient.

SL-59

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

BACILLUS THURIENGIENSIS BIOLOGICAL ACTIVE MOLECULES: DELTA-ENDOTOXINS, BACTERIOCINS AND ENZYMES OF BIOTECHNOLOGICAL INTERESTS

Samir Jaoua and Roda Al-Thani

Biological & Environmental Sciences Department, College of Arts and Sciences. Qatar University, Doha, Qatar; E-mail: samirjaoua@qu.edu.qa

Biopesticides are biotechnology-products that have been developed for the safe and efficient control of mainly insects, bacteria and fungi, in agriculture, food and environment.

During the last years, we have been developing several ***Bacillus thuringiensis*** biotechnological pesticides (bioinsecticides, bacteriocins and biofungicides) that could be directly applied in agriculture, food and pharmaceuticals:

1- *Bioinsectides*: *Bacillus thuringiensis* (Bt) is a Gram positive bacterium characterised by the production, during sporulation, of various toxins having insecticidal activities, among which the delta-endotoxins and the vegetative insecticidal proteins. Our main objective is the production of Bt bioinsecticides using strains showing originalities, for the development of local biopesticides industry. Hundreds of strains of *B. thuringiensis,* isolated mainly from Tunisia [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 18 and 19] but also from different countries and particularly from Qatar [11], were studied and their bioinsecticides coding genes *cry*, were cloned and characterized. Among the Tunisian strains, we evidenced the abundance of the *kurstaki* subspecies active on Lepidoptera and particularly the lepidopteran olive tree pathogenic insect *P. Oleae*, whereas from the Qatari soil samples, no *kurstaki* strains could be isolated until now, but other strains were evidenced, harbouring different plasmid profiles and producing amorphous crystals, probably containing novel insecticides. The majority of the strains produce delta-endotoxins acting on different insects families and particularly on mosquitoes, vectors of several diseases.

The bacterium *Bacillus thuringiensis* produces, at the vegetative stage of its growth, Vip3A proteins with activity against a broad spectrum of lepidopteran insects. Considering the fact that the Egyptian cotton leaf worm (*Spodoptera littoralis*) is one of the pathogenic insects causing several damage in agriculture, the mode of action of Vip3Aa16 toxin of *B. thuringiensis kurstaki* strain BUPM95 in *S. littoralis* midgut was investigated [20, 21].

In an applied part, we produced such bioinsecticides by fermentation (scales 2 l, 7 l, 20 l and 300 l) [5, 6, 7, 8] and successufly applied in the field for the control of the olive tree pathogenic insect *P. oleae* with a very high efficiency.

2-***Bacteriocins*** [12, 13, 14 and 15]*:* Bacteriocins are proteins or peptide antibiotics produced by Gram-positive bacteria. They have attracted great interest because of their potential use as food preservatives, and because they are considered as therapeutic agents against Gram-positive bacteria. *B. thuringiensis*, producer of bacteriocins, can be used in coculture to prevent the establishment of pathogens. We describe the biochemical properties of a new bacteriocin produced by a local strain of *B. thuringiensis.* This bacteriocin has a molecular weight of 3160.05 Da, different from those of other Bt published bacteriocins. It has bactericidal activities mainly on Gram positive bacteria, and could be used for food and seed preservation. We describe also the investigation of coding genes.

3-**Biofungicides** [16, 17]*:* ***B. thuringiensis chitinases:*** One chitinase was characterized by both its high chitinolytic and antifungal activities. The cloning and sequencing of the corresponding gene *chi255*, showed an open reading frame of 2031 bp, encoding a protein of 676 amino acid residues with a calculated molecular mass of 74 kDa. Both nucleotide

and amino acid sequences similarity analysis revealed that Chi255 is a new chitinase, presenting several differences from the published chitinases of *B. thuringiensis*. Heterologous expression in *E. coli* was performed by cloning the *chi255* ORF downstream a strong promoter in the vector pBAD. Identification, by HPLC analysis, of chitin hydrolysis products issued from the activity exhibited by Chi255, revealed that this enzyme is a chitobiosidase.

Keywords: Biofungicides, B. thuringiensis chitinases, Bacteriocins, Bioinsectides, Bacillus thuringiensis.

SL-107

Track: *Medical Biotechnology*

HIGH PREVALENCE OF OCCULT HEPATITIS B VIRUS INFECTION IN CHILDREN BORN TO HBsAG-POSITIVE MOTHERS USING HIGH TECH MOLECULAR APPROACHES

S. Shahmoradi, Y. Yahyapour, M. Mahmoodi, S.M. Alavian, Z. Fazeli and S.M. Jazayeri

Hepatitis B Molecular Laboratory, Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; Email: jazayerism@tums.ac.ir

Background: Occult hepatitis B virus (HBV) infection is a well-recognized clinical entity characterized by the detection of HBV DNA in serum and/or in liver in the absence of detectable hepatitis B surface antigen (HBsAg). The frequency of the diagnosis depends on the relative sensitivity of both HBsAg and HBV DNA assays used.

Objective: To determine the prevalence of occult HBV infection in a high risk group of children who developed HBV infection despite immunoprophylaxis.

Methods: Sera from 75 children born to HBsAg-positive mothers immunized by HBIG and vaccine prophylaxy regimen were tested for HBV DNA by real-time Polymerase Chain Reaction. Subsequently, the samples were tested with a sensitive standard PCR employing independent sets of primers for all HBV genes and, were analyzed by direct sequencing.

Results: HBV DNA was detected in 21/75 (28%) of children, ranged between 77 and 9240 copies/mL. No one had anti-HBc alone. 5 (24%), and 16 (76%) of 21 were found positive for both anti-HBc and anti-HBs, and isolated anti-HBs antibodies, respectively. 13 infected children (62%) contained at least one mutation in regions known to beinvolved in functional and/or immune epiotpe activity.

Conclusion: Routine tests including serology are not effective for the diagnosis of occult hepatitis B infections. Molecular tests based on high-tech should be used to detect small amount of HBV DNA. Occult HBV infection terminology and diagnosis seem to be meaningless in the absence of application of such high-tech instruments.

Keywords: Occult hepatitis B infection, HBsAg vaccine, Escape mutants, HBV real time PCR.

SL-61

Track: *Other areas: Bio-safety, Nanobiotechnology…*

THE CYTOTOXICITY OF ZERO-VALENT IRON NANOPARTICLES

Vladimir Jirku, Lucie Homolova, Alena Cejkova and Jan Masak

Institute of Chemical Technology, 166 28 Prague 6, Czech Republic;
E-mail: vladimir.jirku@vscht.cz

The extra ordinary properties of nano particles and their increasing use necessitate to assess their toxicity / biological effects. In this context, both the chemical nature and the physicochemical interactions occurring between a nano particle and model cell must determine the primary focus of each nano toxicology study. Zero – valent iron (ZVI, Fe0) – based nanoparticles increasing usage in there mediation of ground waters contaminated with halogenated hydrocarbons / heavy metals is such a case when the casual mechanism linking this remediation tool with it spotential cytotoxicity toward water and soil microflorais missing. This work is focused on preliminary inve stigations of potential cytotoxicity of NANOFER 25 (NANOIRON®, Future Technology, Czech Republic) ZVI nanoparticles, usedfor a variety of applications in groundwater and soil decontaminations. In this connection, bacterial and fungal cell populations were tested to elucidate

the effect of Nanofer 25 application conditions on the cell/ZVI contact, cell reproduction, lipid peroxidation and protein carbonyl content of cells exposed to nano-Fe0. The cytotoxicity of Nanofer 25, related to the markers of oxidative stress, is affected both bythe physiology of growth phase, Nanofer 25 dose or pH and by pre-adaptive oxidative stress or by the effect of iron chelators and oxidant scavengers.

SL-38

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

DEVELOPMENT OF STRESS-TOLERANT TRANSGENIC PLANTS VIA THE REGULATION OF RNA METABOLISM

Hunseung Kang, Hyun Ju Jung, Kyung Jin Kwak, Joo Yeol Kim, Min Kyung Kim and Xu Tao

Department of Plant Biotechnology, College of Agriculture and Life Sciences, Chonnam National University, Gwangju, 500-757 Korea; E-mail: hskang@jnu.ac.kr

Environmental stresses including high and low temperatures, drought, and high salinity are major factors that severely limit crop productivity worldwide. To overcome yield loss due to these environmental stresses, a large number of researches have been conducted to understand how plants respond to and adapt these environmental stresses. Posttranscriptional regulation as well as transcriptional regulation of gene expression is recognized as a key regulatory process in the response of plants to various environmental stresses, and these cellular processes are regulated by diverse RNA-binding proteins (RBPs). Over the last years, several classes of RBPs that harbor an RNA-recognition motif at the N-terminal half and a glycine-rich region at the C-terminal half (glycine-rich RNA-binding proteins, GRPs), zinc finger-containing GRP, and cold shock domain proteins (CSDPs) have been implicated in the response of plants to diverse environmental stresses. To develop stress-tolerant transgenic plants, we characterized the functional roles of GRPs and CSDPs in *Arabidopsis thaliana,* rice (*Oryza sativa*), and wheat (*Triticum aestivum*), and found that a specific family members of GRP and CSDPs contribute to enhance stress tolerance in plants and they display RNA chaperone function during stress adaptation process in monocotyledonous plants as well as in dicotyledonous plants. These findings point to the importance of the regulation of mRNA metabolism in plant response to environmental stresses and shed new light on the practical application of GRPs and CSDPs to develop stress-tolerant transgenic crops.

Keywords: RNA metabolism, stress-tolerant transgenic crops, glycine-rich RNA-binding proteins.

SL-110

Track: *Industrial and Manufacturing*

BIOGENERATOR – THE FIRST BIOTECHNOLOGICAL PROCESS FOR LARGE-SCALE STATIONARY POWER GENERATION

D. Karamanev

Department of Chemical and Biochemical Engineering,University of Western Ontario, London, Ontario N6A5B9, Canada; E-mail: dkaraman@uwo.ca

The conventional fuel cells have been considered a promising technology since their discovery 170 years ago. Unfortunately, there is still no truly commercial fuel cell after decades of intensive R&D worldwide.

The root cause is the extreme slowness of the cathodic oxygen reduction (electron consumption) reaction.

The respiratory oxygen reduction reaction in living organisms is several orders of magnitude faster than in fuel cells.

Therefore, our idea was to develop a unique (bio)technology where the oxygen reduction in the electricity generation process is performed by the respiration of living microbial cells. We named it “BioGenerator”. We do not consider it a biofuel cell because the BioGenerator has a power density which is between 100* and 25000** times higher than in any known biofuel cell.

The unique features of the BioGenerator include:

- It is the first practical bio-technology for electrical power generation in megawatt scale.
- It is the most efficient convertor of hydrogen to electricity.
- It is the only known power generation technology of any kind which consumes CO_2 from the atmosphere.

- It produces high-quality protein as a co-product.
- It is expected to be commercialized in less than 4 years, producing electricity at ~US$0.10/kWh.
- It will be used for:
  - smoothing of intermittent renewable power (solar, wind).
  - baseload power generation.

SL-73

CHEMOTROPHS, PHOTOTROPHS, … ELECTROTROPHS?

Dimitre G. Karamanev

Department of Chemical and Biochemical Engineering, The University of Western Ontario, London, Ontario N6G 5B9, Canada; E-mail: dkaramanev@eng.uwo.ca

It is well known that currently there are two groups of living organisms in terms of their direct energy source: chemotrophs and phototrophs. This presentation shows that theoretically, some living organisms, and in particular, microorganisms, could use electrical current as both a direct sole energy source and as an electron donor. I have proposed to name this, third group of organisms, electrotrophs. The experimental results reported here show that the microorganism *Acidothiobacillus ferrooxidans* can grow in electrotrophic mode.

SL-68

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology, Clinical Research/clinical trials; bioethics; nanobiotechnology.*

IN VITRO EVALUATION OF *BARLERIA PRIONITIS* L EXTRACT ON URINARY TRACT INFECTION (UTI) CAUSING MULTIDRUG RESISTANT *E. COLI* MEMBRANE BY FLUORESCENCE STUDY

C.N. Khobragade and Rashmi M. Bhande

School of Life Sciences, Swami Ramanand Teerth Marathwada University Nanded 431 606, India; E-mail: profcnkbt@rediffmail.com

Resistance to antibiotics is a ubiquitous clinical problem and is compounded by a dearth of new therapeutic agents. A variety of compounds from natural sources that modify membrane permeability are employed in management of multidrug resistant organisms. In the present investigation total phenol, flavonoid contents (0.33±0.1 mg of Gallic acid and 0.9±0.5mg of Quercetin equivalent per gram of dry extract respectively), anti-oxidant (IC_{50} 0.3±0.02 mg/ml compared to standard Ascorbic acid 0.5±0.01 mg/ml) and anti-inflammatory activity (in terms of albumin percent inhibition was 85.77% as compared to standard Ibuprofen 90.0% at 50µg/ml) of the extract were evaluated along with the effect of extract on membrane potential of *E.coli* by fluorescence spectroscopy method. Extract of *B.prionitis* L effectively disrupted *E.coli* cell membrane by depolarization peak at 516.62 nm with intensity 210.91 a.u. suggesting decrease in membrane potential after the treatment with extract. The presence of reactive oxygen scavenging agents in the extract effectively exerted their antibacterial action through membrane perturbations. Thus *B.prionitis* L extract containing bioactive compounds can be studied as future alternative to treat UTI infections caused by *E. coli*.

SL-71

Track: *Medical Biotechnology*

STRUCTURAL DYNAMICS AND EPIGENETIC MODIFICATIONS OF HOXC LOCI ALONG THE ANTEROPOSTERIOR BODY AXIS IN DEVELOPING MOUSE EMBRYOS

Myoung Hee Kim

Professor, Embryology Laboratory,Department of Anatomy, BK21 Project for Medical Science,Yonsei University College of Medicine,50 Yonsei-ro, Seodaemun-gu, Seoul 120-752, Korea;
E-mails: mhkim1@yuhs.ac; myoungheekim1@gmail.com

Hox genes are organized as clusters and specify the regional identity along the anteroposterior body axis by expressing sequentially at the specific time and region during embryogenesis. However, the precise mechanisms underlying the sequential spatio-temporal, collinear expression pattern of *Hox* genes were not fully understood. Since the epigenetic modifications like chromatin architecture and histone modifications have become crucial mechanisms for highly coordinated gene expressions, we examined such modifications. E14.5 mouse embryos were dissected into 3 parts along the anteroposterior axis: brain, trunk-anterior, and trunk-posterior. Then, the epigenetic modifications were analyzed along the *Hoxc* cluster using chromosome conformation capture and c*hromatin i*mmunoprecipitation-PCR methods. *Hox* non-expressing brain tissues had more compact, heterochromatin-like structure together with the strong repressive mark H3K27me3 than trunk tissues. In the trunk, however, more loose euchromatin-like topology with reduced amount of H3K27me3 modifications were observed along the whole cluster. The active mark H3K4me3 was closely associated with the collinear expression of *Hoxc* genes; anterior and posterior genes tested were modified with H3K4me3 only in the trunk-anterior and -posterior tissues, respectively. Altogether, these results indicate that loosening the chromatin architecture and removing H3K27me3 were not sufficient, but the concomitant acquisition of H3K4me3 drove the collinear expression of *Hoxc* genes.

SL-162

Track: *Medical Biotechnology*

NEW APPROACHES CONTROLLING CANCER: ZINC FINGER AND WATER MEMORY

Won H. Kim

Department of Biochemistry, Yonsei University Wonju College of Medicine, Korea

1. Protein Gene Therapy using Zinc Finger Targeting Telomerase: Telomerase activation is a key step in the development of human cancers. Artificial zinc finger protein (ZFP)s were designed using ZFP modules derived from human genome to repress expression of telomerase in human cancer cell lines at the transcriptional level. Variety of 4-finger ZFPs were constructed to recognize 12 bp sequential sequences within the promoter of the telomerase gene and were fused with KRAB repressor domain to create potent transcriptional repressor. Luciferase activity was decreased >80% in all of the transcriptional repressors. When they were transfected into the telomerase-positive HEK293 cell line, a decrease of mRNA level and telomerase activity together with shortening of telomere length could be observed. Actual growth of HEK293 cells was also inhibited by transfection. The repression of telomerase expression by artificial ZFP-TFs targeting the promoter region of telomerase presents a new promising strategy for inhibiting the growth of human cancer cells.

2. Information Wave of p53 showed Anti-cancer Effect: It was revealed that every matter has its accompanying matter wave. The wave part of the matter contains information (information wave), and can be transferred to water physically by shaking or tapping, and thus serially diluted water have been used to stimulate natural healing power in traditional homeopathy. This way of transferring the information of the mater to water has been already proved by Benveniste and other scholars. In this study, instead of traditional homeopathic method a new electronic machine was devised to transfer the information of matter to any medium including water. p53 (53KD) functions as a potent tumor suppressor. However, there is no practical way to utilize the function of p53. If the information wave of p53 could be transferred to water or any medium producing water, various strategies could be possible. In this study, information wave of p53 was first transferred to UM (meaning 'healing mineral' in Korean, mix of ceramic balls which makes alkaline reduced water), and then UM produced alkaline reduced water containing the information of p53 by contacting water. The water containing information wave of p53 inhibited cancer proliferation, showed anti-metastasis, and increased apoptosis. Water memory effect could be very useful for future cancer therapy.

SL-119

Track: *Industrial and Manufacturing*

A THERMOPHILIC B-GLUCOSIDASE FROM THERMOTOGA MARITIMA EXTENDS THE UPPER LIMIT OF THERMOTOLERANCE; SOLUTION TO GLOBAL CHALLENGES IN BIOETHANOL PRODUCTION

Farooq Latif, Iza Shahid , Muhammad Aamer Mehmood, Khadim Hussain and Muhammad Ibrahim Rajoka

National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan;
E-mail: farooq_latif@yahoo.com

β-Glucosidase (bgl) is one of the most important industrial enzyme in the plant biomass to bioethanol as biofuel. The present study was focused on β-glucosidase gene from Thermotoga maritima: Its cloning, heterologous expression in E. coli Top 10, purification and molecular characterization. The recombinant β-2 and β-5 were selected on LB medium with ampicillin using IPTG (0.1mM) as inducer. The shake flasks were run at high speed of 250 rpm and kept at 37 ºC. The induction period of 6 hrs was given and cells were harvested by centrifugation. The intracellular β-glucosidase activity showed a maximum of 4.0 U/ml, whereas, the protein amounts was 0.25 mg/ml. In 10 L bioreactor the β-glucosidase production was almost the same as in shake flasks. The bgl gene on SDS-PAGE showed a capacity to encode a 51 kDa β-glucosidase enzyme. The recombinant enzyme was purified using Ni-NTA column chromatography. Different parameters including melting temperature (tm), activation energy (Ea), enthalpy (ΔH*), Gibb's free energy (ΔG*) and entropy (ΔS*) were measured to determine the thermostability of the enzyme. It was found that enzyme activity was remarkably stable at pH range of 5.0-7.0 with a peak activity at 80-100oC. Structural studies and molecular techniques would further reveal the mechanisms involved in thermostability of the enzyme.

Keywords: Thermotoga maritima, β-Glucosidase, heterologous cloning, expression, molecular characterization.

SL-51

Track: *Industrial and Manufacturing*

BEYOND THE BARRIERS: MODIFICATION OF LIGNOCELLULOSIC BIOMASS FOR BIOFUEL PRODUCTION

Laigeng Li

Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Science, Chinese Academy of Science, 300 Fenglin Rd, Shanghai, 20032, PR China; E-mail: lgli@sibs.ac.cn

Lignocellulosic biomass is the most abundant renewable resource and raw material for biofuels. However, one of the barriers in converting lignocellulosic feedstock to fuels is that lignocellulosics are difficult to process. The resistance of lignocellulosics to component separation/processing arises primarily from high lignin quantity, low lignin reactivity (or low syringyl/guaiacyl (S/G) lignin constituent ratio) and high cellulose crystallinity and DP. At the same time, low cellulose content in natural populations represents another intrinsic barrier for the industry to capturing new and substantial strides in improving the process economics. Using a novel technique to simultaneously manipulate multiple lignin biosysnthetic genes, we produced transgenic trees with multi-trait modifications, including an up to 50% less lignin, a 64% higher S/G ratio and, remarkably, 30% more cellulose. Metabolic engineering of multiple traits in lignocellulosics provides a promising possibility to breakdown the barriers faced in the lignocellulosic biofuel conversion.

SL-43

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

IMPROVEMENT OF SEED YIELD AND ENHANCEMENT OF TOLERANCE TO SOFT ROT DISEASE IN TRANSGENIC BRASSICA

Yong Pyo Lim

Department of Horticulture, Chungnam National University, Gung-dong, Yuseong-gu, Daejeon 305-764, Korea; E-mail: yplim@cnu.ac.kr

Genetic transformation of Brassica is important for its high economic value and potential in expanding crop usefulness. Previously, we developed transgenic rapeseed plants (*Brassica napus* L.) overexpressing AtGRF1 and AtGRF2 genes and transgenic Chinese cabbage (*Brassica rapa* L.) with high tolerance to soft rot disease. AtGRF proteins have been shown to be positive regulators of cell proliferation in leaves of Arabidopsis. Quantitative and microscopic analyses showed that leaf area of both AtGRF1 and AtGRF2 overexpressors in both inbred lines became larger than those of wild-type plants, which was due to increases in both palisade cell size and number in leaves, indicating that enhancement of both cell expansion and proliferation led to larger leaves. Determination of fluorescence induction kinetics and other photosynthetic parameters showed that AtGRF1 and AtGRF2 gene products exert a stimulatory effect on overall electron transport rate (ETR). This result showed that overexpression of AtGRF1 and AtGRF2 in *B. napus* improves seed yield via expansion of leaf photosynthetic area and an enhancement of photosynthetic ETR. Soft rot disease tolerance was conferred by expression of N-acyl-homoserine lactonase (AHL-lactonase) in Chinese cabbage. To synthesize and express the AHL-lactonase in Chinese cabbage, the plant was transformed with the aii gene (AHL-

lactonase gene from Bacillus sp. GH02) fused to the PinII signal peptide (protease inhibitor II from potato). Soft rot disease tolerance was evaluated at tissue and seedling stage. Transgenic plants showed a significantly enhanced tolerance (2–3-fold) to soft rot disease compared to wild-type plants. Thus, expression of the fusion gene pinIISP-aii reduces susceptibility to soft rot disease in Chinese cabbage.

Keywords: *Brassica rapa* L., *Brassica napus* L., Soft rot disease tolerance, AtGRF1, AtGRF2.

SL-22

Track: *Plant and Environment Biotechnology*

SATELLITE RNA-MEDIATED RESISTANCE TO BAMBOO MOSAIC VIRUS INFECTION IN TRANSGENIC PLANTS

Kuan-Yu Lin, Hsin-Chuan Chen and Na-Sheng Lin

Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan 115 and Graduate Institute of Biotechnology, National Cheng Kung University, Taiwan 700; E-mail: nslin@sinica.edu.tw

Although satellite RNAs (satRNAs) depend on helper viruses for replication and encaspidation, they share little sequence homology. Some satRNAs can modify virus-induced symptoms and interfere with helper virus replication, becoming potential agents for the bio-control of viruses. In our study, we isolated a satellite RNA associated with Bamboo Mosaic Virus (BaMV) from the field, which can greatly reduce the accumulation of BaMV RNA in co-inoculated plants. We generated transgenic *Nicotiana benthamiana* and *Arabidopsis thialiana* expressing full-length interfering satBaMV. Four transgenic N. benthamiana lines were selected: lines 1-4 and 2-4 exhibited delayed and mild symptoms, while lines 3-2 and 3-3 showed no symptoms after challenge with BaMV. The BaMV accumulation levels were greatly down-regulated by the highly accumulated transgene-derived satBaMV in all transgenic *N. benthamiana* lines. By ELISA, after infection with BaMV RNA, transgenic lines showed only 5% BaMV accumulation when compared with wild type amounts. Nevertheless, those transgenic lines were more resistant to BaMV viral RNA than to virion infection. Transgenic *A. thaliana* expressing satBaMV were also generated; they showed high resistance to both BaMV virion and viral RNA. Through microarray analysis, many defense genes were upregulated by BaMV infection in transgenic *A. thaliana*. The possible satRNA-mediated resistance mechanism will be discussed.

Keywords: satellite RNAs, helper virus replication, transgenic N. benthamiana.

SL-8

WORST INVASIVE OR MOST VALUABLE: WILL BIOSORPTION TILT THE BALANCE FOR WATER HYACINTH?

Courtie Mahamad and Tichaona Nharingo

Chemistry Department, Bindura University of Science Education, P. Bag 1020, Bindura, Zimbabwe; E-mails: courtiema@yahoo.com; cmahamadi@buse.ac.zw

Water hyacinth (*Eichhornia crassipes*), has attracted significant attention as the world's worst invasive aquatic plant due to its extremely rapid proliferation and congest growth, presenting serious challenges in navigation, irrigation, and power generation. Attempts to control the weed have proved to be energy intensive and costly with minimum results. However, the same plant has demonstrated an amazing ability to absorb and concentrate many toxic metals from aquatic environments. Consequently, significant research activity focusing on possible utilization of the plant in a beneficial way has been registered over the last few decades. Our paper reviews recent research related to the utilization of dried/processed *E. crassipes* in the biosorption of toxic metals from aquatic environments and presents our recent findings and ideas on the competitive adsorption of Pb2+, Cd2+ and Zn2+ onto acid-treated biomass.

Keywords: Water hyacinth, biosorption, toxic metal, sorption capacity, competitive adsorption.

SL-113

THE POTENTIAL USE OF ACETONE LEAF EXTRACTS OF BREONADIA SALICINA AND UROLIC ACID AGAINST PENICILLIUM SPP. INFECTING ORANGES

Salome Mamokone Mahlo

Department of Life & Consumer Sciences, School of Agriculture and Life Sciences, College of Agriculture & Environmental Sciences, UNISA, PO Box 392, UNISA, 0003 Florida Campus, c/o Christiaan De Wet & Pioneer Ave, Block B, Room 217, South Africa;
E-mail: mahlosm@unisa.ac.za

Acetone leaf extracts of Breonadia salicina and the main isolated antifungal compound ursolic acid were active against three important plant fungal pathogens (Penicillium expansum, P. janthinellum and P. digitatum). Acetone extracts had good *in vitro* antifungal activity against P. janthinellum (MIC 0.08 mg/ml). Penicillium digitatum and P. expansum were more resistant (MIC 1.25 mg/ml). The potential use of the acetone extract and ursolic acid against these fungal pathogens in post-harvest infections of oranges were determined. The crude leaf extract (1 mg/ml) gave the same level of protection as ursolic acid (1 mg/ml) indicating synergistic activities within the crude extract. The acetone extract (MIC of 0.16 mg/ml) had similar activity as amphotericin B (MIC 0.08 mg/ml) against P. digitatum. Cytotoxicity of the crude extract and ursolic acid was determined using the tetrazolium-based colorimetric assay (3-(4,5-dimethylthiazol)-2,5-diphenyl tetrazolium bromide (MTT)) against Vero monkey kidney cells. The acetone extract had sufficient antifungal activity against these organisms to consider its use in the citrus industry. The results show the potential use of plant extracts to combat plant fungal infections if extracts with lower cellular toxicity can be found or if the toxicity of the extract can be decreased without changing the antifungal activity.

Keywords: Antifungal activity, Breonadia salicina, Minimum inhibitory concentration (MIC), Penicilium digitatum, P.expansum, P. janthinellum, Citrus sinensis, lethal concentration (LC 50).

SL-18

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

TOXICITY AND SYNERGIC EFFECTS OF VANADIUM AND NICKEL ON HEAVY METAL-TOLERANT MICROBIAL SPECIES IN WASTEWATER SYSTEMS

I. Kamika and M.N.B. Momba

Department of Environmental, Water and Earth Science, Tshwane University of Technology, 175 Nelson Mandela Drive, Pretoria 0002, South Africa; E-mail: mombamnb@tut.ac.za

Pollution caused by chemical pollutants including heavy metals is a global issue. In recent years, increasing concern has been expressed about the disposal of these heavy metals into the environment, and especially into water. As single-cell organisms, microorganisms are the first to be affected by the heavy metal pollution generated by human activities. Essential or not, heavy metals have toxic effects on the growth and survival of microorganisms by interfering with their metabolic functions even at moderate concentrations. The presence of heavy metals in wastewater treatment has been probably one of the biggest challenges to water source pollution due to their toxicity and especially when a synergistic action between two or three metals is possible. In high concentrations, heavy metals may have acute effects on microorganisms and in turn reduce the effectiveness of the biological processes in wastewater treatment plants. The present study assessed the toxicity and synergic effects of vanadium and nickel on four heavy metal-tolerant microbial isolates, two bacterial species (*Bacillus licheniformis and Pseudomonas putida*) and two Protozoan species (*Peranema* and *Trachelophyllum*). The experimental study was conducted in a laboratory-scale unity containing modified domestic wastewater mixed liquors. The isolates were exposed to various concentrations (10, 20, 30, 40, 50, 60 ppm) of V^{5+} and Ni^{2+}. The effects of V^{5+} and/or Ni^{2+} on microbial species were assessed at 30°C and various pHs (4, 6, 7 and 8) in the modified wastewater mixed liquor for four days of exposure. In addition, the bioremediation of metals by test organisms was measured using ICP-OES.

The results revealed that Ni^{2+} was more toxic than V^{5+} to microbial isolates. Target organisms with the exception of *Trachelophyllum* sp. were able to grow in the presence of V^{5+} up to 60 ppm for 4 days at pH 7(range for bacterial growth rates: 0.72 to 5.07 d^{-1}; range for prototozoan .growth rate: -1.73 to 1.20 d^{-1}; while in the presence of Ni^{2+} at the same concentration, there was no growth. Significant die-off rates were, however, noted for protozoan species even at

lower Ni^{2+} concentrations compared to bacterial species ($p < 0.05$). *Bacillus licheniformis* and *Pseudomonas putida* showed *die*-off rates of 1.05 (16%) and 2.26 d^{-1} (37%), respectively when exposed to 60ppm Ni^{2+} at day 4. For *Peranema* sp. and *Trachelophyllym* sp. the die-off rates of 2.70 (56%) and 2.39 (35%) were recorded when exposed at 40 ppm and 30 ppm N^{2+}, respectively from day 3. Complete die-off of protozoan species occurred at 50 and 60 ppm Ni^{2+} from the first day of exposure. Despite the toxic effects of V^{5+} and Ni^{2+} at high concentrations, the results of this study revealed that the test organisms were able to remove V^{5+} (percentage removal range for bacteria: 57 to 81%, for protozoa: 21 to 49%) and Ni^{2+} (16 to 63%; for bacteria; 9 to 28% for protozoa). Earlier inhibition of microbial growth and metal removal capability were revealed at 20 ppm when V^{5+} and Ni^{2+} were present in the same wastewater system. This situation proves that there is a synergistic action between V^{5+} and Ni^{2+} and this is related to the pH in the wastewater system. The study therefore revealed that this synergistic action affects the removal efficiency of heavy metals by the target organisms in wastewater systems.

Keywords: Toxicity, vanadium, nickel, wastewater systems, metal-tolerant microbial species.

SL-44

Track: *Plant and environment Biotechnology*

ANTIBACTERIAL EFFECTS OF SOME ESSENTIAL OILS ON THE GROWTH OF RALSTONIA SOLANACEARUM (RACE 3, BIOTYPE 2)

M. Hosseini Nezhad, L. Alamshahi and N. Panjehkeh

Khorasan Research Institute for Food Science and Technology (KRIFST), Iran; E-mail: Hosseinynejad@yahoo.com

Biological control methods are considered as the best approaches to reduce plant diseases which include utilizing essential oils among the most important antibacterial agents. In this study, the efficacy of the essential oils from 5 plant species was evaluated *in vitro* against *Ralstonia solanacearum* (race 3, biotype 2) causing potato wilting incidence.

Essential oils of *Thymus vulgaris*, *Rosmarinus officinalis*, *Coriandrum sativum*, *Cuminum syminum* and *Eucalyptus globulus* were extracted by hydrodistillation and tested against the bacterium species by paper disc diffusion method at different concentrations between % 0.01 to % 100 (v/v). The minimum inhibitory concentration and minimum bactericidal concentration was determined by twofold broth dilution method. Means were compared using Duncan's Multiple Range Test at the 1% level of significance by MSTATC software. The most inhibition zone (34.8 mm) and MIC (1 µl/ml) were resulted by *Thymus vulgaris* while this values were higher than positive controls of streptomycin and erythromycin. Thyme followed by *C. syminum=C. sativum* > *R. officinalis*. The efficacy of essential oil from *E. globulus* (inhibition zone 6.5 mm) was insignificant. MIC and MBC values of essential oils were 1-250 µl/ml. Results indicate that thyme essential oil can be used as against bacterial wilting disease in potato.

Keywords: *Ralstonia solanacearum*, essential oil, antibacterial, inhibition zone.

SL-97

Track: *Medical Biotechnology*

USING CUTTING-EDGE COMPUTATIONAL MASS SPECTROMETRY TO IDENTIFY MICROORGANISMS- A MICROBIAL MICROSCOPE

Ali Pervez

Sage-N Research, Inc., 1525 McCarthy Blvd, Milpitas CA 95035, Suite 1000, USA;
E-mail: apervez@sagenresearch.com

Detection and identification of pathogenic microorganisms continues to be an area of high concern. Particularly important is the identification of pre-existing or newly arising strains of infectious agents such as bacterial, viral or other disease organisms. Current diagnostics efforts are hampered by either the inability or length of time required to identify the presence or absence of pathogenic organism in the host.

Most current methods rely on PCR (RT-PCR). The real shortcoming of such an approach is that it requires known sequence information for detection. For example, RT-PCR uses probes that hybridize to a known sequence. If a significant mutation occurs in a viral target, the known effective probes are rendered useless, as they will be unable to hybridize to a mutant sequence.

The session talk will discuss a mass spec proteomics approach that does not require any knowledge of what is contained within the mixture, using the power of computational proteomics. The power of such an approach is increased by its ability to be "culture-less" based on the limit of detection of the instrument used and is also based on "PhyloProteomics" that enables the micorganism to be classified by its origin.

SL-120

Track: *Medical biotechnology*

CLONING AND EXPRESSION THE STREPTOCOCCUS PYOGENES HYALURONIDASE IN *E. COLI*

Iraj Pakzad, Hamid Abtahi and Azar Moradkhani

Department of microbiology, Ilam University of Medical Sciences, Ilam, Iran; E-mail: pakzad_i2006@yahoo.com

Background: extracellular hyaluronidase of Streptococcus pyogenes is a spreading factor (hyaluronate lyase) which composed of 868 amino acid protein with a molecular size of 99 636 Da, the aims to clone and express extracellular hyaluronidase of Streptococcus pyogenes in E.coli Methods: the hyal A gene inserted in pTz57R/T vector. Subsequently, the vector was purified and later digested using SacI and BamHI restriction endonuclease. Following insert in to pET32a vector, the recombinant plasmid was introduced in to Ecoli DH5α and the transferred in to the E.coli. BL21 (DE3) pLysS bacteria, to monitor its expression by use IPTG and Protein analysis was carried out by SDS-PAGE electrophoresis. The new recombinant protein antigenicity was evaluated by Immunoblot analysis.

Results: PCR product was a fragment with about 2608 bp, and complete similarity with gene bank accession number af218838. Recombinant protein in expression vector pET32a was produced by 1mM IPTG. In SDS-PAGE several bands was seen. The 110 kD band belongs to Hyl A recombinant protein together His -tag. An others bands was 50-110 kD in molecular weight range. This recombinant protein in western blotting was confirmed by use serum from mouse immunized with supernatant culture of S. pyogenes.

Conclusion: the several molecular weight of recombinant protein possibility is due to heavy weight of Hyl A protein or may be has two start codon in hylA gene . In other hand, western blotting results indicate all bands of this recombinant protein have antigenic property, and can used for diagnostic assay.

Keywords: extracellular, hyaluronidase, recombinant.

SL-9

Track: *Pharmaceutical Biotechnology*

PROS AND CONS OF A PLANT-DERIVED ORAL VACCINE AGAINST HBV

Tomasz Pniewski, Józef Kapusta, Piotr Bociąg, Marcin Czyż, Anna Kostrzak, Justyna Wojas, Magdalena Milczarek, Elżbieta Pajtasz-Piasecka, Joanna Wietrzyk, Egbert Piasecki, Sławomir Samardakiewicz, Jacek Wojciechowicz, Paweł Krajewski, Bogdan Wolko and Andrzej Płucienniczak

Institute of Plant Genetics PAS, Strzeszyńska 34, 60-479 Poznań, Poland; E-mail: tpni@igr.poznan.pl

Oral plant-based vaccines can induce immune response against both mucosal pathogens and blood-borne diseases as hepatitis B. Efficient anti-HBV plant-derived vaccines requires appropriate plant expressor as well as vaccine composition and administration protocol.

We have engineered herbicide-resistant lettuce expressing HBV antigens, HBsAg subunits (S, M, L) and HBcAg, at levels 5 - 200 μg/g FW. Expression of HBV antigens was confirmed by ELISA, western blot and microscopic observation of S-HBsAg assembled into VLPs. Vegetative propagation of superior expressors enabled scaling-up multiplication of antigen-bearing material.

The technology for potent vaccine production has been developed, based on lyophilisation of plant material containing HBV antigens. The particular antigens were preserved at a half or equivalent level during freeze-drying and long-term storage. Powdered lyophilised tissue facilitated controlled oral administration and enabled conversion into tablets for possible human vaccination.

Freeze-dried tissue containing S-HBsAg induced systemic humoral response in mice at minimal protection level (≥10 mIU/ml of anti-HBs antibodies). Combination of injection priming and oral boosting triggered significant reaction (hundreds mIU/ml). Relatively low antigen dosage and extended timing between immunizations correlated with an increased responses.

The study provides some basics regarding immunization strategy and an efficacious and convenient formula of plant-derived oral vaccines against hepatitis B.

Keywords: vaccine against HBV, blood-borne diseases as hepatitis B, ELISA.

SL-39

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

MICROBIAL VOLATILES INDUCED ACCUMULATION OF EXCEPTIONALLY HIGH LEVELS OF STARCH INVOLVES A PROCESS WHEREIN STARCH SYNTHASES CLASS III AND IV AND NTRC-MEDIATED CHANGES IN REDOX STATUS OF PLASTIDIAL ENZYMES PLAY IMPORTANT ROLES

J. Pozueta-Romero

Institute of Agrobiotechnology, National Research Council of Spain (CSIC), Spain; E-mail: javier.pozueta@unavarra.es

Microbial volatiles promote growth and the accumulation of exceptionally high levels of starch in leaves (Ezquer et al., 2010). Time-course analyses of starch accumulation in Arabidopsis leaves exposed to fungal volatiles (FVs) emitted by Alternaria alternata revealed that microbial volatiles induced starch accumulation process (MIVOISAP) is due to stimulation of starch biosynthesis during illumination. This phenomenon was inhibited by cordycepin, and accompanied by drastic changes in the Arabidopsis transcriptome. MIVOISAP was also accompanied by enhancement of the total 3-phosphoglycerate/Pi ratio, and a 2-3 fold increase of the levels of the reduced form of ADP-glucose pyrophosphorylase. Using different Arabidopsis knockout mutants we investigated the impact in MIVOISAP of down-regulation of genes directly or indirectly related with starch metabolism. These analyses revealed that the magnitude of the FVs-induced starch accumulation was low in mutants impaired in starch synthases (SS) class III and IV, and plastidial NADP-thioredoxin reductase C (NTRC). The overall data thus showed that Arabidopsis MIVOISAP involves a transcriptionally and post-translationally regulated network wherein photoreceptors, SSIII, SSIV and NTRC-mediated changes in redox status of plastidial enzyme(s) play important roles.

Keywords: Plant-microbe interaction, starch.

SL-46

Track: *Industrial and Manufacturing*

CRYSTALLIZATION OF THERMOPHILIC LIPASE

Rashidah Abdul Rahim, Fan Choy Fong, Fatimah Azzahrah Abdul Rashid and Anuradha Balan

School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden Penang, Malaysia;
E-mail: rashidahrahim@gmail.com

One of the most crucial method in which biologists determine protein's shape, function, structure and character is through its three-dimensional molecular visualization, which involves protein crystallization techniques. In this research, we are interested to crystallize lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) from thermophiles due to its broad potential of applications in biotechnological industries. The methods used for lipase crystallization were hanging drop and microbatch method. Theoretically, 2 different regions are divided in standard phase diagram. That is undersaturation region and supersaturation region. Lipase crystal will form in the supersaturation region and no crystal forms in undersaturation region. Screening process using Hampton Research Crystal Kits was carried to determine the suitable formulation for crystallization of lipase. Once it is obtained, the formulation and crystallization parameters such as concentration of salt, concentration of buffer, concentration of protein and temperature were manipulated in characterization of lipase crystal to obtain a well-defined protein crystal for further structure determination analysis using X-ray diffraction system. Lipase crystals were obtained from both hanging drop and microbatch methods with

good lcrystal produced by using calcium chloride dihydrate as the salting agent and Tris HCl as the buffer at 20ºC. The protein crystal was confirmed by using Izit crystal dye.

Keywords: Protein crystallization, lipase, thermophiles.

SL-53

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

CEREAL GRAIN MODIFICATION FOR IMPROVED HUMAN HEALTH

Ahmed Regina, Tony Bird, Behjat Kosar-Hashemi, Zhongyi Li, Stephen Jobling, Sadequr Rahman, David Topping and Matthew Morell

Department of Plant Industry, Commonwealth Scientific and Industrial Research Organization, Canberra, Australia; E-mail: regina.regina@csiro.au

The impact of resistant starch (RS) on human health is well demonstrated through its potential to enhance bowel health indices thereby reducing the risks of life-style diseases such as colon cancer. RS is the type of starch that enters the large bowel escaping digestion in the small intestine, where it gets fermented by resident microflora into short chain fatty acids (SCFA). Modifying cereal grains to produce starch with higher proportion of amylose (the predominantly linear component of starch) is a potent way of incorporating RS into human food chain. RNA mediated gene silencing technology was utilized to concomitantly reduce the expression of starch branching enzyme (SBE) IIa and SBE IIb in wheat leading to increasing the amylose content in the endosperm starch to >70%. Structurally, the starch was further altered in its chain length and molecular weight distributions. As a consequence there were significant changes in the functional properties with regard to its thermal and pasting behaviours. In vitro studies have revealed significant increase in the resistant starch content in this wheat, which was further substantiated through in vivo studies in rat model. Rats fed high amylose wheat revealed improved indices of bowel health such as lowered gut pH and increased levels of SCFA.

Keywords: Resistant starch, short chain fatty acid, health benefit, cereal.

SL-100

Track: *Pharmaceutical Biotechnology*

IMMUNE RESPONSE TO NEW HEPATITIS B VACCINATION PROTOCOLS IN PATIENTS ON HEMODIALYSIS – SINGLE CENTER EXPERIENCE

Halima Resić, Nihad Kukavica, Fahrudin Mašnić, Nejra Prohić, Selma Ajanović and Aida Ćorić

Clinic for Hemodialysis, Clinical Center University of Sarajevo, Bolnička 25, 71000 Sarajevo, Bosnia and Herzegovina; E-mail: halima.resic@undt.ba

Patients with end stage renal disease (ESRD) have a reduced response to vaccination against hepatitis B infection.

Aim: The aim of the study was to determine the adequacy of immune response with new protocol of vaccination against hepatitis B infection in patients on hemodialysis.

Patients and Methods: The study included incident hemodialysis patients since 2008th until 2011, at the Clinic for Hemodialysis, Clinical Center University of Sarajevo. We started the new vaccination protocol in September 2009. Old vaccination protocol included vaccination at the start of renal replacement protocol. New protocol implied vaccination six months before starting renal replacement therapy (RRT) and “ic” (intracutaneously) application of vaccine vs. “sc” (subcutaneously) application. Vaccination was carried out for over 12 months. The follow-up period was from 2009 to 2011.

Results: The study included 64 patients. Males were represented with 57,81% (37), and females with 42,19% (27), divided in two groups. The first group included patients from the period 2008 to 2009, which have been vaccinated under old vaccination protocol, and second group included patients with the new protocol from September 2009 to beginning of 2011. The first group had 28 patients, mean age 55,17±11,84 years and mean duration of hemodialysis 24,65±5,32 months. The second group had 36 patients, with mean age of 62,79 ± 15,88 years, and mean duration of hemodialysis 22,16 ± 24,53 months. None of the patients in second group has been previously vaccinated, nor had

positive antiHbs antibody in serum before vaccination. 5 patients received a booster dose of vaccine, after which 4 patients showed adequate responses with antiHbs titre.

In the first group of patients, from the total of 28 patients, 15 patients didn't response adequately with antiHbs titre at the end of vaccination. Out off 36 patients, in the second group of patients, 31 of them have had response to vaccination with the new protocol, which was statistically significant ($p<0,005$). The total percentage of patients with adequate titre of antiHBs after vaccination was 97.43% with a new protocol. And the percentage of patients who required booster dose of vaccines was 12.82%.

Conclusion: Vaccination of patients with ESRD six months before starting renal replacement therapy and with intradermal application of vaccine vs. subcutaneous application, improved immune response with antiHbs titre in our patients.

Keywords: hemodialysis, vaccination, antiHBs, hepatitis B.

SL-49

Track: *Plant and Environment Biotechnology*

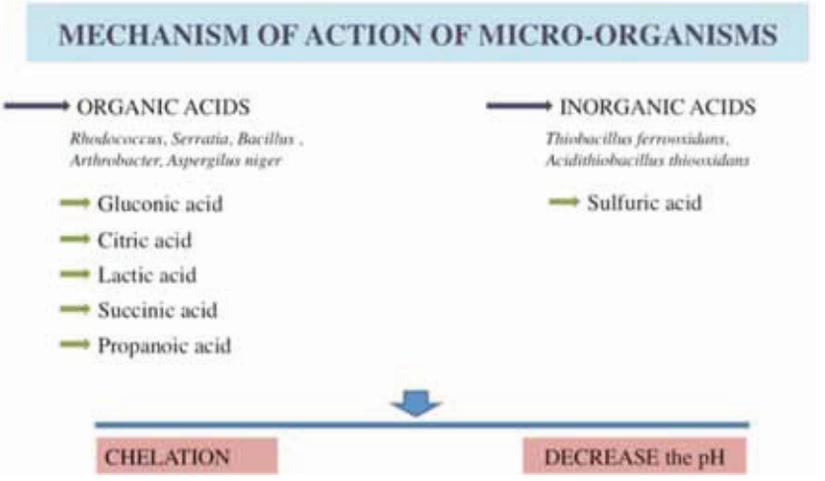
BIOTECHNOLOGICAL PRODUCTION OF PHOSPHORUS FERTILIZERS

A. Saeid, K. Chojnacka and H. Górecki

Institute of Inorganic Technology and Mineral Fertilizers, Wrocław University of Technology, Wrocław, Poland;
E-mail: agnieszka.saeid@pwr.wroc.pl

Problem of decreasing resources of main raw material for phosphorus fertilizers-phosphate rock, can be solved by application of biological solubilization of low quality substrates, by bacterial species able to produce mineral and low molecule organic acids.

The following bacteria were found to be able to slolubilize phosphorus:



It was found that *Bacillus megaterium* and *Acidithiobacullus ferroxidans* are able to solubilize phosphorus bound in organic matter, like bones or sewage sludge form third step of waste water treatment.

Using the microbial treatment (by acids produced by microorganisms) it is possible to produce liquid fertilizers. At the same time the problem with utilization of agricultural wastes can be solved. After bone meal was identified as a vector for bovine spongiform encephalopathy (BSE, or "mad cow disease") among livestock the utilization of bones creates the problem.

Keywords: FERTILIZERS, organic acids, phosphorus.

SL-99

Track: *Business Development*

LATERAL ISSUES OF MODERN BIOTECHNOLOGY: SOCIAL, REGULATORY, ETHICAL ASPECTS AND COMMUNICATION

George Sakellaris

National Hellenic Research Foundation, 48, Vas. Constantinou Ave. 11635 Athens, Greece;
E-mail: g.sakellaris@eie.gr

Social legal and communication aspects have been proved important issues in many applications of modern technology the last 15 years. A systematic analysis of the public perceptions is definitely and guideline influencing decisions and priorities.

Modern Biotechnology offers a broad existing knowledge-base to support a wide spectrum of various applications with promising results. However, in many cases, a number of non-technical, social barriers continue to prevent the realization of this potential. Public opinion significantly influences technological adoption, the market uptake of novel bio-products, and the development of the appropriate regulatory framework.

It is an interesting task to identify public attitudes on the use of modern Biotechnology. The findings suggest that Europeans are sceptical and ambivalent versus the new technologies, while their knowledge and trust deficits are often leading to exaggerated judgments on the risks involved.

Besides the fears and insecurities for safety, the general public and the stakeholders often are raising specific concerns of ethical or legal nature.

The global goals of the world food availability, the necessary conditions in order this availability to be achieved, the use of GMO's and the environmental safety, the need for agricultural intensification under sustainable conditions, the freedom from hunger, the use of agriculture for non-food purposes, are some of the reasons raising these concerns.

A minimal consensus across society is necessary in order these issues to be managed. The first step in establishing such consensus is a common dialogue platform including all involved actors. To do so, science needs a supportive climate in terms of public esteem. To achieve such goal, particular communication strategies are necessary. It is well known that problems arose from claims about safety that turned out to be politically non-realistic. Scientists increasingly communicate with the general public, but what scientists say does not always meet a friendly perception.

A special attention should be devoted in the case of risk communication. Risk analysis is a systematic way to more fully assess risks, to get transparency into complexity and to address uncertainties or knowledge gaps. It is composed of three activities: risk assessment, risk management and risk communication. While all effort is made to minimize hazards occurring, food safety is not an absolute and hazards can occur. Risk assessment follows a structured approach to estimate the risk and to obtain insight in the factors that influence the risk in a positive or negative sense.

In order to benefit an efficient communication, three main parameters have to be taken in consideration:

- Increasing public awareness
- Increasing risk tolerance
- Considering a democratic context of communication

Modern society depends on scientific discovery and applying this new knowledge through technology. However, the role that science plays in our daily lives is often overlooked or taken for granted and public opinion is often only mobilized when research and new discoveries raise ethical questions. Besides the above conditions a new generation of young scientists with good communication skills will be crucial.

A wide number of studies of social, ethical and regulatory aspects, applicable on Modern Biotechnology have been conducted in Europe and in North America. Sometimes the findings seem to be controversial or incompatible, depending on the country or a specific audience. It would be therefore extremely useful to set up a profile of other regions such as Africa, South America or Asia, collecting the same type of data and having a concrete and scientifically based element of comparison. Such an investigation offers the guidelines for the development of a balanced regulatory frame and an effective communication strategy, tailored to the needs of specific target publics and markets.

SL-36

Track: *Industrial and Manufacturing*

EFFECT OF ONE POINT MUTATION ON PROTEIN STRUCTURE

Abu Bakar Salleh, Mahiran Basri, Mohd. Basyaruddin Abd Rahman, Adam Leow Thean Chor, Bimo Ario Tejo, Mohd Shukuri Mohd Ali, Arilla Sri Masayu Abd Rahim, Norhayati Zakaria and Raja Noor Zaliha Raja Abd. Rahman

Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor; E-mail: abubakar@biotech.upm.edu.my

The role of each amino acid in a protein sequence towards protein function has been continuously studied. A single point mutation can occur spontaneously and naturally in nucleotide replication. However, it is possible to get specific single point mutation via site directed mutation. Earlier we studied, one point mutation on F1 protease. The mutant W200R and D58S showed the importance of ion pairs and secondary structure towards conformational stability of the F1 protease. Studies on L2 lipase, with mutation at Ser2 to Phe2, located at the N-terminal resulted in the alteration of the optimal temperature and pH. The analysis showed that S2F mutation was not directly involved in catalysis since there was no changes in the substrate specificity. In another work, mutation from ARM lipase R157S showed an increase in temperature stability but the S236R mutant showed the reverse effect. Apparently single point mutation can influence the conformational structure and properties of protein.

SL-28

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

HEAVY METAL RESISTANT PGPR FOR BIOCONTROL OF PHYTOPATHOGENS AND BIOREMEDIATION OF HEAVY METAL CONTAMINATED SOIL

R.Z. Sayyed, A.S. Patil and P.R. Patel

PG Department of Microbiology, PSGVP Mandal's Arts, Science and Commerce College Shahada, Maharashtra, 425 409, India. E-mail: sayyedrz@gmail.com

Heavy metal resistant Plant Growth promoting Rhizobia (PGPR) represent unique feature of plant growth promotion, phytopathogen suppression and absorption of heavy metal ions from metal contaminated agriculture fields. In the present work we report in vitro studies of PGPR mediated suppression against plant pathogenic fungi and bacteria. PGPRs are effective and offers a natural mechanism for biological control of plant disease. Meanwhile, it also provides practices compatible with the goal of a sustainable agricultural system. PGPR strains differ in their ability to protect plants by producing the array of secondary metabolites. The main PGPR determinant of plant protection includes siderophores. In present research work we have observed antagonistic action of siderophore producing heavy metal resistant PGPR against common phytopathogens. Heavy metal resistant *Alcaligenes* sp. STC1 and *Pseudomonas aeruginosa* RZS3 SH-94B were isolated from soil, against $MnCl_2.4H_2O$, NiCl.$6H_2O$, $ZnSO_4$.7HO, $FeSO_4$, $CuSO_4$, $HgCl_2$, $FeCl_3$, $AgNO_3$ and $CoCl_2$. Maximum growth was observed at 100µM concentration of each heavy metal concentration. Siderophore rich broth and supernatant exhibited potent and superior antifungal activity as compared to chemical fungicides kitazin, carbistin and bilcop 50.

Keywords: PGPR, siderophore, phytopathogens.

SL-115

CHARACTERIZATION OF FOULING ATTRACTANTS TO ENHANCE THE RECRUITMENT OF BENTHIC ASSEMBLAGES ON ARTIFICIAL SURFACES

M. Sidharthan, Sang Mok Jung, H. S. Lee, Jinwook Noh and Myeong Jin Song, Deo Han and Hyun Woung Shin

Department of Marine Biotechnology, Soonchunhyang University, Asan City, South Korea 336 745; E-mail: hwshin@sch.ac.kr

Marine pollution and over exploitation of marine living resources are eventually resulted in reduction of benthic vegetation cover. Benthic flora and fauna are the basic life forms that nurture the fish and other macrobenthic forms, reduction in their coverage area directly affects the overall fishery production. In recent years, artificial reef surfaces constructed with various materials have failed to function in many parts of the world. Therefore, an attempt has been made to develop fouling attractant-coatings in order to enhance the recruitment of initial colonizers as well as macrobenthic communities on artificial surfaces that are used to extend or recover the benthic vegetation. In benthic ecosystem, recruitment of both algal and animal propagules is mediated by various surface and environmental cues. In this study, fouling enhancing efficiencies of six chemical candidates (soybean oil, corn oil, grapeseed oil, furfuryl alcohol, ferrous sulfate and ferrous lactate) were characterized. Test substances were subjected to preliminary laboratory screening against slime forming diatom species and spores of a fouling alga, *Ulva* sp. In order to demonstrate the nontoxic nature of these test substances, they were tested against *Tetraselmis suecica* and *Artemia salina*. Results showed that at concentrations ≤100 mg l^{-1} levels these chemical candidates had no significant inhibitory effects on *T. suecica* and *A. salina*. Two best performing fouling attractant-candidates, corn oil and soybean oil were further investigated in field trials. Controlled depletion coatings (rosin based matrix) prepared with these two fouling attractant-candidates were screened using panel immersion tests at Gangneung (east coast) and Mijo harbor (south coast), South Korea. Both corn oil and soybean oil based fouling attractant-coatings showed significant increase in total biomass of benthic assemblages, whereas corn oil-based fouling attractant-coating found to enhance the recruitment of macroalgae. Recruitment of major fouling organisms and fouling coverage (%) on corn oil and soybean oil based fouling attractant-coatings are discussed.

SL-14

Track: *Pharmaceutical Biotechnology: biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering.*

RECOMBINANT *SALMONELLA FLAGELLIN* PROTEIN AS AN ADJUVANT TO PLASMID SHIV – DNA

Dinesh K. Singh

Winston Salem State University, 217 WBA Science Bldg. Cromartie Street,Winston Salem, NC 27110, USA; E-mail: singhd@wssu.edu

The intramuscular injections of non-infectious SHIV-DNA protected macaques from AIDS but not from infection on intravaginal challenge of heterlogous SHIV89.6P. Our attempt to use this DNA as mucosal vaccine failed to elicit a strong immune response. We decided to evaluate a Samonella recombinant flagellin protein as an mucosal adjuvant to potentiate SHIV-DNA at vaginal surface. A total of 80 female BALB/c mice divided in four groups were used in this study. First group received 200µg of vaccine DNA, second received 200µg of vaccine DNA+ 1µg Flagellin, third received only 1µg Flagellin, and fourth received only PBS and used as control. The vaccine and Flagellin was administered intravaginally. The DNA alone, and DNA Flagellin groups were g of Flagellin as booster at 21µg of DNA with or without 1 µadministered 100 day after the first immunization. Splenocytes from all vaccinated and controlled group animals at different time points were subjected to ELISPOT and ELISA tests to evaluate immune responses. The group administered with DNA and Flagellin has demonstrated significantly higher titers of gag/env/nef specific immune-spots and ELISA titers than DNA alone group. These and other results will be discussed.

Keywords: SHIV-DNA, AIDS, BALB/c mice, ELISPOT.

SL-57

Track: *Other Areas: Bionanotechnology*

TIP-ENHANCED RAMAN FINGERPRINTING OF MEMBRANE PROTEINS OF GENETICALLY MODIFIED YEAST CELLS

Denys Naumenko, Valentinas Snitka, Elena Serviene, Ingrida Bruzaite, Andrius Stogrin, Vitas Lendraitis and Boris Snopok

Research Center for Microsystems and Nanotechnology, Kaunas University of Technology, Studentu 65, 3031 Kaunas, Lithuania; E-mail: vsnitka@gmail.com

Recent advances in cellular imaging techniques based on Scanning probe microscopy, micro-Raman and fluorescence imaging at the diffraction limit tracking single mo lecules and exploiting super

resolution imaging have now reached a stage where they can provide fundamentally new insights and can be efficiently used to reveal the peculiarities of molecular composition of cell wall without the need for labeling. The development of the Tip-enhanced Raman spectroscopy (TERS) and microscopy open the new possibilities for topographic and chemical mapping of cell membranes with molecular resolution. The challenge now is to combine the information obtained using these different methods and on different cells to obtain a coherent view of the cell surface, what is a key importance problem for the future developments of bionanotechnology and nanomedicine. In the present work we discuss the scanning probe microscopy coupled with micro- and nano-Raman spectroscopy for cell-line individualization by fingerprinting of composition of subcellular components of wild-type and genetically modified cells. The utility of the approach is exemplified by the detection of a specific protein generated in the cell membrane of genetically modified *Saccharomyces cerevisiae* yeast cells. The yeast secretion plasmids pYEsec1-GDH and pYEsec1-GDHab, bearing *Acinetobacter calcoaceticus* glucose dehydrogenase gene *(gdh)* alone (GenBank accession number GC657400.1) or fused with human amyloid-β (αβ40) sequence (DAEFRHDS GYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV), were obtained by the insertion of a GDH and GDH:Aβ40 sequences into pYEsec1 plasmid [1] downstream the GAL-CYC1 promoter and in frame with Kluyveromyces lactis toxin signal sequence. For this purpose, GDH was PCR-amplified from bacterial plasmid pAI3-PT15 (Patent No. WO2004099399) and GDH:Aβ40 from pTAbEcoI by forward primer $GDHsec_{Fw}$ 5'- GCATGGATC CAATAAACATTTATTGGC-3' and reverse primer $GDHab_{Rw}$ 5'-AACACGGTCTCAGCGCTCTGAGCTTTATATG-3', cut by *BamHI* and *PstI* restriction enzymes and ligated to the receiving vector pYEsec1. General procedures for the construction and analysis of recombinant DNAs were performed according to [2].

The cell membrane of genetically modified yeast cells is enriched by GDH protein as revealed by tip-enhanced Raman spectra and AFM imaging. Comparative analysis of tip-enhanced Raman spectra of normal and modified cells allow us to detect biochemical changes in intracellular metabolism driven by genetic modification. Changes in Raman spectra bands (500-2000 cm^{-1}) distribution (cell fingerprints) of the modified cells were assigned preferably to proteins incorporated into membrane. It has to be noted, that micro-Raman was not able to show the differences between the two lines of cells. In contrast the TERS revealed a clear amide bands on the membrane surface. In the TERS spectrum the amide I band can be assigned to the band centred at above 1655-1667 cm^{-1} and the amide III band with weaker intensities was centred at 1245 cm^{-1} (Fig. **1**), indicating that random coils are the major secondary structures. Peak positions of amide I and amide III are different from the ones obtained by micro-Raman for the proteins inside the cell where the secondary structure composition of native glucose dehydrogenase was preferably presented by random coils and α-helixes.

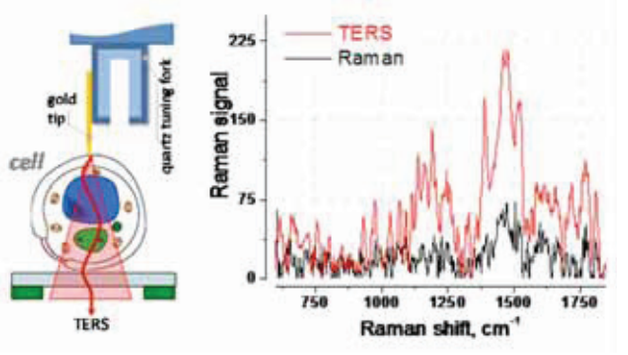


Fig. (1). Measurement configuration and Raman spectra (500–2000 cm^{-1} region) of genetically modified yeast cells.

Our results demonstrate the possibility to recognize the genetically induced topographic changes in yeast cell membranes by Atomic force microscopy and to provide the chemical mapping and fingerprint of the proteins enriched cell membrane by tip-enhanced Raman spectroscopy.

Keywords: tip-enhanced Raman spectroscopy, fingerprinting, yeast cells, genetic modification, nanobiotechnology.

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SL-48

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

GENE EXPRESSION PROFILINING UNDER ALUMINUM STRESS IN SOYBEAN

Khairy Mohamed Soliman

Natural Resources and Environmental Sciences, Alabama A&M University, 4900 Meridian Street North Normal, AL 35762, USA; E-mail: khairy.soliman@aamu.edu

Aluminum (Al) is the third- most abundant metal in the earth's crust. Some soil in Southern Mississippi Valley have 1g of Al per kg of soil at a depth of 60-75 cm. It is well documented that Al toxicity is the most important constraint of crop production on acid soils. Many commercial soybean cultivars and advanced breeding lines have been evaluated for Al tolerance. Al tolerance is quantitatively inherited trait in soybean making it difficult for genetic improvements. Further, there is a dearth of genetic diversity among improved cultivars and a wealth of discrepancies among screening methods. Understanding the molecular and genetic mechanisms of tolerance is crucial for developing efficient and effective breeding programs aimed at improving Al tolerance trait. Magnesium (Mg) is known to ameliorate Al toxicity in soybean. The objective of this study was to discover putative genes which are differentially regulated by Al and Mg in soybean using DNA microarrary technology. Two soybean genotypes Young (Al sensitive) and PI 416937 (Al tolerant) were used in the study. Soybean seedlings were exposed to zero (control) or 10 µM Al and in a separate experiment to 10 µM Al or 10 µM Al plus 50 µM Mg in growth chamber under hydroponic conditions for four time spans of 2 hrs, 12 hrs, 48 hrs and 72 hrs in the Al experiment and for two time spans of 12 hrs and 72 hrs in the Al plus Mg experiment in randomized complete block design with three replications. Microarray analysis was made on mRNA isolated from 1 cm long tap root tips using Affymetrix soybean array with 62,000 probe sets. Gene expression change was varied with genotype, time point and treatment. The results indicated that Al and Mg induced major transcriptional changes in many genes. Aluminum most up-regulated genes whereas Mg mostly down-regulated genes. The predicted protein products of the changed genes include oxidative stress proteins, toxin extrusion and sequestration proteins, pathogenesis related protein and transcription factors among others all of which are relevant to Al tolerance and toxicity. The putative genes identified herein are potential molecular signatures for screening soybean population for Al tolerance or creating transgenic soybean for Al tolerance.

Keyword: Heavy metals, barley, barrier function.

SL-98

Track: *Other Areas: Systems Biology*

DEALING WITH REALISTIC KINETIC MODELS: NEW DEVELOPMENTS IN GLOBAL OPTIMIZATION TECHNIQUES

Albert Sorribas, Carlos Pozo, Gonzalo Guillen-Gosalbez, Laureano Jimenez and Rui Alves

Departament de Ciències Mèdiques Bàsiques, IRBLLEIDA. Universitat de Lleida (Spain) Montserrat Roig 2, 25008-Spain; E-mail: albert.sorribas@cmb.udl.cat

Mathematical models and optimization tools have become central for successful metabolic engineering applications within biotechnological industry. However, the available tools for addressing complex models are limited. Our group has developed a series of new approaches that overcome much of the dif ficulties that one face when using general purpose tools. By capitalizing on a recasting technique, we transform a non-linear detailed kinetic model into a standard generalized mass action model (GMA). Using the resulting model, we can apply optimization tools that are extremely efficient for GMA models. We show the performance of this method on several examples and discuss the extension of these ideas to deal with multiobjective problems.

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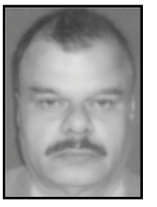
SL-93

Track: *Other Areas : Systems Biology*

IDENTIFICATION OF SNP MARKERS LINKED TO GENES CONTROLLING COCOON AND POST-COCOON TRAITS IN THE MULBERRY SILKWORM, *BO*

S. Sreekumar, S.K. Ashwath, A.K. Saha , B.B. Bindroo and Keiko Kadono-Okuda

Central Sericultural Research and Training Institute, Srirampura, Mysore-570 008, Karnataka, India; E-mail: sreecsrti@yahoo.co.in



Though India today is the second largest producer of silk in the world, the twin problems of low productivity and poor fibre quality continue to impair an increase in production. To overcome these drawbacks, new breeding strategies adopted in the early '90s have resulted in the production of a number of productive silkworm strains. However, these strains are popular only with those farmers who can provide adequate inputs and managerial skills. Even today, the bulk of the silk in India is produced by a polyvoltine × bivoltine cross strain and is not of the required International standard. As a result, India imports large quantities of silk from China. The available bivoltine strains that produce quality silk are not hardy. Hence the polyvoltine strains need to be improved by incorporating genes carrying the desired traits so that they produce high quality silk. Molecular markers associated with quantitative trait loci (QTL) are reported for many crops and many important traits. After linkage between QTL and a molecular marker is determined, the QTL can be transferred to a different genetic background by marker assisted selection. For the identification of DNA markers closely linked to a selected character, one of the strategies adopted to identify the few that are tightly linked to the target gene from a large number of markers is bulked segregant analysis (BSA).

Silkworm breeds viz., Pure Mysore (PM) and CSR2 which show contrasting features for cocoon traits were selected for the present study. F1 generation of PM x CSR2, F2 by selfing of F1 progeny, backcross progeny viz., (PM x CSR2) x PM and (PM x CSR2) x CSR2 were raised. The frequency distribution of the cocoon and shell weight in the F2 was analysed. The cocoon weight and shell weight data was sorted in the descending order and the top ten (high bulk) and the bottom ten (low bulk) samples were selected in the F2 as well as the BC progeny. In addition, the cocoons of high and low bulks of F2 and BC progeny were subjected for reeling analysis. The data on filament length, denier, size deviation and boil of loss was recorded after cold reeling and the DNA from the respective pupae was extracted and purified for screening by PCR amplification with SNP primers. Two hundred and forty EST SNP primers representing all the 28 linkage groups in silkworm were screened with genomic DNA of parents, PM, CSR2 and their F1 progeny. 48 primers showed polymorphism between the parents showing clear size difference in the amplified products and the co-dominant expression of these polymorphic bands were observed in the F1 where two bands were detected. A single SNP primer no. 04124 of LG 4 showed close linkage with the quantitative trait loci (QTL) controlling the cocoon traits. Likewise, the primer No. 09082 of LG 9 showed close linkage with the QTL controlling filament length character. We were able to identify a SNP primer no. 1205 of LG 12 closely linked to QTL controlling the denier trait.

The results of the present study has shown the prospects of using SNP markers in the silkworm breeding programme for improving cocoon traits in the low yielding polyvoltine breeds of India leading to the maximisation of silk yield and quantity.

SL-79

EFFICACY OF CERTAIN PLANT EXTRACTS AGAINST PULSE BEETLE *CALLOSOBRUCHUS CHINENSIS* L.

Meera Srivastava

Laboratory of Entomology, P.G. Department of Zoology, Govt. Dungar College, Bikaner-334 001, Rajasthan India

The pulse beetle *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) is one of the major pests infesting stored pulses. Plants and plant products are traditionally used by people in different parts of the world to control insect infestation as

they possess secondary metabolites, which can be an effective source of insecticides. This characteristic feature was made use of and certain plants were screened for their insecticidal efficacy against *Callosobruchus chinensis* L. by treating the pest insect with different plant extracts and recording its mortality. The plants included *Peganum harmala* (Family: Zygophyllaceae), *Trigonella foenum-graecum* (Family: Leguminosae), *S. surratense* (Family: Solanaceae). All the three plants are found in the desert region of Rajasthan, India.

The pulse beetle *C. chinensis* was raised on green gram *Vigna radiata* in incubators maintained at 28 $\pm$ 2^0C and 70% RH. The plant material was collected from Bikaner and its vicinity, cleaned and shade dried after separating different parts selected for the study. The plant derivatives were applied in three forms namely aqueous suspension, aqueous extract and ether extracts of 10, 5, 2.5 and 1% dose concentrations. For comparison normal and controls sets were also kept under observation. Specific number of adult male and female insects were released in muslin cloth covered beakers containing weighed green gram grains and treated with different dose concentrations (w/v). Each experimental set comprised of five replications. Observations were recorded after three days of treatment.

P. harmala was found to be most effective resulting in 80-90% adult mortality while some of the treatments of plant *Trigonella foenum-graecum* caused significantly ($p<0.01$) high mortality of 70% and above and many of the treatments of plant *S. surratense* also resulted in significantly ($p<0.01$) high mortality of up to 80 per cent of the pest insect. The present findings therefore suggest that the plants screened possess certain chemicals, which, result in the mortality of the pest insect and, therefore could be a potent source for checking the population build up by *C. chinensis*.

Keywords: Callosobruchus chinensis L. (Coleoptera: Bruchidae) , Trigonella foenum-graecum, Peganum harmala.

SL-37

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology, Clinical Research/clinical trials; bioethics; nanobiotechnology.*

EX-ANTE EVALUATION OF BIOTECHNOLOGY INNOVATIONS IN THE CASE OF FOLATE GM BIOFORTIFIED RINCE IN CHINA

Hans De Steur, Xavier Gellynck, Dominique Van Der Straeten and Willy Lambert

Ghent University, Faculty of Bioscience Engineering, Department of Agricultural Economics, Division Agri-Food Marketing and Chain Management, Coupure links 653, B-9000 Ghent, Belgium; E-mail: Hans.Desteur@UGent.be

Folate biofortification, i.e. enhancing the folate (vitamin B9) content of staple foods through transgenic breeding, could be a pro-poor, pro-rural, agriculture-based intervention to reduce the health burden of folate deficiency. While previous research already demonstrated the large potential to implement folate biofortified rice (FBR) in China, this study is the first to put such results into broader perspective.

First, the ex-ante health impact and cost-effectiveness of FBR in China are benchmarked against the currently existing studies on biofortified crops and target countries. Second, the costs to introduce FBR in China are compared with other folate interventions, i.e. folic acid fortification and supplementation.

The findings show that, based on a pessimistic and optimistic impact scenario, FBR is ranked, respectively, 15th and 20th out of 27 cost-effectiveness analyses. Moreover, nearly all biofortification strategies achieve the World Bank threshold of highly cost-effective interventions. The implementation costs to reduce folate deficiency in China lend further support to FBR ($31.6M), compared to a country-wide folic acid supplementation ($428.8M), a folic acid fortified wheat ($184M) and soy sauce program ($240M).

This study demonstrates the importance to incorporate ex-ante impact analyses into innovative biotechnology research and provides a benchmark tool to examine the potential of agriculture-based technologies.

SL-111

Track: *Medical Biotechnology*

HUMAN BONE MARROW AND WHARTON JELLY MESENCHYMAL STEM CELLS AND CARTILAGE ENGINEERING

J-F. Stoltz, C. Huselstein and J. Schiavi-Tritz

UTCT (Unity of Cell Therapy and Fabrics), Hospitals of Brabois, Street of the Morvan 54 500 Vandoeuvre Lès Nancy,UMR-CNRS 7561 - Faculty of Medicine, France; E-mail: jf.stoltz@chu-nancy.fr

Injuries of cartilage are one of the most challenging issues of musculoskeletal medicine due to the poor intrinsic ability of this tissue for repair.

Despite progress in orthopaedic surgery, cell-based surgical therapies such as autologous chondrocytes transplantation (ACT) have been used for cartilage repair for over a decade but this approach has shown mixed results. Moreover, the lack of efficient modalities of treatment for large chondrale defects has prompted researches on tissue engineering combining chondrogenics cells, scaffold materials and environmental factors like mechanical parameters.

This paper is focused on the main parameters in cartilage engineering and on the potential of mesenchymal stem cells (MSCs) of bone marrow or Wharton jelly as an alternative to cells derived from patient tissues in autologous transplantation.

The main parameters to construct functional cartilage are discussed: origin of stem cells, scaffold, bioreactive molecules, mechanical environment, grafting and safety problems.

In other respect a new strategy based on a stratified scaffold with cellular hydrogel suspension and polyelectrolyte multilayers, to repair whole cartilage is presented.

Keywords: Cartilage, Chondrocytes, Stem cells and Bioengineering.

SL-50

Track: *Medical Biotechnology*

NANOPEPTIDE BIOREGULATORS INDUCE REACTIVATION OF "AGED" HETEROCHROMATIN

Lezhava Teimuraz, J. Monaselidze and T. Jokhadze

Department of Genetics, Tbilisi State University, 380028 Chavchavadze 1, Tbilisi, Georgia;
E-mail: lezhavat@yahoo.com

Background & Aim of the study: Nanopeptide bioregulators (tetrapeptide Ala-Glu-Asp-Gly and Lys-Glu-Asp-Ala; and dipeptide -Lys-Glu) stimulates lowering for the risk of premature aging, increased average life span, has a antitumor activity and stimulates functioning of immune system and reparative processes. The effect of synthetic nanopeptide bioregulators on structural and facultative heterochromatin of cultivated lymphocytes have been studied.

Materials and Methods: The level of total heterochromatin (including of telomere) - identified by the method of differential scanning microcalorimetry; level of C-banded; Ag-positive NORs and association of acrocentric chromosome; unscheduled DNA synthesis and the frequency of sister chromatid exchanges (SCE) under the single, single and combined effect of nanopeptide bioregulators and CoCl2 have been studied in lymphocyte cultures from individuals at the age of 80 and over.

Results: The data obtained indicate that nanotetrapeptide Ala-Glu-Asp-Gly and Lys-Glu-Asp-Ala; and nanodipeptide - Lys-Glu:

1) Induce unrolling (deheterochromatinization) of total heterochromatin;

2) Activate synthetic processes, caused by reactivation of ribosomal genes as a result of deheterochromatinization (decondensation) of nucleolus organizer regions;

3) Release genes repressed by heterochromatinization of euchromatic regions forming facultative heterochromatin;

4) Nanotetrapeptides (Ala-Glu-Asp-Gly and Lys-Glu-Asp-Ala) induce deheterochromatinization (decondensation) of pericentromeric structural heterochromatin of the chromosomes 1 and 9. However, nanodipeptide (Lys-Glu) does not induce deheterochromatinization of pericentromeric structural heterochromatin;

5) Nanopeptide bioregulator (Lys-Glu-Asp-Ala) in combination with the heavy metal salt (CoCl2) induces heterochromatinization of telomeric heterochromatin in lymphocytes of old individuals.

Conclusions: These results indicate that peptide bioregulators nanotetrapeptide (Ala-Glu-Asp-Gly and Lys-Glu-Asp-Ala) and nanodipeptide (Lys-Glu) cause activation (deheterochromatinization) of heterochromatin in lymphocytes of old individuals.

Our data can be important for the revealing new information about the remodeling of constitutive and facultative heterochromatin induced the heavy metal and bioregulators in aging and aging pathology. Chromosome deheterochromatinization is an area where one should seek the ways for prolonging the lifespan.

Keywords: Ageing, Heterochromatin, Nanopeptide, Heavy metal, Telomere.

SL-16

Track: *Food; Marine; Bio-safety; Systems Biology; Bioethics*

SUSTAINABLE CHITIN AND CHITOSAN EXTRACTION AND THEIR APPLICATION IN THE FOOD INDUSTRY

Claudia Troeger and Keshavan Niranjan

Department of Food and Nutritional Sciences, University of Reading, Whiteknights, P.O. Box 226, Reading RG6 6AP, UK; E-mail: c.troeger@reading.ac.uk

Chitin, a natural biopolymer contained in invertebrates, and chitosan, its deacetylated form, can be used as pharmaceutical products, food additives, biodegradable polymer films, clarification and stabilising agents and edible multifunctional films. Another property of chitosan is its antimicrobial activity which has applications in the food and pharmaceutical industries.

Crustacean processing produces about 40% of shell waste. Since biodegradation of this waste is slow, accumulation of large quantities is a major concern in the seafood industry. The production of renewable products to minimise waste is a challenge for current research and development. Crustacean waste can be used as raw material for chitin production. Currently, chitin extraction and chitosan modification are done chemically which has a number of disadvantages such as use of toxic solvents which not only makes the product unsuitable for medical and food applications but also leads to expensive waste disposal problems. Biological extraction methods are cost effective and environmentally friendly. This presentation will give an insight in the current biological extraction methods, such as microbial fermentation and enzymatic reactions, and compare them to chemical extraction methods. Furthermore, possible applications of chitin-derived products, especially regarding antimicrobial activity, will be discussed.

Keywords: Chitin, chitosan, extraction, food packaging, antimicrobial.

SL-47

Track: *Other Areas: Food; Marine; Bio-safety; Systems Biology; Bioethics*

MUSHROOMS: A FUNCTIONAL FOOD AND SOURCE OF VALUABLE COMPONENTS

Senka Vidovic, Zoran Zekovic and Marija Radojkovic

Department of Biotechnology and Pharmaceutical Engineering, Faculty of Technology, University of Novi Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia; E-mail: senka.curcin@yahoo.com

From historical point of view, especially in China, Japan and Korea, mushro oms have been considerate as a high quality food and medicine. The use of mushrooms in many forms of immunity disorders have been recorded in Traditional Oriental Medicine. Also, the medicinal roles of mushrooms have been documented in Chinese Pharmacopoeia which describes the use of more than 100 species in treatment of many diseases.

Until recent, beside in some Asian countries, consumption and use of mushrooms in other world regions was on very low level. In the modern world, as knowledge on mushrooms is growing, the production and the use of mushrooms in everyday life is increasing. This is encouraged by scientific confirmation of positive medicinal effect of mushrooms and their products. From nutritional point of view mushrooms have been considerate as a food rich in proteins and carbon hydrates, with a low amount of fats and high amount of almost all essential amino. Mushrooms contain important micro and macro elements, and are especially valuable for high content of iron, calcium, phosphorus, magnesium, zinc and selenium.

Beside β glucans, pharmacologically active key compound responsible for immunomodulatory and anticancer activity, some others components present in mushroom, mushroom extracts and other products are of importance. These valuable compounds, among many, are nucleosides, antioxidant components, micro elements Zinc and Selenium, sterol compounds and others. To encourage and increase the use of different species from Balkan region, in wild mushrooms collected in Istra region in Croatia, some of these compounds have been investigated. According to that in some wild edible delicious mushrooms from *Boletus* species high content of selenium has been determined. In extract of *Lycoperdon saccatum* important amount of nucleoside adenoside has been found. Many of investigated species for example *Boletus, Clavaria, Armilaria* and *Daedaleopsis* posses high antioxidant activity. For some of mushroom extracts, such as *Macrolepiota procera* and *Collybia platyphylla*, it has been proven capability to increasing fluidity of erythrocytes membrane, so they may serve as a beneficial component of diet for patients suffering from hypertension. In China mushrooms are considerate as elixir of life. According to obtained results we could considerate them as a valuable functional food, a source of pharmacologically important compounds and as important factor of human diet.

Keywords: Mushrooms, functional food, zinc, selenium, adenoside.

SL-21

Track: *Others : Food ; Marine ; Bio-safety ; Systems Biology ; Bioethics*

PROTEIN FROM LACTIC ACID BACTERIAL AND EM-FERMENTED PRAWN WASTE FOR APPLICATIONS IN AQUACULTURE DIET

Zainoha Zakaria, Nor Masdiana Zulkeple, Nurzahwani Mohd Nor, Sallehhuddin Hamdan and Mohammad Suhaimee Abdul Manaf

Chemistry Department, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM, Johor, Malaysia; E-mail: zainoha@gmail.com

Waste products from shellfish processing industries which constitutes up to 55 perc ent of the waste are generally considered to be highly polluting, putrifiable waste products which have to be disposed of and a major concern to many producing countries. Undoubtedly, the crustacean waste (head, claws and shells), is an excellent source of many potentially valuable constituents, namely chitin, protein, pigments (astaxanthin) and flavours. While protein is an excellent source of animal feed ingredient, the chitin, a nitrogen-containing polysaccharide, is the most valuable, as it has many current and potential applications. The adoption of fermentation process to prawn waste to recover protein and chitin are being discussed. Lactic acid bacteria (LAB) and effective microorganism (EM) were used as starter cultures in the fermentation process of prawn waste and their performances for fermentation and for producing protein were compared. Trials were conducted at laboratory using 10 % (v/w) of inoculums (LAB and EM), 10% carbohydrate source and at different temperatures. Prawn waste were fermented for 72 hours using suitable bioreactor and their pH values, titratable acidity, glucose and protein content were monitored. Fermentations were also compared for long term storage of up to sixty days. Results showed that both fermentations using LAB and EM gave comparable successful fermentation results. Temperatures should a slight effect on the fermentation process where a higher temperature of 60^{o}C showed a slower decrease in the pH drop as compared to 37^{o}C and ambient temperature. Stability study conducted using both types of inoculums for a period of 60 days at ambient temperature showed stable pHs and no signs of spoilage. Long term storage is important for sustainability of a larger scale processes. Feeding trials conducted using Brackishwater species showed that protein from fermented prawn waste is suitable to partially replace fishmeal in aqauculture diet. Large scale projection will also be briefly discussed.

SL-26

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology*

STERILIZATION OF DIFFERENT PACKED MEAL BY ELECTROMAGNETIC INDUCTION

Nazanin Zand

Department of Food Industrial Engineering, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran; E-mail: n_zand2008@yahoo.com

The effect of high frequency electromagnetic induction (EMI) and combination of EMI with various condition of pre-heating for Sterilization of different packed cooked meal has been studied. All samples of meal (cooked meat, cooked chick) were filled in pouches then EMI sterilization which discharges square-wave pulses with variable voltage 1-20kV/cm and different frequency (2-4GHz, 4-6GHz, 6-8GHz, 8-10GHz) have been used in step one. The effect of EMI on Clostridium and Bacillus is not adequate because spore of these bacteria were practically resistant in electric field's, so pouches have been put in water bath chamber, different condition of pre heating (80 ^{o}C 5min, 80 ^{o}C 10min, 80 ^{o}C 15min, 85 ^{o}C 5min, 85 ^{o}C 10min, 85 ^{o}C 15min) have been combined with 8-10GHz. If cells bacteria of are cultivated at higher temperature, increasing tendency which can permanently keep fluidity viscosity of the cell membrane before electromagnetic field so EMI efficiency is increased. The populations of mesophile microorganisms depended on type of culture, type of treatment and type of meal. The death ratio of mesophilic microorganisms increases in cooked chick and cooked meat 13500-14200% more than cooked chick meal and cooked meat meal. Other hand chance of negative mesophilic microorganisms growth in every treatment compares with last treatment increased 46-54%. But in every conditions growth of thermophile microorganisms have not been reported.

Keywords: Electromagnetic induction (EMI), flexible pouch, mesophilic microorganisms, thermopile microorganisms, thermal processing, cooked chick, cooked chick meal, cooked meat, cooked meat meal.

SL-80

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

VALUE-ADDED CASSAVA FOR BIO-INDUSTRIAL DEVELOPMENT USING BIOTECHNOLOGICAL APPROACHES

Peng Zhang

Institute of Plant Physiology & Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 300 Fenglin Road, Shanghai 200032, China; E-mail: zhangpeng@sibs.ac.cn

Cassava (Manihot esculenta Crantz) accumulates a lot of starch in its storage roots that can be processed to food, feed, modified starches and bio-fuels. The high yield potential and robustness against unfavorable environmental conditions make cassava suitable crop for marginal lands, which will not highly compete with other food crops on arable lands. There are several key biological constraints in cassava for bio-industrial development. For example, the post-harvest physiological deterioration (PPD) of cassava storage roots is the biggest disadvantage during starch and bio-ethanol process world-wide; Different types of starches from cassava are also demanding by starch companies; Stable and high yield under different environmental conditions is also required. Conventional breeding efforts have attempted to address these constraints, but with limited success due to the nature of heterozygousity and inbreeding depression in cassava. New biotechnological tools can change this situation by offering various approaches to the challenges of cassava. These new technologies have the potential to make it much more productive, a better source of bio-industrial products, and profitable to grow. Here we present our recently research progresses, for example, on genetic transformation (Liu *et al.,* 2011), modification of starch biosynthesis (Zhao *et al.,* 2011), prolonged leaf life (Zhang *et al.,* 2010) and delayed PPD of cassava storage roots.

To further extend application potential of cassava starch, development of novel starches with different amylose/amylopectin ratio has been achieved by the down-regulation of granule bound starch synthase I (GBSSI) or branching enzymes (BE) expression in cassava. Amylose-free and high-amylose cassava will provide novel feedstock for industrial application. Another successful example of transgene-mediated cassava improvement is to prolong the leaf life in cassava. Cassava sheds its leaves during growth, especially within the tropical dry season. With the production of SAG12-IPT transgenic cassava, we have proved that senescence-inducible expression of isopentenyl transferase could extend leaf life, increase drought stress resistance and alter cytokinin metabolism in cassava. Recently, we have also

investigated the protein expression profiling during the PPD process and several key enzymes have been identified. Since PPD in cassava is associated with wound induced oxidative burst in its roots, over-expression of enzymes that regulate reactive oxygen species (ROS) is necessary. Transgenic cassava co-expressing ROS scavenging enzymes superoxide dismutase (MeCu/ZnSOD) and catalase (MeCAT1) or ascorbate peroxidase (MeAPX2) showed improved plant antioxidant defenses and delayed PPD occurrence.

Our ultimate objective is to promote cassava as the major feedstock for bio-industrial applications through the generation of novel germplasms by genetic engineering.

Keywords: Cassava, SAG12-IPT transgenic cassava, enzymes superoxide dismutase, catalase, ascorbate peroxidase.

SL-65

MOLECULAR CHARACTERIZATION OF ENDOPHYTIC *STREPTOMYCES KEBANGSAAN* SP. NOV. THAT PRODUCES PHENAZINE-1-CARBOXYLIC ACID

Nurul 'Izzah Mohd Sarmin, Annie Geok Yuan Tan, RuAngelie Edrada-Ebel, Jalifah Latip, Christopher M.M. Franco and Noraziah Mohamad Zin

Deparment of Biomedical Science, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur, Malaysia, E-mail: nora@medic.ukm.my

A spore-forming streptomycetes designated strain SUK12 was isolated from a Malaysian ethnomedicinal plant and its taxonomic position established using a polyphasic approach- indicates that it is a new species of the genus *Streptomyces*. Morphological and chemical characteristics of the strain were consistent with those of the genus *Streptomyces*. Analysis of the almost complete 16S rRNA gene sequence placed strain SUK12 in the genus *Streptomyces* where it formed a distinct phyletic line with recognized species of this genus. The strain exhibited highest sequence similarities to *Streptomyces chrestomyceticus* NRRL B-3310^T (98.3%), *Streptomyces olivaceoviridis* NBRC 13066^T (98.0%), *Streptomyces bungoensis* NBRC 15711^T (97.8%) and *Streptomyces capoamus* JCM 4734^T (97.8%). The G+C content of the genomic DNA was 74.35%. Chemotaxonomic data [MK-9(H_8) as major menaquinone; LL-DAP as component of cell wall peptidoglycan; $C_{12:0}$, $C_{14:0}$ and $C_{16:0}$ as the major fatty acids] supported the affiliation of strain SUK12^T to the genus *Sreptomyces*. The results of the phylogenetic analysis and phenotypic data derived from this and previous studies, allowed the genotypic and phenotypic differentiation of strain SUK12^T from the related *Streptomyces* species. This micro-organism produces phenazine-1-carboxylic acid known as Tubermycin B, an antibacterial agent. It is proposed, therefore, that strain SUK12^T be classified in the genus *Streptomyces* as *Streptomyces kebangsaan* sp. nov.

Keywords: *Streptomyces kebangsaans* sp. nov., ethnomedicinal plant, 16S rRNA, polyphasic taxonomy, Tubermycin B

POSTERS

PO-149

Track: *Plant and Environment*

GENETIC ANALYSIS OF GRAIN YIELD OF WHEAT UNDER DROUGHT STRESS CONDITION BY GENERATION MEAN ANALYSIS

S. Abbasi[1], G. Mohammadi-nejad[2], A. Baghizadeh[3], B. Nakhoda[4], G. Abedi[5]

Department of Plant Breeding, Kerman Graduate University of Technology, Iran;
E-mail:abbasi.shahrbanoo06@gmail.com

Increasing yield is the most important aim in any breeding program. In order to study the genetics control of yield of drought tolerance in bread wheat a cross was made between Carchia (tolerance) × Gaspard (sensitive) and F2, F3, F4 progenies as well as their parent were grown under field condition in dry environment , and using a Randomized Complete Block Design (RCBD) with two replications.. Generation mean analysis (GMA) was performed by scaling test which essays all generations simultaneously. result revealed four parametric model using weighted least square showed Additive– Additive model is effective model. This analysis indicated that gene effects including mean effect, additive, dominance, epistasis effect of additive × additive, wereimportant in controlling the trait.

Keywords: Generation Mean Analysis, drought tolerance, Wheat.

PO-10

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

ANTIBACTERIAL β-AMYRIN ISOLATED FROM *LAURENCIA MICROCLADIA*

Nevein Abdel-Raouf

Botany Department, Faculty of Science, Beni-Suef Unersity, Beni-Suef, Egypt; E-mail: neveingalal@yahoo.com

The present study aimed to isolate β-amyrin for first time from *Laurencia microcladia* distributed at Dahab Coast, Aqaba Gulf, Egypt. The successive extraction of the ethanolic extract of the alga showed that, the petroleum ether extract was the best extractive solvent which contains the isolated active compound. Based on IR, MS and ^{1}H-NMR analyses, the active principal is proposed to be triterpens having the empirical formula $C_{30}H_{50}O$ with a melting point range 191-194 °C. The importance of this study was to draw attention and study the economic importance of the marine algae that grow with different types on the Egyptian shores as it contains many types of antimicrobial effectiveness. β-amyrin which obtained in this study from *Laurencia microcladia* was known for its potent antibacterial activity and commonly used medically in many areas. These results provide evidence, to intensify the study on this *Laurencia microcladia* to be used as a source of β-amyrin.

Keywords: Marine algae, *Laurencia microcladia* , triterpens, β-amyrin, antibacterial activity.

PO-116

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology; Bioethics*

CYTOCHROME C OXIDASE SUBUNIT-1(COX1) GENE IN TILAPIA (*OREOCHROMIS NILOTICUS*): CLONING, IDENTIFICATION AND CHARACTERIZATION

Iman Mohamed Kamel Abumourad and P. Nie

National Research Centre, Veterinary Division, Hydrobiology Department, Cairo, Egypt;
Email: imankam_2@yahoo.com

The current study aimed to identify and examine gene expression of cytochrome c oxidase subunit 1 (ONCOX1) in Tilapia (Oreochromis niloticus) immunized by formalin-killed *Flavobacterium columnarae*. Suppressive subtractive hybridization (SSH) was utilized to construct a cDNA library and a semi-quantitative RT-PCR analysis used to examine *Oreochromis niloticus* cytochrome c oxidase subunit 1 (COX1) gene expression. COX1 cDNA is composed of 1139 bps with a 1107 bps open reading frame, the predicted gene product is 369 amino acids with molecular weight of 40.16 kDa. The amino acid sequence revealed high identity with subunit 1 from *Oreochromis mossambicus*. Compared to ß-actin,

the semi-quantitative RT-PCR revealed that ONCOX1 expressed in tissues of stimulated fish as up-regulated gene suggesting that this member of COX genes is probably involved in the general immune response against the pathogenic bacterium.

Keywords: Tilapia, cytochrome c oxidase, protein prediction, gene characterization, tissue expression.

PO-34

Track: *Industrial and Manufacturing*

EFFICIENT USE OF *GALEGA ORIENTALIS* LAM. AND GALEGA-GRASS BIOMASS FOR BIOGAS PRODUCTION

Aleksandrs Adamovics, Silvija Strikauska and Vilis Dubrovskis

Latvia University of Agriculture, Liela iela 2, Jelgava, LV-3001, Latvia; E-mail: aleksandrs.adamovics@llu.lv

Biogas can be used for heat and power generation or as a vehicle fuel and thus can help to fulfill Latvia's obligations to increase its share of renewable energy up to 40% by 2020. Usage of perennial forage plants, especially legumes, for biogas production can be an important alternativefor farmers, due to the unstable animal breeding market in Latvia.

Galega (*Galega orientalis* Lam.) is a fodder legume with a long productive lifetime of more than 30 years, having a high productivity and the capacity to fix atmospheric nitrogen in the range of 200-453 kg ha^{-1} [1, 2].

Swards of 5 galega-grass mixtures (three binary- and two multi-species) were developed on a stagnic luvisol without usage of commercial nitrogen fertilizer. Galega mixed swards with cocksfoot (*Dactylis glomerata* L.), perennial ryegrass (*Lolium perenne* L.) or meadow fescue (*Festuca pratensis* Huds.) increased dry organic matter yield by 2.5% - 8.5% compared to pure galega. Biogas yield from galega and cattle manure substrates was investigated in four laboratory scale digesters of 5 l volume operated in batch mode at a temperature of 38±1.0^{o}C.Data of the variance analysis showed that in the 3-year period of utilization, DM yield for galega-grasses mixed swards was reliably ($P < 0.05$) dependent on the sward botanical composition.ODM yield of pure galega was 12.16 Mg ha^{-1}, which was 1.44 Mg ha^{-1} or 0.68 Mg ha^{-1} lower than most successful two-component or three-component galega-grass mixtures, respectively. Average biogas yields strongly varied between digesters, dependingon galega to manure ratio in substrates. The lowest biogas and methane output was observed from cow manure, due to strong previous digestion of fodder organic matter by domestic animals. Average methane content in biogas from galega-cow manure substrates was 58%, and was acceptable for biogas usage for electricity cogeneration. Average biogas yield per unit of degraded organic matter (DOM) was 533 m3 $MgDOM^{-1}$ and average methane yield was 313 m^3 $MgDOM^{-1}$. These average values were used for estimation of biogas potential obtainable per unit of galega or galega-grass area, for long-term planning purposes, helped by equation (1). Technically possible biodegradation ratio of organic matter of 0.75 was presumed the same both for galega-grass mixtures or pure galega in calculations. Fodder galega significantly surpasses other forage legumes in terms of productive longevity, but investigated galega-grass mixed swards, despite their shorter productive longevity, have higher organic matter and biogas yields. Substrate of galega 75% and cow manure 25% in anaerobic fermentation process produced the highest biogas yield of 628 m^3 $MgDOM^{-1}$ with a methane content of 61.2% . Galega in pure stands or in mixtures with grasses is an acceptable plant for fodder or biogas production.

Keywords: biogas, cocksfoot, galega, meadow fescue, perennial ryegrass.

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PO-30

Track: *Plant and Environment*

AMENDMENT OF SOIL WITH AFRICAN MARIGOLD AND SUNN HEMP FOR THE MANAGEMENT OF MELOIDOGYNE INCOGNITA IN SELECTED LEGUMES

Ojo Kolawole Adekunle

Crop Production and Protection Obafemi Awolowo University, Ile-Ife, Nigeria; Email: kolaade2002@yahoo.co.uk

Field experiments were conducted in 2008 and 2009 in the tropical rainforest zone of Nigeria to investigate the effects of amendment of soil with seedlings of African marigold (Tagetes erecta) and sunn hemp (Crotalaria juncea) incorporated singly in plots on Meloidogyne incognita and yield of cowpea and soybean. The experimental field, which was naturally free of plant-parasitic nematodes, was inoculated with chopped roots of M. incognita race 2-infected Celosia argentea roots and planted to tomato to increase M. incognita population at the site.

Eight week-old marigold seedlings were incorporated in cowpea or soybean field and eight week-old sunn hemp seedlings were also incorporated in cowpea or soybean field. At the ends of the experiments, M. incognita population densities was significantly higher in control plots than those of the plots amended with marigold or sunn hemp with correspondingly higher grain yield in the amended plots in both cowpea and soybean fields in both years. A significantly higher population of the nematode and consequently, lower yield was associated with Ife Brown than Ife Bimpe cultivar of cowpea for each treatment whereas in soybean cultivars, the pattern was not definite. Also twelve seedlings of marigold or sunn hemp per plot incorporated into the soil produced significantly higher grain yield in Ife Brown cultivar of cowpea and TGX 1440 of soybean compared to six seedlings per plot. The results of this study suggest that incorporating marigold or sunn hemp in M. incognita-infected cowpea or soybean field has potentials to suppress M. incognita population and reduce nematode damage on yield of the associated leguminous crops.

PO-164

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

ISOLATION AND CHARACTERIZATION OF OIL DEGRADING AND BIOSURFACTANT PRODUCING BACTERIA FROM OIL CONTAMINATED SITE

Safia Ahmed, Muneer Ahmed, Zulfiqar Ali Malik and Abdul Hameed

Department of Microbiology, Quaid-i-Azam University, Islamabad, Pakistan; E-mail: safiamrl@yahoo.com

Crude oil is composed of thousands of different pollution producing health hazard hydrocarbon molecules, and soil indigenous bacteria have the ability to degrade and utilize them as carbon source. 15 different bacterial isolates of Fimkassar oil field Chakwal, Pakistan showed maximum growth with crude oil and other petroleum products like petrol, diesel, grease and Mobil oil. The crude oil degradation efficiency of the isolates was analyzed by gas chromatography. The GC analysis of the test samples showed a maximum removal of aliphatics (94.64%) and aromatics (93.75%). The lab scale study of crude oil bioremediation in soil by bacterial consortium showed maximum removal of oil components, i.e. C_{12} (90.7%), C_{17} (87.7%), C_{16} (83.8%), C_{11}, C_{24} and C_{28} (40-55%), remaining alkanes (20-30%), and pyrene (73.1%). In later experiments, biosurfactant producing ability of *Pseudomonas putida* SOL-10 and *Bacillus licheniformis* DW3 was checked and optimized. The biosurfactants produced by the isolates significantly reduced the surface tension of water upto 29.9 $m.N.m^{-1}$ and efficiently emulsified many hydrocarbons and oils including η-hexane, hexadecane, kerosene, xylene, glycerol, olive oil, corn oil, soyabean oil and used cooking oil. The bacterial isolates of the current study have a great potential for oil-related fields.

Keywords: biodegradation, crude oil, biosurfactants, gas chromatography.

PO-96

Track: *Plant and Environment*

STUDY OF SOME IRANIAN CUMINUM CYMINUM BASED ON MORPHOLOGICAL AND MOLECULAR MARKER

Hossein Rostami Ahmadvandi, Kianoosh Cheghamirza, Denial Kahrizi

Biotechnology for Drought Tolerance Department, Razi University, Kermanshah, Iran;
E-mail: h.rostami83@gmail.com

Cumin (cuminum cyminum) is native to Mediterranean regions, belongs to Apiaceae family that has grown by people of Egypt and India since ancient time. Diversity is bases for selection in plant breeding. Selection is required enough and by rising up genetic diversity in community, the choice widens. In this present study, genetic diversity among different collected cumin population were assessed based agronomic treats and molecular markers such as RAPD and ISSR. The farm and laboratory sections of this research were conducted in a field located 10 km west of Kermanshah, road of Sarab Niloofar and biotechnology laboratory, campus of agriculture and natural resources, Razi university of Kermanshah, Iran in 2011, respectively. The result extracted from analysis of variance and mean comparison showed significant differences between accessions regard to all treats. Correlation analysis between various treats showed that there was a significant correlation among plant high with other treats including number of miniumbel in umbel (0.344) and 1000 weight seed (0.555). Cluster analysis based on agronomic treats placed all accessions into three groups. In molecular section of this study, 25 ISSR primers (18 primers as a single and 4 of them as a double primer) and 13 RAPD primers were used for evaluation of polymorphism in the genome. Total polymorphism percentages for ISSR and RAPD were 67.32% and 55.16%, respectively. the accessions were grouped into 7 and 6 cluster by cluster analysis based on RAPD and ISSR markers, respectively. For the ISSR marker, Mashhad and Gorgan ecotypes were more similar based on jaccard similarity coefficient (0.946); this situation was correct with Boushehr and Gonabad accessions by (0.921) but based on RAPD markers. Finally, mantel correlation test between similarity matrix of molecular markers and this markers in comparison to agronomic treats were significant except RAPD markers.

Keywords: Multivariate statistical analysis, Genetic diversity, Morphologic and agronomic treat, RAPD, ISSR, Mantel.

PO-99

Track: *Plant and Environment*

RECOGNITION TESTING FOR A NOVEL BACTERIOCIN PRODUCED BY NATIVE SERRATIA MARCESCENS DGH1

Dariush Gholami, Saeed Aminzadeh, Nasrin Kazemipour, Seyed Mehdi Alavi and Zeinab Emruzi Tubkanlu

Department of Pharmacognosy, University of Lagos, Nigeria; E-mail: gbendedada@yahoo.com

In recent years, the approach of using innovative strategies such as probiotics or bacteriocins for the prevention or treatment of bacterial infections has come into focus. Bacteriocins are bacterial ribosomally synthesized, extracellular peptides or proteins with an antibacterial activity usually against bacteria closely related to the producer. Proteinaceous character of the bacteriocin produced by Serratia marcescence DGH1 was confirmed (its inhibitory activity was lost after its treatment with proteases), it was found to be non- stable after heating (75 0 C, 90 °C and 130 °C after 30 min). The bacteriocin activity of the cell free culture supernatant was analyzed at different pHs. The Data was shown Serratia marcescence DGH1 inhibitory component is a proteiaceous bacteriocin.

Keywords: Antibacterial activity, bacteriocin, probiotics, serratia marcescence DGH1.

PO-122

Track: *Others - Animal Production*

NEW TECHNIQUES FOR SEMEN COLLECTION AND ARTIFICIAL INSEMINATION IN QUAILS

Hazim Jabbar Al-Daraji

Department of Animal Resource, University of Baghdad, College of Agriculture, Abu-Ghraib, Baghdad, 9646, Iraq; E-mail: prof.hazimaldaraji@yahoo.com

This study was conducted in order to find appropriate and easy techniques to collect semen from the male and artificial insemination of females in quail birds, so one can use these techniques on a commercial scale in breeding these birds. After a series of experiments lasted for several years, we at the end found a new technique for collecting semen from the male bird quail, which are summarized by intercept the natural mating between male and female and before the completion of this process by using female just to stimulate the male, and before the arrival of male to the stage of ejaculation of semen, they remove from back of female and the semen was collected by pressing both sides of the male cloaca. Even reluctant males in mating with females, it was found convenient way to collect semen from these males which is summarized by but the male for a certain period on the back of the female for the purpose of encouraging them and then lifted up and make the process of collecting semen from them. Have also been finding appropriate and easy technique for artificially inseminate females which summarized by the creation of moderate pressure on the abdominal area of female for the purpose of the bringing out the vagina of female and when the vagina of female was get out the oviduct the syringe containing the semen that diluted 1: 1 with Lake diluent was entered at depth of 1 cm inside the vagina and then the pressure on the abdominal region was stopped to ensure the oviduct back to normal state before the deposition of semen inside the oviduct of female. In the present study also find a solution to the problem of the existence of an egg with solid shell which may be found inside the oviduct of female during a process of artificial insemination and represents one of the important problems facing the process of artificial insemination in birds. This procedure summarized by making a slight pressure behind the egg in the uterus and pushes it quietly and very gently to get out of the oviduct and then the syringe containing the semen is inserted inside the female's vagina and artificial insemination for this female would be completed.

It was also found in this study that rates of semen characteristics (whether in quantity or quality) is better than the rates for these traits that have been obtained by using conventional method (dorsal – abdominal massage method) to collect semen from males. It was also obtained by using these new techniques better rates of fertility, hatchability, and chicks livability than those obtained from the original flock of birds, which the birds used in our study randomly selected from this flock and the birds in this original flock of birds were naturally matted. However, by using these new techniques we can increase the number of females that can be inseminated with one male, so these techniques can be use on broad commercial scale because of the great positive results that obtained by using these new technique as regards significant improvement in semen quality and fertility and hatchability and chicks livability rates and significant decrease in the number of males that used for artificially inseminate the quail females. Furthermore, these positive results will increase the economic profit of breeder of birds.

Keywords: New techniques, semen collection, artificial insemination, quail.

PO-89

Track: *Other Areas: Clinical Research/Clinical Trials*

APPLICATION OF HORMONAL AND SINGLE MULTIPLEX PCR ASSAYS FOR DETECTION OF FREEMARTINISM IN A HORNED GOAT WITH 60XX/60XY CHIMERISM

Chalmeh Aliasghar, Pourjafar Mehrdad, Badiei Khalil, Sharifiyazdi Hassan, Naghib Mojtaba

Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran, Postal Code 71345, P.O. Box 1731, Shiraz, Iran; E-mail: achalmeh81@gmail.com

Freemartinism is a condition that occurs in twins of different sexes, where an imperfect masculinised sterile female twin is born with a male. In an adult 7-year-old horned, mixed Lori breed phenotypically doe, signs of masculinization were evident in both the behavioral patterns and body conformation, but there was no structure similar to penis and hypospadias was observed. External genitalia were similar to that of a female one. In this study, a single multiplex PCR

assay was used to determine the freemartinism status (XX-XY blood chimerism) based on semi-quantitatively co amplify a sex-based polymorphism in the amelogenin locus (AMX and AMY). In addition, serum concentrations of anti-Müllerian hormone (AMH), testosterone and progesterone of this horned intersex goat were evaluated. Serum AMH levels in the present case (0.2 ng/ml) were similar to male (0.2 ng/ml) and lower than female (0.6 ng/ml) goat. The serum concentrations of testosterone (0.2 ng/ml) were remarkably high in comparison to normal control male and female ones (0.02 ng/ml). Progesterone level in the intersex goat (0.4 ng/ml) was close to control buck (0.7 ng/ml) and remarkably lower than control doe (10 ng/ml). The results of the present study showed that the use of hormonal pattern, especially AMH level, and single multiplex PCR technique provide an easy and alternative approaches for indication of XX/XY chimerism or mosaicism in intersex freemartin goats.

Keywords: Freemartin. XX/XY Chimerism. Intersex. Horned goat. Single multiplex PCR. Anti-Müllerian hormone.

PO-165

Track: *Other areas: Food*

EFFECT OF DIFFERENT LEVELS OF SUPPLEMENTAL PROBIOTIC ON PERFORMANCE INDICES AND SERUM BIOMARKERS OF BROILER CHICKENS

T. Aluwong, M.A. Raji, B.F. Hassan, M.U. Kawu, P.I. Kobo and J.O. Ayo

Department of Veterinary Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria; E-mail: aluwong_tagang@yahoo.co.uk

The aim of this present study was to investigate the effect of supplemental probiotic preparation on performance indices and serum biomarkers of broiler chickens. This experiment was carried out on hybrid broiler chickens Marshall (n=200). Two hundred day-old chicks were randomly selected and distributed into four groups of 50 day-old chicks each, (Control, C; $E_1$0.5%, $E_2$1.5% and $E_3$2.0%, experimental groups). Birds were housed in an environmentally controlled poultry house with floor covered with wood shavings. The shavings were kept dry throughout the experimental period by replacing the spoiled litter as when due. The feeding lasted for 42days. Chicks were fed commercial broiler starter diet for the first 28 days of age followed by pelleted finisher diet from 29-42 days. Feed and water were provided *ad libitum*. Body weight (BW), feed conversion ratio (FCR), feed intake (FI) and mortality were recorded on weekly basis for comparative evaluation and interaction effects of all treatment groups. For body weight measurement, birds were weighed individually at weekly intervals and the body weights were recorded to calculate body weight gains. Feed conversion ratio (FCR) was calculated by the standard formula using total feed intake (g)/bird divided by total body weight gain (g) for each period. Feed intake (FI) was calculated as the difference between the amount of feed supplied to the birds and the amount of feed that remained at the end of each feeding period. To know the status of mortality, daily observations were made to record the occurrence of deaths in different experimental groups. On day 42, blood was collected from ten birds randomly selected from each experimental group. Blood was collected through the brachial vein and drained into polythene tubes. Serum was collected by, first, allowing the blood to clot, followed by centrifugation at 5,000 revolutions per minutes. Serum enzymes, albumin, total proteins and cholesterol were determined using Ecoline kits and automatic analyzer Microlab 300 (Merck®, Germany) and spectrophotometer Beckman Coulter DU 520 (Voigt Distribution Inc., USA). Chickens fed 2.0% probiotic had higher ($p<0.05$) body weight gain and a significant ($p<0.05$) feed conversion ratio. Serum albumin level was significantly increased in the experimental group with higher percentage of probiotic supplementation. Also, serum levels of alanine aminotransferase and alkaline phosphatase decreased in the same group. Cholesterol concentration and total proteins level in broiler chickens were found to decreased and increased with the values of 2.5308±1.56mmol/L and 2.46±0.18g/L respectively, in the experimental group with higher dose of probiotic supplement. In conclusion, supplementing broiler feeds with yeast probiotics may contribute to improvement of welfare, health status and performance of broiler chickens thereby providing good quality meat for human consumption. More research is warranted to be able to establish a reliable dose for yeast probiotics in order to enhance production efficiency of broiler chickens industries.

Keywords: Body weight, Feed conversion ratio, Blood biochemistry, Lipid profile, *Saccharomyces cerevisiae*

PO-59

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

CYANOBACTERIAL EXTRA-METABOLITES AGAINST SOME PATHOGENIC BACTERIA

Neveen Abdel-Raouf, Monera Al-Othman and Ibraheem B. M. Ibraheem

Botany and Microbiology Department, Faculty of Science and Medical Studies, Women Students Medical Studies and Sciences Sections, King Saud University for Girls, Riyadh, KSA; E-mail: malothman@ksu.edu.sa

Ten cyanobacterial species (Nostoc calcicola , N. commune, N. entophytum, N. minutum, N. palndosum, N. passerianum, N. punctiforme, Anabaena ambigua, A. amomala, and A. doliolum) were isolated from the mangrove region of Ras Mohammed (Sinai, Egypt) and have been tested for their allelopathic activity that of inhibitory and / or promoting effects against two Gram positive bacteria (Bacillus subtilis and Staphylococcus aureus) and two Gram negative bacteria (Escherichia coli and Pseudomonas aeruginosa). Data suggested two types of allelopathic effects: one type which always appeared in cyanobacterial medium as in the case with Nostoc minutum (medium that inhibits the growth of all tested bacterial species). The other type is induced only when Cyanobacteria are in contact with bacteria; this is the case when the growth of both Bacillus subtilis and Staphylococcus aureus were inhibited in co-culture with Nostoc commune. On the other hand, promotion effects of bacterial growth were observed when grown in cyanobacterial metabolites in most of studied cyanobacterial species. The biological assays for aqueous and methanolic extracts of the two Nostoc species revealed that both extracts for each species were not toxic at concentrations of 0.52 and 0.59 g L^{-1} water extract for Nostoc commune and N. minutum, respectively and 0.31 and 0.425 g L^{-1} for methanolic extract for Nostoc commune and N. minutum, respectively. No mortality was observed in tested mice within 72 hours.

Key words: Allelopathic activity, Cyanobacteria, Pathogenic Bacteria.

PO-92

Track: *Medical Biotechnology*

DEVELOPMENT OF MOLECULAR PROTOCOLS ON FOOD BORNE *VIBRIO PARAHAEMOLYTICUS* PATHOGENS STUDIED IN MALAYSIA

Saleh Mutahar Y. Al-Othrubi

International Medical School (IMS), Department Medical Microbiology, Management and Science University (MSU), Malaysia; E-mail: salehoth_2000@yahoo.com

Vibrio parahaemolyticus is a gram negative curved-rod bacterium that is widely distributed in the marine environment. This organism is frequently isolated from raw seafoods, particularly shellfish. Consumption of raw or undercooked seafood contaminated with *V. parahaemolyticus* may lead to development of acute gastroenteritis characterised by diarrhoea, headache, vomiting, nausea, and abdominal cramps. This pathogen is a common cause of food poisoning in many Asian countries, including China, Japan and Taiwan. This study was done to shed light on some molecular aspects of the pathogenic *V. parahaemolyticus* isolates found in seafood and seawaters of Malaysia. The bacterial isolates studied include 144 *V. parahaemolyticus* isolates collected from 2004 to 2007 plus 5 isolates as reference. All isolates were confirmed to be *V. parahaemolyticus* by culture on CHROMvibrio agar and biochemical tests (API 20NE). Antibiotic susceptibility of the isolates to a panel of antibiotics was determined using E-test. Polymerase chain reaction (PCR) was done to detect the *toxR* species-specific regulatory gene and *tlh* family-species gene, and the *tdh* and *trh* hemolysin genes; whilst the Enterobacterial repetitive intergenic consensus sequence (ERIC) PCR was performed to differentiate and to study the relatedness of the isolates and Pulsed-field gel electrophoresis (PFGE) was performed as well for epidemiological profile. PCR showed that the *toxR* and *tlh* genes were detected in all study isolates, and showed consistency with the API 20NE results, suggesting PCR as a potential diagnostic test for *V. parahaemolyticus*. The virulence gene *tdh*+ was negative in all the isolates except one strain isolated from shrimp, whereas the *trh*+ virulence gene was found in 8.5% shrimp and 10.7% cockle isolates respectively. The findings indicate a low prevalence of pandemic *tdh*+ *V. parahaemolyticus* (1/144; 0.69%) while a higher prevalence was found for *trh*+ strains (12/144; 8.3%). The PFGE of *Not*I restriction patterns revealed that shrimp isolates from Perak are very similar in their genetic origin, while isolates from cockles and sea water have a more diverse PFGE profile. In contrast, ERIC-PCR patterns produced by strains isolated from the three sources (Perak, Penang and Selangor) were very diverse, a distinct pattern for any particular cluster was not observed. As a conclusion, *toxR* and *tlh* species-specific PCR is a reliable molecular approach for rapid detection of *V. parahaemolyticus,* while PFGE remains the gold standard in determining the genetic

relatedness and epidemiology profile of the isolates. Most environmental isolates sampled in this study did not possess the virulence genes that are associated with acute gastroenteritis. This might be the reason behind the low incidence of *V. parahaemolyticus* associated seafood poisoning in Malaysia. The findings in this study indicate that the occurrence of pathogenic *V. parahaemolyticus* in seafood sell in the local retail market means the potential risk of *V. parahaemolyticus* outbreak or infection through seafood in Malaysia that should not be neglected.

PO-151

Track: *Industrial and Manufacturing*

BIO ETHANOL PRODUCTION FROM CORN-MASA-FLOUR-WASTEWATER

Aceves Diez Angel, Mateos Díaz Juan Carlos and Asaff Torres Ali

CIAD A.C., Km 0.6 carretera a la Victoria, 83000, Hermosillo, Sonora, México; E-mail: asaff@ciad.mx

In Mexico, EEUU and Mesoamerican countries, nixtamal and corn masa flour are made commercially by the traditional method of cooking whole corn kernels in a mixture of water and lime. The cooking liquor is then drained and the corn washed to remove lime and other solubilized materials. Wastewater from the process is high in biochemical oxygen demand (BOD) and total dissolved solids (TDS), resulting in a complex mixture that cannot be efficiently treated by conventional methods. We developed a novel and efficient enzymatic treatment that allows, in a first stage, separating completely total suspended solid (TSS) by decantation. In a second stage, the TSS-free wastewater is treated by ultra and nanofiltration obtaining, as permeate, water for reuse in the process and a concentrate, rich in fermentable material (around of 85% of the retained material). In a third stage, decanted TSS and the concentrated material are treated with glucoamylases and hemicellulases, obtaining around 20-25 g/L of glucose, 14-18 g/L of xylose and 10-14 g/L of arabinose. Finally sugars are transformed into ethanol using a *Kluyveromyces* sp. yeast strain, able to metabolize hexoses and pentoses, obtaining around 18-25 g/L of ethanol.

PO-4

Track: *Others - nanotechnology*

CHROMATOGRAPHIC FRACTIONATION AND ANALYSES OF CDS NANOPARTICLES SYNTHESED BY SCHIZOSACCHAROMYCES POMBE

N. Krumov, V. Gotcheva, C. Posten and A. Angelov

Department of Biotechnology, University of Food Technology, 26, Martiza, Blvd. 4000 Plovdiv Bulgaria;
E-mail: angelov@uft-bio.com

The fission yeast Schizosaccharomyces pombe were successfully cultivated in a fed-batch process at cadmium levels up to 100 mg l−1. S. pombe incorporated 20 mg Cd g−1 dry biomass within 24 h. The UV-vis signals of proteins of S. pombe after size exclusion chromatography (SEC) showed that the retention times were comparable to those of protein standards with a known molecular weight. This technique allowed fractionation of the sample and selection of the most promising fractions in a cross-check of the specific protein absorption and fluorescence emission spectra. SEC showed that Cd is associated to a protein fraction between 25 kDa (chymotrypsinogen) and 67 kDa (BSA) which corresponds to the theoretical molecular weight, based on Dameron's model of CdS nanoparticles of 35 kDa coated with phytochelatins. This fraction contained 80% of the cadmium in the sample. The obtained results give evidence that cadmium is associated with small peptides, probably phytochelatins.

Keywords: Yeast, nanoparticles.

PO-31

Track: *Industrial and Manufacturing*

PURIFICATION, CHARACTERIZATION AND APPLICATION OF POLYGALACYURONASE FROM *ASPERGILLUS NIGER* CSTRF

D.J. Arotupin, F.A. Akinyosoye and A.K. Onifade

Department of Microbiology, Federal University of Technology, PMB 704, Akure, Nigeria;
E-mail: daniel_juwon @yahoo.com

Polygalacturonase (PG) from *Aspergillus niger* CSTRF isolated fromcultivated soils was purified by ammonium sulphate precipitation and dialysed. The resulting fraction of the enzyme was further separated by molecular exclusion and ion exchange chromatography. The enzyme molecular weight was 79,430 daltons. Enzyme activity was markedly influenced by temperature, pH and substrate concentrations of the reactions with optimum PG activity of 35^0C, pH 4.0 and 8mg/ml respectively. The PG was heat stable over a broad range of range of temperatures. Line weaver-Burk plot for the apparent hydrolysis of pectin showed approximately 2.7mg/ml. The activity of PG was enhanced by Na^+, K^+, Ca^{2+}, Mg^{2+} and Zn^{2+}, while EDTA, $PbCl_2$, $HgCl_2$ and IAA were inhibitory. This purified enzyme brought about an increase in the flow of juice from pineapple must. The PG produced by *A. niger* in this study possess properties of applications particularly in fruit juice industries.

Keywords: Polygalacturonase, fractions, activity, optimum, enhanced, fruit juice.

PO-147

Track: *Plant and Environment*

SALINITY TOLERANCE EVALUATION OF DIFFERENT INBRED LINES OF WHEAT IN KERMAN-IRAN

M. Asadi[1], G. Mohammadi-Nejad[2], B.Nakhoda[3] and H.Naghavi[4]

Department of agronomy, Islamic Azad University – Jiroft branch, Jiroft, Iran; E-mail: Mohammadinejad@uk.ac.ir

Wheat is the first staple crop in the world and most important crop in rotation in the slain land of Kerman province. Salinity is one of the most important constraints in agricultural lands, since amelioration of saline soils is so expensive, achievement to genotypes with tolerant and high yield potential is essential. 378 promising lines of wheat as well as their parent Roshan and Superhead were grown in salinity and normal conditions based on lattice experiment with two replications at Kerman-Iran. In this study some traits such as total dry weight, shoot dry weight, root dry weight, plant height, 1000-grain weight and grain yield were measured. The results of combined analysis revealed significant difference among genotypes. Salinity stress decreased all the measured traits. Grain yield at normal condition showed significant correlation with total dry weight, shoot and root dry weight, plant height and 1000-grain weight, whereas it significant between grain yield and shoot dry weight, number of seed per ear in salinity condition. There was a significant interaction effect on shoot and root dry weight, 1000-grain weight and grain yield.

PO-83

Track: *Medical Biotechnology: stem cells; gene therapy; tissue engineering; biopharmaceutical manufacturing; cell based therapy; cell cultivation; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers*

EXPRESSION OF THE EXTRACELLULAR DOMAIN FOR THE LOW-AFFINITY SURFACE PROTEIN RECEPTORS FcγRIIa (CD32a) AND FcγRIIIa (CD16a) IN PICHIA PASTORIS

Dana Naeem Ashoor, Sonia Bourguiba-Hachemi and M. Dahmani Fathallah

Department of Biotechnology, Arabian Gulf University, Bahrain; E-mail: danana@agu.edu.bh

FcγRIIa (CD32) and FcγRIIIa (CD16) are low- affinity cell surface receptors for the Fc region of immunoglobulin G (IgG). They are part of a large group of receptors present on monocytes, macrophages, neutrophils, natural killer (NK) cells, T and B lymphocytes. They participate in diverse functions such as phagocytosis of immune complexes and modulation of antibody production by B cells. Studies showed that CD16 and CD32 are responsible for antibody-dependent cell-mediated cytotoxicity (ADCC) which has been the focus, nowadays, in developing cancer therapeutic monoclonal antibodies (mAb). In this paper we report on the production of a recombinant soluble form of the extracellular domain of CD16a and CD32a secreted by Pichia pastoris KM71H, a highly efficient successful system for the production of a wide variety of recombinant proteins. we cloned the sequence encoding the extracellular domain of CD16a and CD32a in the expression vector pPICZαA, downstream of the saccharomyces cerevisiae Alpha factor signal sequence and in phase with a c-myc epitope and an additional 6X Histidine (His) tag at the 3`-end. The engineered plasmid was introduced in

P. pastoris by electroporation and the recombinant clones selected on increasing concentrations of zeocin. 50 clones were selected and grown on different culture medias (BMGY, BMMY, MM and BMMY with glycerol) in shaking incubator (250 rpm) at 30°C. Protein expression was under the control of tightly regulated alcohol oxidase 1 (AOX1) promoter and induced by different concentrations of methanol. Supernatants were collected at different times after induction, and protein expression was evaluated by SDS-page and western blot. Anti-myc and anti-His antibodies were used to detect secreted protein on western blot. The highest expression level was obtained in presence of 1% methanol and 0.8% glycerol in the cultural medium BMMY after 96 h of induction. The secreted recombinant human CD16a and CD32a consists of 218 and 212 amino acids, with a predicted molecular mass of ~25 kDa and ~23 KDa respectively. SDS-PAGE, under reducing conditions, and western blot showed a molecular mass of ~25 KDa for CD32 and a ~45 KDa for CD16a. The difference in size in the case of CD16a can be explained by posttranslational modification (glycosylation). This data are the first data to report the expression of extracellular domain of human CD16a and CD32a receptors using P. pastoris expression system.

Keywords: Pichia pastoris, FcγRIIa (CD32a), FcγRIIIa (CD16a).

PO-166

Track: *Plant and Environmental*

EFFECT OF POURING SEWAGE WATER IN THE RED SEA COAST ON THE ACCUMULATION OF HEAVY METALS IN WATER, SOIL AND COASTAL PLANTS

H.S. Al-Zahrani and S.A. Zahrani

Department of Biology, King Abdulaziz University, KSA; E-mail: hmobarak2002@yahoo.com

The huge increase in the population of Jeddah Government caused an increase in water consumption, which caused a consequence increase in wastewater quantities discharged. Large amounts of these waters (treated & untreated) is discharged into the shores of the red sea daily cause harmful effects to environments and their biota. This research aims to clarify some of these negative effects of the wastewater on the red sea coast south of Jeddah City, in which the accumulation of different ions - especially the heavy elements - in the water, soil and plant tissues have been studied. Two locations were selected for study: the first one, which receives a huge daily influx of sewage water, is located near Al-Khumra treatment plant; while the other one (control) is about 50Km away (Near Al-shua'iba), in unpolluted area. Seawater, Soil and three plant species were chosen for analysis: Suaeda aegyptiaca, Zygophyllum album and Cyperus jeminicus. Some Nutrient elements and heavy metals were measured for all collected samples while soil and water samples were measured also for EC and pH. The results show that there were high concentrations of ions in the water, soil and plant samples in the polluted area, in comparison with the control.

Keywords: Pollution, heavy metals , sewage water, Halophytes, Ions, sea coast.

PO-148

Track: *Plant and Environment*

EVALUATION OF GENETIC PARAMETERS RELATED TO SALINITY TOLERANCE IN WHEAT BY GENERATION MEAN ANALYSIS

N. Babaahmadi[1], G. Mohammadi-Nejad[2], M. Khodarahmi[3], R. Abdolshahi[4] and B. Nakhoda[5]

[1] Plant breeding, Shahid Bahonar University of Kerman, P. O. Box, 76169-133-Iran;
Email: N.babaahmadi98@yahoo.com

In order to study the genetic control of salinity tolerance in bread wheat, different generation of Carchia (tolerance) × Gaspard (sensitive) containing F1, F2, F3 as well as their parent, were grown under field condition in salinity environment (EC of water and of soil respectively, 10 and 8.2 ds/m) at Kerman (N 30.15, E 56.58, MALS 1740) based on RCBD with tree replication. Result revealed three parametric model using weighted least square showed Additive–dominant model is non effective model, subsequently Scaling test showed significant D test, which it means there is a significant Additive × Additive epistatice effects. Also it showed there is a relative dominance towards the tolerant parent, finally based on joint scaling test it was clear that dominant by dominant is significant, and based on four parameters, goodness of fit was done and it significant which can be due to three allelic epistatic effect, there for it can be concluded that toward breeding program based on selection this epistatic effects showed be considerate.

Keywords: Generation Mean Analysis, Salinity tolerance, Wheat.

PO-29

Track: *Pharmaceutical Biotechnology*

BIOREMEDIATION OF CHROMIUM IN TANNERY EFFLUENT BY MICROBIAL CONSORTIUM

S. Saravana Babu and S. Paulsamy

Department of Botany and Plant Biotechnology, Chikkiah Naicker College, Erode, India;
E-mail: ssbabuflora@yahoo.com

Chromium is the most toxic and common among the heavy metal pollutants of industrial effluents particularly tannery. In the present work the chromium remediation ability of Bacillus subtilis, Pseudomonas aeruginosa and Saccharomyces cerevisiae in consortia and in their immobilized forms was studied and their efficiencies were compared. Flame Atomic Absorption Spectroscopy and diphenyl carbazide method was used to quantify chromium in the effluent. The chromium content of the effluent was around 770 mg/l before remediation, after which it reduced to 5.2 - 5.7 mg/l. The best activity was observed by S. cerevisiae - P. aeruginosa consortia, followed by immobilized beads of S. cerevisiae and S. cerevisiae - B. subtilis consortia.The results are discussed.

Keywords: Consortia, Bioremediation.

PO-120

Track: *Plant and Environment*

FIELD EVOLUTION OF SALINITY TOLERANCE BASED ON AGRONOMIC TRAITS IN BREAD WHEAT CULTIVARS

Mollaheydari Bafghi[1], G. Mohammadi-Nejad[2], A. Baghizadeh[3], S.Hajizadeh[4], M. T. Tabatabai[5] and B. Nakhoda[4]

Department of Plant Breeding. Kerman Graduate University of Technology, Kerman, Iran;
E-mail: S_molaheydari@yahoo.com

To study the effects of salinity stress on morphological traits of 12 bread wheat cultivars containing: Roshan, Sorkhtokhm, Sistan, Carchia, Mahuti, Bolani, Arta, Gaspard, Moghan-3, Arg, Shotordandan, Sholea which were most extreme cultivars, an experiment was conducted in salinity Research center of Yazd under Normal and saline condition in growing seasons of 2009-2010. Experimental design was randomized complete block with three replications. Electric conductivity in normal conditions for water and soil was 3.5 and 4.5 dS/m respectively) while it was 10.2 and 11.9 ds/m respectively in saline environment. In this study different traits including plant height, stem height, tiller number, spike number/m^2, spike length, spike weight, awn length, peduncle length, peduncle diameter, the weight of flag internodes, flag internodes' length, total seed weight,1000 seed weigh , economic yield, biological yield and harvest index were measured. Analysis of variance showed that salinity decreased all the measured traits except plant height and harvest Index. Combined Analysis showed highly significantly interaction effect just for 1000 seed weight and HI ($P<0.01$) whilst the genotypes showed significant difference in all the measured traits. According to correlation Analysis in stress condition, grain yield showed high relationship with plant height, peduncle length, flag leaf internodes length ($p<0.05$) and highly significant correlation with biological yield and HI ($P<0.01$). Bolani was distinguished as the high yielding genotypes in both condition and Mogh-3 and Arta were introduced as the most Iranian Salt sensitive genotypes.

Keywords: Field Evaluation, Salinity tolerance, Wheat.

PO-121

Track: *Plant and Environment*

GENETIC DIVERSITY ASSESSMENT OF BREAD WHEAT GENOTYPES USING SSR MARKERS

R. Mollaheydari Bafghi[1], G. Mohammadi-Nejad[*2], A. Baghizadeh[3], B. Nakhoda[4]

Department of Plant Breeding. Kerman Graduate University of Technology. , Kerman, Iran;
E-mail: S_molaheydari@yahoo.com

SSR Markers are effective tools for evaluation of genetic diversity. In this study genetic diversity of 20 Iranian extremes wheat *(Tritium aestivum L.)* genotypes was evaluated using 50 SSR markers. Subsequently polymorphism assessment was performed after scoring of obtained bands. A total of 631 alleles variants were detected for 50 SSR loci. The number of alleles per locus ranged from 4 to 19 and the allelic polymorphism information content (PIC) varied from 0.66 (Xgwm429) to 0.94 (Xgwm212).The highest number of polymorphic alleles was belonged to Xgwm212 with 19 alleles, while the lowest ones were belonged to xgwm429 with 4 alleles. dendrogram was constructed using Jaccard similarity coefficient and UPGMA algorithm by software NTSYSpc2.0.The result revealed genotypes were categorized in to six groups, tolerant genotypes such as Roshan, Sholea, Shotor-dandan and Bolani were arranged in the first group. While on the sixth group tolerant genotypes such as Mahuti were placed in same groups with sensitive ones such as Arta and Moghan-3. It showed the low correlation of phenotyping and molecular data, there for extensive genomics coverage must be applied to get a highest relationship of attributed to stress tolerant traits with controlling chromosome regions. Any way obtained result can be effective in germplasm management of wheat and other breeding aspects.

Keywords: Genetic diversity, Microsatellite Markers, wheat.

PO-108

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology; Bioethics*

BENEFICIAL EFFECTS OF A STRAIN OF *LACTOBACILLUS PARACASEI* SUBSP. *PARACASEI* IN *STAPHYLOCOCCUS AUREUS*-INDUCED INTESTINAL AND COLIC INJURY

Farida Bendali, Nassim Madia and Djamila Sadoun

Applied Microbiology Laboratory, Nature and Life Sciences Faculty, A. Mira University, Bejaia, Algeria; E-mail: kamelea03@hotmail.com

The goal of this work was the investigation of a Lactic Acid Bacterial strain anti-staphylococcal activity *in vitro* and *in vivo* and its effect on the intestinal histological damage caused by th e *S. aureus* infection. *Lactobacillus paracasei* subsp. *paracasei* was isolated in our laboratory from breastfed newborn faeces and identified phenotypically and genotypically. The strain was analysed for the production of bacteriocins by the spot on lawn and the well diffusion assays against five antibiotic-resistant *S. aureus* strains isolated from faeces of hospitalized patients with antibiotic-associated diarrhoea. The anti-staphylococcal activity of this strain was evaluated in fermented milk and *in vivo* using holoxenic rabbits. The strain was able to produce a bacteriocin-like substance active towards the staphylococcal strains. A reduction of 2 Log in *S. aureus* cells number was registered in co-culture with *L. paracasei* in fermented milk. Administration of skimmed milk containing *S. aureus* (10^7cells/ml) to healthy rabbits induced a persistent diarrhoeal state five days after the challenge. Dissection of the rabbits and the consequent histological observations showed damage and an atrophy of the intestinal and colic mucosa of the diarrhoeal rabbits, whereas an arrest of the diarrhoea concomitant to recovery of the intestinal villi and the colonic crypts was observed in the rabbits treated with *L. paracasei*-fermented milk. Furthermore, the diarrhoeal state persisted in spite of a decrease in the *S. aureus* cells level in the faeces of the rabbits receiving sterile milk contrary to the rabbits treated with *L. paracasei*-fermented milk, where the decrease in the *S. aureus* faecal number was associated with the arrest of the diarrhoea. *L. paracasei* could act as a potential barrier to prevent *S. aureus*-associated injury and might exert its effect on the staphylococcal enterotoxins or their sites.

Keywords: *L. paracasei* subsp. *paracasei*, *S. aureus*, antibiotic-associated diarrhoea, antibiotic resistance, bacteriocins, *in vivo* study.

PO-63

Track: *Medical Biotechnology: stem cells; gene therapy; tissue engineering; biopharmaceutical manufacturing; cell based therapy; cell cultivation; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers.*

DIAGNOSIS OF INVASIVE CANDIDIASIS BY HIGH-RESOLUTION MELTING ANALYSIS (HRMA) BY USING 65 KDA MANNOPROTEIN GENE PRIMERS

F. De Bernardis, S. Arancia, S. Graziani and S. Sandini

Department of Infectious Parasitic and Immune-Mediated Diseases , Istituto Superiore di Sanità, Rome, Italy; E-mail: flavia.debernardis@iss.it

Invasive candidiasis has increased significantly over the last years and remains an increasing source of morbidity and mortality in immune-compromised patients. Early diagnosis and initiation of antifungal therapy is essential for improving patients outcomes. Blood cultures are often negative or become positive too late.

Consequently, the application of Real Time-PCR technology represents the most recent advance in the diagnosis of fungal infections.

We developed and evaluated a High-Resolution DNA Melting Analysis (HRMA) for the detection of medically relevant Candida spp. using primers encoding a 65 KDa mannoprotein (MP65) and differentiating the various Candida spp. (C. albicans, C. glabrata, C. kefyr, C. parapsilosis and C. guilliermondii) and Saccharomyces spp. Melting curves from different amplicons can be differentiated on the basis of shape. We tested the sensitivity and the specificity of the HRMA method on DNAs extracted from simulated serum samples spiked with different strains of Candida species and successfully differentiated the Candida species and S. cerevisiae.

In conclusion, HRMA is an effective method to easily and rapidly identify different Candida spp. The risk of contamination is far lower than in a multi-step procedure. It is less expensive and consequently more readily adaptable to Candida detection in clinical laboratories.

Keywords: Candidiasis Diagnosis High -Resolution Melting Analysis (HRMA) technology.

PO-133

Track: *Other Areas: Food; Marine; Bio-safety; Systems Biology, Clinical Research/clinical trials; bioethics; nanobiotechnology*

PHYTOCHEMICAL MEDIATED SYNTHESIS OF SILVER AND GOLD NANOPARTICLES

Ya. Pirko, I. Danylenko, O.Kolomys, P. Smertenko, O. Lytvin, V. Strelchuk and Ya. B. Blume

Institute of Food Biotechnology and Genomics, Natl. Acad. of Sci. of Ukraine, Osipovskogo str., 2a, Kiev, 04123, Ukraine; E-mail: cellbio@cellbio.freenet.viaduk.net

The chemical and physical methods for nanoparticle synthesis are most developed now. However, chemical approaches for nanoparticle synthesis are not always spared from the necessity to use toxic substances and physical methods are often very expensive. Biological methods of nanoparticle synthesis (using microorganisms, plant cell and tissue culture or phytoextracts) were proposed as alternative and environmentally friedly approaches to existing chemical and physical methods. While microorganisms and fungi have long been used for synthesis of metal nanoparticles, the use of plants for similar purposes is relatively unexplored. In this research we developed synthesis of silver nanoparticles from solutions of $AgNO_3$ using phytoextracts of *Magnolia denudata* and *M. stellata*. Respectively, gold nanoparticles were synthesized from solutions of $NaAuCl_4$ using the phytoextracts from tea (mix of *Thea sinensis* var. *sinensis*+*Thea sinensis* var. *assamica*). Nanoparticles were purified from phytoextracts by centrifugation. The sloping peak absorption of the mixtures corresponded to absorption of silver nanoparticles (425-435 nm) and to absorption of gold nanoparticles (530-550 nm). Both types of nanoparticles obtained were studied by Raman and FTIR spectroscopy as well as AFM. The size of silver nanoparticles was identified as 25-30 nm, and gold nanoparticles as 4-10 nm.

PO-15

Track: *Pharmaceutical Biotechnology: biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering*

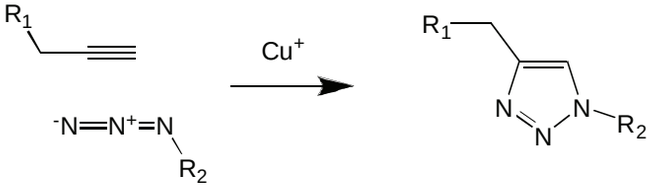
CLICK CHEMISTRY – A MODERN BIOCONJUGATION TECHNOLOGY APPLICABLE IN MANY DIFFERENT FIELDS

Thomas Bruckdorfer

Iris Biotech GmbH, Waldershofer Str. 49-51, D-95615 Marktredwitz, Germany;
E-mail: thomas.bruckdorfer@iris-biotech.de

Azido and Alkyne functions can cyclise by an intramolecularCu^{I}-catalyzedazide-alkyne 1,3-dipolar cycloaddition (CuAAC). This so-called Click Reaction, developed by K. Barry Sharpless and Morton Meldal, has meanwhile developed to a widely used type of reaction orthogonal to many

other types of reactions in different kind of applications. Both residues R_1 and R_2 can be used either as conjugation partner or as substrates. Due to its high thermodynamic driving force, usually greater than 20 kcal/mole, the click reaction normally proceeds rapidly to completion and also tends to be highly selective for a single product.



A variety of Azido and Alkyne Building Blocks is available, where some can be incorporated into biomolecules by recombinant syntheses, in particular by non natural protein translation using the amber-suppression-based orthogonal system or by chemical reactions, for example by solid phase synthesis. Then the conjugation with a second molecule carrying the appropriate other function can be done.

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PO-16

Track: *Pharmaceutical Biotechnology: biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering*

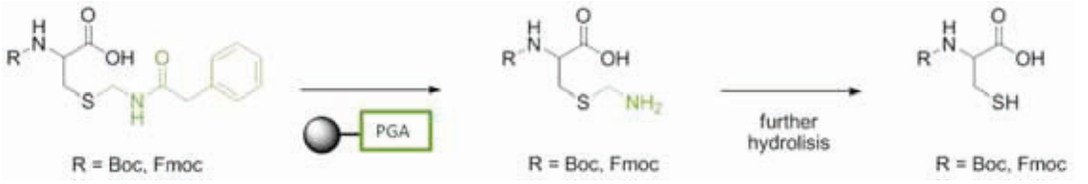
A GREEN STRATEGY FOR THE SYNTHESIS OF CYSTEINE-CONTAINING PEPTIDES

M. Góngora-Benítez, A. Basso, T. Bruckdorfer, J. Tulla-Puche and F. Albericio

Iris Biotech GmbH, Waldershofer Str. 49-51, D-95615 Marktredwitz, Germany;
E-mail: thomas.bruckdorfer@iris-biotech.de

Cysteine rich and disulfide bridged peptide fragments are found in nature in many enzymes, natural toxins and biologically active peptides. Their efficient and economic synthesis is a crucial factor for developing this class of biopharmaceuticals to robust and affordable drugs.

The appropriate choice of cysteine-protecting groups and its corresponding removal conditions is often crucial for the formation of disulfide bridges as a final stage in peptide synthesis. Phenylacetamidomethyl (Phacm) is a protecting group compatible with both Boc and Fmoc strategies, which can be removed in similar conditions than acetamidomethyl (Acm) and, in addition, by the action of the enzyme penicillin amidohydrolase (PGA). As PGA is selective to the phenylacetyl moiety, Phacm can be smoothly deblocked, while the resulting thioaminal is hydrolyzed to formaldehyde and free Cys. This green strategy is a promising alternative to conventional Acm protection, which will prevent undesired side products due to the concomitant Acm addition to the aromatic rings of Tyr and Trp residues. Moreover, it avoids the use of oxidizing agents which are not suitable for all sequences. Furthermore, Phacm group is orthogonal with the common cysteine-protecting groups and may be wisely used for the regioselective disulfide bridges formation.



The Phacm removal by an immobilized-PGA from E. Coli on amino acrylic resin and the subsequent disulfide bridge formation will be studied. The reliability and versatility of this strategy will be tested.

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PO-37

Track: *Industrial and Manufacturing*

SELECTION AND IDENTIFICATION OF TANNASE-PRODUCING FUNGI ISOLATED FROM BRAZILIAN CAVES

Patricia Gomes Cardoso, Alessandra Gonçalves de Melo, Patrícia Nirlane da Costa, Natália da Costa Maia, Ariela Betsy Thomas, Luís Roberto Batista and Rodrigo Lopes Ferreira

Department of Biology, Federal University of Lavras, 372000, Lavras, Minas Gerais, Brazil; E-mail: patricia@dbi.ufla.br

Tannase is an extracellular inducible enzyme of great biotech nological interest. It catalyzes the hydrolysis of ester and depside bonds in hydrolysable tannins, as tannic acid, releasing glucose and gallic acid. Tannase is extensively used in the preparation of instant tea, wine, beer, coffee, soft drinks and also as additive for detannification of food. The most important source for tannase production is microbial such as species from the Aspergillus and Penicillium genus. The objective of this study was to isolate, identify and select strains of filamentous fungi present in caves located in the Brazilian biome named Caatinga (Brazilian xeric shrubland) for tannase production. Five hundred and forty-four fungal isolates were isolated and three hundred eighty-six had the ability to grow in plates with tannic acid-containing medium the sole carbon source. A total of 48 strains were considered to be good tannase producers. Morphological characterization indicated 28 Aspergillus and 8 Penicillim species. Tannase activity was also measured in liquid culture medium (Czapek-Doxs agar modified) at 30oC for 5 days by submerged fermentation technique. The highest tannase activity were obtained by Aspergillus japonicus strain 246A (26,41 U.mg-1) and Aspergillus tamarii (20,76 U.mg-1).

Acknowledgement: Financial Support by CAPES and FAPEMIG.

Keywords: Fungi, Tannase, Caves.

PO-81

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

SCENARIO CONSTRUCTION FOR TRANSGENIC AGRICULTURE

D.D. Carvalho, L. Pessôa and N. Pereira Jr.

Cidade Universitária, Centro de Tecnologia, Bloco E, Ilha do Fundão - CEP: 21949-900 – Rio de Janeiro, RJ, Brasil; E-mail: denize@eq.ufrj.br

Despite the rapid diffusion of transgenic crops, there are still few environmental impact studies capable of supplying a conclusive scientific response in regard to its technical and economic advantages and disadvantages.Prospective scenarios were developed based on the evaluation of environmental indicators, using techniques emerging from the SWOT analysis (Strength, Weakness, Opportunity, Threaten), and the relationships between indicators from the model DPSIR (Driving force - human activity, Pressure, State, Impact, Response). Due to the high value of agro-business to the

global economy it is crucial to have a more specific means of analyses. The advantages and disadvantages of genetically modified technology applied to agriculture were classified as pressure indicators, making the internal dimension of the issue at hand, and the benefits and risks of the use of transgenic agriculture were classified as indicators of impact, making the external dimension. We identified control measures and environmental management (response indicators) through the relationships between indicators of pressure and impact of the use of transgenic agriculture, in order to integrate aspects of the transgenic biotechnology, biodiversity, biosafety and intellectual property. The application of the model DPSIR showed that it is a tool for integrated environmental assessment.

PO-93

Track: *Medical Biotechnology*

THREE YEAR STUDY OF A PRIVATE CORD BLOOD AND MESENCHYMAL CORD- BANK CRYOBANKS OF IASO MATERNITY PEDIATRICS RESEARCH AND GENERAL HOSPITAL- THE GREEK EXPERIENCE

Eirini Mitrou, Konstantinos Ntallis, Nikos Panagiotopoulos, Emmanouil Bougioukas, Vaso Kalodimou, Ekatherina Charvalos

Central Labs , IASO Hospital, Kifissias 37-39, Athens Greece 151 23; E-mail: directorcentralabs@iaso.gr

CRYOBANKS is one of the most reputated stem cell bank of Greece. Historically, Cryobanks International Services of Athens-IASO is the 1st stem cells bank kicked off in 2005 and the 1st accredited bank from AABB (American Association of Blood Banks) in Greece. CRYOBANKS is divided in two units, namely, the laboratory and storage unit (cord blood and cord mesenchymal bank) and the regenerative, research and flow-cytometry unit. Two major projects are under consideration /accomplishment, the adipose tissue development technique for esthetic surgery gynecology-oncology purposes and the development of flow-cytometry for purposes other than for testing of cord blood bank products.

The stored 22.000 cord blood units as well as the 7000 umbilical cord- mesenchymal stem cells units translate into years of experience in the field of cellular therapy products and a frame of continuing effort for excellence. We briefly report here, a study of CRYOBANKS cord blood collection and storage during a period of three year 2008-2010. The whole procedures have been accomplished according to AABB Standards for Cellular Therapy Products (4th Edition) and taking in consideration regional ethical and cultural issues. Most of the samples have been collected at IASO from deliveries or ceasareans. From a total number of 11482 units collected, 11111 have been processed by the AABB approved protocol. Five hundred forty six were with low volume and from those 364 have been discarded for this reason. Twenty seven samples have been discarded because of client's request.

From a number of 4368 of units processed in 2008, 3644 in 2009 and 3099 in 2010, 0.69%, 0.93% and 1.32% cultured specimen respectively were positive for the presence of bacterial /fungal pathogens after inoculation and culture by the BACT T alert system. Ceasarean collections mostly were free of contaminants. By routine culture procedures we identified (descending order), Enterobacteria, *Staph aureus, Enterococci, Bacilli, Diptheroides, Streptococcus spp, Pseudomonas sp and Candida sp.* Antibiotic resistance profiles have been identified by using the Vitek 2 system (Biomérieux, France). The strains in all the cases showed usual antibiotic sensitivities. The above mentioned contamination rates, kind of bacteria and antibiotic resistances are negligible, confirming thus the capacities of our premises.

Cord stem cell banking started in October 2010 using a classical cell extraction protocol, but with major improvements (results presented at the 1st International conference on Stem cell research, Turkey, 28- September- 2 October 2011). Private vs public banks, new products for cellular therapy are among our priorities. We would like to open the dialog with private banks in the Mediterranean and Middle East region to create bridges, exchange ideas and promote our capacities.

PO-66

Track: *Industrial and Manufacturing: bio-fuels; energy crops (cellulosic ethanol industry); Industrial Enzymes; Bioprocess Engineering and Optimization*

PROCESS OPTIMIZATION FOR ENHANCED BIOGAS PRODUCTION FROM RICE STRAW

Gopinathan Chengalath and Tayi Karthikey

Department of Biotechnology, University of Calicut, Tenjhipalam.P.O-673635, Malappuram District, Kerala, India; E-mail:shinuu@rediffmail.com

Lignocelluloses are often a major or sometimes the sole components of different waste streams from various industries, forestry, agriculture and municipalities. Hydrolysis of these materials is the first step for either digestion to biogas (methane). However, enzymatic hydrolysis of lignocelluloses with no pretreatment is usually not so effective because of high stability of the materials to enzymatic or bacterial attacks. Rice straw is available in huge quantities, especially in tropical countries, which are at present not properly utilized.

Pretreatment of Rice straw exposes the cellulose making it accessible to cellulase enzyme.Both chemical and biological pretreatments are possible.

Exposing rice straw to 1 % alkali(sodium hydroxide) and mixing with urea enhances biogas production.

Similarly growing pleurotus florida fungi in the presence of urea, lactose and cupric chloride improves biogas production from rice sraw. Urea is used to optimize the carbon :nitrogen ratio of the substrate. Lactose and cupric chloride enhances secretion of laccase enzyme, which delignifies rice straw, without sodium hydroxide.

Keywords: Ligno cellulose, pretreatment, cupric chloride, urea, laccase, delignification

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PO-41

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

Application of Plant Endophytic Bacterium, *Burkholderia cenocepacia* 869T2, on Biocontrol of Fusarium Wilt and Plant Growth Promotion *in planta*

Hsing-Mei Chiang, Ying-Ning Ho and Chieh-Chen Huang

Department of Life Sciences, National Chung Hsing University, Taichung, Taiwan;
E-mail: cchuang@dragon.nchu.edu.tw

Fusarium wilt (Panama disease) of bananas caused by *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4) is a soilborne vascular disease. It cannot be controlled by chemical pesticide and traditional biocontrol agents. Endophytic bacteria are bacteria living intercellular in plants but won't be harmful to plants obviously. Recentrly, endophytic bacteria are widely studied on biocontrol and plant growth promotion. Therefore, in this study, we tried to use endophytic bacteria inoculating on banana tissue culture for biocontrol of Fusarium wilt *in planta*. Previously, we isolated the endophytic bacterium, *Burkholderia cenocepacia* 869T2, from roots of vetivers. *B. cenocepacia* 869T2 inhibited the growth of Foc TR4 100% on media for at least 10 days. After inoculating on bananas (*Musa sapientum* cv. Cavendish, Pei-chiao, AAA), *B. cenocepacia* 869T2 decreased the disease incidence of Fusarium wilt and promoted the growth of bananas effectively both in green houses and fields. The height, girth, and healthy leaves of inoculated bananas were increased significantly about 20-40%. We also found *B. cenocepacia* 869T2 could produce the plant growth hormone, indole-3-acetic acid

(IAA). Results suggested that endophytic bacterium, *B. cenocepacia* 869T2, had good potential for biocontrol of Fusarium wilt and plant growth promotion of bananas.

Keywords: Banana, endophytic bacterium, *Burkholderia cenocepacia* 869T2, biocontrol, Fusarium wilt, plant growth promotion, IAA.

PO-98

Track: *Industrial and Manufacturing*

OPTIMAL CONDITIONS OF BOTRYOCOCCUS BRAUNII CULTIVATION TO PRODUCE LIPIDS, CARBOHYDRATES AND PROTEINS

Belkacem Guenachi[1], Amall Ezzanega[1], and Chang Won Choi[1,2*]

Department of Biology & Medicinal Science, Pai Chai University, Daejeon 302-735, Korea;
E-mail: choi5617@leading.or.kr

Biodiesel is an alternative form of petroleum-based diesel fuel, which can be derived from a unicellular green colonial alga Botryococcus braunii that is characterized by the ability to produce and accumulate large amounts of lipids in its cells. Therefore, we investigated the effects of pH, organic and inorganic carbon sources (including glucose, citric acids and sodium carbonate) and nitrogen sources (sodium nitrate and potassium nitrate) on the B. braunii cell growth and its biochemical composition (lipids, carbohydrates and proteins). To do this, B. braunii was cultured in BG11 medium under different pH values. The highest value of proliferous rate (K=0.130±0.002) and the fastest value of average generation time (G=2.31±0.04) were marked at pH 7.5. However, no significant difference found in lipids production among different pH values. A basal medium, BG11, was modified into sixteen new media (from MM1 to MM16) by adding different sources of carbon and nitrogen. Among the modified media, MM7 medium containing sodium carbonate (0.01 g/l) was the best medium in the cell growth showing the highest (K=0.150±0.003) and the fastest (G=2.01±0.04) values. When the concentration of sodium carbonate (2 g/l) in MM9 medium increased 10 times more than that in MM7 medium, the algal growth in MM9 medium was lower (K=0.119±0.000) and slower (G=2.56±0.20) than that in MM7 medium. Among the modified media, however, the highest content of lipids (48.27±1.09), carbohydrates (15.29±0.03) and proteins (27.20±0.24) was obtained, suggesting that the cell growth is not proportional to the biochemical composition of B. braunii cells.

Keywords: Botryococcus braunii, cell growth, biochemical composition, proliferous rate; Average generation time.

PO-107

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

EFFECTS OF PIPE MATERIALS AND WATER VELOCITIES ON THE BIOFILM FORMATION IN A SIMULATED DRINKING WATER DISTRIBUTION SYSTEM

Sung-Chan Choi, Ji-Hye Seo, Jung-Chul Suh, Hun-Joo Cha and Yeong-Kwan Kim

Department of Environmental Science and Biotechnology, Hallym University, Chuncheon, Gangwon 200-702, South Korea; E-mail: scchoi@hallym.ac.kr

The aim of this study was to compare the effects of water flow rates on com munity level physiological profiles(CLPP) of the biofilm and to examine the effects of pipe materials on the biofilm formation in simulated water distribution system. Two simulated distribution systems fed with continuous tap water were equipped witha number of coupons made of stainless steel (STS) 304, polyvinyl chloride (PVC), and galvanized steel (GS) were operated to induce biofilm development. Each system was operated at a flow rate of 2 cm/sec and 8 cm/sec, respectively. Heterotrophic plate counts in the biofilm formed for 90 days of operation on the surfaces of PVC, STS, and GS at 2 cm/sec was 3.01×10^4 CFU/cm^2, 3.80×10^4 CFU/cm^2, 6.56×10^3 CFU/cm^2, respectively. BIOLOG test demonstrated that the functional potential of communities with respect to the utilization of each of 95 carbon sources was significantly higher, especially on STS, at the flow rate of 8 cm/sec than at 2 cm/sec (P<0.01). Similar result was obtained by the principal component analysis based on the sole carbon substrate utilization pattern of the biofilm-forming microorganisms on STS surfaces. The CLPP analysis performed for investigating microbial community structure of the biofilm revealed a less metabolic diversity on

GS compared to that of PVC and STS at the flow rate of 8 cm/sec.[This subject is supported by Korea Ministry of Environment as "Projects for Developing Eco-Innovation Technologies (GT-11-G-02-001-3)"].

PO-21

Track: *Pharmaceutical Biotechnology: biopharmaceuticals discovery (cancer)*

PRODUCTION OF ANTIMICROBIAL BIOSURFACTANTS BY PSEUDOMONAS AERUGINOSA AND BACILLUS SUBTILIS

Kim G. Clarke, Francis Ballot and Keenan Bence

Department of Process Engineering, University of Stellenbosch, Private Bag X1, Stellenbosch 7602, South Africa; E-mail: kclarke@sun.ac.za

Biosurfactants, especially rhamnolipids and lipoproteins, have enormous potential for antimicrobial activity against human and plant pathogens. These are biologically produced molecules with surface tension lowering properties which facilitate lysis by disrupting cell membranes. The non-specificity of this mechanism further suggests functionality against antibiotic resistant pathogens.

Rhamnolipids and lipoproteins were produced by *Pseudomonas aeruginosa* ATCC 9027 and *Bacillus subtilis* ATCC 21332 respectively in a laboratory bioreactor. Maximum concentrations of 4.3 g rhamnolipid /L and 1.7 g surfactin /L were attained during batch culture. Rhamnolipids were accumulated during nutrient limited growth while the accumulation of the lipoprotein, surfactin, occurred under conditions of nutrient excess. Nutrient feeding to extend surfactin production facilitated a 50% increase in the total amount of surfactin in the first 3h at a dilution rate (D) =0.4 h^{-1}. This rate of increase was 29 to 34-fold higher than at D=0.05–0.15 h^{-1}, suggesting fed batch operation with a high D as a preferred process strategy for enhanced surfactin production.

Antimicrobial activity of the *P. aeruginosa* and *B. subtilis* culture supernatants were confirmed against *Mycobacterium aurum*. Since *M. aurum* is a surrogate for *M. tuberculosis*, the causal agent of most forms of tuberculosis, this suggests effective use as disinfectants in the health industries, especially as the ability of biosurfactants to adhere to interfaces reduces pathenogenic biofilm formation. Studies are currently directed towards application of *B. subtilis* lipopeptides to target crop spoilage organisms in the agricultural industry.

Keywords: biosurfactant, antimicrobial, rhamnolipid, surfactin.

PO-22

Track: *Industrial and Manufacturing*

ACID HYDROLYSIS VERSUS ENZYMATIC HYDROLYSIS FOR ETHANOL PRODUCTION FROM LIGNOCELLULOSIC WASTES

Pratima Khadoo, Romeela Mohee and Kim G. Clarke

Department of Process Engineering, University of Stellenbosch, Private Bag X1, Stellenbosch 7602, South Africa; E-mail: kclarke@sun.ac.za

Alcohol manufacture from biomass is attracting attention globally in view of its use as an alternative source to petrol or in blends with petrol. To explore low-cost feedstock for ethanol production and seek environmental-friendly alternatives in waste disposal, acidic and enzymatic hydrolysis of cellulose from five types of wastes most easily and readily available in Mauritius were studied: peels of cane stalk (PCS), cane tops and leaves (CTL), elephant grass (EG), coconut husk (CH) and acacia leaves and twigs. The optimum conditions for maximum fermentable sugars from each feedstock were determined for each hydrolysis. A comparison in the yields between acidic hydrolysis and enzymatic hydrolysis was also performed.

Concentrated sulphuric acid hydrolysis gave a maximum ethanol yield (in liter per ton) of 65 for EG, 63 for CTL, 52 for PCS, 45 for acacia and 35 for CH, all hydrolyzed at 25^0C with 60% acid. With dilute sulphuric acid hydrolysis, maximum ethanol yields (in liter per ton) of 145 for CTL (121^0C, 4% acid, 2 hours), 118 for PCS (100^0C, 2% acid, 1 hour), 104 for EG (121^0C, 4% acid, 2 hours), 87 for acacia (100^0C, 2% acid, 3 hours) and 82 for CH (121^0C, 6% acid, 5 hours), were obtained.

Enzymatic hydrolysis was carried out with ACCELERASE TM 1000 cellulase enzyme at 50^0C and pH 5.0. PCS required an optimum enzyme loading (in ml per gram dry biomass) of 0.10 for a hydrolysis period of 48 hours, CTL and EG a loading of 0.20 for a hydrolysis period of 72 hours, CH a loading of 0.25 for a hydrolysis period of 96 hours and acacia a loading of 0.15 for a hydrolysis period of 72 hours. Maximum ethanol yields (in liter per ton) of 361, 259, 228, 208 and 196 were obtained from PCS, CTL, EG, acacia and CH respectively.

To attain the maximum ethanol yields for the three hydrolysis technologies, pH 5 and 72 hours fermentation were required for CTL and EG, pH 6 and 72 hours fermentation for acacia, pH 6 and 24 hours fermentation for PCS and pH 4 and 72 hours fermentation for CH.

PO-126

Track: *Other Areas: Food Science*

BIOLOGICALLY ACTIVE PEPTIDES DERIVED BY HYDROLYTIC DEGRADATION OF CASEIN WITH PLANT PROTEASES

Dąbrowska A., Szołtysik M., Babij K., Pokora M., Niedbalska J., Chrzanowska J.

Wrocław University of Environmental and Life Sciences, Department of Animal Products Technology and Quality Management. Chełmońskiego Str. 37/41, 51-630 Wrocław, Poland; Email: anna.dabrowska@up.wroc.pl

The aim of the study was the determination of biological activities of peptides obtained after proteolytic degradation of casein with the use of noncommercial enzyme - serine protease isolated from Asian pumpkin *(Cucurbita ficifolia).*

The hydrolysis, with the use of three doses of enzyme 50, 150 and 300U/ml, was conducted for 24 hours in 37^oC on 1% isoelectric casein as substrate. The analyzed samples, taken after 0, 0.5, 1, 3, 5 and 24h of hydrolysis, were qualiativaly and quantitivaly analyzed to determine the level of casein degradation (DH-degree of hydrolysis). The obtained peptide fractions were separated on the RP HPLC. The biological activities were determined with the use of three different tests.

Antimicrobial activity against three different pathogen species: *E. coli, B. cereus* i *P. fluorescens* was performed by the standard plate test. The antioxidant activity in obtained hydrolyzates was determined by the free radicals DPPH scavenging activity, reduction of Fe(III) and chelating Fe (III) activities.

It was showed that the hydrolysis of 1% isoelectric casein conducted by the serine protease from Asian pumpkin caused deep and time dependent degradation of casein. In all analyzed samples antimicrobial or antioxidative activities were detected. The hydrolyzates obtained with the use of 150 and 300U/ml of enzyme, showed the antimicrobial activity against *B. cereus* and *P. fluorescens*. The antioxidative acivities were positively correlated with the time of hydrolysis.

Acknowledgement:

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PO-132

Track: *Pharmaceutical Biotechnology: biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering.*

MULTIPLE EFFECTS OF A PORCINE PLACENTA AND UMBILICAL CORD EXTRACT, IMML-PC01, ON CANCER, ARTERIOSCLEROSIS, DEGENERATIVE NERVOUS DISEASE, IMMUNOLOGIC DISEASE, AND INFLAMMATION

Katsuaki Dan, Akiyoshi Kohata and Shuichi Kohata

KEIO University School of Medicine, 35 Shinanomachi shinjyuku-ku,Tokyo, Japan; E-mail: kdanmrw@sc.itc.keio.ac.jp

Introduction: In new drug development, chemotherapeutic agents including synthetic compounds cause side effects and become ineffective, raising an issue. We have searched for stably active substances with a low incidence of side effects from natural substances and biomaterials over many years. As a result, we found that a porcine placenta and umbilical cord extract, IMML-PC01 (PC01), which was prepared using a specific manufacturing method, exhibited extensive physiological activities. Based on the results of a study with various disease models and cultured cell lines, PC01 showed physiological activities against cancer, arteriosclerosis, degenerative nervous disease, and inflammation.

Materials and Methods: Extracts of the porcine placenta and umbilical cord: The frozen placenta and umbilical cord were directly purchased from contracting farmers meeting the regulations established by IMML, Inc., in accordance with the SPF. The extraction process is shown below: (1) After thawing, corrupted and unnecessary sites were removed, and blood was washed off with water. (2) The specimen was crushed finely using a mincer. (3) The specimen was sterilized at a low temperature for 14 hours. (4) Hormonal removal was performed (secret of IMML, Inc.). (5) The supernatant was separated by centrifugation. Neither enzyme-decomposing treatment nor hot-water extraction was conducted.

The effects of PC01 on several diseases were examined using the following methods: (1) Cancer model: Balb/c mice were used to prepare leukemia, breast cancer, and lung metastasis models. The anti-tumor effects (life-prolonging effects, tumor-reducing effects) of PC01 were investigated using various tumor models. (2) Arteriosclerosis: Mice, as a diabetes model, were divided into PC01-treated and non-treated groups. In each group, a high-fat diet was given for 4 weeks, and the blood LDL level was measured. (3) Degenerative nervous disease: The inhibitory effects of PC01 added to the amyloid fiber elongation response system of β2-microglobulin, on elongation responses were investigated using the thioflavin T fluorescence level as an index. (4) Immunologic disease: Macrophages were collected from the abdominal cavity of mice treated with PC01 for 2 weeks and control mice, and cultured with fluorescent Latex beads. The ability to ingest a foreign substance was examined under a fluorescence microscope. Macrophage fluorescence was quantified employing image processing. (5) Inflammation: Adjuvant arthritis was prepared in mice treated with PC01 for 2 weeks and control mice to measure serial changes in images of the inflammation site and foot capacity. Furthermore, serum IL-1β was quantified using ELISA.

Results: (1) Among mice with leukemia, the survival rates 28 days after cancer-cell implantation in the control and PC01 groups were 18 and 60%, respectively. The breast tumor capacity was reduced to 1/2. Furthermore, there was a significant decrease in the number of metastatic foci of the lung (colonies). In addition, tumors were implanted at 2 points of the mouse dorsal region, and PC01 was infused into the intra-tumor area only on the unilateral side. Not only the treated tumor but also contralateral tumor reduced.

(2) The administration of PC01 significantly decreased the blood LDL level to the normal range. (3) PC01 inhibited the amyloid deposition of β2-microglobulin. (4) PC01 enhanced the phagocytosis of intraperitoneal macrophages in mice. (5) PC01 inhibited an adjuvant arthritis-related increase in the foot capacity. Furthermore, the serum IL-1β level was low.

Conclusion: The extract PC01 was effective for various diseases. However, it may function to maintain homeostasis rather than as a special-efficacy drug. In the future, the usefulness of PC01 as an anti-aging agent should be investigated.

PO-53

Track: *Other Areas: Nanobiotechnology*

LIVER TARGETED SILYMARIN NANOPARTICLES AN ANTIDOTE IN ACETAMINOPHEN OVERDOSE

Suvadra Das, Partha Roy and Arup Mukherjee

Department of Chemical Technology, University of Calcutta, Kolkata-700009, India; Email- Suvadra.tech@gmail.com

Silymarin (Sm) exert hepatoprotection for multitude of effects including hepatocytes RNA polymerase interactions towards upregulation of protein synthesis, restoration of glutathione content and facilitation of ATPase activity. Therapeutic success of Sm is hindered for biopharmaceutical limitations like low aqueous solubility, short absorption half life (0.17 hour), and degradation in gastric environment. Nanoparticles for Sm were conceived to improve biological distribution and systemic half life. Sm-nanoparticles in Eudragit RS100 (Smnp), were synthesized following nanoprecipitation technique. The PCS particle size recorded was 120nm and the zeta potential was +35mv. Smnp's were layered with heparin in polyethylene glycol (HepSmnp) for targeted delivery to the liver. HepSmnp particle size was 138nm and zeta potential +25mv. Biological efficacy of HepSmnp was evaluated as a protective and as an antidote in Acetaminophen induced hepatic necrosis in mice. Acetaminophen hepatotoxicity was observed in the rise of serum enzymes (SGOT 4943.3± 220.5 IU/L, SGPT 5161.7 ± 339.8 IU/L, ALP 2161.7± 129.1 IU/L) and in depletion of hepatic GSH level to 6.9±1.21µmole/gm of tissue. FITC co-loaded HepSmnp were localized in liver within 15 mints of intravenous injection as observed in confocal microscopy of liver-lobe sections. HepSmnp exerted significant hepatoprotection by restoration of hepatic GSH store to near normal level of 15.8±1.71µmole/gm of tissue.

PO-94

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

EVALUATION OF GENETIC DIVERSITY IN *CALLIGONUM* SPECIES FROM IRAN USING RAPD MARKERS

H. Dashti[1], H.Azarnivand[2], MR.Naghavi[3]

Natural resource Department, University of Tehran, Iran; E-mail: dashti@vru.ac.ir

Calligonum species are distributed in Centeral Asia desert and are effective to stabilize sand fields. RAPD is a useful genetical marker to determine polymorphism and relationship among plant species. In order to evaluate genetic diversity within and between species of *Calligonum* genus by using 15 RAPD primers, 26 samples of 11 species collected from different parts of Iran. According to diversity indices, Dice similarity, cluster analysis and band information a total of 191 bands was amplified and 94% of these bands were polymorphic. Cluster analysis revealed a high amount of diversity among samples. The most similar samples with 0.81 similarity was found within *C. bungei* species, while the least similarity (0.45) obtained for samples from different species. All samples clustered in four groups, the samples *C. polygonoides* and *C. leucocladum* located in two groups separately. However the other species were distributed in the two other groups. There was little relationship between genetic divergence and geographical origins that can be because of seed migration between places by wind and human.

PO-127

Track: *Industrial and Manufacturing: bio-fuels; energy crops (cellulosic ethanol industry); industrial enzymes; bioprocess engineering and optimization.*

OPTIMIZATION OF THE BIOMASS PRODUCTION OF ARTHROSPIRA (SPIRULINA) USING TAGUCHI METHOD

Monchai Dejsungkranont, Natapas Phoopat and Sarote Sirisansaneeyakul

Department of Biotechnology, Kasetsart University, Chatuchak, Bangkok, 10900, Thailand;
E-mail: monchaibiot@hotmail.com

Arthrospira (*Spirulina*) is cyanobacteria that used as protein-rich health food for a long time. The potential health benefits of *Arthrospira* associated with antioxidant, immunomodulation, anti-virus and anti-cancer effect, which are mainly due to three bioactive constit uents such as phycocyanin (a biliprotein pigment), the sulfated polysaccharide spirulan and polyunsaturated fatty acid (gamma-linolenic acid: GLA). The objective of this study is to optimize the environmental growth factors for maximizing the biomass yield and productivity of *Arthrospira* under the photoautotrophic cultivation in a microalgal culture tube. An optimization of the algal biomass production involved experiments that were statistically designed using the Taguchi method. Six factors varied at either three or two levels, which were as follows; three light intensities (klux), three initial culture pHs, two strains of the cyanobacteria, three concentrations of Zarrouk´s medium (%), three rates of aeration mixed with 1–2% v/v carbon dioxide (vvm) and two temperatures (°C). The optimal conditions obtained from this study helps maximizing the biomass cultivation of *Arthrospira* with using the Zarrouk´s medium.

Keywords: *Arthrospira* (*Spirulina*), photoautotrophic, biomass, Taguchi method.

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PO-152

Track: *Business development: strategic alliances; partnering trends; product opportunities; growth; business models and strategies; licensing; merger and acquisitions; outsourcing; venture capital and financing; intellectual property*

CREATION OF A NEW BEER BRAND: METHODOLOGICAL APPROACH

S.G. Davydenko, A.T. Dedegkaev and D.V. Afonin

JRC "Baltika breweries" Russia 194292 St Petersburg, 6 Verkhny per., 3, Russia; E-mail: davydenko@spb.baltika.ru

JRC "Baltika breweries" is the leader of Russian beer market and one of the biggest beer producers in Europe. Creation of new beer brands is based not only on effective business processes, but also on operational perfection of all production phases for the purpose of stable quality of production taking into account requirements of consumers. Quality function deployment (QFD) is the flexible decision-making method used in new products creation. QFD transforms requirements of clients (a voice of clients) to engineering characteristics of technology, places priorities for each product / services and simultaneously defines problems in the field of production or service development. Yeast basically defines taste and aroma properties of beer. As a result of auxotrofic segregant crossing with the parental strain lacking, mitochondrial DNA we selected the new hi-tech yeast strain, possessing a number of distinctive properties: good technological properties, decrease sulphur substances production, high speed of diacetyl reduction, high colloidal stability of beer. This strain was used to produce new sort of beer, serving for client requirements. The newly designed sort of beer got several International prices and brought 250 million dollars in 2007 for JRC "Baltika Breweries".

PO-1

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology, Clinical Research/clinical trials; bioethics; nanobiotechnology*

PRECISE TOOL FOR GENE EXPRESSION STUDY IN *STAPHYLOCOCCUS AUREUS*

Kateřina Demnerová and Lukas Valihrach

Department of Biochemistry and Microbiology, Institute of Chemical Technology, 166 28 Prague, Czech Republic; E-mail: demnerok@vscht.cz

Background: The reverse transcription quantitative real-time PCR (RT-qPCR) is often described as a "gold" standard of methods using for gene expression study, although it is far from being a standard assay.

Objectives: The aim of this study is to offer a complete and optimized protocol for gene expression study in *S. aureus* involving actual knowledge and approaches. This protocol could serve to precise measuring gene expression of theoretically any *S. aureus* gene under any experimental setting.

Methods: The method RT-qPCR was used for gene expression study including steps - RNA extraction, reverse transcription and quantitative PCR. The appropriate normalization method was evaluated using algorithm GeNorm1 and NormFinder2. The optimum sampling plan to minimize sources of variability was determined with help of Pilot nested study3.

Conclusions: The protocol for gene expression study in *S. aureus* was prepared. All steps were optimized for the best possible result. The designed protocol was evaluated with experiment determining the gene expression of enterotoxin G during different growth phases in *S. aureus*.

Keywords: *Staphylococcus aureus*, enterotoxins, gene expression

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PO-105

Track: *Other Areas: Food; Marine; Bio-safety; Systems Biology, Clinical Research/clinical trials; bioethics; nanobiotechnology*

METHOD FOR DEVELOPMENT OF STRAIN-SPECIFIC DNA MARKERS FOR PROBIOTIC BIFIDOBACTERIA BASED ON AFLP

Zhechko Dimitrov

LB-Bulgaricum Plc., ELBY Research Development and Production Center, 12-A Malashevska str., Sofia 1202, Bulgaria; E-mail: zhechko.dimitrov@lbbulgaricum.bg

Several health-promoting effects are attributed to Bifidobacteria as a major part of the normal intestinal microbiota. Because of the probiotic properties are strain-specific, the use of reliable and discriminative molecular methods is very important.

Aim of study: The goal of the present work is to present a new method for development of strain-specific DNA markers, as well as AFLP genotyping for Bifidobacteria with satisfactory discriminative power and reproducibility.

Results: AFLP genotyping is based on restriction cleavage of DNA with enzyme couple Xho I and Taq I, specially designed adapters, preselective and selective PCR primers. AFLP derived ampliphicants were used successfully as a source of strain-specific markers towards one probiotic Bifidobacterium longum strain, and subsequently, based on their specific sequences - for design of strain-specific probe. It's specificity was confirmed upon 70 Bifidobacteria strains. The strain specific markers and primers were successfully applied for quantitative detection of the same strain by help of real-time PCR in faeces of volunteers after consumption of probiotic product.

Conclusions: The developed AFLP for Bifidobacteria proved to be at least as discriminative as PFGE. The approach for design of strain-specific markers would be used to confirm the presence and quantify certain strain in complex bacterial communities.

Keywords: Bifidobacteria, probiotics, DNA markers.

PO-114

Track: *Medical Biotechnology*

CICATRIZING ACTIVITY AND POSSIBLE ADVERSE EFFECTS OF PISTACIA LENTISCUS FRUIT'S FATTY OIL

Zouhir Djerrou, H. Djaalab, F. Riachi, M Serakta, Z. Maameri, A. Belkhiri and Y. Hamdi Pacha

Department of Veterinary Sciences, Mentouri Constantine University, Constantine, Algeria;
E-mail: zouhir21265@yahoo.fr

Skin wound healing is a complex and dynamic process involving a sequence of integrated events including inflammation, granulation tissue formation, matrix deposition and remodeling [1, 2]. The search for natural compounds which can stimulate tissue repair has gained importance in the recent years aiming the development of non-toxic formulations for wound treatment1. Pistacia lentiscus fatty oil (PLFO) is one of the well-known natural products used in the management of cutaneous burns in the Eastern Algeria folk medicine.

The present study was undertaken to assess the effect of this oil (PLFO) on healing process and its safety to the skin and mucous membranes in the rabbit model. The oil was extracted traditionally from black fruits collected from Tamalous region, located in East of Algeria. The cicatrizing activity was evaluated in 6 male New Zealand rabbits; 4 equal burns were realized on the back of each animal. The wounds treated with oil were compared to these treated in Vaseline, Madecassol® and untreated wounds. PLFO has shown promising healing properties by reducing the inflammatory phase, stimulation of wound contraction and reducing the epithelization period compared to the different controls. Regarding possible adverse effects of PLFO, some toxicological tests were undertaken according to OECD Testing Guidelines [3-5]. The test of the Eye irritation / corrosion and the dermal irritation/corrosion showed that this oil is only slightly irritating to the eye and to the skin, either intact or abraded after a single dose.

The repeated dermal toxicity test showed that PLFO produced a slight erythema after the second week of application. The erythematic index is growing and regressing alternately until the end of the experiment. One third of rabbits had

presented a phenomenon of local sensitization with skin thickening. This phenomenon had been perfectly reversible and the skin has found its normal texture in a few days after cessation of oil application.

Regarding repeated toxicity via rectal route, PLFO was well tolerated. No anatomical or functional disturbance was observed in biochemical analysis, pathological examinations and histology.

The study concludes that Pistacia lentiscus fatty oil is effective for healing burns. It is tolerable in the short term, but may cause skin sensitization after prolonged use.

Keywords: Pistacia lentiscus, fatty oil, burns, wound healing, irritation, toxicity

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PO-64

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

RESEARCHES ON GENOTYPE CARRY BY EMBRYO CULTURE FROM SEEDLESS X SEEDLESS GRAPE CROSSES

Ayşe Yalçin Elidemir and H. İbrahim Uzun

Department of Horticulture, Faculty of Agriculture, Akdeniz University, Finike Vocational School, Turkey; E-mail: ayseyalcin@akdeniz.edu.tr

In this research, Yalova Seedless, Barış and Tekirdağ Seedless grape species' being crossed with 6 different seedless kinds (Superior Seedless (SS)), Perlette (P), Sultani Seedless (S), Yalova Seedless (Y), Barış (B) and Tekirdağ Seedless (T), cross embryos are gained as a result of crossing them and determining the best sample taking time in ovule-embryo culture (4, 6 and 8 weeks later from totally blooming), ovules' germination rates and plant developing facilities are specified. The highest rate of alive embryo was gained from the 8th week samples (%44) as the consequence of Yalova Seedless's crossing with itself in 2005, in the research. From the 8th week samples of equipments of Barış species, %88, 88 rates of embryo germination was gained. The highest rates of little plant was gained from the 6th week samples in the sums of both two years in Yalova Seedless species equipments (%38, 09).

In the consequence of the research, it was stated that plant carry's being possible from stenospermocarpic seedless grapes' crosses' embryos by using embryo culture.

Keywords: Vitis vinifera L. hybridization, ovule-embryo culture, seed, embryo.

PO-82

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology; Bioethics*

SELECTION OF STRAINS ACIDIFYING OF LACTOCOCCUS ISOLATED FROM RAW GOAT MILK

S. Hamma Faradji, S. Dj Sadoun and F. Leriche

Laboratory of Applied Microbiology, Department of Microbiology, Faculty of Natural and Life Sciences, University A.MIRA of Béjaia, 06000, Algeria; E-mail: hamma_samia@yahoo.fr

The lactic acid bacterium *Lactococcus* is used in the manufacture of several kinds of cheeses and fermented milks, in which its main functions are the production of lactic acid, aroma compounds, and exopolysaccharides. Lactic acid assists

in milk coagulation and curd draining, imparts a fresh acid flavor to fermented milks, and helps to suppress the growth of pathogens and spoilage microorganisms. In contrast to the other lactic acid bacteria used as starter cultures A total of 300 lactic acid bacteria isolates from raw sheep's milk samples obtained from 25 different region of Bejaia in Algeria. The cultures were identified according to their morphological, cultural, physiological and biochemical characteristics. 85 strains are the *Lactococcus*, this a total of isolated strains were examined for their acidification activity and for the growth in milk. 15 strains were selected for further investigations: Acidification activities, as determined by the Cinac system. Acidification of milk by pure cultures largely varied ,with a final pH of 4.3+- 0.05 after 10 h, the lowest final ph (5.2+-0.05) was obtained. These strains will select for identification génotipique PCR.The results suggest that wild bacterial populations should be preserved in order to protect the traditional raw milk cheeses, and to select new specific strains for the dairy industry.

Keywords: *Lactococcus* bacteria, acidification activity, goat's milk, PCR.

PO-130

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology; Bioethics*

ACCEPTANCE OF GENETICALLY MODIFIED PRODUCTS IN ISLAM (SHIA)

Najaf Allahyari Fard

Department of Plant Genetic, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran; E-mail: allahyar@nigeb.ac.ir

Genetically Modified (GM) crops have great potential to improve food production. They are increasingly used for food production, food quality and many industrial applications. GM crops will dramatically change the agricultural world. They have many abilities such as: the advancements in agricultural crop production, the enhancement of the quality and quantity of crops to increase their micronutrient content, the decreasing use of costly chemicals harmful to the environment, the reduction in the maturation time of seedlings, the enhancement of plant resistance to pests, disease, drought and salinity, the improvement of the adaptability of crops to nutrient deficient soil and the protein production for human and animal medicine. Due to mentioned benefits, the areas under GM cultivation have growth from 1.7 million hectares in 1996 to 148 million hectares in 2010.

FAO has predicted that food preparation of 9.1 billion world population in 2050 requires 70 percent increase food production in world and 100 percent increase food production in developing countries. Report of International Service for the Acquisition of Agri-biotech Applications (ISAAA) indicates biotechnology can be a key solution to growing demand for food in the world. In many countries, public acceptance of GM crops is rooted in religious views. However, Islamic view about GM crops and GM products has not been systematically examined. This view is important and has influence on Muslims" decisions to consume GM products.

Islamic view about GM crops and GM products was explained in this article. Islam is made up of two major branches, Sunni and Shia. In this article views of grand ayatollahs in Shia about various aspects of direct and indirect consumption of GMO products have described. All of them permit consumption of GMO products, also some permit with reservation 1- they haven't losses on the present and future, 2- annunciation of product information. Based on their views, Islam (Shia) accepts consumption of GM crops and GM products with some reservation.

Keywords: GMO, consumption, Jurisprudential views, Islam (Shia).

PO-144

Track: *Plant and Environment*

EVALUATION OF YIELD STABILITY OF TRITIPYROM AND TRITICALE BASED ON GEI PARTITIONING

S. Farokhzadeh[1], G. Mohammadi-Nejad[2]* and H. Shahsavand-Hassani[3]

Plant Breeding, Department of Agronomy and plant breeding, college of Agriculture, Shahid Bahonar University of Kerman, Kerman 76169-133, Iran; E-mail: Mohammadinejad@uk.ac.ir

In order to study genotype-environment interaction of grain yield in six primary tritipyrum lines in compare to five triticale lines, An experiment was conducted in a randomized complete block design with three replication in three locations (Kerman, Sirjan, Fars) during growing season (2001-2002, 2002-2003, 2005-2006 and 2010-2011). Totally eight different experiment were performed in terms of grain yield in different environments. Combined analysis of variance for grain yield showed significant differences between genotypes, environments and genotype × environment interactions. Results analysis based on Muir method for partitioning of GEI to change in rank & change in value showed the highest percentage of change in rank interaction was related to 4115 & 4116 (triticale lines) and the least amount was related to (St/b)(Cr/b)F4, Ka/b tritipyrum and M45 triticale lines respectively. (Ka/b)(Cr/b)-5, 4103, La/b, (Ma/b)(cr/b)-4, La (4B,4D)/b, 4108, 4116, M45, Ka/b lines had changes in value or environmental sensitivity and (St/b) (Cr/b)-4, 4115 lines had the highest change in value and according to Muir's methods, 4116 and (St/b)(Cr/b)F4 line are considered as unstable and stable genotypes respectively. Result revealed that 86 percent of interaction sum of squares was belonged to change in rank and only 14 percent was change in value.

Based on the present results and the Muir criteria SS(IC)i and SS(HV)i, it can be concluded that M45 tritipyrum Was introduced as stable and high yielding genotype for entering to the next breeding programs or recommending to farmers.

PO-145

Track: *Plant and Environment*

PATH ANALYSIS IN HEXAPLOID AND PRIMARY TRITIPYRUM LINES IN COMPARE WITH NEW TRIRICALE LINES AND IRANIAN BREAD WHEAT VARIETIES

S. Farokhzadeh[1], G . Mohammadi-Nejad[2]* and H. Shahsavand-Hassani[3]

Plant Breeding, Department of Agronomy and plant breeding, college of Agriculture, Shahid Bahonar University of Kerman, Kerman 76169-133, Iran; E-mail: Mohammadinejad@uk.ac.ir

In order to analysis the correlations in to direct & indirect effect in tritipyrum in compare to triticale and wheat, an experiment was conducted by a Randomized complete block design with three replications for 13 tritipyrum lines as well as 9 Iranian local wheat varieties and 5 triticale lines in growing season 2010-2011 years. Totally 32 traits was measured in the mentional genotypes. Results analysis of variance showed mainly there is a significant different between cultivares &. Pearson correlation coefficint was significant between yield and harvest index, 1000 grain weight, number of grain per spike, number of Spikelet per spike and number of Plant traits ($p<0.01$). According to stepwise multiple linear regression seed yield is considered as dependent variable and other traits as independent variables.The highest R^2 = 0.95 was belonged to spike weight. Result of stepwise regression showed harvest index, 1000 grain weight, plant height, fertility (%), number of tiller and spike weight have the most effective in yield.

Path coefficients analysis showed harvest index (0.608) and 1000-grain weight (0.493) traits has the highest direct effect than other traits on grain yield. Also Indirect effect of harvest index through 1000-grain weight and Indirect effect of 1000-grain weight through harvest index were 0.190 & 0.234 on grain yield respectively.

Principal component analysis and cluster analysis was confirmed above results.Therefore the most important traits for making selection index for grain yield improvement were 1000-grain weight and harvest index.

PO-110

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

ISOLATION RAHNELLA AQUATILIS FROM RHIZOSPHARE AND ITS INHIBITORY EFFECT ON AGROBACTERIUM

Adel Tari Farrokhrun and Lotfi Hajie

Plants Technology Group, Institute of Technical and Vocation Higher Education Jahad – e – Agriculture, Tabriz, East Azerbaijan, Iran; E-mail: tarifarrokh@yahoo.com

Introduction: Rahnella is commonly found in plant rhizospheres, roots, soil samples or water. Rahnella is a bacterium of considerable agro-economical interest. Several Rahnella strains have been reported to be efficient antagonists of the plant pathogens such as Erwinia amylovora , Xanthomonas campestris and Agrobacterium. Bell et al. demonstrated that certain grapevine endophytes can control population numbers of Agrobacterium in situ and provide significant

biocontrol of crown gall. In addition, Chen et al (2007) reported Rahnella aquatilis strain HX2 and its antagonistic activity on crown gall of sunflower and grape induced by Agrobacterium vitis. Here we report the isolation Rahnella aquatilis from rhizosphare and its inhibitory effect on Agrobacterium.

Material and methods: Rahnella strains TL20 was isolated from rhizosphere soil of Vitis vinifera from Iran. TL20 was identified by routine biochemical tests. The 16S rDNA was polymerase chain reaction (PCR) amplified from TL20 genomic DNA with the primers TF (5′GGCTTAACACATGCAAGTCG3′) and TR (5′ACCTTGTTACGACTTCA CCC3′) and then sequenced. In vitro, antagonist activity of the TL20 against pathogenic agrobacterial strains was studied according to Chen et al (2007). Native Agrobacterium strains were isolated from grape crown gall samples.

Results: According to the biochemical identification TL20 was identified as Rahnella aquatilis . The results were negative for different test including: Catalase, Citrate utilization, Methyl red, Arginine dihydrolase, Lysine decarboxylase, Cytochrome oxidase, Amylase, H2S production , Utilization of tartrate and acid from D-Arabitol and Ethanol . Also, positive result related to Growth on 2% NaCl, Voges-proskauer, Growth at 4C or 41 C, Gelatin liquefaction (20 0C), Gas from D-glucose and acid from D-Sorbitol, Sucrose, D-glucose. In addition,the 16S rDNA sequence was showed 99% similarity to R. aquatilis.(accession number: 1449702). TL20 can inhibit five native Agrobacterium which they isolated from crown gall and identified as Agrobacterium vitis acording to biochemical tests.

PO-14

Track: *Industrial and Manufacturing: bio-fuels; energy crops (cellulosic ethanol industry); industrial enzymes; bioprocess engineering and optimization*

ENZYMATIC SACCHARIFICATION UNDER HIGH HYDROSTATIC PRESSURE

Érica Dutra Albuquerque, Patricia Machado Bueno Fernandes and Antonio Alberto Ribeiro Fernandes

Department of Núcleo de BiotecnologiaUniversidade Federal do Espirito Santo, Brazil; E-mail: aarfernandes@gmail.com

The use of cellulosic biomass for bioethanol production has been largely pursued and a feasible process to improve the saccharification process, thus increase the hydrolysis of cellulosic materials to low molecular weight products such as hexoses, is the main focus. Even though enzymatic disruption of cellulose is considered the best environmental practice, cellulase is the most expensive step during ethanol production from cellulosic biomass. Significant cost reduction is required in order to enhance the commercial viability of cellulase hydrolysis technology. We propose the use of high hydrostatic pressure (HHP) to increase cellulase activity. Using coconut husk powder as cellulosic substrate and a blend of cellulase from soil microorganisms at HHP of 100 to 600 MPa and temperature among 30 °C for 20-40 min, we were able to double the efficiency of enzymatic hydrolysis. Enzymatic hydrolysis was calculated as the amount of enzyme required to release 1 μmol of reducing sugar/min-1/ml (UI). HHP technology increased cellulase enzymes efficiency and enzymatic hydrolysis of cellulose of coconut husk, releasing about twice more reducing glucose per milligram of protein than hydrolysis at room pressure. Thus, hydrolysis of cellulose performed by a blend of cellulase under HHP could feasibly be applied to the development of scaled-up processes.

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Keywords: Cellulase, hydrolysis, bioethanol, Aspergillus niger, Trichoderma, coconut husk, lignocellulosic wastes, cellulose.

PO-7

Track: *Pharmaceutical Biotechnology*

IMMUNOGENICITY OF BACTERIA-EXPRESSED HEMAGGLUTININ OF AVIAN INFLUENZA VIRUS H5N1

K. Florys, V. Sączyńska, J. Kapusta, M. Kęsik-Brodacka, A. Romanik, V. Cecuda-Adamczewska, G. Płucienniczak and A. Płucienniczak

Bioengineering Department, Institute of Biotechnology and Antibiotics, Poland; E-mail: florysk@iba.waw.pl

Different expression systems, mainly eucaryotic, are being used to develop subunit vaccine against avian influenza virus (AIV) based on hemagglutinin (HA) - an immunodominant antigen of AIV capable to elicit neutralizing antibodies. Here, the vaccine potential of bacteria-derived recombinant HAH5 was studied in the mouse model. Two various length fragments of HAH5 were expressed in Escherichia coli system. Mice immunization was performed with varied doses, adjuvants, routs and timing of antigen delivery. All of parenteral immunization schedules applied evoked antibodies, recognizing recombinant HAH5 of mammalian origin, which is known to preserve essential epitopes of native protein and oligomeric structure. Using ISCOMATRIX as adjuvant, strong Th1 fashion immune response could be obtained with only single subcutaneous immunization of mice. Moreover, some booster effect was observed in parenterally primed mice after delivery of antigen via the mucosal route. Preliminary immunization studies show possibility to use bacteria-expressed hemagglutinin to construct subunit vaccine against AIV H5N1.

Keywords: Recombinant hemagglutinin, avian influenza virus, immunization, vaccine.

PO-52

Track: *Plant and Environment: Transgenic Plants and Crops; Bioremediation; Microbial Diversity; Bio-monitoring*

EVALUATION OF THE EFFICIENCY OF ENZYMES SYNTHESIS BY MOLDS OF THE FUNGAL STRAINS ISOLATED FROM DAIRY SEWAGE SLUDGE

Magdalena Frac and Anna Pawlik

Institute of Agrophysics PAS, Department of Plant and Soil System, Laboratory of Molecular and Environmental Microbiology ul, Doswiadczalna 4, 20-290 Lublin 27, Poland; Email: m.frac@ipan.lublin.pl

Fungi play a vital role as the decomposers of different materials and are capable of producing different enzymes, important in the environmental protection field. The fungal strains and enzymes preparation could be used in waste biodegradation, such as dairy sewage sludge.

The aim of the study was the fungal strains isolated from the dairy sewage sludge as well as the evaluation of the efficiency of the enzymes synthesis by selected fungi isolates and their applicability in waste degradation.

The fungal strains used in this study were isolated from a dairy sewage sludge sample collected at Krasnystaw, Poland. The strains were isolated on bengal rose medium and then cultured on potato dextrose agar (PDA). The fungi were identified with molecular technique using comparative rDNA sequencing of the LSU D2 region. The MicroSEQ ID software were used for assess raw sequence files, performs sequence matching to the MicroSEQ ID validated reference database and creates Neighbor-Joining trees. The identified strains belong among others to the genus: *Penicillium*, *Trichoderma*, *Aspergillus* and *Fusarium* were applied in evaluation of the efficiency of the enzymes synthesis used in the biodegradation studies. The fungal strains were cultured in 250 Erlenmeyer flask containing dairy sewage sludge extract medium (DSS). For ten days at 28 °C. The proteases, lipase, α-amylase, β-glucosidase, poligaracturonase, pectinoesterase, glucose oxidase, laccase and peroxidase activities were examined in the after-cultured supernatants in time interval.

Individual mold strains varied in their ability to attack various substrates. Using DSS medium the ability to hydrolyse caseine, starch and PNPG occurred frequently whereas no laccase, peroxidase and glucose oxidase activities were detected. Pectinolytic enzymes were produced by very few fungal isolates. The results of our research can be useful in the agriculture and environment protection sector, indicating the possibility of various enzymes synthesis by mold fungi used in this research and their promising biotechnological application.

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PO-54

Track: *Plant and Environment*

DETECTION OF HUMAN PATHOGENIC VIRUSES IN THE UMGENI RIVER OF DURBAN, SOUTH AFRICA

Atheesha Ganesh and Johnson Lin

Department of Microbiology, University of kwaZulu-Natal, South Africa; E-mail: 204007017@ukzn.ac.za

Microbial contamination of our aquatic environments poses a potential public health risk when improperly managed. Public water systems rely on bacterial indicators for monitoring water quality, and it has been shown that bacterial indicators are often poorly correlated with the presence of other microorganisms, such as viruses, which can be found in various water sources. Viruses are a group of particular concern because they include highly stable pathogens that can be resistant to standard wastewater treatment processes. Compared with most pathogens, the minimal infectious dose of viruses is extremely low. It is thus important to consider viruses in water quality because of their incidence as causal agents for diarrheal disease, and due to their characteristics, which allow them to survive in the changing environmental conditions indefinitely. The extent of our knowledge on the occurrence of these viruses in the Umgeni River of Durban South Africa appears to be relatively limited, since the detection of viruses is relatively expensive and most microbiological laboratories in South Africa do not possess the necessary facilities and expertise for routine virological monitoring. The current study assessed the microbiological including viruses and physico-chemical quality of the Umgeni River in Durban South Africa seasonally. A setup for the concentration of viruses from large (20 l) volume water samples using a two-step tangential flow filtration (TFF) process was established, with 95% recovery. Virus like particles (VLPs) was detected using electron microscopy, traditional cell culture and molecular techniques. Viral abundance and diversity was found with the virus populations at the different points along the river. Recovery of indicator bacteria and phage was determined by membrane filtration plate count and plaque assay respectively. The cytopathic effect of the concentrated viruses from the water was determined by spiking the virus into various cell lines and incubated over 7 days. The concentrated viruses from water displayed the cytopathic effect on all cell lines, indicating that Adenoviruses and other enteric viruses are present in the water samples. This study has lead to the need for developing an effective and efficient method for the simultaneous collection and recovery of low levels of human viruses that can then be rapidly identified and quantified.

Keywords: Pathogen, tangential-flow filtration, cytopathic effect.

PO-70

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology; Bioethics*

PHYSICOCHEMICAL CHARACTERIZATION OF SODIUM CASEINATE SOLUTIONS AS A FUNCTION OF PH

S. Ghorbani Gorji, E. Ghorbani Gorji and M. A. Mohammadifar

Department of Food Science and Technology, Faculty of Nutrition and Food Science, Shahid Beheshti University of Medical Sciences, P.O.Box 19395-4741, Tehran, Iran; E-mail: sarah.ghorbani@gmail.com

Proteins are widely used in the food and cosmetic industries as emulsifying agents because of their amphiphilic nature, i.e., they contain both charged hydrophilic and hydrophobic regions [1] which leads to self assembly of proteins in low pHs.

Na-caseinate is broadly used in a wide range of food products due to its nutritional and functional importance. In milk casein is present in the form of spherical complexes with a radius of approximately 100 nm that are called casein micelles. Casein micelles are stabilized by colloidal calcium phosphate (CCP), which can be removed by precipitation and washing at pH 4. The precipitate can be redissolved in the form of sodium caseinate (SC) by adding NaOH [2]. SC associates into small particles with a hydrodynamic radius (Rh) that depends somewhat on the temperature, the ionic strength and the pH (Rh ≈ 11 nm at 20° C, 0.1M NaCl and pH 7) [3]. The characterization of sodium caseinate solutions as a function of pH was determinedusing titration with HCL and slow acidification in situ with glucono-δ-lactone (GDL) through turbidumetry in different concentration (0.03%w/w, 0.045%w/w, 0.06%w/w, 0.09% w/w, 0.2%w/w, and 0.3% w/w). Additionally, rheometry(steady and unsteady) methods and particle size distribution analyzer have been used to determine the behavior of sodium caseinate solutions in different pHs.

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PO-168

Track: *Medical Biotechnology*

BONE MARROW MESENCHYMAL STEM CELLS REDUCE APOPTOSIS BY DECREASE OF C-FOS EXPRESSION DURING THEIR REPAIR ON SPINAL CORD ISCHEMIA-REPERFUSION INJURY

Li Guo, Chun-yang Meng, Yu-bo Zhou, Hao Chen and Fei Yin

Department of Toxicology, School of Public Health, Jilin University, Changchun 130021, China;
E-mail: : gli@jlu.edu.cn

Background: In modern society, spinal cord ischemia-reperfusion injury (SCII) is a devastating problem, up to now, it has not yet resolved.

Objectives: To explore the repair effect of BMSCs on SCII and the relative mechanism.

Methods: BMSCs were transplanted into SCII rats by retro-orbital intravenous injection. The hindlimb motor function evaluation was performed by BBB locomotor rating scale after cell transplantation. The pathohistological examination, nerve cells apoptosis and the expression of c-Fos were detected by HE staining, TUNEL staining, immuohistochemistry, respectively.

Results: compared with model group, rats motor function in cell transplantation group significantly recovered during the first week after reperfusion. HE staining result showed that in model group rats, some injured motor neurons with nucleus psychosis were observed, blood vessels congestion was significant, whereas the pathohistological changes in cell transplantation group were improved with the near-normal cell morphology. In addition to, in model group the rate of apoptotic cells and the rate of c-Fos-positive expression were significantly higher than those in the cell transplantation group (P <0.05).

Conclusion: The above results demonstrated that BMSCS could repair SCII by anti-apoptosis, which may be mediated by decrease of c-Fos protein expression.

Keywords: bone marrow mesenchymal stem cells, repair, spinal cord ischemia-reperfusion injury, apoptosis.

PO-109

Track: *Plant and Environment: Transgenic Plants and Crops; Bioremediation; Microbial Diversity; Bio-Monitoring*

BIOREMEDIATION OF CARPET INDUSTRY EFFLUENT USING SPECIES OF PSEUDOMONAS

Anil Kumar Gupta

Department of Botany, S.B.P.G. College, Baragaon, Varanasi – 221204 (U.P.), India;
Email: akgupta52@rediffmail.com

Eastern Uttar Pradesh, especially Varanasi and surrounding districts are the largest designer and manufacturer of carpets, using variety of threads and colours. Carpet industry, especially their dyeing plants are the greatest polluters of the water bodies in which they discharge their effluents. Lot of changes in the physico-chemical characteristics of these water bodies are observed. Their colour, odour, pH, EC, TSS, TDS, COD, BOD, Sulphide, Chloride Sulphate, Phosphorous, Nitrogen and Chromium content show drastic changes, therefore in the present study, three prominent Carpet industries of Varanasi were selected for the present study and above mentioned parameters were analysed. Considering high increase in their values crossing the prescribed limits of Central Pollution Control Board, a need was felt for their remediation, Bioremediation is considered to be very safe and economic process, therefore in the present study, an attempt was made using three species of *Pseudomonas* for bioremediation of carpet industrial effluent. Three species namely *Pseudomonas aeruginosa, Pseudomonas desmolyticum and Pseudomonas putida* were selected and obtained from culture collection and then their mass culture was developed. The Carpet industry effluent was treated with all the three species separately and after 15 days, drastic changes were observed in the characteristics of effluent. It was concluded that the phytoremediation is very effective to degrade pollutants of carpet industries. Out of three species, *P, putida* was observed to be most effective in phytoremediation of carpet industrial effluent. In the present study, an attempt was made using three species of *Pseudomonas* for bioremediation of carpet industrial effluent. Three species namely *Pseudomonas aeruginosa, Pseudomonas desmolyticum and Pseudomonas putida* were selected and obtained

from culture collection and then their mass culture was developed. The Carpet industry effluent was treated with all the three species separately and after 15 days, drastic changes were observed in the characteristics of effluent. It was concluded that the phytoremediation is very effective to degrade pollutants of carpet industries. Out of three species, *P, putida* was observed to be most effective in phytoremediation of carpet industrial effluent.

Keywords: Bioremediation, carpet industry effluent, phytoremediation, pseudomonas.

PO-65

Track: *Industrial and Manufacturing: bio-fuels; energy crops (cellulosic, ethanol industry); industrial enzymes; bioprocess engineering and optimization.*

EXTREMOPHILES IN SUPERCRITICAL CARBON DIOXIDE

Maja Čolnik, Mateja Primožič, Nina Gunde Cimerman, Polona Zalar, Željko Knez and Maja Habulin

University of Maribor, Faculty of Chemistry and Chemical Engineering, Laboratory for Separation Processes and Product Design, Smetanova ulica 17, 2000 Maribor, Slovenia; E-mail: maja.habulin@uni-mb.si

Extremophiles are organisms or microorganisms which thrive in an environment with very extreme conditions. They fall into a number of different classes that include thermophiles, acidophiles, alkalophiles, psychrophiles, barophiles and others. Some organisms are able to survive in an environment with extremely low and high temperatures, as well as the pH level scale can be very low or high. The temperature range which extremophilic organisms can survive extends of -20°C to +118°C. Other organisms can survive in an environment, where there is almost no water and in an environment with extremely high hydrostatic pressure. Extremophilic biomass production is very important to provide sufficient material for enzyme and biomolecule isolation and characterization, eventually revealing particular features of industrial interest.

Supercritical carbon dioxide (SC CO_2) is often discussed as an alternative, environmentally benign reaction medium for chemical synthesis, due to its non-toxic and nonflammable nature, and its relatively low critical pressure and temperature which allows preservation of thermally unstable compounds. The effects of SC CO_2 on microbial cells are probably due to the decrease in intracellular pH and the extraction of vital components from the cells. The decreased intracellular pH can affect the cell viability by inactivation of enzymes or inhibition of specific metabolism.

The sensitivity of the cell suspension of different extremophiles to treatment with SC CO_2 was determined under varying pressure and temperature for different treatment durations. The suspensions of extremophiles were incubated in SC CO_2 in order to use the enzymes from them for biotransformation. Activities of the enzymes in cell suspension were measured on UV-Vis spectrophotometer before and after incubation in SC CO_2. The total proteins concentration in cell suspension was determined by the Bradford method.

Keywords: Extremophiles, SC CO_2, enzymes, proteins.

PO-84

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology; Bioethics*

SELECTION OF STRAINS ACIDIFYING OF *LACTOCOCCUS* ISOLATED FROM RAW GOAT MILK

S. Hamma, S. Dj. Sadoun and F. Leriche

Laboratory of Applied Microbiology, Department of Microbiology, Faculty of Natural and Life Sciences, University A.MIRA of Béjaia, 06000, Algeria; E-mail: hamma-samia@yahoo.fr

The lactic acid bacterium *Lactococcus* is used in the manufacture of several kinds of cheeses and fermented milks, in which its main functions are the production of lactic acid, aroma compounds, and exopolysaccharides. Lactic acid assists in milk coagulation and curd draining, imparts a fresh acid flavor to fermented milks, and helps to suppress the growth of pathogens and spoilage microorganisms. In contrast to the other lactic acid bacteria used as starter cultures A total of 300 lactic acid bacteria isolates from raw sheep's milk samples obtained from 25 different region of Bejaia in Algeria. The cultures were identified according to their morphological, cultural, physiological and biochemical characteristics. 85

strains are the *Lactococcus*, this a total of isolated strains were examined for their acidification activity and for the growth in milk. 15 strains were selected for further investigations: Acidification activities, as determined by the Cinac system. Acidification of milk by pure cultures largely varied, with a final pH of 4.3+- 0.05 after 10 h, the lowest final ph (5.2+-0.05) was obtained. These strains will select for identification génotipique PCR.

The results suggest that wild bacterial populations should be preserved in order to protect the traditional raw milk cheeses, and to select new specific strains for the dairy industry.

Keywords: *Lactococcus* bacteria, acidification activity, goat's milk, PCR.

PO-111

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

PRODUCTION OF GLADIOLUS (*GLADIOLUS GRANDIFLORUS* HORT.) VITRO PLANTS BY NEWLY FORMED BUDS IN SALT STRESS CONDITIONS

Faouzi Haouala[1] and Ismahen Salhi[2]

National Institute of Agronomy of Tunisia, 43 avenue Charles Nicolle, 1002 Tunis Belvédère Tunisia;
E-mail: faouzi.haouala@laposte.net

Apical buds of corms of gladiolus (*Gladiolus grandiflorus* Hort. cv 'Ben Venuto' and 'Chinon') were cultivated on Murashige and Skoog (1962) medium supplemented with 0.5 $mg.l^{-1}$ IBA, 2 $mg.l^{-1}$ BA and various concentrations of NaCl (0, 50, 100 and 150 mM). On control medium without salt, the number of newly formed buds was 5 buds per explants for each cultivar. Salinity affects this parameter and on NaCl 150 mM, the number of buds represented only 13 and 19% of control, respectively for 'Chinon' and 'Ben Venuto'. The number of shoots per explants longer than 0.5 cm was the highest for the control, it was respectively 0.5 and 1.2 shoot. The rooting of these shoots reached 100% as well as in absence and in presence of IBA and until NaCl concentration did not exceed 100 mM. On NaCl 150 mM, the rate of rooting varied from 46.6 to 100%, depending on the cultivar and the concentration of IBA in the medium.

Keywords: Plant, gladiolus, *in vitro*, bud, salinity, rooting.

PO-60

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

GrIGS; GRAFT INDUCED GENE SILENCING FOR HORTICULTURAL CROP IMPROVEMENT

T. Harada, S. Bai, A. Kasai, H. Xu and T. Li

Faculty of Agriculture and Life Science, Hirosaki University, Hirosaki 036-8561, Japan;
E-mail: tharada@cc.hirosaki-u.ac.jp

Transcriptional gene silencing (TGS) can be induced by promoter-targeted siRNA. The long-distant transmission of TGS in plants has been reported with virus-induced gene silencing in which case not only the siRNA but also the virus moves over long-distance. In contrast, the transmission of TGS has not been observed in the case of siRNA derived from inverted repeat transgene as the silencing trigger. We reported here that the mobile siRNA produced from the hairpin-structure transgene controlled by companion cell specific promoter can also induce the transmissible TGS in both our grafting system. Although the transmissible TGS occurred only in the cells in the vicinity of leaf vein in the scion, very strong silencing in the root system was observed, especially lateral root, including the root apical meristem. The transmissible TGS can also be maintained through tissue culture and then inherited to progeny. Our results suggest the potential application using the mobile promoter-targeting siRNA to improve horticulture plants cultivars by grafting.

PO-169

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

PRODUCTION OPTIMIZATION AND BIOCHEMICAL CHARACTERIZATION OF AN EXTRACELLULAR PROTEASEPRODUCED BY THEMODERATELY HALOPHILIC BACTERIUM, *SALINIVIBRIO* SP. STRAIN MS7

Mahnaz Shahbazi and Hamid Reza Karbalaei-Heidari

Molecular Biotechnology Lab., Department of Biology, Faculty of Sciences, Shiraz University, Shiraz, Iran;
E-mail: karbalaei@shirazu.ac.ir

Microbial proteases are among the most important hydrolytic enzymes which represent oneof the three largest groups of industrial enzymes and account for approximately 60% of thetotal enzyme sales in the world. In present study, we report isolation, purification andbiochemical characterization of an alkaline protease produced by the moderately halophilic bacterium strain MS7.The strain MS7 was isolated from the Maharlo salt lake in Iran and cultivated aerobically at30°C and 180 rpm in nutrient broth containing 5% (w/v) NaCl. 16S rRNA sequence analysisplaced MS7 in the *Salinivibrio* genus. A basal medium of the following composition (g/l) wasused for the production of protease: peptone 10; yeast extract 10; maltose 5; NaCl 81; $MgCl_2$7; $MgSO_4$ 9.6; $CaCl_2$0.36;KCl 2; $NaHCO_3$ 2; and pH 8.0. To determine the effects ofvarious carbon sources on protease production, glucose, lactose, sucrose and maltose wereinvestigated and maximum production of the enzyme was obtained in basal medium (pH 8.0)containing maltose as a carbon source. Kinetics of bacterial growth and protease productionwere conducted in the basal medium. Maximum growth and protease activity (25.95 U/ml)was achieved after 48 hours at 30°C and 180 rpm.The extracellular protease of MS7 waspurified by a combination of acetone precipitation and ion exchange chromatography onDEAE cellulose. The characterization of the purified protease revealed that the enzymeexhibited optimum temperature and pH at 50°C and 8.0, respectively.

Keywords: Alkaline protease;*Salinivibrio*sp.; Optimization; Moderately halophilic bacterium.

PO-170

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

ISOLATION OF A NEW ACHROMOBACTER SP. STRAIN CAR1389AS A *CARBAZOLE DEGRADINGBACTERIUM*

Zahra Farajzadeh and Hamid Reza Karbalaei-Heidari

Molecular Biotechnology Lab., Department of Biology, Faculty of Sciences, Shiraz University, Shiraz, Iran;
E-mail: karbalaei@shirazu.ac.ir

One of the important nitrogen-containing organic compoundsexisting in crudepetroleum oil is carbazole. Carbazole possess mutagenic and toxicactivitieswith serious environmental problems if it released into the environment. Forthisreason, aromatic compounds biodegradation has been extensively studied asan importantmethod in environmental cleanup process.In this work, several samples were collected fromdifferent oil contaminated regions in Iran,and carbazole utilization in the enrichment cultureswas examined as a result ofclearing in the medium compared with the control. Based on thismicrobialscreening study, we isolated a Gram-negative, rod shape bacterium with theabilityof using carbazole as the sole source ofcarbon, nitrogen, and energy.Biochemical analysis and phylogenicstudy by determining DNA sequence of aportion of the 16S rRNA gene revealed that thenewly isolated bacterium placesin *Achromobacter* genus and identified as an *Acromobacter*sp. strain CAR1389.The biodegradation of carbazole by the *Acromobacter*sp. CAR1389wasperformed with growing cell cultures in a 250 ml conical flask containing 50 mlminimalmedium supplemented with 6 mM carbazole at 30 ^{o}C with an agitation rateof 180 rpm. Thepercentage of carbazole degradation during 7 days was analyzedby C18 reverse-phase HPLCexperiments. The primary results show high degradationactivity of the strain CAR1389 towardcarbazole and revealed approximately 90%Carbazole consumption during 96 hours. Our findings introduce this strain as apotent microorganism forbioremediation of environments contaminated by suchheterocyclic nitrogen compounds.

Keywords: Carbazole; *Achromobacter* sp.; Bioremediation; Biodegradation; heterocyclic nitrogencompounds.

PO-131

Track: *Industrial and Manufacturing*

ENGINEERING OF PURIFIED ALPHA AMYLASE THROUGH CHEMICAL MODIFICATION TO INCREASE THERMO STABILITY

Tayyaba Huma

Department of Bioinformatics and Biotechnology, GC University,Faisalabad Pakistan;
E-mail: tayyabashahbaz@gmail.com

Alpha amylase (EC 3.2.1.1) is an important industrial enzyme which has got second place (next to proteases) in world's distribution and sales among industrial enzymes. These enzymes account for about 25 % of the world's enzyme production. Amylases generally stand out for useful applications in the food, brewing, textile, detergent and pharmaceutical industries. Thermo-stable enzymes can withstand extreme environmental conditions and can resist against detergents, organic solvents, temperature & pH. The operational stability of enzymes is of paramount importance for any bioprocess, which can be improved through various protein engineering techniques. With the development of novel procedures that exploit selective and efficient protein chemistry, chemical modification, either alone or combination with other mutagenesis techniques, could make a significant contribution to the development of enzymes that cope with the industrial demands. A number of new possibilities for industrial processes have emerged with the maximum availability of thermostable enzymes. To economize industrial processes, it is necessary that α-amylases should be active at the high temperatures of gelatinization (100-110°C) and liquefaction (80-90°C), therefore, there is always need for more thermophilic and thermostable α-amylases. To cope with the current industrial demands it is necessary to produce alpha amylase with improved catalytic and kinetic properties which can withstand the robust industrial environment. The current report deals with chemical modification of purified α-amylases (Isoform-I & Isoform-II) of parent and mutant strains of *phialocephala humicola*. The enzyme engineering was done with aromatic (aniline) and aliphatic (dimethylamine) nucleophiles for carboxyl group modification in the presence of EDC. Isoform-I of mutant strain showed great potential for industry due to its high turnover number. The maximum values of half life ($t_{½}$) 568 and 324 min at 60°C for Isoform-I & Isoform-II, respectively, proved that α-amylase of Isoform I was comparatively more stable than Isoform II. Aniline also improved the thermostability of α-amylase towards higher temperatures (60-65 ºC). The resistance of protein structure towards higher temperatures is one of the most important criteria in the applications of enzymes at industry.

PO-142

Track: *Pharmaceutical Biotechnology: biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering*

PERIPHERAL ADMINISTRATION OF PEPTIDE 234 (KISSPEPTIN ANTAGONIST) DOES NOT ALTER BASAL PLASMA TESTOSTERONE LEVELS, BUT DECREASES PLASMA ADIPONECTIN RELEASE IN ADULT MALE RHESUS MONKEY (*MACACAMULATTA*)

Tanzeel Huma, Farhad Ullah, Farnaz Haneef and Muhammad Shahab

Reproductive Neuroendocrinology Laboratory, Department of Animal Sciences Faculty of Biological Sciences, Quaid-i-Azam University, 45320 Islamabad, Pakistan; E-mail: tanzeelhuma@gmail.com

Kisspeptin-GPR54 signaling has recently emerged as a potent regulator of reproductive axis and elicitor of GnRH and gonadotropin secretion. Augmentation in the hypothalamic KiSS1 (encoding kisspeptin) and GPR54 gene expression have been noted in different stages of puberty, at the time of ovulation and seasonal breeding and in a variety of mammalian species including primates. Recently peptide 234, a compound reported with the ability to block kisspeptin action *in vivo* and *in vitro*, has provided impetus for physiological studies of kisspeptin action. Central administration of the peptide 234 has been recently shown to inhibit HPG-axis/block LH surge in a number of species. Present study was therefore carried out to assess effect of peripheral administration of peptide 234 on testosterone release in the adult male rhesus monkey. A set of five chair-restraint habituated intact adult male rhesus monkeys under normal conditions, was employed for in vivo administration of peptide 234. Sequential blood samples were obtained at 15min interval. Plasma was separated and assayed for testosterone, adiponectin and GH levels. Systemic administration of p234 did not affect the basal plasma testosterone release but the decrease was observed in pulse frequency and amplitude. It also delayed

kisspeptin dependent testosterone release. No effect was observed on plasma growth hormone release but decreased the plasma adiponectin levels for short period of time (60min) and kisspeptin dependent adiponectin release was inhibited.

PO-115

Track: *Industrial and Manufacturing: bio-fuels; energy crops (cellulosic ethanol industry); industrial enzymes; bioprocess engineering and optimization*

GENETIC EVOLUTION OF THE 5S RIBOSOMAL DNA IN THE GENUS *PAEONIA*

Sung Geun Park[1], Yan-Lin Sun[1], Dong Wang[1] and Soon-Kwan Hong

[1]Department of Bio-Health Technology, Kangwon National University, Chuncheon, Kangwon, 200-701, Korea and [2]Institute of Bioscience and Biotechnology, Kangwon National University, Chuncheon, Kangwon, 200-701, Korea; E-mail: soonkwan@kangwon.ac.kr

The ribosomal RNA 5S gene is considered to be one of the most variable regions commonly used in the identification of herbal medicinal materials. Due to its high variability and discrimination ability, the 5S region has been show to successfully identify many medicinal species. To clarify the evolutionary dynamics of ribosomal RNA genes in the genus *Paeonia* (Paeoniaceae), we investigated the 5S rDNA genes through phylogenetic analyses using the 5S rDNA combined with the nontranscribed spacer of 5S rDNA (5S-NTS) and the internal transcribed spacer between 18S and 26S rDNAs (ITS1 and ITS2). The 5S-NTS and rDNA ITS1 and ITS2 have been used globally as molecular markers for resolving species-level phylogenetic relationships. In this study, we performed three *Paeonia* species (*P. lactiflora*, *P. obovata*, and *P. suffruticosa*) and four *Paeonia* accessions in all, collected from different regions of Korea. Based on sequence analyses of 5S and ITS rDNA gene, the obtained results of phylogenetic trees were similar to each other. Among these four accessions, *P. lactiflora* and *P. obovata* belonging to the same section *Paeonia*, did not show predicted, more closed evolution compared with *P. lactiflora* and *P. suffruticosa*. These results were not congruent with previous hypothesis of the clear phylogeny between section *Paeonia* and section *Moutan* of genus *Paeonia*. To further understand the phylogenetic relationships of the genus *Paeonia* and the subsections, more samplings and more available DNA marker sequence sources were required to investigate.

[Following are results of a study on the "Human Resource Development Center for Economic Region Leading Industry" Project, supported by the Ministry of Education, Science & Technology(MEST) and the Natinal Research Foundation of Korea(NRF).]

Keywords: *Paeonia*, rDNA ITS, 5S-NTS, genetic divergence, phylogeny.

PO-103

Track: *Medical Biotechnology: stem cells; gene therapy; tissue engineering; biopharmaceutical manufacturing; cell based therapy; cell cultivation; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers.*

ESTABLISHMENT OF *IN VITRO* M CELL MODEL AND EVALUATION OF GENETICALLY MODIFIED BACTERIA AS VACCINE DELIVERY VEHICLES TARGETING M CELLS

Kazuya Masuda and Shizunobu Igimi

Biomedical Food Research, National Institute of Health Sciences, Japan; E-mail: igimi@nihs.go.jp

It is generally accepted that M cells, which are located in the follicle-associated epithelium (FAE) of Peyer's patch, play a major role in the uptake of luminal antigens. Therefore, it is expected that development of an antigen delivery vehicle targeted to M cells will lead to efficient induction of intestinal immune responses against vaccine strains. It is necessary that an evaluation system for the interaction of antigen delivery vehicles and M cells. Therefore, developing an in vitro evaluation system for interaction with M cells is highly desirable. This study developed an *in vitro* human M cell model and evaluated recombinant bacteria as delivery vehicles targeting M cells using the M cell model we developed.

To evaluate the function of M cell targeted genetically modified (GM) bacteria using this human M cell model, *Escherichia coli* and *Lactobacillus casei* IGM393 expressing Yersinia Invasin, which is an invasion factor to M cell, were generated. The difference in results obtained with GM *E. coli* and GM *L. casei* in the M cell model suggests that the M cell model shows different reactions to objective bacteria.

Keywords: M cell model, vaccine, GM bacteria.

PO-43

Track: *Business development: strategic alliances; partnering trends; product opportunities; growth; business models and strategies; licensing; merger and acquisitions; outsourcing; venture capital and financing; intellectual property*

THE SCIENTIFIC CULTURE OF BIOTECHNOLOGY: A STUDY FROM THE PERSPECTIVE OF ACTOR NETWORK THEORY

Ana Silvia Rocha Ipiranga and João Paulo Da Silva Costa

Curso de Mestrado Acadêmico em Administração, Universidade Estadual do Ceará, Brazil; E-mail: anasilviaipi@uol.com.br

Considering the approach Actor - Network Theory (ANT) that originate d from the need for a new social theory adapted to the study of Science and Technology, this research aimed to understand the reality of scientific studies, describing the practices of biotechnology culture science in the context of relations between academia (universities and research centers), industrial science (business and market) and government. Based on the ethnographic method of research, interviews and observations were performed in a laboratory of Northeast Biotechnology Network (RENORBIO) located in Brazilian Northeastern. The results led to a peculiarity of the biotechnology scientific culture to highlight an event complex, heterogeneous and contingent (technical, scientific, institutional, discursive, cultural), pointing to the emergence of a set of scientific practices built by a network of actors. However, it was found that in the context of RENORBIO this flow of information has not happening in a broad and diversified over the network of actors, not only in the laboratory, but also and above all of that with the market, which should have intended to approximate the R & D performed in the laboratories of companies and potential customers. In this sense, the results raised questions about the predominance of a linear model of technological development in the context of network actors RENORBIO, highlighting the gap between those doing research and development (R & D) and those that promote innovation (companies and other research institutes and innovation).

Keywords: Actor - Network Theory; Scientific Culture; Biotechnology.

PO-155

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

EFFECT OF NEEMARIN ON SOME PHYSIOLOGICAL ASPECTS OF THE COMMON PISTACHIO PSYLLA, AGONOSCENA PISTACIAE UNDER LABORATORY CONDITIONS

R. Sadeghi, H. Izadi and K. Mahdian

Department of Plant Protection, Vali-e-Asr University of Rafsanjan, Iran; E-mail: izadi@vru.ac.ir

The common pistachio psylla, Agonoscena pistaciae Burckhardt and Lauterer (Hem: Psyllidae) is known as the key pest of pistachio orchards in Iran. In this study, effect of neemarin (a toxic extract from neem plant) was investigated by measuring glycogen, glucose, lipid and protein contents of the 5th instar nymph of A. pistaciae. The 5th instar nymphs dipped in insecticide for 3s. Treated nymphs allowed to air dry and then located on the pistachio leaves in controlled conditions (27±2 oC, 60±5 RH and 16L:8D). The effect was studied in 24, 48, 72 hours after treatment. Low molecular weight carbohydrate (glucose) as well as glycogen and lipid contents were measured by methods described by Warburg and Yuval (1996). Protein content of whole body was measured by Lowery method (Lowery et al., 1951). Glycogen content of treated nymphs 48 after treatment (11.603 mg/g fresh body weight) and 72 hours after treatment (8.324 mg/g fresh body weight) was significantly lower than control (21.061 and 41.733 mg/g fresh body weight).but no significant difference was observed between protein and lipid contents of treated nymphs and control ($P>0.05$). Glucose content in nymphs treated with neemarin 72 after treatment (35.011 mg/g fresh body weight) was significantly different from control (62.506 mg/g fresh body weight). This study revealed that neemarin is able to significantly decrease carbohydrate level of treated 5th instar nymphs in relation to control.

Keywords: Physiology, neemarin, Agonoscena pistaciae.

PO-156

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

EFFECT OF OVER WINTERING ON THE ADULT INSECTS OF COMMON PISTACIAE PSYLLA AGONOSCENA PISTACIAE

R. Sadeghi, H. Izadi and K. Mahdian

Department of Plant Protection, Vali-e-Asr University of Rafsanjan, Iran; E-mail: izadi@vru.ac.ir

The common pistachio psylla, Agonoscena pistaciae Burckhardt and Lauterer (Hem: Psyllidae) is known as the key pest of pistachio orchards in Iran. This pest passes the winter as adult insects under the lo ose bark on the trunks of pistachio trees, soil and leaves. In this study, effect of overwintering was investigated by measuring food reserves changes of the adult insects of A. pistaciae. Overwintering adult insects of A. pistaciae were collected from infested pistachio garden in Rafsanjan from October to March 2011. Low molecular weight carbohydrate (glucose) as well as glycogen and lipid contents were measured by methods described by Warburg and Yuval (1996). Protein content of whole body was measured by Lowery method (Lowery et al., 1951). No significant difference in protein and lipid contents of adult insect was observed during overwintering, but glycogen content in October was at highest level and fell to lowest level in January with decrease in environment temperature. Decrease in glycogen content was proportional to increase in total carbohydrate content. In October, total carbohydrate content was at lowest level and rose to highest level in December. Our results indicated adverse relation between glycogen and total carbohydrate contents. The carbohydrate content changes during overwintering period with a concomitant decrease in glycogen content. Decrease in ambient temperature in October to January is associated with a remarkable decrease in glycogen and increase in carbohydrate content which most probably acts as cryoprotectant.

Keywords: Physiology, Overwintering, Agonoscena pistaciae.

PO-138

Track: *Medical Biotechnology: stem cells; gene therapy; tissue engineering; biopharmaceutical manufacturing; cell based therapy; cell cultivation; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers*

A NEW RFID MIDDLEWARE FOR BIOINFORMATIC APPLICATIONS

Reza Javidan and Farahnaz Vahdati

Department of Computer Engineering and IT, Shiraz University of Technology, Shiraz, Iran;
E-mail: Reza.Javidan@gmail.com

Bioinformatics is the application of computer engineering and information technology to the field of biology and medicine. Today RFID technology as a part of bioinformatics is used in medical applications for tracking and automated data entry for aiding in the elimination of medical mistakes. Middleware usually is computer software that connects software components or data and their applications especially in distributed networks. RFID middleware, however, is a kind of application software which facilitates data communication between automatic identification equipments such as RFID readers and enterprise applications. Medical RFID data, on the other hand, contains false readings and duplicates. Such data cannot be used directly by applications unless they are filtered and cleaned. To compensate for the inherent unreliability of RFID data streams, most RFID middleware systems employ a "smoothing filtering". In this paper, a new RFID middleware is proposed based on a kind of smoothing filtering which is based on the concept of saving the status of tag's readings. The proposed method for middleware uses some rules for solving false positives and false negatives to provide accurate RFID data for medical applications. The simulation results on prototype data show the effectiveness of the proposed method.

Keywords: RIFD, bioinformatics, middleware, data redundancy, smoothing filter.

PO-79

Track: *Plant and Environment*

ASSESSING PROTOZOAN ABILITY ON THE BIOREMEDIATION OF HEAVY METALS IN THE INDUSTRIAL WASTEWATER COLLECTED FROM WITBANK, SOUTH AFRICA

Momba MNB and Ilunga Kamika

Environmental, Water & Earth Sciences, Tshwane University Of Technology, South Africa; Email: alainkamika2@yahoo.com

In the course of the past century, the ever-growing population, industrialisation and urbanisation have resulted in a world-wide increase in environmental pollution. The environmental damage caused by chemical pollutants such as heavy metals has become a global issue due to their effect on humans and their persistence in wastewater during the treatment processes. Moreover, anthropogenic resources are putting pressure on the larger resource base (water, soil, air) that supports our life. In this study, the heavy metal removal efficiency of two protozoan species (Peranema sp. and Trachelophyllum sp.) was determined and compared to the one of Pseudomonas putida, which is repeatedly reported to be a heavy-metal tolerant bacterial species. Specific variables such as the chemical oxygen demand, pH and the dissolved oxygen and the microbial growth/die-off were measured using standard methods. Heavy metal removal was determined in both biomass and supernatant by the Inductively Couple Plasma Optical Emission Spectrometer (ICP-OES). Results revealed that the industrial wastewater sample was highly polluted with heavy metal concentrations exceeding by far the maximum limit of 0.05 mg-Co/ℓ, 0.2 mg-Ni/ℓ, 0.1 mg-Mn/ℓ, 0.1 mg-V/ℓ, 0.01 mg-Pb/ℓ, 0.01 mg-Cu/ℓ, 0.1 mg-Zn/ℓ and 0.005 mg-Cd/ℓ, prescribed by the UN-FAO and the National Water Act of South Africa. Although the inhibitory effect of heavy metals to microbial isolates, a significant heavy metal removal was revealed after 5 days of incubation. When compared to Pseudomonas putida heavy-metal removal efficiency (Co-71%, Ni-57%, Mn-45%, V-83%, Pb-96%, Cu-49%, Zn-25%, Cd-24%), the result revealed Peranema sp. with the highest removal efficiency of both Zn (45%) and Cd (42%) and Trachelophyllum sp. as the most sensitive isolate with the lowest heavy metal-removal efficiency (Co_19%, Ni-27%, Mn-33%, V-32%, Pb-47%, Cu-41%, Zn-27%, Cd-38%) for all the heavy metals in the industrial wastewater. But when compared to Peranema sp. alone, Trachelophyllum sp. showed higher removal of Ni (27%), Mn (33%), V (32%) and Cu (41%). Considering the above, the results highlighted the role of protozoan isolates in the bioremediation of heavy metal and also the importance of microbial interaction in the bioremediation process.

Keywords: Industrial wastewater, heavy metal, bioremediation, bacteria, protozoa.

PO-139

Track: *Medical Biotechnology*

SIMULTANEOUS AND RAPID DETECTION OF BACILLUS ANTHRACIS, SALMONELLA TYPHI AND YERSINIA PESTIS BY MULTIPLEX PCR

Karami Ali, Fateme Pourali

Research Center of Molecular Biology, Baqiyatallah Medical Science University, Tehran-Iran; P.O.Box: 19945- 581; E-mail: karami@bmsu.ac.ir

Background: Rapid detection of biological agents are prime importance in countering the emerging infectious disease and bioterrorism events.

Methods: We have tested Bacillus anthracis Vaccine strain and Yesrinia pestis from recombinant clone containing F1 gene for Vaccine purposes but for salmonella typhi we used DNA extracted directly from strain obtailed from reference laboratory that is isolated from clinical sample.

Results: We report here for the first time the development of a rapid PCR method for simultaneous detection of the Bacillus anthracis, salmonella typhi and Yersinia pestis with Multiplex mixture of 6 specific primer sets designed and tested specifically for these agents.

These methods may provide a rapid tool for the simultaneous detection and identification of the three category A bacterial species listed as biological threats and can be used in reference laboratories, clinical and diagnostic labs and specially for filed analysis of samples or contaminated letters in mobile labs.

Keywords: Simultaneous detection. Rapid Detection, PCR. Bacillus anthracis, Yersinia pestis and Salmonella typhi.

PO-116

Track: *Plant and Environment*

FILD EVALUATION OF SALINITY TOLERANCE BASED ON MORPHOLOGICAL AND BIOCHEMICAL TRAITS IN MOST EXTREME VARIETIES OF IRANIAN BREAD WHEAT

M. Aram Kasmayie[1], G. Mohammadi-Nejad[2], A. Baghizadeh[3], M. Mansouri[4], B. Nakhoda[5], G. Hosseini Salekdeh[6]

Department of Plant Breeding, Kerman Graduate University of Technology, Iran; E-mail: m_aram_k@gmail.com

In order to evaluate the Na^+, K^+ and Mg^{2+} absorbtion and their accumulation in different part of extreme wheat cultivares as well as their agronomic performance, six different extreme wheat cultivars were grown at research field of Shahid Bahonar University of Kerman (N 56°: 58′, E30°:15′ and 1755 MASL) on growing seasons of 2010-2011. Ion distribution patterns was asseced by measuring of Na^+, K^+ and Mg^{2+} concentration by ICP(Inductively Coupled Plasma- optical emission spectrometer) during the Anthesis and Flowering stages, subsequently Na^+/K^+ ratio as well as their translocation from roots to spikelet and flag leaf was calculated in 10ds / m^{1-} condition. Analysis of variance showed significant effects for all biochemical and morphological traits. Sensitive plants accumulated more Na^+ and less K^+ in their leaf tissue than the tolerant plants. Dry matter showed the lowest amount in Moghan3 (standard sensitive). According to correlation analysis negative relation was seen between Na^+ with dry matter, grain yield and yield components. Stepwise regression analysis showed the maximum grain yield in different genotypes can be attributed to the characteristics such as transferring of potassium from root to flag leaf and the amount of K^+ in spikelets. Salinity affects plant growth by the osmotic stress of the salt around the roots as well as toxicity caused by excessive accumulation of salt and ions in leaves and spikelet. Finally it is determined there is significant genetic variation in tolerance to osmotic stress that can be useful in improving the salinity tolerance of crop plants.

Keywords: Bread wheat, Field evaluation, Ion distribution, Salinity stress.

PO-11

Track: *Others - Pharmacogenetics*

THE IMPACT OF 3 GLUCOCORTICOID RECEPTOR GENE POLYMORPHISMS ON RISK OF RELAPSE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IN IRANIAN POPULATION

Iman Karimzadeh, Soha Namazi, Soheila Zareifar, Ahmad Monabati and Shahla Ansari

Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran; E-mail: karimzadehiman@yahoo.com

Objectives: To evaluate the possible association between 3 prominent glucocorticoid receptor gene polymorphisms including BclI, N363S, and ER22/23EK and risk of relapse in children with acute lymphoblastic leukemia (ALL).

Methods: We conducted a case-control study on 100 children with ALL aged 0-15 years including 50 non-relapsed and 50 relapsed subjects. Genotyping of BclI, ER22/23EK, and N363S polymorphisms was carried out by polymerase chain reaction restriction fragment-length polymorphism.

Results: Sixty five (65%) patients were wild-type, 31 (31%) heterozygous, and 4 (4%) homozygous carriers of mutant allele of BclI polymorphism. One (1%) subject was the heterozygous carrier of the mutant allele of ER22/23EK polymorphism and 99 (99%) were non-carriers. Four (4%) children were heterozygous for the N363S polymorphism and 96 (96%) were wild-type. No case or control individuals were homozygous for ER22/23EK and N363S polymorphisms. No significant association between studied polymorphisms and risk of relapse was observed ($P > 0.05$). We didn't find any significant association between different genotypes of BclI and N363S polymorphisms and relapse properties including number, site, and time interval from date of initial diagnosis ($P > 0.05$).

Conclusion: Our data suggest that BclI, ER22/23EK, and N363S polymorphisms have no prognostic value for prediction of relapse in childhood ALL.

Keywords: Acute lymphoblastic leukemia, Childhood, Glucocorticoid receptor gene, Polymorphism, Relapse.

PO-25

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology; Bioethics*

DEGRADABILITY AND ALLERGENIC POTENTIAL OF KUNITZ-TYPE PROTEASE INHIBITORS IN TRANSGENIC POTATOES EXPRESSING A STRUCTURAL HOMOLOGUE FROM TOMATO

Moustafa Khalf, Ismaïl Fliss and Dominique Michaud

Université Laval, Faculté d'agriculture et d'alimentation, Institut des nutraceutiques et des aliments fonctionnels (INAF), Pavillon des services, 2440, Boulevard Hochelaga, Québec (Québec) G1V 0A6, Canada ; E-mail: moustafa.khalf@fsaa.ulaval.ca

We reported earlier that potatoes expressing a Kunitz-type cathepsin D inhibitor (CDI) from tomato were substantially equivalent to untransformed or transgenic comparators not expressing the inhibitor. Here we assessed the impact of tomato CDI expression on the degradability of endogenous -and potentially allergenic- Kunitz-type protein homologues in raw and processed potatoes, using in vitro digestion assays with simulated human gastric and human intestinal fluids. Unlike the major storage protein patatin but similar to most allergenic proteins, the Kunitz inhibitors were resistant to simulated digestive fluids or heat treatment but highly sensitive to the gastric fluid after heat treatment. Likewise, potato protein extracts strongly triggered splenocyte proliferation and accumulation of cytokines such as interferon gamma and interleukin-4 *in vitro*, but significantly weaker inductions were observed after simulated digestion. Despite a robust IgG response in *Balb/c* mice ingesting the potato proteins, no IgE antibody response was observed, thereby suggesting a type-1 immune response *in vitro*. Overall, these data suggest a high digestibility of endogenous Kunitz inhibitors in cooked or processed potatoes, a limited allergenic potential of these proteins in processed potato tubers, and a null impact of CDI expression on both their digestive stability and eventual allergenicity in CDI-expressing potatoes.

Keywords: Cathepsin D inhibitor, simulated human gastric, human intestinal fluid, splenocyte proliferation, cytokines, transgenic potatoes.

PO-90

Track: *Other Areas: Clinical Research/Clinical Trials*

ENZYME LINKED IMMUNOSORBENT ASSAY OF CORONAVIRUS ANTIGEN IN DIARRHEIC CALVES OF HIGH AND AVERAGE PRODUCING HOLSTEIN DAIRY COWS

Badiei Khalil, Pourjafar Mehrdad, Ghane Mohsen

Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran, Postal Code 71345, P.O. Box 1731, Shiraz, Iran; E-mail: badiei33@gmail.com

Diarrhea in newborn farm animals, especially calves under 30 days of age is one of the most familiar types of disease complexes that the large-animal clinician encounters in practice. Over a one-year period (from January to December 2009), 661 fecal samples from natural cases of diarrheic calves were taken by veterinary stuff in Department of Large Animal Internal Medicine of Shiraz Veterinary School and veterinary practitioners in Fars province (Iran). The samples were taken from the 267 diarrheic calves of high (HPDCs) and 394 diarrheic calves of average producing Holstein dairy cows (APDCs). Fecal samples were collected and submitted for the laboratory diagnosis of coronavirus antigens. Herd selection was based on geographical location and density of cattle in the region. All herds had HPDC and APDC coronavirus infected diarrheic calves in their population. Diarrheic coronavirus infected HPDC calves in northern region of Fars province were at much lower risk of diarrhea than APDC calves ($P<0.05$). The rate of coronavirus infection in diarrheic APDC calves in northern region was highest when compared to other geographical locations. When considering the effect of age, diarrheic coronavirus affected APDC Holstein calves of younger dams (>2 to 3years) showed a higher rate of infection when compared to diarrheic HPDC coronavirus infected ones ($P<0.05$). The proportion of infected coronavirus diarrheic HPDC and APDC calves decreased with increasing the parity of their dams. There was no difference among the occurrence of coronavirus infection in diarrheic HPDC and APDC calves of different herd size groups.

Keywords: Coronavirus, diarrheic calves, dairy cows.

PO-140

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology; Bioethics*

EVALUATING DIFFERENT THERMAL IMAGING TREATMENTS TO CLASSIFY HEALTH AND FUNGAL INFECTION PISTACHIO BY COMPARE MEAN ANOVA

K. Kheiralipour[a*], H. Ahmadi[a], A. Rajabipour[a], S. Rafiee[a], M. Javan-Nikkhah[b]

Mechanical Engineering of Agricultural Machinery Dept, University of Tehran, Karaj, Iran;
E-mail: kamrankheiralipour@gmail.com

Human health is extremely affected by food safety evenly more than food type, quality and value. Thermal imaging (TI) is a new beneficial technique used for inspectional aims in agriculture and also in food safety. In this research, different conditions of thermal imaging were investigated to detect fungal infection in pistachio kernel. In this regard, pistachio thermograms were captured from pseudo-image stream given by a thermal imaging camera. The different treatments to obtain thermograms of health and manually infected pistachios by Aspergillus flavus were included heating temperature (2 levels), heating times (3 levels), cooling times (3 levels) and emissivity (2 levels). The required features were extracted using MATLAB 2009[A] software and were compared using ANOVA in SPSS 18 environment. The analysis result the best treatment with 60˚ as heater temperature, 90 s as heating time, 10 s as cooling time, and 0.95 as emissivity to inspect fungal infection of pistachio kernel.

Keywords: Thermal imaging; Pistachio kernel; fungal infection; Heating; Cooling; Emissivity.

PO-13

Track: *Industrial and Manufacturing*

EFFECTS OF COMPRESSIVE FORCES AND HARVEST TIME ON THE EXTRACTION OF JUICE FROM SWEET SORGHUM AND SWEET PEARL MILLET FOR BIOETHANOL PRODUCTION

Marianne Crépeau, Mohamed Khelifi, Anne Vanasse, Philippe Seguin and Gaëtan Tremblay

Université Laval, Faculty of Agriculture and Food Sciences, Department of Soil Science and Agri-Food Engineering, Pavillon Paul-Comtois, 2425, rue de l'Agriculture, Local 2305, Québec (Québec) G1V 0A6, Canada;
E-mail: Mohamed.Khelifi@fsaa.ulaval.ca

Bioethanol is an interesting alternative to fossil fuel. Many studies have been carried out on the use of sweet sorghum for biofuel production since its stems contain a large amount of fermentable sugars. Few research studies indicate that sweet pearl millet is also a good source of fermentable sugars. Both crops can grow on marginal soils. While the juice would be fermented, pressing residues of both crops could be used as forage for cattle feeding. The main objective of this research study was to investigate the effects of three compressive forces on the juice extraction and its sugar content. Also, the impact of the harvest time was explored. A specific screw press was used to extract the juice. Results showed that the increase of compressive forces positively affected the extraction of juice, especially for sweet pearl millet. The average rates of extracted juice were 0.475 and 0.595 litre per kilogram of biomass for sweet pearl millet and sweet sorghum, respectively. Also, the harvest time of the day did not have any effect on the juice extraction. However, pressing in PM was found to be better in terms of sugar content of the extracted juice.

PO-12

Track: *Industrial and Manufacturing*

MECHANICAL RELEASE OF INSECT PREDATORS FOR THE BIOLOGICAL CONTROL OF THE COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA* (SAY)

Saad Almady and Mohamed Khelifi

Université Laval, Faculty of Agriculture and Food Sciences, Department of Soil Science and Agri-Food Engineering, Pavillon Paul-Comtois, 2425, rue de l'Agriculture, Local 2305, Québec (Québec) G1V 0A6, Canada;
E-mail: Mohamed.Khelifi@fsaa.ulaval.ca

The Colorado potato beetle (CPB) is the major insect pest of potato crops in North America, Europe, and Asia. Large amounts of chemical insecticides are used to control this pest. However, heavy reliance on these chemicals can result on serious health and environmental problems. Also, the CPB has developed over the years a resistance to most of the registered chemical insecticides, including those that were very effective at one time. The biological control of the CPB using its natural enemies is a promising alternative to chemical insecticides. The use of the predator *Perillus bioculatus* to control the CPB has been successful at small scale. However, hand release of this natural enemy at large scale is not realistic. The main objective of this research study was to test a mechanical distributor designed to release predators in potato fields. In this distributor, masses of predators are placed in small containers and mixed with a carrier material. In the field, the containers are mechanically opened at different locations, based on a source-point mass release option. The success of this release system with *Perillus bioculatus* in potato crops could be generalized to other predator insects to protect other crops such as strawberry and lettuce.

PO-102

Track: *Medical Biotechnology: stem cells; gene therapy; tissue engineering; biopharmaceutical manufacturing; cell based therapy; cell cultivation; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers.*

CAT FERTILIZATION BY MOUSE SPERM INJECTION

Yong-Xun Jin, Xiang-Shun Cui and Nam-Hyung Kim

Department of Animal Science, Chungbuk National University, Korea; E-mail: nhkim@chungbuk.ac.kr

Interspecies intracytoplasmic sperm injection has been carried out to understand species specific differences in oocyte environments and sperm components during fertilization. While sperm aster organization during cat fertilization requires a paternally derived centriole, mouse and hamster fertilization occur within the maternal centrosomal components. To address the questions of where sperm aster assembly occurs and whether complete fertilization is achieved in cat oocytes by interspecies sperm, we studied the fertilization processes of cat oocytes following the injection of cat, mouse, or hamster sperm. Male and female pronuclear formations were not different in the cat oocytes at 6 h following cat, mouse or hamster sperm injection. Microtubule asters were seen in all oocytes following intracytoplasmic injection of cat, mouse or hamster sperm. Immunocytochemical staining with a histone H3-m2K9 antibody revealed that mouse sperm chromatin is incorporated normally with cat egg chromatin, and that the cat eggs fertilized with mouse sperm enter metaphase and become normal two cell stage embryos. These results suggest that sperm aster formation is maternally dependent, and that fertilization processes and cleavage occur in a non-species specific manner in cat oocytes.

Keywords: Fertilization, ICSI, sperm, mammal.

PO-72

Track: *Industrial and Manufacturing: bio-fuels; energy crops (cellulosic ethanol industry); industrial enzymes; bioprocess engineering and optimization*

CLONING AND EXPRESSION OF CELLULASE GENE FROM *MONOCHAMUS SALTUARIUS*

Hyuk-Min Kwan[1], Hyun-Jun Ko[1] and Yong Chul Park[1,2]

Hyun-Jun Ko

[1]Department of Bio-Health Technology, Kangwon National University, Chuncheon, Kangwon, 200-701, Korea; [2]Institute of Bioscience and Biotechnology, Kangwon National University, Chuncheon, Kangwon, 200-701, Korea; E-mail: ycpark@kangwon.ac.kr

Yong Chul Park

Cellulose is the most abundant carbohydrate polymer in nature. Plants produce cellulose of 10^{11}kg every year and it is one of the most commonly used food source by organisms including insects. Most insects using cellulose for their food can digest the compound with the help of symbiotic microorganisms which produce cellulolytic enzymes called cellulase. Because cellulose is composed with β-1, 4-glucosidic bond between each D-glucose so it is too solid to be decomposed by heat or polar solvent. However, recent works confirmed that some insects produce cellulase for their digestion. There are four types of currently known cellulase genes of originated by insect; Glycosyl Hydrolase Families (GHFs): GHFs1, GHFs5, GHFs9,

GHFs45. This study is performed for confirming the existence of endogenous cellulase in *Monochamus saltuarius* and analysing its characteristics.

To determine the presence of cellulase in *Monochamus saltuarius* we carried out Native-PAGE, containing the Carboxy-Methyl-Cellulose(CMC) which is a substrate of cellulase about digestive juice of larva and adult *Monochamus saltuarius*. Then we confirmed 3 bands of cellulase genes both larva and adult. We determined that 628bp of cellulose nucleotides by performing Gradient-PCR. The full sequence of GHF5, 978bp nucleotides and 326 amino acids was analysed as a result of Race-PCR and Nested-PCR with partial cDNA. Homology analysis of the deduced polypeptide sequence with long-horn beetle families showed correspondence of 93.2% with A*priona germari* cellulaseIII and 92.9% with *Psacothea hilaris*. Expression of the cellulase by the transformed *Escherichia coli* in SDS-PAGE was as expected. To make sure that there is expressional distinction among the tissues we peformed Semiquantitive-PCR and Western-blot and we can confirmed the only expressed tissue is gut. From these results we found that cellulase is related to disgestion.

[Following are results of a study on the "Human Resource Development Center for Economic Region Leading Industry" Project, supported by the Ministry of Education, Science & Technology(MEST) and the National Research Foundation of Korea(NRF).]

Keywords: *Monochamus saltuarius*, MsGHF5, insect endogenous cellulose.

PO-123

Track: *Plant and Environment*

THE DEVELOPMENT OF HIGH-THROUGHPUT SCREENING SYSTEM BASED ON DEFENSE RELATED GENE EXPRESSION TO SELECT THE PLANT ACTIVATORS IN ARABIDOPSIS THALIANA

Masahiro Kusama, Nobuaki Urata, Go Banzashi, Rieko Ogura, Shin-ichi Ogata and Kazuyuki Hiratsuka

Graduate School of Environment and Information Sciences, Japan; E-mail: kusama-masahiro-tz@ynu.ac.jp

Plant activators (PAs) are a group of pesticides that can induce plant immunity to pathogens by activating plant defense systems. They are considered to be low environmental impact pesticides, because unlike conventional fungicides PAs induce disease resistance in plants without antibiotic activity, In addition, PA-mediated defense induction is also effective for control of viral diseases. Although PAs are considered to be ideal agro-chemicals for plant disease management, the selection of compounds based on induction of plant defense systems requires tedious and time-consuming steps such as the analysis of plant defense related gene expression. To overcome the problem, we developed a high-throughput screening (HTS) system using luciferase reporter assay and 96-multiwell plates. As a result of estimating the reporter activity of several defense related gene promoters in transgenic Arabidopsis seedlings, we found that the Pathogenesis Related protein 1a promoter from tobacco BY-2 and the Vegetative Storage Protein 1 promoter from Arabidopsis thaliana, show clear induction of Fluc activity in response to treatment with chemicals. To improve the in planta system for the HTS of PAs, we are currently introducing the dual-color luciferase assay system using click beetle luciferase reporter genes.

Keywords: Arabidopsis thaliana, plant activator, high-throughput screening, luciferase.

PO-68

Track: *Industrial and Manufacturing: bio-fuels; energy crops (cellulosic ethanol industry); industrial enzymes; bioprocess engineering and optimization.*

A MODULATION OF THE BIVALENT METAL INTRACELLULAR ISOTOPY TO STIMULATE EXPRESSION OF HUMAN RECOMBINANT PROTEINS IN INDUSTRIAL CELL CULTURES

Anatoly Buchachenko and Dmitry Kuznetsov

Department of Medical Nanobiotechnologies, IsoBioTech (in association with Pirogov Russian State Medical University), Moscow, Russia; E-mail: isobiotech@googlegroups.com

Special cell culture additives were developed in a way to design solutions of water soluble cation exchanging nanoparticles (NPs) loaded with bivalent ions of paramagnetic isotopes ($67Zn2^{+}$, $25Mg2^{+}$, $43Ca2^{+}$). This additives allow to stimulate the expression of various human recombinant proteins (e.g. IFN-β, erythropoietin, moAb, hEGF, somatropin, etc.) in prokaryotic and eukaryotic cells (*E. coli, Saccharomyces cerevisiae,* CHO, SF-9, plasmatic-myeloid hybridoma, etc.). NPs stands for the 2.0 - 2.4nm particles consisting of heavily acetylated oligoheterocyclic and/or oligoaromatic adducts of fullerne-C60 which were found to be water-soluble and metabolically stable with a marked membranotropic properties. Being nanocationites as well, these novel types of NPs provide the efficient cross-cell wall transfer of NP-metal complexes inside the cell cultures. Thus, once the NPs loaded with paramagnetic ions saturate the cell culture sample, the NP-Me complexes shift the intracellular magnetic metal abundance point from its natural level to the increased one inside the cell. This shift, in turn, leads to a substantial increase (e.g. ＞60% for hEGF) in the recombinant protein production level, compared to the production levels achieved in the water control samples or in samples saturated by either non-magnetic ions or natural metal ions manifested under the same conditions. This novel technology offers an opportunity to increase efficiency (reducing cost, accelerating proteins expression) of a wide range of biotechnological manufacturing processes.

Keywords: Magnetic isotope effect, proteins expression, cost efficiency.

PO-80

Track: *Other areas: Food, Bio-safety*

NOVELL MICROFLUIDIC SYSTEM TO INVESTIGATE ADHESION AND BIOFILM FORMATION OF MICROORGANISMS ON Ta-C COATED SURFACES

S. Friedrich, F. Lenk, T. Bley and E. Boschke

Institute of Food Technology and Bioprocess Engineering, Dresden University of Technology, Bergstraße 120, 01069 Dresden, Germany; E-mail: felix.lenk@tu-dresden.de

Biofouling is one of the major risk factors in the food processing industry with respect to product safety and the resulting health threat to consumers. In particular, contaminants from biofil ms being in contact with the raw materials and products pose threats to processed food. Thus, food producers undertake big efforts to eliminate biological fouling including strategies to prevent adhesion of contaminants as well as efficient cleaning and disinfection routines to shorten shut-down periods.

In this context various surface systems are under development with respect to their anti-adhesive properties like ta-C coatings. With their diamond like hardness and so called “easy to clean” properties they are more and more applied as wear protection coatings for components and tools.

In the present study, a newly developed flow cell was established to compare bacterial biofilm formation and adhesion on these innovative surfaces to conventional materials. Therefore, growth experiments were performed in this flow system, where especially the substrate and the design of the fluidic layer is variable adaptable. The laminar flow contributes to highly controlled conditions for biofilm growth and allows getting information about biofouling tendency of the studied ta-C coatings.

It was possible to monitor dynamic, living biofilms in-situ in the flow chamber under the fluorescence microscope. With image analysis software the biomass was calculated from the acquired biofilm images to show the differences between biofilm growth on coated and uncoated surfaces.

PO-82

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

MOSQUITOCIDAL EFFECT OF THREE PLANT SPECIES AGAINST THE CHIKUNGUNYA VECTOR, *AEDES AEGYPTI* (DIPTERA: CULICIDAE)

K. Logankumar and D. Rajmohan

PG and Research Department of Zoology, Kongunadu Arts and Science College, Coimbatore – 641 029, Tamilnadu, India; E-mail: klogankumar@yahoo.com

Mortality values of egg, larvae and pupae treated with different concentrations of the crude and partially purified extract of three experimental plants (*Tridax procumbens, Annona squamosa* and *Tagates erecta)* at the end of 24 hrs of exposure of Chikungunya vector, *Aedes aegypti* were recorded. Based on the probit analysis, the LC_{50} values and 95% upper and lower fiducial limit and chi- square value of the crude and partially purified plant extracts on *Aedes aegypti* were also noticed. The malformation particularly on larval- pupal intermediates, half- ecdysed adult and partially melanized cuticle of egg and larva were observed. The effective response of mosquito in terms of malformations and mortality to partially purified extracts of these three plants exhibited that the mosquito, *Aedes aegypti* is highly susceptible to the active principles present in these three plants. Embryonic eggs were taken as a source to determine the β-N-acetylglucosaminidase enzyme activity, during different hours of development. The present work shows that egg, developmental stages and ovary of *Aedes aegypti* showed significant changes in total protein level under the treatment of plant extract. The tested doses of extracts of experimental plants exhibited detectable effects on total protein in *Aedes aegypti*. Many of the defensive components of plants are biodegradable with non-residual effects on the biological environment. Hence, an attempt has been made in the present investigation to identify plants with potential to control vector mosquitoes.

Keywords: *Aedes aegypti, Tridax procumbens, Annona squamosa, Tagates erecta,* LC_{50}.

PO-137

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

THE EXPRESSION OF CHITINASE AND RIP ENHANCED RESISTANCE TO *RHIZOCTONIA SOLANI* IN TRANSGENIC POTATO (*SOLANUM TUBEROSUM* L.)

M. M'hamdi, N. Boughalleb, T. Bettaieib, H. Chikh-Rouhou and J.I. Ruiz de Galarreta

Higher Institute of Agronomy- Chott Mariem, Biological Sciences and Protection of Plants Department. 4042 Sousse. Tunisia; Email: mhamdimahmoud@yahoo.fr

Potato (*Solanum tuberosum* L.) is sensitive to many fungal pathogens including *Rhizoctonia solani*. In the present study, the potato cultivar Desirée was transformed via *Agrobacterium tumefaciens* strain LBA4404 containing the plasmid pBIN19 which harbor the Ribosome Inactivating Protein (*rip30* gene) and strain GV3101 containing the binary plasmid pGJ132 which harbor a double genes the chitinase (*chiA*) and *rip30*. The potato leaf disc was used as an explant for transformation. PCR, Southern blot and Western blot were used for characterization of the transgenic plants. In this study it was shown that the regenerating callus, the developed shoots and the percentage of transgenic plants were influenced by the type of plasmid vector used for the transformation. Not all the plants developed in selective medium were positive for the corresponding gene using the PCR technique. Southern blot analysis confirmed that transgenic plants integrated 2-3 copies of *rip30* and *chiA* genes into their genome. Greenhouse assay was carried out to evaluate the resistance to the pathogen *R. solani* of transgenic clones expressing the transgenes. Transgenic potato plants expressing *rip30* gene showed only partial resistance to *R. solani*. However higher levels of resistance were improved when *rip30* was associated to *chiA*.

Keywords: Chitinase, ribosome inactivating protein, potato, *Rhizoctonia solani*, fungal disease, resistance.

PO-42

Track: *Business development: strategic alliances; partnering trends; product opportunities; growth; business models and strategies; licensing; merger and acquisitions; outsourcing; venture capital and financing; intellectual property*

THE ROLE OF BIOTECHNOLOGY NETWORKS IN THE PRODUCTION OF INNOVATIONS: A CASE STUDY OF RENORBIO

Diego de Queiroz Machado and Ana Silvia Rocha Ipiranga

Curso de Mestrado Acadêmico em Administração, Universidade Estadual do Ceará, Brazil;
E-mail: diegoqueirozm@yahoo.com.br

With the growth of the biotechnology industry in Brazil, the role of research and innovation networks that act as support for the development of this sector have been gaining momentum. Therefore, seeking to understand how relationships are developed between the agents within this performance dynamic in networks, as well as the major research areas and

markets to which their efforts drive innovation is essential for significant progress in this industry. Thus, the Northeast Biotechnology Network (RENORBIO), which involves 30 institutions in Northeastern Brazil, was chosen for this study because of its importance at a national level. In its few years of operation, RENORBIO has produced a significant number of patents (more than 140 by mid-2011). For this purpose, we used data obtained from surveys and desk research of research project reports, patents and other institutional documents. These data were analyzed with the aid of UCINET software (version 6.2) and NETDRAW (version 2.0). Finally, it was possible to characterize the relationships between the research groups, scientific laboratories, the member institutions of the network and to identify the key research areas and market focus of their innovations.

Keywords: Biotechnology; Innovation; Networks.

PO-24

Track: *Plant and Environment*

DEGRADATION OF HIGH LIPID-CONTAINING WASTE FROM GREASE TRAP BY USING YEAST COCULTURE

Hironori Maeda, Hiroo Uchiyama, Nobuhiko Nomura, Toshiaki Kambe

Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan; E-mail: koutoku.m@gmail.com

Grease trap system is installed in commercial kitchens for collecting waste oil. This waste oil and scum after collection, in disposed off at high cost and energy. Microbial treatment of the edible oil is a cheap source for the management of waste oil. However, reported strains do not always show their high lipid-degrading ability owing to changes in temperature and pH. Some kind of strains lead to high fatty acid accumulation after degradation. In this study, two yeasts strains, Meyerozyma guilliermondii TY-89 and Rhodotorula mucilaginosa TY-92 have been isolated from flowers (rose and dantelion), capable of degrading edible oil. TY-89 has the capability to degrade fatty acid, whereas TY-92 has high lipase activity. Both the strains have higher rate of lipid-degradation in the form of consortium. The TLC analysis of degradation products revealed that no fatty acid remained after degradation. Both the strains were actively degrading lipid at wide temperature and pH range. The results indicated that both the yeast strains can be applied for treatment of waste oil under various environmental conditions.

PO-135

Track: *Medical Biotechnology: stem cells; gene therapy; tissue engineering; biopharmaceutical manufacturing; cell based therapy; cell cultivation; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers*

SERODIAGNOSIS BY IMMUNOBLOTTING IN THE LEISHMANIA- HIV CO-INFECTION AT THE LABORATORY OF PARASITOLOGY. CHU ANNABA

R. Mansouri, F. Bachi, Z. Boudiaf and B. Hamrioui

Laboratoire de Parasitologie-Mycologie, Hôpital Ibn Sina CHU d'Annaba, Algeria;
E-mail: r.mansouri@facmed-annaba.com

Introduction

The occurrence of visceral leishmaniasis during HIV requires the most sensitive and specific immunological techniques for detection antibodies. The western blot, immunoblot technique satisfies these two criteria and reveals the presence of antibodies directed against a large number of antigenic fractions of *Leishmania* including 14 and 16 kDa antigen The diagnosis of visceral leishmaniasis is conventionally done by immunofluorescence or ELISA but these techniques are not sufficient during the immunosuppression. In this case, the Western blot finds its indication.

Objective

Improve the diagnosis of visceral leishmaniasis during HIV by immunoblot in the laboratory of Parasitology - Mycology, Hospital of Annaba.

Materials and Methods

Among 36 patients with HIV Leishmania serology is requested in front of a clinical presentation with predominance of fever. It was made by indirect immunofluorescence, ELISA and Western blot.

Results

The Western blot has corrected the diagnosis in 14 patients revealing specific bands of Leishmania, 14 kDa 16 kDa, 30 kDa 46 kDa and 90 kDa. The diagnosis of visceral leishmaniasis is based on the characterization of three groups of antibodies corresponding to antigens of molecular weight between 14 and 16 kDa, 30 and 46 kDa and 90 kDa.But the presence of the band of 14 kDa and/or 16 is sufficient to confirm the diagnosis.

Conclusion

The remarkable specificity and sensitivity of the immunoblot led us to propose this technique for the diagnosis of visceral leishmaniasis in immunocompromised patients.

PO-157

Track: *Others - Clinical Research*

ISOENZYME CHARACTERIZATION OF LEISHMANIA STRAINS RESPONSIBLE FOR CUTANEOUS AND VISCERAL LEISHMANIASIS IN THE AREA OF ANNABA, NORTH EAST ALGERIA.

R. Mansouri, F. Pratlong, F. Bachi, B. Hamrioui and J.P. Dedet

Laboratoire de Parasitologie-Mycologie, Hôpital Ibn Sina CHU d'Annaba, Algeria; E-mail: r.mansouri@facmed-annaba.com

INTRODUCTION: Leishmaniases are endemic prevalent in Algeria. Accurate identification of parasitic species involved allows specifying the clinico-epidemiological data and the geographic distribution of each of the forms produced. The area of Annaba is explored for the first time during this study.

MATERIALS AND METHODS: Cutaneous and visceral strains isolated in culture on NNN medium are identified by the reference method of electrophoresis of isoenzymes.

RESULTS: The isoenzymatic characterization revealed the presence of three species of Leishmania, *L. infantum*, *L.major* and L.killicki, responsible of the chronic cutaneous leishmaniasis. In addition, it is a new zymodeme in the taxonomy of Leishmania, L. killicki MON-306.

CONCLUSION: The results obtained show the interest to isolate and identify different strains of Leishmania circulating in an endemic focus.

PO-91

Track: *Plant and Environment*

CHANGES OF SEED STORAGE PROTEINS AND PROTEIN PROFILE IN CHICKPEA (*CICER ARIETINUM* L.) VARIETIES UNDER DROUGHT STRESS AND N FERTILIZER

M. Shaban[2], C. Mansourifar[1], M. Ghobadi[1] and M. Lak[3]

Department of Crop Production and Plant Breeding, School of Agriculture, Razi University, Kermanshah, Iran; E-mail: cyrusamf@yahoo.com

Chickpea is an important crop in the cropping pattern supplying cheap protein diet especially for poor people. Over the years, however, low yields are more prominent declining acceptability of this crop. this study was planned to examine effect of drought stress and N fertilizer on protein content and protein banding pattern of chickpea cultivars. The experiment was laid out in a split-factorial design with drought stress in main plots and cultivar with nitrogen fertilizer in subplots with three replications.

The experimental treatments consisted of three levels of drought stress [sever drought stress (S2), moderate drought stress (S1) and no drought stress(S0)] and four cultivars of chickpea (*Cicer arietinum* L.) , Azad, Bivanij, Hashem and

ILC482 and 2 N levels. Plants were either not given any N fertilizer (0N), or supplied with N fertilizer at the rate of 25 kg ha-1 (25N).

The results showed that the effects of drought stress on seed storage proteins and protein yield, effect of cultivars on protein yield were significant($P<0.01$). With increase drought stress seed storage proteins was increased and protein yield decreased. Also, results showed that No effects treatments (Drought stress and nitrogen fertilizer) on protein banding patterns. Also, results indicated that not obvious any new band and not deleted any bands.

Keywords: Chickpea, drought, electrophoresis, nitrogen and protein.

PO-151

Track: *Pharmaceutical Biotechnology: biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering*

MOROCCAN PLANTS ESSENTIAL OILS AS POTENTIAL INHIBITORS OF EFFLUX PUMPS IMPLICATED IN BACTERIA RESISTANCE

Fadli Mariam

Université Cadi Ayyad, Marrakech, Morocco. E-mail: fadlimariam@yahoo.fr

Bacterial drug resistance is a worrying problem of public health. Antibiotic efflux is the major non-specific resistance mechanism used by bacteria and efflux pumps are involved in the low level susceptibility of various important Gram-negative pathogens. The use of molecules that can block bacterial pumps is an attractive strategy, but several researches report only a partial efficacy due to limits of these molecules (stability, selectivity, bioavailability, toxicity...).

Our objective is to search natural sources of molecules able to inhibit efflux pump systems of resistant Gram-negative bacteria (*Escherichia coli*, Enterobacter aerogenes, Klebsiella pneumoniae, Salmonella enterica Typhimurium and Pseudomonas aeruginosa). The results indicate that the studied essential oils exhibit an interesting activity against the tested bacteria. This activity is significantly enhanced in the presence of efflux pump inhibitor such as phenylalanine arginyl β-naphthylamide. The role of lipopolysaccharide (LPS) structure in the effect of essential oils was also reported in Salmonella LPS deep rough mutants. In addition, essential oils of Thymus maroccanus and Thymus broussonetii, used at a low concentration (a fraction of the Mimimum Inhibitory Concentration), are able to significantly increase chloramphenicol susceptibility of several resistant isolates. These results demonstrate that these essential oils can alter the efflux pump activity, and may be attractive candidates to develop new drugs for chemosensitizing multidrug resistant strains to clinically used antibiotics.

PO-46

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology; Bioethics*

DETECTION OF GENETIC VARIATION IN MOZAMBICAN COWPEA LANDRACES

Célia Marilia Martins, Orlando a. Quilambo, Karl J. Kunert

Department of Plant Sciences, University of Pretoria, Mozambique; E-mail: celiabio@yahoo.com.br

Characterization of genetic variation within natural populations and among breeding lines is crucial for effective conservation and exploitation of genetic resources for crop improvement programs. The objective of this study was to evaluate if already existing simple sequence repeats (SSRs), which have been previously used in cowpea genetic diversity studies, are applicable to differentiate Mozambican cowpea landraces. The potential of the SSR technique was further compared to morphological (seed weight and color) and biochemical characteristics (protein and amino acids content). From the eleven SSRs evaluate, primer pairs VM68 and VM70 showed a high level of polymorphism between the four landraces and these primers could differentiate between these landraces using SSR sequencing. Selected morphological and biochemical characteristics were less suitable for differentiation. Only slight differences were found between landraces in total protein content, ranging from 22.5 to 24.3%. Overall, results have shown that cowpea landraces can be differentiated by application of phenotypic and genetic characteristics, such as SSRs, but with genetic characterization superior to phenotypic characterization.

Keywords: Cowpea, genetic characterization, landraces, phenotypic characterization, SSRs.

PO-69

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

DEGRADATION OF PBSA BY PBSA DEPOLYMERASE FROM LEPTOTHRIX SP.TB-71, AND ANALYSIS OF DEGRADATION PRODUCTS

Yui Matsumoto, Hiroo Uchiyama, Nobuhiko Nomura and Toshiaki Kambe

Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan; E-mail: chris_6221@ybb.ne.jp

Plastics cause severe environmental problems in terms of waste after disposal. Therefore, the use of biodegradable plastics is the only solution for the existing problem of waste. To evade the mass production and volume consumption, recycling of biodegradable plastics is required. Some novel processing methods using specific microbial enzymes for plastics depolymerization and monomer recovery can be beneficial. In our previous study,PBSA depolymerase production and purification from Lepothrix sp.TB-71 has already been reported.The enzyme has been tested against different polyesters and was found active against PBSA,PES,PCL whether no active was observed PBS,PLA and PHBV. In this study ,degradation of PBSA by PBSA depolymerase and analysis of products of degradation by LC-MS ,have been carried out ,keeping in view of the future biochemical monomer recycling.

Keywords: PBSA , Leptothrix sp TB-71, PBSA depolymerase , LC-MS analysis.

PO-88

Track: *Other areas: Clinical Research/clinical trials*

PHYLOGENETIC ANALYSIS OF TRYPANOSOMA EVANSI IN IRANIAN CAMELUS DROMEDARIUS

Pourjafar Mehrdad, Badiei Khalil, Sharifiyazdi Hassan, Chalmeh Aliasghar, Naghib Mojtaba, Babazadeh Dezfouli Marzieh, Moutabi Alavi Amir

Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran, Postal Code 71345, P.O. Box 1731, Shiraz, Iran; E-mail: dmp4m@yahoo.com

Trypanosma evansi is the most widespread pathogenic trypanosome in the world. Genetic characterization of phylogenetic classification of the members of *Trypanosoma* spp. based on ITS sequence have not been studied in Iran so the present study was devoted, for the first time, to the molecular identification of *T. evansi* derived from naturally infected camels in Iran. In November 2010, whole blood samples were collected from 117 male clinically healthy *Camelus dromedaries* aged between 6-month to 18-year old from several farms in Yazd province of Iran. Affected camels were detected by Giemsa stained blood smears and the positive blood samples (4 out of 117) were submitted to PCR examination and phylogenetic analysis for *Trypanosoma evansi*. BLAST data of the obtained complete internal transcribed spacer (ITS) sequences revealed that they were corresponded to those of *T. evansi*, Thailand cattle isolate (AY912276) with the homology of 99%. Both trees of ITS1 and complete ITS showed low bootstrap values among clades that were significantly unable to discriminate the genetic diversity of these regions in *T. evansi* isolates. The phylogenetic tree inferred from the ITS2 nucleotide sequences (569 bp) clearly showed the genetic diversity of the parasites. According to the tree, two main groups of ITS2 region in *T. evansi* of Iranian camel isolates were separate with bootstrap value 61%. In contrast to ITS1 and ITS2 regions, multiple alignment of the nucleotide sequence of the 5.8S rRNA showed a high degree of sequence conservation during evolution in varies *Trypanosoma spp*.

Keywords: Phylogenetic analysis, Trypanosoma evansi, Camelus dromedarius, Iran.

PO-143

Track: *Plant and Environment*

MOLECULAR CHARACTERIZATION OF CUMIN (*CUMINUM CYMINUM* L.) BASED ON MICROSATELLITE MARKERS

Alireza Bahraminejad[1], Ghasem Mohammadi-Nejad[*2] and Mihdzar Abdul Kadir[3]

Horticultural research Institute, Shahid Bahonar University of Kerman, P.O.B. 76169-133 Kerman –Iran; E-mail: Mohmmadinejad@uk.ac.ir

Cumin (*Cuminum cyminum* L.) is from the family Apiaceae, and second most popular spice in the world. In this study SSR markers were used for investigating of genetic variation of forty nine cumin ecotypes which they are sub-populations belonged to nine provinces of Iran. Polymorphism information content (PIC) of thirteen pairs of primers was varied between 0.18 - 0.37. The highest amount of PIC (0.37) indicated by Elap017, Elap1340, and the lowest was belonged to the primer Elap040, Elap1493, Elap1479 (0.18). Dendrogram obtained from cluster analysis delineated all cumin populations into three clusters at the level of similarity (0.71); the first group was Semnan and Southern_ Khorasan populations and the second includes: Pars, Kerman, Northern_Khorasan, Khorasan_Razavi, Esfahan, Golestan and the third class was containing Yazd population. Also high variation was found among the ecotypes within the populations. It is concluded that the high variation of Iranian germplasms showed their worth in breeding strategies.

Keywords: cumin, genetic variation, SSR.

PO-158

Track: *Industrial and Manufacturing*

THE IDENTIFICATION AND SUBCLONING OF THE ALKB GENE OF A BACTERIAL CONSORTIUM DEGRADING DIESEL FUEL FROM SOIL OF RIBEIRAO PRETO, SAO PAULO, BRAZIL

Silvana Pompeia do Val de Moraes, Eliamar Aparecida Nascimbem Pedrinho, Douglas Antonio Alvaredo Paixao and Eliana Gertrudes de Macedo Lemos

Universidade Estadual Paulista – UNESP (FCAV), Via de Acesso Prof. Paulo Donato Castellane Zona Rural, Jaboticabal - São Paulo – Brasil. Postal Code 14884-900; E-mail: valmoraes-silvana@gmail.com

The alkB gene has been reported as one of those responsible for the bioconversion of hydrocarbons, and microorganisms are the main agents of biodegradation of contaminants. An alternative would be environmentally feasible to increase the speed of degradation, using recombinant microorganisms able to act quickly on this bioconversion. Currently, the genome projects and several surveys that generate biological data are increasing exponentially each year. The bioinformatics tools tend to solve impossible problems to be addressed in the past decades. The location of the gene, characterization and analysis of proteins encoded by it was based on the use of data from GeneBank, PROSITE, CDART, CDD, InterPro, SWISS-PROT, TOPPRED2 and ProtParam program, which provides the biochemical characteristics of proteins and Blast-P (Basic Local Alignment Search Toll), which allows the verification of the similarity between the protein of interest and its orthologs. The present study allowed the identification of organisms which, isolated or associated in consortium, could potentially be explored in biotechnological processes for bioremediation which involve the degradation of hydrocarbons. Although no new group of microorganisms was identified in the processes for decomposing hydrocarbon residue, it was possible to quantify, in sampling terms, the taxons which were represented in more significant numbers.

PO-49

Track: *Medical Biotechnology: stem cells; gene therapy; tissue engineering; biopharmaceutical manufacturing; cell based therapy; cell cultivation; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers*

COLORECTAL CANCER: PERIPHERAL BLOOD mRNA SIGNATURE CAN DISCRIMINATE CANCER PATIENTS FROM COLONOSCOPY-NEGATIVE PATIENTS

B. Mougin, Y. Xu, Q.H. Xu, F. Liu, F. Wu, X. Ye, X. Meng, X. Du and S.J. Cai

bioMérieux, Chemin de l'Orme, 69280 Marcy l'Etoile, France;
E-mail: bruno.mougin@biomerieux.com

Background: Colorectal cancer (CRC) is the third most prevalent cancer worldwide. Screening among medium-risk people is a widely adopted strategy. However, screening programs based on detection of faecal occult blood or colonoscopy are difficult to implement, mainly due to low compliance and logistics issues. Searching biomarkers in peripheral blood using gene expression analysis with microarray, we have identified a candidate signature composed of 7 genes, and now are

confirming our results using quantitative real-time polymerase chain reaction (qRT-PCR).

Materials and Methods: We have analysed peripheral blood samples from 152 CRC patients and 153 Colonoscopy Negative Controls (CNC) collected in PAXgene^{TM} tubes. qRT-PCR experiments were performed on ABI 7900 using SYBR detection, for 7 genes previously identified using Affymetrix GeneChip® U133Plus2.0 array, and 3 selected reference genes.

Results: For the 7 selected genes, qRT-PCR experiments confirmed our previous microarray results (correlation of fold change values). The performances of the 7 gene-signature assessed using 152 CRC and 153 CNC showed 84.6% accuracy, 80.9% sensitivity and 88.2% specificity.

Conclusion: We have identified a combination of 7 genes for which measurement of their expression in peripheral blood, using a simple PCR-based method, represents a novel approach for early detection of colorectal cancer.

PO-159

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

LEAF N, P, MG AND K CONCENTRATIONS OF GROUNDNUT VARIETIES AS INFLUENCED BY PLANT POPULATION AND BASIN SIZES UNDER IRRIGATED CONDITIONS

A.A. Mukhtar, B. Tanimu, H. Mani, S. Ibrahim and C.P. Shinggu

Department of Agronomy, Ahmadu Bello University, Zaria, Nigeria; E-mail: yayaaishang95@gmail.com

Plant analysis has been used as a method for determining the relative quantities of mineral elements in plants. In order to measure the leaf N, P, K and Mg of groundnuts grown under irrigation, an experiment was conducted during 2006 dry season at the Irrigation substation of the Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria. The results showed that there was no significant influence of the plant populations used on the leaf N, P, K and Mg concentration although Leaf N concentration was slightly higher at 100,000 plants ha-1 than at other populations. The leaf P, Mg and K concentrations were slightly and non-significantly higher at 50,000-plant ha-1. Basin size did not have significant effect on leaf concentrations of N, P, K and Mg. It was observed that leaf N concentration was high in the 3m x 3m basin while leaf P Mg and K concentrations were higher in the 3m x 5m basin than others. However, among the varieties used, Samnut 21 and 11 had significantly higher leaf Mg concentration than Samnut 23.

Keywords: Leaf concentration, nitrogen, phosphorus, plant populations

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PO-2

Track: *Plant and Environment: Transgenic Plants and Crops; Bioremediation; Microbial Diversity; Bio-monitoring*

COMPLEX APPROACH FOR BIOREMEDIATION AND RECULTIVATION OF CONTAMINATED SOILS

Olga Muter, Baiba Limane, Katrina Potapova, Solvita Stelmahere and Andrejs Grinbergs

Institute of Microbiology & Biotechnology, 4, Kronvalda Blvd., LV1586, Riga, Latvia; E-mail: olga.muter@inbox.lv

Technological solutions on soil remediation embrace a wide spectrum of approaches in various combinations to achieve the most efficient biodegradation result and to prevent further contaminant dissemination. Our research is focused on the biodegradation of hydrocarbons and nitroaromatics in soil and water.

Prior to soil treatment, specific conditions of the contaminated site are evaluated, e.g. soil type, C/N ratio, moisture, climatic conditions, level of contamination, toxicity status etc.

The complex approach for in situ soil bioremediation in our study includes the following activities: 1) Bioaugmentation. Collection of microorganisms-degrades isolated from contaminated soils was tested under laboratory, pilot scale and field conditions. Our results demonstrated that addition of specific biodegradative bacterial strains to soil resulted in an enhanced biodegradation activity as well altered microbial community structure. The metabolic versatility in soils demonstrated the ability of soil microorganisms to tolerate the pressure caused by contamination (1-2).

2) Biostimulation. Nutrient amendments added to contaminated soil can considerably stimulate the process of biodegradation by autohtonous or/and allohtonous microbial communities (3). However, in some cases this activity can pose additional ecological problems, e.g. an increase of the contaminant mobility and it's leaching through the deeper layers of soil.

3) Phytoremediation. In the bioremediation experiments, higher plants, e.g. ryegrass and clover, noticeably stimulated microbial activity in contaminated soil (2,4).

4) Application of surfactants. Most of the organic contaminants are hydrophobic. Two surfactant formulations synthesized from different plant oil and yeasts were shown to be efficient and biodegradable by soil microorganisms. Combination of different methodical approaches provides a tool for monitoring of their degradability under various environmental conditions (5).

Summarizing our experience and current results obtained in the field of soil remediation, it can be concluded that the efficiency of soil remediation depends on the proper characterization of contaminated site prior to the treatment, as well as optimal combination of remediation methods discussed above.

Keywords: Bioaugmentation, biostimulation, phytoremediation, soil bioremediation, surfactants.

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PO-125

Track: *Medical Biotechnology*

RESPONSE OF T REGULATORY CELLS TO THE IMMUNIZATION WITH SOLUBLE EGG ANTIGEN IN MURINE SCHISTOSOMIASIS*MANSONI*

Eman El-Ahwany[1], Faten Nagy[1], Ibrahim Rabie[2], Rabab Zalat[2], Ola Mahmoud[3],Suher Zada[4]

Theodor Bilharz ResearchInstitute. P.O.BOX: 30 Imbaba, Imbaba Giza, Egypt; E-mail:fatennagy@hotmail.com

Objectives: In acute and chronic schistosomiasis, survival of the host requires a carefully balanced immune response against highly immunogenic parasite eggs. The aim of the study is to characterize the phenotype of $CD4^+$ $CD25^+$ T regulatory cells within the liver granulomas of both infected and immunized groups in order to characterize their function and association with both Foxp-3 gene expression and the levels of the different splenic cytokine in the regulation of egg-induced immunopathology.

Material and Methods: Naïve C57BL/6 mice were intravenously injected with multiple doses of the Soluble Egg Antigen (SEA) 7 days before cercarial infection. The immunized and infected control groups were sacrificed 8 and 16 weeks post infection (p.i.). Histopathological studies, parasitological parameters, splenic phenotype for T regulatory cells, the FOXP3 expression in hepatic granuloma using real time PCR and the associated splenic cytokines were assessed.

Results: The percentage of T regulatory cells ($CD4^+$ $CD25^+$) was increased significantly ($p<0.01$) in the immunized group compared to the infected control at 8 and 16 weeks p.i.The FOXP3 expression in hepatic granuloma was increased from 10 at 8 weeks to 30 fold at 16 weeks p.i. in the infected control group. However, its expression in the immunized group showed increase from 30 at 8 weeks to 70 fold at 16 weeks p.i. in immunized group.

Conclusions: The magnitude and phenotype of the egg-induced effecton T helper response were found to be controlled by a parallel response within the T regulatory population which provides protection in worm parasite-induced immuopathology.

PO-112

Track: *Industrial and Manufacturing*

ONESIA DENITRIFICANS BN13 AN ACTINOMYCETE ISOLATED FROM ALGERIAN SOIL

Boucherba Nawel, Benallaoua Said , Copinet Estelle, Hebal Hakim and Duchiron Francis

Department of Microbiology, University of béjaia, Algeria; E-mail: boucherbanawel@yahoo.fr

Fifty strains were isolated from samples collected from Algerian soil Among these, 20 isolates were found positive for xylanase production, the strain BN13 showed very high capacity in producing xylanase (10.81 U/ml),. Physiological and biochemical tests were done using the substrate panel for Jonesia identification, the growth temperature (4,10, 15, 20, 25, 30, 35, 40, 45, 50°C), pH values (4, 5, 6, 7, 8, 9, 10, 11, 12) and salt tolerance at 2, 5, 7.5, 10, 12, 15, 17.5% (w/v) NaCl were determined. This isolate was motile, rod-shaped, catalase-positive and aerobic.Optimal growth temperature was 37°C; optimal pH was 7;optimal salt concentration was 2-12 (w/v) NaCl. From the analysis of the almost-complete 16 S rRNA gene sequence, this strain was found to be similar to Jonesia denitrificans X83811 (99.6% sequence similarity).According to the biochemical criteria and comparison of 16s r RNA gene sequence, the strain BN 13 was identified as strain of Jonesia denitrificans and named Jonesia denitrificans BN 13.

Keywords: Jonesia denitrificans, identification, xylanase.

PO-17

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology; Bioethics*

ISOLATION AND IDENTIFICATION OF FUNGI FROM RAISINS VARIETIES IN KHORASANE RAZAVI

Mahboobe Sarabi Jamab, Marzieh Hosseini Nejad, Masoomeh Mehraban Sangatash, Fakhri Shahidi, Ahmad Reza Bahrami, Seyed Ali Mortazavi and Mohammad Reza Nassiry

Department of Agriculture and Food Science,, Khorasan Research Institute for Food Science and Technology (KRIFST), Mashhad, Iran; E-mail: hosseinynejad@yahoo.com

Raisins are dried grapes that may be eaten raw or used in cooking, baking and brewing. Iran is one of the major exporters of raisins in recent years and Iranian raisins are being exported to many countries for decades. It is important to ensure of microbiological quality and safety of raisins. The aim of this study was isolation and identification of mycoflora from main raisin's varieties (Poloie, Pikami & Teiphi) produced in Khorasan Razavi Province. Four types of culture medium (Yeast Extract Glucose Chloramphenicol Agar (YGC), Potato Dextrose Agar (PDA), Czapek Dox Agar (CA) and Dichloran Glycerol Agar (DG18)) were used for isolation. The highest contamination was found in Teiphi raisin samples. For Fungi Identification, Macroscopic and Microscopic features were investigated. The Result showed that frequently isolated fungi were *Aspergillus* and *Penicillium* species. Research on identification of mycoflora using molecular methods are under going.

Keywords: Raisin, Fungi, Isolation, Identification, Khorasan Razavi.

PO-33

Track: *Industrial and Manufacturing*

PRODUCTION OF METALLOPROTEASE IN SOLID STATE FERMENTATION AND PARTIAL CHARACTERIZATION

Youssef Ali Abou Hamin Neto and Hamilton Cabral

Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil;
E-mail: youssef@fcfrp.usp.br

Peptidases are enzymes that can be produced through fermentation process by microorganisms and have great importance in industrial field, as pharmaceutical, detergents and bioremediation. Changes in some parameters such as time, nitrogen source and temperature can influence the rate of production of these enzymes. This work evaluate the influence of these parameters in the production of peptidases by the fungus Eupenicillium javanicum submitted to solid state fermentation (SSF), and partial biochemistry characterization of crude enzymatic extract, aiming for a possible industrial application of this enzyme. Our studies have indicated that the best conditions for the production of peptidases were 10% of nitrogen source (albumin) and 90% of agro-industrial waste (wheat bran), when incubated at 30°C, with peak production at 72 hours. The enzymatic biochemistry characterization shown that the enzyme is a metalloprotease, with optimum pH in 5,5 and optimum temperature in 60°C. The enzymatic stability decreases when it is submitted to high temperatures and alkaline pHs.

Keywords: Metalloprotease, solid state fermentation, Eupenicillium javanicum.

PO-18

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology; Bioethics*

EFFECT OF INULIN EXTRACTED FROM CHICORY AND JERUSALEM ARTICHOKE ON THE SURVIVAL OF *LACTOBACILLUS CASEI* AND *LB. RHAMNOSUS* UNDER ACIDIC CONDITIONS

S. Kamali, M. Hosseini Nezhad and M. Elahi

Department of Agriculture and Food Science,, Khorasan Research Institute for Food Science and Technology (KRIFST), Mashhad, Iran; E-mail: hosseinynejad@yahoo.com

Probiotics are microbial food supplements that their consumption in specific amounts can lead to maintenance the microbial balance of gastrointestinal tract and health benefits thereof. Probiotics should be acid tolerant to survive through intestinal lumen and reach the colon to exert their effects. Therefore, some attempts like addition of prebiotics are made to improve their stress resistance and may result in suitable symbiotic pairs. In this study in vitro effects of inulin extracts from *Cichorium intybus* and *Helianthus tuberosus* was investigated on growth and viability of two probiotic strains, *Lactobacillus casei* and *Lactobacillus rhamnosus* at different pH values (2.5 , 4 and 6.2) compared to glucose. Our results indicated that the inulin fructans, from different sources having various characteristics like reducing sugar, total ash content and degree of polymerization significantly extended the survival and viability of lactobacilli strains under acidic conditions.

PO-86

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology; Bioethics*

ERYTHROCYTE TOXICITIES OF GERMABEN II®

Halla Noureddine, Boucherit Kebir, Boucherit Zahia and Seddiki Sidi Mohammed Lahbib

Department of Biology, Faculty of Sciences; Abou bekr Belkaïd University of Tlemcen, Algeria;
E-mail: halla.nour@yahoo.fr

The addition of antimicrobial preservatives to cosmetic and toiletry products is necessary to prevent microbial growth. However, the use of preservatives can also produce other undesirable effects. For several years, researchers have been investigating the use of alternative methods in safety assessment of cosmetic ingredients and formulations by means of variety methods. The aim of this study was to evaluate the erythrocyte toxicities of commercial preservative Germaben II® (GB). Germaben II® is a combination of Propylène Glycol, Diazolidinyl Urea, Methylparaben and Propylparaben. Relatively few studies about the cytotoxicity of this preservative are available. The determination of its cytotoxicity is an essential step to warrant their safe use. The erythrocyte toxicities were evaluated by assessment of the amount of hemoglobin released by red blood cells after their lysis. We tried to determine the toxicity of a range of increasing concentrations with an arbitrary contact time (5 to 120 min). In this study, Germaben II® showed cytotoxic activity against red blood cells. Based on the results of Germaben II®, it appears that lysis of red blood cells increases with the concentrations of preservatives and the cytotoxicity is elevated after 120 minutes compared with the first 60 minutes of incubation. The Germaben II® is recommended to be used in the range of 0.3% to 1.0% in final formulations. These

concentrations induced the release of hemoglobin from red cells to the rate of: 32-66.58% after 120 minutes of incubation.

Keywords: Erythrocyte toxicity, Germaben II®, Preservative, Cosmetic.

PO-100

Track: *Medical Biotechnology*

PRELIMINARY STUDY WITH LABVIEW SOFTWARE TO ASSESS THE STEATOSIS AND SINUSOIDAL CELL DAMAGE FOR SUITABILITY OF THE LIVER FOR TRANSPLANTATION

F. Marinozzi, S. Novelli, M. Rossi and G. Novelli

General Surgery and Organs Transplant, Sapienza University of Rome, Italy; E-mail: vinmorabito@aol.com

Graft viability is of the utmost importance for successful orthotopic liver transplantation(OLT). Steatosis of the donor liver is known to impact on patient and allograft outcome after OLT. Sinusoidal cell damage can be considered another aspect that can influence the success of OLT. Functionally, sinusoidal cell damage leads to plate let trapping, increased vascular resistance, and secondary hepatocyte ischemia reflected by transaminase elevations. The initial sinusoidal injury is not detectable by routine microscopic or clinical tests. Steatosis values may differ depending on which type of test is used, either histologic or the application of labview software. Our first end point was therefore, to evaluate the differences in results of these two methodologies. Our second objective was to evaluate sinusoidal histomorphometric modifications and local hydraulic resistance in the various stages of steatosis.

Materials and Methods:

For this study 15 OLT patients were enrolled. Preperfusion biopsies of liver were obtained in all patients. Each image was then processed with a custom program written utilizing the Vision® toolbox of the Labview® platform following a semi-automated procedure. The portions of each image occupied by sinusoids or hepatocities with steatosis were outlined and the number of pixels of each sinusoid was calculated. Finally, we analyzed the form of sinusoids approximating them to an ellipse in order to be able to define the relationship between the two axes with the aim of proposing a parameter that is proportional to the resistance to the blood flow within the bounds of the hystologic specimen known as "local hydraulic resistance" (LHR).

Results:

In evaluation of steatosis we observed a difference between histological response (steatosis 10%-40%) and Labview software (steatosis 20%-60%) in 12 patients. In these patients the images studied showed a difference in size of sinusoidal areas and LHR. There was evidence of a reduction in the area of the sinusoids with steatosis 20% - 40%, with an average value of 0.0039 mm2. While in patients with steatosis 50% the average area of sinusoids was 0.0022 mm2 with a further reduction in subjects with steatosis grade 60% (mean 0.0019mm2). In six patients showed no correspondence between percentage of stetaosis, sinusoidal cell damage and LHR. In fact at 6 month follow up these six patients with increase of LHR, sinusoidal damage and percentage of steatosis (20%-40%) showed delayed graft function.

Conclusion:

In conclusion, our study leads us to determine the percentage SA/PA and mean SA in sinusoidal hydraulic resistance which could give us great predictability for steatosis evolution. These differences are well worth looking into with larger numbers of patients

Keywords: Sinusoids, steatosis, liver.

PO-97

Track: *Plant and Environment*

YIELD LOSS IN YAM (DIOSCOREA ROTUNDATA) DUE TO INFECTION BY YAM MOSAIC VIRUS (YMV) GENUS POTYVIRUS

B.O. Odu, M.O. Adeniji, T. Ikotun, S.A. Shoyinka, R. Asiedu and J.d'A. Hughes

Crop Production and Protection Department, Obafemi Awolowo University, Nigeria; Email: bodu@oauife.edu.ng

An experiment was conducted to investigate the influence of Yam mosaic virus (YMV) genus Potyvirus on the tuber yield of Dioscorea rotundata based on assessment of comparative performance of inoculated and uninoculated plants of two genotypes (TDr 93-31 and TDr 95-127).

Symptoms of virus infection were evident on inoculated plants especially at 4 weeks after inoculation. Visual virus symptom severity scores had significant ($P<0.0001$) correlations with enzyme-linked immunosorbent assay (ELISA) readings ($r = 0.7$), leaf chlorophyll content ($r = -0.9$), and tuber yields ($r = -0.9$). Stomatal conductance results showed higher values ($P < 0.001$) of diffusive resistance in virus-infected compared to virus-free leaves, indicating slower rates of photosynthesis and transpiration in the former. The leaf area per plant, leaf dry weight, vine dry weight, and tuber dry weight were less ($P< 0.001$) in inoculated compared to uninoculated plants of both genotypes at 10, 18 and 24 weeks after inoculation. Similarly lower values for leaf area index ($P > 0.004$), harvest index ($P < 0.01$), leaf chlorophyll content, and intercepted photosynthetically active radiation ($P = 0.01$) were obtained on plots with inoculated plants.

YMV infection in D. rotundata caused yield loss of 65.4% in TDr 93-31 and 52.6% in TDr 95-127. Reduced capacity for photosynthesis in YMV infected plants, due to increased diffusive resistance of stomata, as well as reduced leaf area and chlorophyll content, contributed significantly ($P < 0.001$) to their reduced tuber yields.

Keywords: Dioscorea rotundata; TAS-ELISA; yield loss; Yam mosaic virus (YMV) genus Potyvirus; White yam

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PO-51

Track: *Plant and Environment*

PIRIFORMOSPORA INDICA - AN ENDOPHYTIC ROOT-COLONIZING FUNGUS WITH MULTIPLE BIOTECHNOLOGICAL APPLICATIONS

Ralf Oelmüller

Department of Plant Physiology, FSU Jena 07743 Jena, Germany; E-mail: b7oera@hotmail.de

Piriformospora indica is an endophytic fungus which colonizes the roots of all plant species tested so far. It promotes growth, seed and biomass production and confers resistance against biotic and abiotic stress. The host range suggests that the interaction is based on general mechanism, which can be targets for biotechnological applications.

We use Arabidopsis as a model plant and have identified genes, mechanisms and components in the plant which are targets of the fungus for improving plant performance. I will discuss several of the identified target genes in the context of agricultural applications.

Keywords: Piriformospora indica, plant performance, grwoth promotion.

PO-113

Track: *Plant and Environment.*

STABLE EXPRESSION OF THE FULL-LENGTH VP2 GENE OF CANINE PARVOVIRUS (CPV) IN NICOTIANA BENTHAMIANA

Sung Oh, Jae Sung Park, Tae-Kyun Oh, Sue Hoon Kim, Sei Chang Kim and Chang Won Choi

Department of Biology, Pai Chai University, Korea, Italy; E-mail: 5star@pcu.ac.kr

The entire virion protein 2 (VP2) gene of Canine parvovirus (CPV) was amplified by PCR, engineered into a binary vector, pPZP212, to be expressed in Nicotiana benthamiana and determined its reaction ability with polyclonal antisera and monoclonal antibody. Initially, the construct was tested for its ability to express VP2 after transient expression by Agrobacterium-infiltration into N. benthamiana leaves. The recombinant VP2 was highly expressed as a 65 kDa band, which was strongly cross-reacted with the polyclonal antiserum against Escherichia coli-produced VP2 recombinant protein. Therefore, N. benthamiana plants were genetically transformed with same Agrobacterium housing a cDNA construct encoding the VP2 protein under the control of CaMV 35S promoter. There is no significant difference in morphological characteristics including shoot and root growth between non-transgenic and transgenic N. benthamiana plants. Genomic DNA and mRNA analyses of the transformed plantlets confirmed that the stable integration of the VP2 cDNA into the N. benthamiana genome, as well as its transcription. The expression of recombinant VP2 was then observed in transgenic plants qualitatively by Western blot and quantitatively by immuno-dot blot analysis. Parenterally

immunized rats with the concentrated plant extract induced a strong immune response and protected rats against challenge infection with CPV.

Keywords: Canine Parvovirus (CPV), recombinant VP2, transient expression, Nicotiana benthamiana, transgenic.

PO-101

Track: *Medical Biotechnology: stem cells; gene therapy; tissue engineering; biopharmaceutical manufacturing; cell based therapy; cell cultivation; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers*

JUSTIFYING THE USE OF THE BLUISH LIQUID FROM THE AFRICAN GIANT SNAIL (ACHATINA MARGINATA) IN TRADITIONAL MALE CIRCUMCISION SURGERY IN WESTERN NIGERIA

S.O. Olagbende-Dada, A.A. Adeniyi, F.O. Adefolaju, A. Adepoju-Bello and M.O. Ologunagba

Department of Pharmacognosy, University of Lagos, Nigeria; E-mail: gbendedada@yahoo.com

In contemporary African tradition, the male child is circumcised. One of the requirements for successfully carrying out this surgical feat by the traditional birth attendants in the western part of Nigeria is to immediately bath the exposed penis surface with the fresh bluish liquid from the African giant snail (Achatina marginata). This study aims at investigating the role(s) that the snail's bluish liquid plays in the operation; is it coagulatory, anti-bacterial or anti-fungal?

The coagulatory effect was assessed through the Prothrombin time (PT) of three categories of people, (a) normal persons, (b) patients on warfarin, (c) hemophilic patients and compared with the PT of calcified tissue thromboplastin (reference) on the same people. The anti-bacterial/anti-fungal effects were studied using three bacteria and three fungi grown on nutrient agar; the inhibitory effect of the bluish liquid on their growth was compared with that of standard antibacterial (gentamicin) and antifungal (clotrimazole) drugs. The studies were carried out using the two known varieties of A. marginata (suturalis and ovum) in order to establish any variation in their effectiveness. Observed results did not reveal any anti-bacterial or anti-fungal property in the bluish liquid, but a stronger (than the reference) coagulatory effect which was also effective on hemophilic blood was revealed in the bluish liquid from the two snail varieties. A. marginata ovum showed higher potency over the suturalis variety. Elemental analysis of the bluish liquid from the two snail varieties were then carried out in order to find explanation for the variation in the observed clotting time. Three elements (calcium, magnesium and zinc) were found in relatively large amount when compared with other detected elements.

This study's result on the prothrombin time justified the use of the snail bluish liquid as a strong blood coagulant useful in preventing excessive blood loss during and after the surgery while the elemental results supported the observed higher potency of the ovum snail variety over the suturalis variety. The result also suggests that the hemophiliacs can benefit from this liquid.

Keywords: Male Circumcision, Blood Clotting, Achatina marginata (African snail), Prothrombin Time, Hemophiliacs.

PO-44

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

STABLE-ISOTOPE PROBING OF PLASTICS FILM FOR INVESTIGATION OF SURFACE MICROBIAL COMMUNITY

Megumi Oshima, Nobuhiko Nomura, Hiroo Uchiyama and Toshiaki Kambe

Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan; E-mail: s1021146@u.tsukuba.ac.jp

Recently, biodegradable plastics that can be degraded into water and carbon dioxide by microbes have attracted attention over the years. There have been many reports regarding the isolation of bacteria and fungi that can degrade these plastics from various environmental sources. However, there is scant finding about microbial community from the surface of biodegradable plastic films. In this study, stable isotope probing of PHB film with 13C in order to explore the PHB degraders from different soil samples.

The DNA Extracted from 13C labeled PHB film was subjected to ultracentrifugation. The resultant DNA was found labeled with 13C. It indicates that most of the microbes present on the surface of film might be PHB degraders. This 13C DNA was analyzed through 16S rRNA gene based T-RFLP and clonal analysis to identify different microbial communities. The results indicated the presence of different microbial communities on the surface of PHB films.

Keywords: Biodegradable plastic, stable-isotope-probing, PHB, T-RFLP.

PO-38

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology; Bioethics*

BIOTIC FOOD CONTAMINANT AND ADMIXTURES: CONTROL AND RESEARCH

Jaroslava Ovesná, Hodek Jan, Pavlátová Lucie, Kučera Ladislav

Crop Research Institute, Drnovská 507, 16106, Prague, Czech Republic; E-mail: ovesna@vurv.cz

Biotic contaminants may represent an obstacle in food and feed production. Efficient control based on comprehensive analytical tools help to prevent introduction of biological agents and admixtures into the food chain.

We will introduce control of non required GMO admixtures in the Czech Republic and analytical approaches and development of alternative assays. Similary we will present tools for monitoring of phytopatagenic fungi. their specification and expression of mycotoxin genes.

Keywords: Detection, GMO, fungi, gene expression, specification.

PO-48

Track: *Industrial and Manufacturing: bio-fuels; energy crops (cellulosic ethanol industry); industrial enzymes; bioprocess engineering and optimization*

GENETIC EVOLUTION OF THE 5S RIBOSOMAL DNA IN DIFFERENT *TOONA SINENSIS* ACCESSIONS IN KOREA

Hak-Bong Lee, Yan-Lin Sun, Dong Wang, Soon-Kwan Hong and Wan-Geun Park

Department of Forest Resources, Kangwon National University, Chuncheon, Kangwon, 200-701, Korea; E-mail: wgpark@kangwon.ac.kr

The ribosomal RNA gene, 5S residing as independent tandem array in a plant genome, is used globally as molecular markers for resolving species-level phylogenetic relationships in combination with the internal transcribed spacer of 45S rRNA gene. Due to its high variability and discrimination ability, the 5S region has been show to successfully identify many medicinal species. To clarify the evolutionary dynamics of ribosomal RNA genes in 16 different *Toona sinensis* accessions in Korea, we investigated and sequenced the 5S rDNA genes through phylogenetic analyses using the 5S rDNA combined with the nontranscribed spacer of 5S rDNA region. Using universal primers, the length of the 5S rRNA varied from 232 bp (CHAM-12) to 408 bp (CHAM-4). Among about 330 bp of commonly existing sequences, a total of 23 variable sites occurred among all the accessions. The nucleotide variation rate reached 6.95% of the whole observed 5S rRNA region. Three monophyletic groups were separated among all the accessions, of which CHAM-2 and CHAM-11 formed one monophyletic group, CHAM-5 and CHAM-16 formed another one, and the others formed the other monophyletic group. CHAM-3, 4, 7, 10 forming a monophyletic subgroup showed relatively high homology of 5S rRNA region sequence with each other, and the similar situation was found among CHAM-6, 8, 12, 13, and 14 forming a monophyletic subgroup. Based on the phylogenetic relationship obtained in this study, the evolution relationship of some accessions was supported by the geographical origin information, however, others did not match with the geographical origin distribution well. This result helped clearly construction of the phylogenetic relationship of *Toona sinensis* species in Korea. However, to further understand the phylogenetic relationships of this species, more samplings and more available DNA marker sequence sources would be required and investigated.

Keywords: *Toona sinensis*, 5S rRNA gene, Phylogeny, Medicinal plant.

PO-67

Track: *Industrial and Manufacturing: bio-fuels; energy crops (cellulosic ethanol industry); industrial enzymes; bioprocess engineering and optimization*

MOLECULAR IDENTIFICATION AND PHYLOGENY ANALYSIS OF PEONY *PAEONIA* SPECIES USING THE *matK* CODING REGION

Sung Geun Park[1], Yan-Lin Sun[1], Dong Wang[1] and Soon-Kwan Hong[1,2]


Sung Geun Park

[1]Department of Bio-Health Technology, Kangwon National University, Chuncheon, Kangwon, 200-701, Korea; [2]Institute of Bioscience and Biotechnology, Kangwon National University, Chuncheon, Kangwon, 200-701, Korea; E-mail: soonkwan@kangwon.ac.kr


Soon-Kwan Hong

As known, the sequence composition of MatK is more variable among different plant species than that of any other chloroplast-encoded protein. Due to its high mutation rate, evolving about three times faster than *rbcL*, and lower structural conservation, *matK* gene sequence has been exploited as phylogenetic marker. *Paeonia* is a phylogenetically and taxonomically complex group, particularly section *Paeonia*. In this study, the chloroplast coding region of the *matK* gene was sequenced to study phylogenetic relationships of four *Paeonia* species. The entire *matK* coding region of *Paeonia* species is identical to be 1494 bp long, and 41 nucleotide substitutions were found among all the peony species, accounting for 2.74% of the full-length *matK* coding region. Among these nucleotide sites with variation, 75.61% (31) of the nucleotide sites were obtained between section *Paeonia* species (CHA, SHAN, CHA1) and section *Moutan* (MO); 12.20% (5) were found between CHA and MO, and SHAN and CHA1; 9.76% (4) were found between SHAN, and other three species; 2.43% (1) were found between CHA, and other three species. Based on these results, we found that even between both different accessions of the same species, *P. lactiflora* (CHA and CHA1), there were also nucleotide substitutions. And more interestingly, these nucleotide substitutions in CHA1 compared to CHA could just be identical to the nucleotide sequence of *P. obovata* (SHAN), indicating that CHA1 investigated in this study might be a hybridization result of *P. lactiflora* (CHA) and *P. obovata* (SHAN). This study not only provided more sequence sources of peony species but would help further understand the phylogenetic relationship of the taxonomically complex *Paeonia* species.

[Following are results of a study on the "Human Resource Development Center for Economic Region Leading Industry" Project, supported by the Ministry of Education, Science & Technology (MEST) and the Natinal Research Foundation of Korea (NRF).]

Keywords: *Paeonia*, DNA barcoding, phylogeny, *matK*.

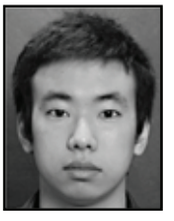
PO-104

Track: *Industrial and Manufacturing: bio-fuels; energy crops (cellulosic ethanol industry); industrial enzymes; bioprocess engineering and optimization*

ENHANCED PLATFORM FOR THE BIODIESEL PRODUCTION FROM CHLAMYDOMONAS REINHARDTII WITH PLANT HORMONES

Won-Kun Park, Gursong Yoo, Chul-Woong Kim, Yoon-E Choi, Ji-Won Yang

Department of Chemical & Biomolecular Engineering, KAIST (Korea Advanced Institute of Science and Technology), Korea; E-mail: pocketgold@kaist.ac.kr



For the production of clean biofuel from microalgae, there have been many trials to design the economical manufacturing system. To achieve that, high biomass productivity and high lipid contents are equally important. But it is hard to get both at the same time. That's because of the inverse relationship between growth rates and lipid contents of a microalga. Usually some spices which have high lipid contents tend to grow slowly and the fast growing species apt to contain little amount of lipid contents.

Among the variety methods to increasing the amount of lipid contents, the most famous engineering method is nitrogen limitation. It gives a stress on the algal cell and makes the cell concentrate on the accumulation of lipid instead of a growth. So, in this study, plant hormone was introduced in the cultivation system which uses the nitrogen deficient

condition (similar with nitrogen limitation) to improve not only the biomass productivity but also lipid contents. When plant hormones were inserted into the modified medium, maximum cell density was increased up to 30% and lipid contents were similar with modified medium. With this additive, we could upgrade the cultivation system to be optimized in high density culture.

Keywords: Chlamydomonas reinhardtii, Plant hormone, biodiesel.

PO-130

Track: *Medical Biotechnology: stem cells; gene therapy; tissue engineering; biopharmaceutical manufacturing; cell based therapy; cell cultivation; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers*

CONTINUOUS INTRAOCULAR PRESSURE MEASUREMENT BY RADIOWAVE TELEMETRY IN A RABBIT MODEL – A PILOT STUDY

Eleftherios Paschalis

Harvard University – MEEI, Cornea Research Group, USA;
E-mail: Eleftherios_Paschalis@meei.harvard.edu

Purpose: To study, by using continuous radio-wave telemetry, the diurnal Intraocular Pressure (IOP) variation, and the lowering IOP effect of two different anti-glaucoma medications (Latanoprost and Dorzolamide) in a rabbit model.

Methods: A micro-electromechanical wireless radio-wave pressure transducer was implanted into a rabbit eye. Continuous measurements were made over a 5-week period. Anti-glaucoma medication was administered for 14 days and its effect was compared to the baseline IOP.

Results: Baseline IOP was 13.73 ± 2.7 mmHg and 12.35 ± 1.8 mmHg for Latanoprost and Dorzolamide groups, respectively ($p>0.05$). Latanoprost and Dorzolamide caused significant reduction in IOP with Latanoprost exhibiting 2 fold greater reduction compared to Dorzolamide (IOP: 12.43 ± 2.18 for Latanoprost; 11.73±1.55 for Dorzolamide; $p < 0.001$ adjusted according to baseline). Absolute IOP reduction was 1.3±3.54 and 0.62±2.2 mmHg for Latanoprost and Dorzolamide, respectively. Circadian IOP variation difference was not significant for both groups ($p>0.15$).

Conclusion: The IOP transducer was efficient in recording the circadian IOP variation during the entire study. Both, Latanoprost and Dorzolamide achieved significant IOP reduction. Animal glaucoma model, enhanced by telemetry IOP acquisition, may help optimizing translational research.

PO-173

Track: *Industrial & Manufacturing*

SELECTION OF EFFICIENT PHB PRODUCING BACTERIA FROM WINE DISTILLERY EFFLUENT AND CLONING OF THEIR PHB SYNTHESIZING GENES IN *E. COLI*

A.S. Patil, N.S. Gangurde, R.Z. Sayyed, Shashi Kiran and A. Gulati

PG Department of Microbiology, S I Patil Arts, G B Patel Science & STSKVS Commerce College, Shahada-425409, Dist. Nandurbar, Maharashtra, India

We reported Poly-β-hydroxybutyrate (PHB) accumulation in the range of 1.35-4.51 gm/L with a recovery yield of 34.35-60.94% (w/w) in six isolates obtained from wine distillery effluents.. Based on by 16S rRNA gene sequencing, biolog characteristics, and FAME analysis these strains were identified as *Alcaligenes faecalis* SM *Alcaligenes faecalis* p.y.g, *Alcaligenes faecalis* p.brown, *Bacillus* sp. AF, *Bacillus cereus* LM2, and *Pseudomonas aeruginosa* p.green. Presence of oxygen and nitrogen limiting conditions enhanced PHB accumulation. Acetone-alcohol proved as suitable method for optimum extraction of PHB.

Significant increase in PHB accumulation was observed by cloning PHB synthesizing genes from efficient isolate into *E.coli.* Recombinant *E.coli* accumulated more PHB vis-à-vis than non-recombinant, *A. faecalis* in LB medium while its level of PHB accumulation was higher in NDMM. The use of recombinant *E. coli* harboring PHB synthesizing genes should make it possible to produce PHB with a high level of economic competitiveness.

FTIR analysis of PHB extracts revealed the presence of functional groups like C-H, =O stretching, =C-H deformation, =C-H, =CH, and =C-O characteristic of PHB. *Pseudomonas aeruginosa* strain p. green came out as potent strain for PHB accumulation.

Keyword: PHB production, 16s rRNA gene sequencing, Phenotypic fingerprinting, FAME analysis, FTIR.

PO-160

Track: *Plant and Environment*

COMPARISON OF MICROBIAL DIVERSITY IN SOILS CULTIVATED WITH SUGAR CANE AND NATIVE FOREST IN THE STATE OF SAO PAULO - BRAZIL

E.A.N. Pedrinho, W.Q. Moreira, L.M.C. Alves, S.P. Val-Moraes and E.G.M. Lemos

FCAV/UNESP, R: Pedro Grotta, 150 Colina Verde , Jaboticabal - São Paulo 148873, Brazil;
E-mail: eliamar.pedrinho@gmail.com

This research is supported by BIOTA, a Brazilian scientific program whose main objective is to recover and study the biodiversity of the State of Sao Paulo. Sugar cane is currently the most important agricultural crop of the State of São Paulo, where the agroclimatic zoning describes the existence of two regions with distinct patterns of environmental conditions suitable for this crop, and a new area recently occupied by this crop. However, with the expansion of agricultural frontiers, greater productivity and sustainability in the production of sugar cane are highly desirable. For this, among other factors, the role of the microbial community present in the soil may have fundamental importance, helping to better development of the plant, is supplying it with nutrients, or reducing the occurrence of diseases and pests. However, little is known about the microbial communities existing in soils cultivated with sugar cane. The metagenomics approach applied to the use of 16S rRNA provides us with a survey of representatives of the two bacterial groups of soil systems in the state of São Paulo: the native forest and soil cultivated with sugar cane. Total DNA was extracted from soil samples and used in PCR using universal primers for 16S rRNA of the bacterial domain. The amplification products generated fragments were purified and cloned into pGEM-T Easy vector system, allowing the DNA sequencing and subsequent analysis. FASTA sequences obtained were analyzed by comparison and classified by the RDP (Ribosomal Database Project). Data analysis revealed a clear difference between the two places, with a change of dominant groups. In the soil of native forest were the main dominant phyla Acidobacteria, Verrucomicrobia, Proteobacteria, Firmicutes and Actinobacteria.Por other hand, the soil cultivated with sugar cane was the predominance of the phyla Proteobacteria and Verrucomicrobia. These data show that the monoculture of sugar cane differs from the bacterial community in soils compared to a native forest. This change can be correlated with soil chemistry, physical properties and sampling.

Supported: FAPESP (proc. 2009/54274-9)

Keywords: diversity, 16S rDNA

PO-75

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

PSEUDOMONAS FLUORESCENCE CAN BE USED FOR BIOREMEDIATION OF TEXTILE

A. Reni Prabha, S. Saravana Babu and P. Anu Priya

Department of Zoology, Chikkaiah Naicker College, 638004, Erode, Tamil Nadu, India;
E-mail: reni_uday@yahoo.co.in

Dyes present in the effluent of textile industries are recalcitrant molecules difficult to be degraded biologically. In this study the ability of *Pseudomonas fluorescens* to degrade Direct Orange - 102 dye was studied. *Pseudomonas fluorescens* was isolated from textile dyeing effluent and was adapted to grow on Direct Orange - 102. The dye was subjected to degradation by the bacterium and its metabolic products were identified by UV, 1HNMR and IR spectrophotometry. The dye was first broken down into 3, 7- diamino- 4 hydroxy - naphthalene - 2 sulfonic acid sodium salt. This compound is further degraded into 7- amino -3, 4- dihydroxy - naphthalene- 2- sulfonic acid sodium salt or 3- amino-4-7-dihydroxynaphthalene -2 sulfonic acid sodim salt or 1,3,4,5,6,7,8 - heptahydroxy naphthalene - 2 - sulfonic acid sodium

salt. These breakdown compounds were non - toxic in nature. Therefore, *Pseudomonas fluorescens* can be used for bioremediation of textile effluent containing Direct Orange- 102 dye.

Keywords: Bioremediation.

PO-129

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

OPTIMIZATION OF HUMUS PRODUCTION FROM ORGANIC WASTES THROUGH VERMICOMPOSTING TECHNOLOGY

Rihards Pulturs

Latvian Earthworm Growers Association, 1-1, Zaubes Str., LV1013, Riga, Latvia; E-mail: rihards.pulturs@slieka.lv

Intensive use of agrochemicals in crop production has lead to environmental degradation. This problem has created a great interest in the use of vermicompost to supply soil with organic matter and minerals. Vermicompost contains plant nutrients, which positively influence the photosynthesis, chlorophyll content etc. The high percentage of humic acids in vermicompost contributes to plant health, as it promotes the synthesis of phenolic compounds.

A new comprehensive technology of biotransformation of organic wastes into biologically active organic fertilizer with a high content of humic acids, is developed in Latvia by LAWR. The first stage is the primary treatment of organic waste by aerobic-anaerobic fermentation and preparation of balanced substrate, accompanied by biogas and heat production.

In the second stage, soil microorganisms and earthworms are cultivated in a specially designed bioreactor, using the substrate obtained in the previous stage. Cultivation is carried out under optimal and controlled conditions. The basic parameter for process control is the oxygen consumption rate, which reflects the overall activity of biological objects in the substrate. Based on this parameter, other parameters are regulated, e.g., temperature, humidity, pH value and addition of the appropriate amendments. The value of the oxygen consumption rate can also determine the overall biological activity of the final product - vermicompost, and, therefore to express the objective quality index.

Some manufacturing operations are encouraged to use elements of nanotechnology and organic nanoparticles, which can increase the intensity (rate and conversion rate) of organic waste transformation.

Duration of the first stage of the substrate preparation from fresh organic waste is up to 30 days, the duration of the second stage of transformation of the substrate to vermicompost is up to 100 days.

The entire process is conducted in a continuous culture with periodic recharge.

The proposed technology can improve process productivity by 2-3 times.

Keywords: Vermicompost; plant growth; organic nanoparticles; bioreactor.

PO-6

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

THE TRANSCRIPTION FACTOR AIM1 MEDIATES CROSSTALK BETWEEN BIOTIC AND ABIOTIC STRESS RESPONSES IN TOMATO

Synan F. Abu Qamar, Hongli Luo, Kristin Laluk, Michael Mickelbart and Tesfaye Mengiste

Department of Biology, Faculty of Science, UAE University, P.O. Box 17551, Al-Ain, UAE;
E-mail: sabuqamar@uaeu.ac.ae

Plants deploy diverse molecular and cellular mechanisms to survive stressful environments. The tomato (Solanum lycopersicum) abscisic acid-induced myb1 (SlAIM1) gene encoding an R2R3MYB transcription factor is induced by pathogens, plant hormones, salinity and oxidative stress. The SlAIM1 RNA interference (RNAi) plants show an increased susceptibility to the fungus Botrytis cinerea, but increased sensitivity to salt and oxidative stresses. Ectopic expression of SlAIM1 is sufficient for tolerance to high salinity and oxidative stress. These responses correlate with reduced sensitivity to abscisic acid (ABA) in the SlAIM1 RNAi, but increased sensitivity in the overexpression plants. Interestingly, with the exposure to high rootzone salinity levels, SlAIM1 RNAi plants accumulate more Na+, whereas the overexpression lines accumulate less Na+ relative to wild-type plants, suggesting that SlAIM1 regulates ion fluxes.

This misregulation of ion fluxes can result in impaired plant tolerance to necrotrophic infection or abiotic stress. Our data reveal a connection between ABA, Na+ homeostasis, oxidative stress and pathogen response, and shed light on the genetic control of crosstalk between plant responses to pathogens and abiotic stress. Together, our data suggest SlAIM1 integrates plant responses to pathogens and abiotic stresses by modulating responses to ABA.

PO-36

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology, Clinical Research/clinical trials; bioethics; nanobiotechnology*

ASSESSING MOLECULAR SIGNATURE FOR SOME POTENTIAL DATE (*PHOENIX DACTYLIFERA* L.) CULTIVARS FROM SAUDI ARABIA BASED ON CHLOROPLAST DNA SEQUENCES *RPOB* AND *PSBA-TRNH*

Fahad Al-Qurainy, Salim Khan, M. Nadeem and M. Tarroum

Department of Botany and Microbiology, College of Science, King Saud University, Riyadh- 11451, Saudi Arabia;
E-mail: fahad_alqurainy@yahoo.com

Phoenix dactylifera L. (date palm), being economically very important, is widely cultivated in the Middle East and North Africa with about 400 different cultivars. Assessment of date cultivars under trading and farming is a widely accepted problem owing to lack of a unique molecular signature for specific date cultivars. In the present study, eight different cultivars of dates viz., *Khodry, Khalas, Ruthana, Sukkari, Sefri, Segae, Ajwa* and *Hilali* were sequenced for *rpoB* and *psbA-trnH* genes and analyzed using bioinformatics tools to establish a cultivar-specific molecular signature. The combined aligned data matrix was of 1147 characters, of which invariable and variable sites were found to be 958 and 173, respectively. The analysis clearly reveals three major groups of these cultivars: (i) *Khodary, Sefri, Ajwa, Ruthana* and *Hilali* (58% BS), (ii) *Sukkari* and *Khalas* (64% BS), and (iii) *Segae.* The economically most important cultivar *Ajwa* showed similarity with *Khodary* and *Sefri* (67% BS).The sequences of the date cultivars generated in the present study showed bootstrap values between 38% and 70% so these sequences could be carefully used as molecular signature for potential date cultivars under trading and selection of genuine cultivars at the seedling stage for farming.

Key words: *Phoenix dactylifera*, dates, molecular signature, Saudi Arabia, *rpoB*, *psbA-trnH*.

PO-86

Track: *Plant and Environment*

ALLELOPATHIC EFFECTS OF PEARL MILLET ON SEED GERMINATION AND SEEDLING GROWTH

Leila Radhouane

Department of Biotechnology and Physiology, Tunisian Institute for Agriculture Research (INRAT), Tunisia;
E-mail: radhouane.leila@iresa.agrinet.tn

Increasing attention has been given to the role and potential of allelopathy as a management strategy for crop protection against weeds and other pests. Incorporating allelopathy into natural and agricultural management systems may reduce the use of herbicides, fungicides, nematicides, and insecticides, cause less pollution and diminish autotoxicity hazards. Pear millet (Pennisetum glaucum (L.)R.Br.) is a potential allelopathic crop, which possesses a number of allelochemicals at maturity. The present study was initiated to investigate the allelopathic effects of autochthonous pearl millet ecotype (CV KS) on the germination and seedling growth of Pennisetum glaucum under laboratory condition. The results suggested that aqueous extracts from shoots and roots significantly inhibited not only germination and seedling growth but also reduced dry mass of the seedlings and the inhibitory effects were increased proportionally with the extract concentration. It was observed that roots were more toxic than stems. It was concluded that autochthonous pearl millet ecotype (CV KS) has strong allelopathic potential and might be candidate for biological control of weeds and insects. However, further studies are required to see its allelopathic behavior under field condition against others species and to identify the toxic principle, their quantification and its efficacy in the soil.

Keywords: Allelopathy, pearl millet, germination, seedling growth, biological control.

PO-71

Track: *Others: Nanobiotechnology*

RELIABILITY AND SAFETY ESTIMATIONS FOR MICRO- AND NANOBIOTECHNOLOGY

M. AboRas, B. Michel, T. Winkler, J. Knüppel and J. Keller

Elektronische Nanosysteme (ENAS), Technologie-Campus 3, D-09126 Chemnitz, Germany;
E-mail: Mohamad.AboRas@enas.fraunhofer.de

The paper presents investigations on advanced Reliability and Safety concepts for components, devices and systems and their applications in the field of biotechnology materials. Special focus of the poster will be given to the behaviour of these materials in the micro-nano transition region. Very often reliability problems may occur because of the existence of thermo-mechanical stresses in the interface regions between the different materials combined (thermal misfit or mismatch). Also gradients of the material properties have to be taken into account. The team of authors will present recent results of reliability and lifetime estimation of testing and simulation of combined materials in components and systems of biotechnological applications. Questions of design for reliability and physics of failure approach will be analysed and discussed for a great variety of practical applications. Thermal and thermo-mechanical stress reduction strategies lead to improved lifetime results (e.g. for modern pacemakers about which will also be reported). We present a new so-called TIMA Tester, an advanced measuring system for experimental determination of thermal interface parameters. These quantities are very important for material characterization and the design of devices in biotechnology applications. In addition new special techniques for characterization of local deformation fields (microDAC, FIBDAC, nanoDAC, lock-in thermography etc.) will be used to determine the deformation fields, temperature fields and related quantities in the critical interface regions of the materials. The obtained data are the input data for further reliability estimations of the investigated systems and optimization of manufacturing processes (e.g. via virtual prototyping etc.). The authors are going to show that the experimental data last not least provide a very good basis for an optimal design of advanced chip-based biotechnological systems. The reason is that also related electronic packages - being included in many biotechnological systems - can be optimized by such a procedure as well.

PO-50

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

IN VITRO CULTURE OF THE MEDICINAL PLANT *ARTEMISIA NILAGIRICA* (C.B. CLARKE) PAMPAN

K. Raju

Department of Botany, Kandaswami's Kandar College, Velore- 638 182, India; E-mail: raju_herbal@yahoo.com

Protocol for *in vitro* culture by using leaf explants of the threatened medicinal plant species, *Artemisia nilagirica* found in open habitats of Nilgiris, the Western Ghats at high altitudes was developed. The MS medium supplemented with growth hormones, like BAP and NAA at concentrations of 2.5 and 0.5mg/l respectively was found to be the optimum for higher frequency of callus formation. Maximum number of shoots (11 shoots/callus) was observed in MS medium fortified with BAP, NAA and IAA at 1.0, 1.0 and 0.3mg/l respectively. MS medium with IBA at 1.0mg/l alone produced higher number of roots during subculturing (15 roots/callus). The plantlets obtained were successfully transferred in the hardening medium containing garden soil, farmyard manure and sand in the ratio of 2:1:1 by volume in which 80% survivability was achieved.

Keywords: *Artemisia nilagirica*, medicinal plant, Nilgiris, the Western Ghats.

PO-124

Track: *Industrial and Manufacturing*

COMPARATIVE STUDY ON CULTIVATION AND YIELD PERFORMANCE OF COPRINUS CINEREUS(SCHAEFF) GRAY ON COW DUNG MANURE SUPPLEMENTED SISAL WASTES

Prosper Raymond, A.M.M Shandete and A.K.Kivaisi

Department of Molecular Biology and Biotechnology, University Of Dar Es Salaam, Tanzania;
E-mail: promosha@yahoo.com

This study aimed at evaluating the suitability of sisal waste fractions (viz. sisal boles and sisal leaf decortication residues, alone or in combination) supplemented with cow dung manure at various rates for Coprinus cinereus cultivation. The periods for spawn running (mycelium development), pinhead and fruit body formation, number of flushes, yield, biological efficiency, mushroom size and loss in organic matter were studied. A substrate combination of 25% sisal leaves + 75% sisal boles supplemented by 20% cow dung manure gave the highest in both mushroom yield (192.60 g) and percentage biological efficiency (B.E; 64%). Least yield (23.31 g) and low B.E (7.3%) were revealed from non-supplemented substrate combination of 75% sisal leaves + 25% sisal boles. The mycelium growth was totally colonized the sisal bole substrates (supplemented and non-supplemented) but no mushroom fruit bodies were formed. The results indicated that, sisal waste fractions supplemented with cow dung manure attributes on increasing yield and productivity of Coprinus cinereus. A further study on the mushroom cultivation using sisal bole substrates is however suggested.

Keywords: Coprinus cinereus, mushroom yield, biological efficiency

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PO-119

Track: *Medical Biotechnology*

RESPONSE OF T REGULATORY CELLS TO THE IMMUNIZATION WITH SOLUBLE EGG ANTIGEN IN MURINE SCHISTOSOMIASIS*MANSONI*

Eman El-Ahwany[1], Faten Nagy[1], Ibrahim Rabie[2], Rabab Zalat[2], Ola Mahmoud[3],Suher Zada[4]

Theodor Bilharz ResearchInstitute. P.O.BOX: 30 Imbaba, Imbaba Giza, Egypt; E-mail:fatennagy@hotmail.com

Objectives: In acute and chronic schistosomiasis, survival of the host requires a carefully balanced immune response against highly immunogenic parasite eggs. The aim of the study is to characterize the phenotype of $CD4^+$ $CD25^+$ T regulatory cells within the liver granulomas of both infected and immunized groups in order to characterize their function and association with both Foxp-3 gene expression and the levels of the different splenic cytokine in the regulation of egg-induced immunopathology.

Material and Methods: Naïve C57BL/6 mice were intravenously injected with multiple doses of the Soluble Egg Antigen (SEA) 7 days before cercarial infection. The immunized and infected control groups were sacrificed 8 and 16 weeks post infection (p.i.). Histopathological studies, parasitological parameters, splenic phenotype for T regulatory cells, the FOXP3 expression in hepatic granuloma using real time PCR and the associated splenic cytokines were assessed.

Results: The percentage of T regulatory cells ($CD4^+$ $CD25^+$) was increased significantly ($p<0.01$) in the immunized group compared to the infected control at 8 and 16 weeks p.i.The FOXP3 expression in hepatic granuloma was increased from 10 at 8 weeks to 30 fold at 16 weeks p.i. in the infected control group. However, its expression in the immunized group showed increase from 30 at 8 weeks to 70 fold at 16 weeks p.i. in immunized group.

Conclusions: The magnitude and phenotype of the egg-induced effecton T helper response were found to be controlled by a parallel response within the T regulatory population which provides protection in worm parasite-induced immuopathology.

PO-167

Track: *Medical Biotechnology*

GINKGO BILOBA L EXTRACT HELPS BMSC IN LOWERING BLOOD GLUCOSE LEVELS

Shujuan Yang, Shuping Ren, Qiang Chen, Yulin Hu, Lu Cai and Qing Wang

School of Public Health, Jilin University, Changchun, 130021, Jilin Province, China; E-mails: rensp@jlu.edu.cn; wangqing5151@126.com

Background: Bone marrow mesenchymal stem cells (BMSCs) are potential therapy for diabetes mellitus. But due to oxidative stress caused by hyperglycemia, transplanted BMSCs will go through high rate of death after transplantation. Ginkgo biloba L extract (EGb) is a potent antioxidative agent which can not only remove free radicals but also improve

blood supply. To investigate whether EGb can enhance the efficacy of BMSCs in lowering blood glucose levels in vivo and provide evidence for antioxidant treatment in BMSC transplantation.

Methods: *In vivo,* diabetes was induced by injection of STZ and rats having blood glucose level over 16.7 mmol/l were included in the study. Diabetic rats received either EGb, BMSCs, or EGb prior to BMSCs transplantation. The serum levels of glucose, insulin, IL-6, TNFα, MDA and SOD, GSH-Px activities were measured in rats from different groups. PKCα expression in kidney was determined by Western Blot.

Results: Diabetic rats receiving EGb injection before BMSCs transplantation had significantly lower levels of blood glucose, serum MDA, IL-6, TNFα and higher levels of insulin, SOD and GSH-Px activities. PKCα expression was significantly inhibited in the kidney in rats treated with BMSC and EGb injection.

Conclusions: EGb administration prior to BMSCs transplantation can enhance the effectiveness of BMSCs in lowering blood glucose levels in diabetic rats induced by STZ.

PO-40

Track: *Medical Biotechnology*

*JAK2*V617F MUTATION PERSISTS IN BLASTS AND MATURE CELLS OF TRANSFORMED-*JAK2*V617F-POSITIVE-MYELOPROLIFERATIVE NEOPLASIA: A EUROPEAN LEUKEMIA NET (ENL) STUDY

Ciro R. Rinaldi, Paola Rinaldi, Luigi Del Vecchio, Bruno Martino, Giorgina Specchia, Anna Candoni, Luigi Gugliotta, Alessandro M. Vannucchi and Tiziano Barbui

Department of Hematology-Oncology, Pilgrim Hospital, Boston, Lincolnshire, United Kingdom;
E-mail: ciro.rinaldi@ulh.nhs.uk

Objectives: Recent retrospective studies reported that in up to 53% of the patients who developed secondary AML from a *JAK2*-mutated MPN the mutation was no longer detectable.

Methods: We collected blasts and mature myeloid cells from BM of 40 newly diagnosed patients with AML secondary to MPN and analyzed the *JAK2* status before and after leukemic transformation by ASO-PCR and (QRT)-PCR assay.

Results: At the time of MPN, *JAK2*V617F was detectable in 28 of 40 patients. No cytogenetic abnormalities or *MPL* and *JAK2*-exone 12 mutations were detected at this stage. A significantly shorter (p= 0.02) time to progression was found in previously *JAK2* mutated MPN patients. In our cohort of patients we found that *JAK2*V617F mutation was still present at the blast transformation in both compartments: CD34+ cells and CD15+ cells in 26 of 28 JAK2 mutated MPN (92%). Two of 28 patients (7%) developed *JAK2*V617F negative AML starting from a mutated PV with a mean TTP of 5.14 yrs. No differences (p= 0.3) in the allele burden were found comparing MNCs from chronic phase with MNCs of leukemic transformations or comparing GRA with blasts in AML phase.

Conclusions: In our work, the loss of *JAK2*V617F mutation during AML progression is a rare event (7%).

PO-56

Track: *Pharmaceutical Biotechnology: biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering*

OBTAINING OF RECOMBINANT, PROPERLY FOLDED NEURAMINIDASE OF INFLUENZA VIRUS H5N1 EXPRESSED IN *ECHERICHIA COLI*

A. Romanik, M. Kęsik-Brodacka, V. Sączyńska, V. Cecuda-Adamczewska, K. Florys, G. Płucienniczak and A. Płucienniczak

The Institute of Biotechnology and Antibiotics, Ul. Starościńska 5, 02-516 Warsaw, Poland;
E-mail: romanika@iba.waw.pl

Neuraminidase (NA) is a surface glycoprotein of influenza A virus known to be the most potent viral antigen next to hemagglutinin (HA). NA promotes influenza virus release from infected cells by removing sialic acids from the host cell surface. Heterologous expression of NA has been performed mainly in insect or mammalian cells. The aim of efforts to

obtain rNA is its usage in the combination with rHA in influenza subunit vaccine or as an antigen in NA antibody tests according to DIVA (differentiating infected from vaccinated animals) strategy. Here, we report expression of major part of NA (34 – 449 aa) serotype N1 (NAN1) of avian influenza virus H5N1 with N-terminal 6-Histidine tag. The protein was produced as inclusion bodies. The agreggates were isolated from cell lysate and solubilized under denaturing conditions. Denatured proteins were loaded on the column packed with Ni-NTA resin to purify and refold recombinant NA. After elution from Ni-NTA column, 46,5 kDa protein was analyzed by ELISA with anti-NA antibodies, which have been shown to recognize NAN1 on particles of influenza virus serotype N1. Positive results obtained in ELISA test indicate that bacteria-expressed rNAN1 is properly folded, thereby may be considered as a candidate for vaccine and/or marker antigen.

PO-28

Track: *Pharmaceutical Biotechnology*

ANDROGRAPHOLIDE NANOPARTICLES INDUCES CELL CYCLE ARREST IN MCF-7 CELLS AND INCREASES LIFE SPAN IN EHRLICH ASCITES CARCINOMA INFECTED MICE

P. Roy, S. Das, U. Chatterji, A. Saha and A. Mukherjee

Department of Chemical Technology, University of Calcutta, Kolkata-700009, India;
Email: Partharoy2502@gmail.com

Phytophamacophores like camptothecin and curcumin have opened vistas in chemotherapy of cancer owing to their wider spectrum and lower toxicity. Solubility and biodistribution remains the major limitations and nanonization is seen as a solution to it. Andrographolide (AG), a diterpenoid lactone, from *Andrographis paniculata* induces apoptosis in a range of cancers including the multidrug resistant types. Chemotherapeutic success of AG is shielded for a short biological $t_{1/2}$ and low aqueous solubility. Nanonization of AG in PLGA (AGnp) and chitosan bedecked multi-component cationic system (CAGnp) were achieved following a modified emulsion solvent evaporation technique. The average PCS particle size for AGnp was 173 nm and that of CAGnp was 219 nm**.** The zeta potential recorded was -34.8 mV for AGnp and that of CAGnp was +35.6 mV. AFM trace revealed a smooth external surface and AG loading efficiency recorded in HPLC analysis was 80%. Both AGnp and CAGnp induced apoptosis and G1 cell cycle arrest in MCF-7 cell line. MTT assay indicated a fall in cell viability from 91% in control to 14% and 9% in case of AGnp and CAGnp. CAGnp reduced the tumor weight to 68.21% with a significant increase in life span in Ehrlich Ascites Carcinoma infected mouse.

PO-95

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology, Clinical Research/clinical trials; bioethics; nanobiotechnology*

PREVALENCE OF PAPI-1 IN CLINICAL ISOLATES OF *PESUDOMONAS AEROGINOSA*

Nourkhoda Sadeghifard, Sobhan Ghafourian, Reza Mohebi and Abbas Maleki

Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran;
E-mail: sobhanghafurian@yahoo.com

Objectives: *Pseudomonas aeruginosa* is a Gram-negative rod-shaped bacterium and an opportunistic pathogen that causes various serious diseases in humans and animals. The aims of this study were to evaluate of PAPI-1 in *Pseudomonas aeroginosa* isolated in References laboratory of Ilam, Milad and Emam Khomaini hospital in Iran and to study frequency of extended spectrum beta-lactamases (ESBLs) among isolates which were positive and negative for PAP-1.

Methods: Forty-eight clinical isolates of *P.aeroginosa* were obtained during April 2010 to Sep 2010. The isolates were evaluated for ESBLs and PAPI-1.

Results: The results of the current study showed that 31.5% (n=15) of 48 isolates of *P. aeruginosa* isolates were positive for ESBLs by screening and confirming disk diffusion methods. In this study, of 48 *P. aeruginosa* isolates in all laboratories, 10 isolates were resistant to azteronam and 3rd generation of cephalosporin and produce ESBLs, While in Imam Khomaini hospital 4 and in References laboratory of Ilam 5 isolates were ESBLs positive. Generally, 15 isolates

were ESBLs positive that also confirmed in the confirming stage. The results of PAPI-1 detection showed 35.4% (n=17) of isolates were positive for PAPI-1, which 42.1% (n=8) were found in isolates in milad hospital, 29% (n=5) of PAPI-1 positive were detected in references laboratory of Ilam and 23.5% (n=4) indicated in Imam khomaini hospital. Interestingly, all the PAPI-1 were ESBLs positive and no PAPI-1 detected in non-ESBLs *P.aeroginosa*

Conclusion: This was first study of prevalence of PAPI-1 in clinical isolates of *P.aeroginosa* which showed most of PAPI-1 positive strains had high levels of resistance and produced ESBLs. PAPI-1 has an important role in virulence and antibiotic resistance of *P.aeroginosa.* This study was the first study of Prevalence of PAPI-1 in *P.aeroginosa* and can explain the role of PAPI-1 in surveillance and incidence of antibiotic resistance among *P.aeroginosa.*

Keywords: *P.aeroginosa*, PAPI-1, ESBLs.

PO-74

Track: *Pharmaceutical Biotechnology: biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering*

A WORKING APPROACH OF GENE SEQUENCE OPTIMIZATION FOR RECOMBINANT PROTEIN PRODUCTION AND DNA VACCINE DESIGN

B.H. Salim, S. Bourguiba-Hachemi, M. Zghal, I. Rabhi and M. D. Fathallah

Biotechnology Department, Arabian Gulf University, Bahrain; Email: janenbs@gmail.com

The demand on recombinant proteins and DNA vaccines that provide excellent therapeutic and preventive solutions has been increasing for the last three decades. The raising demand on such products is due to many aspects involving cost, purity and potency. However the development of such products faces obstacles (purity, cost, efficacy, time consumption) resulting in shortcomings in the production process and efficiency. The use of bioinformatics in gene optimization strategies showed to be promising, especially cod on tuning. However, there are no defined guidelines of how to pick the right software for codon tuning and how to engineer the gene to enhance its yield and quantity. That's why we took the challenge to develop a protocol to serve as a guideline that helps engineering the optimal recombinant proteins and DNA vaccines. Our protocol involves 10 steps that handle different aspects of gene optimization: GC content, number of mRNA hairpin loops and ΔG value, AT content at 5" end, sequences improving translation efficiency such as the Shine Dalgarno [SD] and the downstream box [DB]. Our method is based on the generation of at least three optimized sequences for a given gene using different codon tuning softwares' in combination with various sequence analysis and in silico mRNA secondary structure prediction tools. For developing this protocol, we have used 3 softwares's: JCat, DyNAVacs (based on codon substitution according to a specific codon usage) and GASCO (genetic algorithm-based optimization software). This comprehensive method helps designing the best optimized gene version and cuts down the trial and error time for the development of recombinant proteins and DNA vaccines.

Keywords: DNA vaccines, software, codon tunning, *in silico*.

PO-35

Track: *Plant and Environmental: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

BIOREMEDIATION OF A CRUDE OIL POLLUTED SOIL WITH *PLEUROTUS PULMONARIUS* AND *GLOMUS MOSSEAE* USING *AMARANTHUS HYBRIDUS* AS A TEST PLANT

Abiodun Olusola Salami and Ejiro Anslem Elum

Department of Crop Production and Protection, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria; E-mail: sola1salami@yahoo.com

Fallouts from crude oil pollution of soils are known to have undesirable effects on plant growth. This research attempts to study the growth of *Amaranthus hybridus* grown on a crude oil polluted soil bioremediated with *Pleurotus pulmonarius* (a white rot fungus) and *Glomus mosseae* (a mycorrhizal fungus). Nine different treatment factors were used while three replicates were used for each treatment in randomized Complete Block Design at the same age of the seedlings. These treatment factors include: sterilized and unsterilized soil, crude oil, mycorrhiza, mycelium of the

mushroom and its spent mushroom compost. *Amaranthus hybridus* was cultivated in the nursery for 3 weeks by broadcasting. The seedlings were later transplanted to experimental pots of 12cm depth, containing 1000g of soil. Seedlings were left to establish properly for a week before the soil in the experimental pots were polluted with crude oil (bonny light) in 0%, 2%, 3%, 4% concentrations. These were allowed to grow for six weeks before the experiment was terminated.

Data from greenhouse experiment comprising of *A. hybridus* grown in pots replicated thrice in both sterilized and unsterilized soils were obtained from seedlings of 9-week old *A. hybridus*. Plants grown on crude oil polluted unsterilized soils died within 2 weeks after pollution. The control, sterilized and unsterilized soil showed better growth when compared with the rest treatments. Growth was better in crude oil polluted soil inoculated with mycorrhiza (*Glomus mosseae*) followed by mycelium of the mushroom in both sterilized and unsterilized soil respectively. It was observed that treatment with spent mushroom compost at high % concentration of crude oil grew better than low crude oil concentration. Regression analysis revealed that percent Total Carbon (TC) decreased in crude oil polluted soils treated with mycelium of *P. pulmonarius* and spent mushroom compost of *P. pulmonarius*. Phosphorus level was found to increase in crude oil polluted samples treated with the mycorrhizal fungus eight weeks after inoculation.

Duncan's Multiple Range Test was carried out for comparison of the mean values of plant height, number of leaves and leaf area at the different crude oil concentrations. This study shows that the use of biological and supporting physical treatments to treat contaminated soil and groundwater is effective for improved landuse for vegetation and plant health, yield and growth.

Keywords: *Amaranthus hybridus*, *Pleurotus pulmonarius*, *Glomus mosseae*, Bioremediation, Crude Oil Polluted Soil.

PO-171

Track: *Pharmaceutical Biotechnology*

IMPROVED HUMORAL AND CELLULAR IMMUNE RESPONSES OF A MODIFIED HEPATITIS B PLASMID DNA VACCINE

Mounir M. Salem-Bekhit, Yahiya Jamous, Fares Al-Anazi, Mohsen Bayomi, Mohammed Gad-El-Rab and M. Dahmani Fathallah

Department of Pharmaceutics, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh Saudi Arabia ; E-mail: mounirmsalem@yahoo.com

Immunization responses for different doses of quality controlled modified pDNA (gWizHBs) were evaluated. Four different doses (20, 10, 4 and 2 μg) from the characterized pDNA were used to intramuscularly vaccinate four groups of Balb/c mice compared with positive (unmodified pDNA) and negative controls. IgM antibody level was high at week 3 post injection. As a consequence of a booster dose of 4 μg (injected two weeks later), IgG level appeared at week 3 in all groups. The highest levels of IgM and IgG were noticed with the dose of 4 μg. IgG level was maintained at almost constant level for 6 weeks. Cellular responses were assessed in splenic cells by measuring intracellular cytokines in CD4 and CD8 positive cells. The cytokine profile showed high levels of TNFα, IFN γ, and IL2 and CD69 expression in the group of animal immunized using 4 □g dose. In conclusion, both humeral and cellular responses were induced in mice using a modified plasmid DNA-HBs antigen. The antibody level varied with the concentration of pDNA and the highest level was obtained using 4 μg dose.

Acknowledgement: Project was supported by CEBR, King Saud University, Riyadh, KSA.

PO-161

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

ENVIRONMENTAL PERFORMANCE & HDI: EVIDENCE FROM DEVELOPING COUNTRIES

Ahmad Jafari Samimi and Alireza Kashefi

Department of Economics, University of Mazandaran, Babolsar, Iran; E-mail: jafarisa@umz.ac.ir

The purpose of the present paper is to evaluate the relationship between Environmental Performance and Human Development in Developing countries during 2006- 2010. To do so, we used overall Environmental Performance Index

(EPI) data from the Yale Center for Environmental Law & Policy and Human Development Index (HDI) data from Human Development Report of the World Bank for 86 countries base on the data availability.

The findings of the paper using a panel data regression model support a positive and significance relationship between EPI and HDI for the whole countries. However, in this countries suffering environmental degradation the results indicate that higher human development index does not necessarily improve the Environmental Performances. Perhaps more public awareness and more support of international organizations such as United Nations may play an important role in this regard.

Keywords: Environmental Performance Index (EPI), Human Development Index (HDI), Panel Data. Developing countries

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PO-61

Track: *Industrial and manufacturing: bio-fuels; energy crops (cellulosic ethanol industry); industrial enzymes; bioprocess engineering and optimization*

IDENTIFICATION OF THE NATURAL VARIANT NISIN Z PRODUCED BY *LACTOCOCCUS LACTIS* SUBSP. LACTIS PD6.9 STRAIN ISOLATED FROM FERMENTED SAUSAGE IN BRAZIL

M.A.F. Saraiva, M. J. Magalhães Júnior, M.C. Baracat-Pereira, M.V. Queiroz, I.F. Nes and C.A. Moraes

Federal University of Viçosa, Department of Microbiology, 36570.000, Viçosa, Minas Gerais, Brazil; E-mail: magalice@yahoo.com

The antimicrobial peptide, nisin, produced by several strains of Lactococcus lactis, which belongs to a group of bacteriocins called lantibiotics, is used commercially in food preservation. Lactococcus lactis subsp. lactis PD6.9, strain isolated from fermented sausage in Brazil, produces a bacteriocin that inhibits the growth of food-borne pathogenic bacteria. In this study, bacteriocin produced by L. lactis PD6.9 was identified and characterized.

The presence of nisin gene was identified by using PCR with primers specific to nisin A structural gene. Bacteriocin was purified to homogeneity from culture supernatant by ionic exchange chromatography and reversed-phase chromatography, and molecular weight was determined by MALDI-TOF mass spectrometry. Sequencing of the L. lactis PD6.9 nisin gene showed that it was the natural nisin variant, nisin Z, as indicated by substitution of asparagine residue instead of histidine at position 27. The nisin determinant in strain L. lactis PD6.9 was found to be located in the chromosome. Purification of nisin and mass spectrometry analysis confirmed the genetic finding. The ability of the nisin produced by L. lactis PD6.9 to inhibit a wide range of foodborne pathogens may be useful in improving the food safety of the fermented product.

Keywords: Antimicrobial peptide, Lactic acid bacteria, Lactococcus lactis, bacteriocin.

PO-150

Track: *Plant and Environment*

GENETIC DIVERSITY ASSESSMENT OF BREAD WHEAT LINES USING MICROSATELLITE MARKERS

S. Sardouie-Nasab[1], G. Mohammadi-Nejad[*2], A.R. Zebarjadi[3], B. Nakhoda[4], S.M. Tabatabaie[5] and A. Amini[6]

Department of plant breeding, Razi University, Kermanshah, Iran; E-mail: Mohammadinejad@uk.ac.ir

Genetic variability reduction can be lead to genetic vulnerability of field crops against to environmental stress such as salinity and drought and can cause yield reduction .At this research genetic diversity of 30 diverse lines of bread wheat, which were produced for salinity tolerance were done using 37 SSR markers and different morphological and phonological traits. Number of alleles for each locus was at the range of 6 to 20 bp. Polymorphic information content value was varied from 0.64 to 0.93 for Xgwm445 and Xgwm312 respectively. The size of amplified fragments for all the primers was varied between 90-309 bp. The lowest size was belonged to Xwmc261 (90bp) and the biggest was 309

bp for Xgpw2206. Result showed Xgwm312 SSR marker with the highest PIC value at this research was distinguished as the best marker for genetic diversity analysis.

In the other hand promising lines showed considerable genetic diversity, since they are produced for salinity tolerance so these promising lines may have different genetic mechanism of salinity tolerance.

Finally obtained dendrogram by UPGMA method categorized genotypes in to 3 different groups, and three groups had different salinity tolerance.

Keywords: Genetic diversity, SSR markers, bread wheat lines

PO-76

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

USING RNA SILENCING AS A TECHNIQUE INDUCING RESISTANCE AGAINST CROWN GALL IN ROSE CULTIVARS

Mahmood Khosrowchahli, Zahra Sebghatollahi, Ali Mousavi and Amir Mousavi

Department of Agricultural Biotechnology, Science and Research Branch, Department of Agricultural Biotechnology, Islamic Azad University, Tehran, Iran; E-mail: sebghatolahisepideh50@gmail.com

Recently, crown gall has become one of the most significant plant diseases, especially in rose nurseries and orchards in Iran, which causes significant economic losses in nurseries. In this project, using RNA Silencing technology, we designed two self complementary constructs which induced resistance to crown gall disease in rose plants. iaam and ipt oncogenes were used as gene silencing targets. In this project, for the first time probably, ipt was used as a spacer of iaam construct and iaam was considered as a spacer in ipt construct. After creation of these two self complementary constructs, using PCR based procedure, application of restriction enzymes and DNA sequencing have demonstrated and verified the accuracy of designed cassette. After all required verification the both constructs were transferred to PBI121vector and then to some sensitive cultivars of rose by Agrobacterium mediated transformation. In comparison to non transgenic roses, transgenic ones indicated high level of resistance to crown gall disease.

Keywords: Crown gall, oncogene, PBI121, Agrobacterium tumefacience, transgenic.

PO-45

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

PURIFICATION AND PROPERTIES OF NOVEL ALIPHATIC-AROMATIC COPOLYESTER DEGRADING ENZYMES FROM NEWLY ISOLATED ROSEATELES DEPOLYMERANS STRAIN TB-87

Aamer Ali Shah, Tomoaki Eguchi, Fumie Ichihashi, Daisuke Mayumi, Satoshi Kato, Noboru Shintani and Toshiaki Kambe

Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan; E-mail: aamerali.shah@gmail.com

A novel aliphatic-aromatic copolyester degrading bacterium designated as strain TB-87 was isolated form freshwater. The strain TB-87 has been characterized as aliphatic as well as aromatic copolyester, poly (butylene succinate/terephthalate/ isophthalate)-co-(lactate) (PBSTIL)-degrading microorganism since it grows efficiently and forms clear zones on PBSTIL emulsified NB agar plates. The bacterium was identified through 16SrRNA gene sequencing; it was completely matched with Roseateles depolymerans type strain. PBSTIL depolmerase was purified to homogeneity from strain R. depolymerans TB-87, by column chromatography. This strain produced both high and low molecular weight PBSTIL depolmerases with sizes approximately 31 and 27 kDa, respectively, as determined by SDS-PAGE. The enzymes were determined to be a type of esterase, therefore, designated as EST-H(High) and EST-L(Low). Both the enzymes showed activity against PBSTIL, as indicated by the clear zones of hydrolysis in plate assay as well as disappearance of turbidity in plastic emulsified broth and degradation of PBSTIL film. However, the activity of EST-H was found higher than EST-L. These enzymes were capable of degrading other aliphatic and aliphatic-aromatic copolyesters like poly(butylene succinate)(PBS), poly(butylene succinate)-co-(butylene adipate)(PBSA), Poly(ε-caprolactone)(PCL), and poly(butylene succinate)-co-(butylene terephthalate)(PBST). Our study indicates that this

bacterium can depolymerize aliphatic and aliphatic-aromatic copolyesters, therefore, it can be applied in the process of monomerization for monomers recovery, using its enzymes.

Keywords: Roseateles depolymeranse TB-87, poly[(butylene succinate/terephthalate/ isophthalate)-co-(lactate)], PBSTIL depolymerases, LC-MS.

PO-55

Track: *Plant and Environment*

BIORATIONAL CONTROL PROGRAMME FOR THE GERMAN COCKROACH (BLATTARIA: BLATTELLIDAE) IN SELECTED URBAN COMMUNITIES

Gholam Hossein Shahraki, Yusof Bin Ibrahim, Hafidzi Mohd Noor, Javad Rafinejad and Mohd. Khadri Shahar

Department of Parasitology, Yasuj University of Medical Science, Iran; E-mail: vahabsh@yahoo.com

This study assessed the effectiveness of a biorational control approach using 2% hydramethylnon gel bait on German cockroaches, Blattella germanica (L.) in some residential and hospital buildings in South Western Iran. In total, three buildings consisting of 150 apartment units and 101 hospital units were monitored weekly via sticky trap for German cockroach infestations over a period of eight months. These infested units were randomly subjected to intervention and control treatments. Pamphlets and posters were provided and lectures were given to support the educational programmes as a tactic of the biorational system. Survey on cockroach index for intervention units showed 67-94% recovery to achieve clean level of infestation for intervention units of the residential buildings and 83% for the hospital. Mean percentage reductions for treatment groups throughout the 15-week treatment period were 76.8% for the residential buildings and 88.1% for the hospital, showing significant differences compared to the control groups. Linear regression of infestation rates were recorded weekly after treatment and their negative slope for treatment groups substantiated significant reductions for interventions. The results of this study showed that biorational control method, using gel bait, educational programmes and sanitation, is an effective way to manage German cockroach infestation.

Keywords: German cockroach, Biorational, trap monitoring, Hydramethylnon

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PO-58

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

DETECTION OF RADIATION POLLUTIONS EFFECT ON MICROORGANISM (BACTERIA)

M. Al-Shanawa, A. Nabok, A. Hashim, T. Smith and S. Forder

Material and Engineering Research Institute, Sheffield Hallam University, UK;
E-mail: Maytham.A.Al-Shanawa@student.shu.ac.uk

Recently, radiation pollution becomes the biggest problem threatening the life on the planet. Radiation pollution appears as a result of spreading radioactive isotopes during the nuclear tests, n uclear explosions, and nuclear wastes particularly (depleted Uranium).

Radio-isotopes naturally presents in the environment, e.g. Strontium-90 (^{90}Sr), Technetium-99 (^{99}Tc) and mainly Uranium-238 (^{238}U) appeared as a result of human activity. Radiation is harmful to living organisms because of; (i) the direct effect through the damage of DNA and cells at high radiation causing imminent death, (ii) the indirect effect through the damage of the cell cytoplasm followed by cells division and generation of cancer, as well as through genetic mutations causing congenital malformation The main aim of this study poster is to develop cost-effective methods of Gamma radiation detection using bacteria: *E. coli* (at present) and D. Radiodurans (in future). The samples of *E. coli* bacteria were exposed to ϒ-Ray radiation from Co-57 source with the activity of 330MBq (2000$\frac{\mu S}{h}$), from 1 to 120 hours and referred to the non-exposed samples. Fluorescent microscopy image technique was used to determine the effect of radiation on the living bacteria. The optical absorption (OD-600 nm) technique was also used as an indication of cells density (bacteria cells concentration) versus to the exposure time of radiation.

The result of the Fluorescence Microscope Intensity ratio (FLu_a/FLu_b) that emitted from *E. coli* bacteria depends exponentially on the Gamma radiation dose. The study conclusion is confirmed that all experimental results and techniques show that the live bacteria concentration decreases exponentially with the time of exposure to radiation. This proves the main concept of our research. Further work will be focused on the study of the effect of radiation and other pollutions using different bacteria (e.g. D. Radiodurans) and possibly different experimental techniques.

PO-62

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring.*

CELL SUSPENSION AND *IN VITRO* PRODUCTION OF COLCHICINE IN WILD *COLCHICUM HIEROSOLYMITANUM* FEIB.

Nidal Q. Daradkeh, Rida A. Shibli, Ibrahim M. Makhadmeh, Feras Alali and Tamara S. Al-Qudah

Department of Horticulture and Agronomy, Faculty of Agriculture, University of Jordan, Amman, Jordan; E-mail: r.shibli@ju.edu.jo

Callus was induced from seeds of *Colchicum hierosolymitanum* Feib inoculated on the surface of MS media supplemented with 0.45 µM 2, 4-dichlorophenoxyacetic acid under dark conditions. Then callus was cultured on MS media supplemented with 4.52 µM 2, 4-dichlorophenoxyacetic for growth and maintenance. Friable callus from the fourth generation was transferred to liquid MS media supplemented with 0.54 µM 1-naphthaleneacetic acid to form cell suspension. Cells were successfully subcultured every 27 days on the same liquid media supplemented with 0.54 µM 1-naphthaleneacetic acid. Higher concentration (9 µM) of 6-benzyladenine with 0.45 µM 2, 4-dichlorophenoxyacetic acid resulted in higher cells fresh weight, while 1-naphthaleneacetic acid combinations with 6-benzyladenine had no effect on cell growth. On the other hand, the time for subculturing the cells into new fresh liquid media was determined to be after 27 days of incubation. (-) -Colchicine was identified in callus and cell suspension of *C. hierosolymitanum* by performing HPLC analysis against standard. Different ratios of NH^{+4} : NO^{-3} were used to study their effect on (-)-colchicine content, the highest colchicine content of 0.070 mg g^{-1}DW was obtained at 30 mM NH_4^+ of total nitrogen. Colchicine alkaloid was highest, 0.090 mg g^{-1} DW, at 0.1 M of sucrose after 4 weeks incubation. (-) - Colchicine alkaloid was not detected in callus grown on sucrose free media. Maximum production of colchicine, 0.235 mg g^{-1} DW, was obtained in callus extracts of 60 days old callus grown under dark conditions. Cell suspension had 0.012 mg g-1 DW (-) -colchicine from suspended cells grown under dark. (-) -Colchicine content of callus incubated under dark (0.095 mg g^{-1} DW) was higher than light (0.070 mg g^{-1} DW) condition.

Keywords: Callus, Cell suspension, (-)-Colchicine, HPLC analysis.

PO-73

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology, Clinical Research/clinical trials; bioethics; nanobiotechnology*

MORPHOLOGICAL CHANGES OF NEURONS INVOLVED IN LONG-TERM MEMORY IN DAY-OLD CHICKS HATCHING FROM HYPOMAGNETIC FIELD SPACE

Xuebin Wang, Guang-Zhe Lin, Junfeng Li, Xu Zhang, Muling Xu, Dongfeng Li, Jinchang Jiang, Yan-Lin Sun, Dong Wang, Soon-Kwan Hong, Hyun-Yong Jang and Jong-Suh Shin

Department of Animal Resource Science, College of Animal Life Sciences, Kangwon National University, 200-701, Korea; E-mail: jsshin@kangwon.ac.kr

After the chicks hatched from natural geomagnetic conditions, the 1-day-old chicks were stimulated with MeA (Methyl Anthranilate) and labeled as the control group (CG). We examined whether the chicks formed long-term memory [no-imprinted chicks (n-IC) and imprinted chicks (IC)] following 12 hrs of MeA exposure by comparing their memory to naive chicks (NC). We found that the line density of the dendritic spines (LDds) from the neurons in the memory-related nuclei (IMHV and LPO) treated with MeA was increased by 38.7%, and the total dendritic lengths (TLds) of individual neurons in both the

left and right IMHV were increased significantly by 37.1-45.4%. In addition, the average LDds of individual neurons in both the left and right ICs were increased by 10.8%, and the neurons that had long dendrites (TLd > 1000 μm) were increased by 50%. In contrast, in the experimental group (EG), where chicks were hatched from a hypomagnetic field, the average LDds of each neuron in both the left and right intermediate medial hyperstriatum ventrale (IMHV) and the lobus parolfactorius (LPO) of the NC were similar to the NC of the CG. After a 12 hr exposure to MeA, the LDds from both the left and right IMHV and LPO of the n-IC and IC were similar to the NC but were decreased by 17.4% when compared to the n-IC and IC of the CG. Furthermore, the average TLd of single neurons from the memory nuclei in the NC, n-IC and IC were significantly decreased by 30.9% compared to the CG. However, the LPOs were not different in the n-IC. These results indicate that if the natural geomagnetic environment is disrupted, the development of the dendritic spines from the neurons in the related memory nuclei is unchanged, but the hyperplasia of the dendritic spines in the neurons involved in the formation of long-term memory in the 1-day-old chicks was ablated by stimulating the neurons with MeA. Further, the development and growth of the dendrites were significantly reduced. This study demonstrates an impairment of the ability to form long-term memories using a gustation avoiding model.

Keywords: Hypomagnetic field space, day-old chicks, memory-related nuclei (IMHV and LPO), neuron morphology.

PO-8

Track: *Medical Biotechnology: stem cells; gene therapy; tissue engineering; biopharmaceutical manufacturing; cell based therapy; cell cultivation; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers*

TRANSORAL ROBOTIC SURGERY AND RADIATION THERAPY FOR OROPHARYNGEAL CANCERS

Farzan Siddiqui, Scott Kramer, Arnab Chakravarti and Enver Ozer

Department of Radiation Oncology, Ohio State University, USA; E-mail: farzansid@gmail.com

PURPOSE: Treatment of oropharyngeal cancer represents a unique challenge because the organs at risk are critical for providing a high quality of life. Traditional open surgical approaches preclude adequate organ preservation, therefore primary radiation therapy (RT) or chemoradiation therapy (CRT) have been the standards of care. However, transoral surgical approaches spare oropharyngeal organs and can be combined with a lower adjuvant radiation dose, avoiding some RT side effects. This study compares functional and oncological outcomes of patients treated with or without transoral surgery.

METHODS: 154 patients were treated for tonsillar cancer (ICD-9 141.0-141.9) and tongue cancer (141.0) from 2008 to 2010. The patients were stratified as having received transoral surgery (SURG) or not (NONSURG). All patients received radiation therapy as part of treatment. The two treatment groups (SURG v. NONSURG) were compared by seven fixed variables: age, gender, RT dose, RT fractions, total days of RT, TNM stage, and chemotherapy; and by seven outcome variables: short-term radiation side effects (mucositis and skin reaction), weight loss during RT, PEG placement, locoregional recurrence, distant metastasis, and status at last follow-up. Mucositis and skin reaction were graded weekly by a physician during RT (min = 0, max = 3).

RESULTS: SURG patients received a significantly smaller RT dose than NONSURG patients (65 v. 70 Gy, $p < .001$) and in fewer fractions (34 v. 36 fractions, $p < .01$). SURG patients were more likely to have T1/T2 stage disease (73% v. 58%, $p = .05$). SURG patients had lower grade mucositis at RT completion than NONSURG patients (1.69 v. 1.96, $p = .03$) and experienced a lower maximum grade mucositis during RT (2.02 v. 2.25, $p < .03$). Also, fewer SURG patients had a PEG placed (69% v. 85%, $p = .01$).

CONCLUSION(S): Surgical treatment of oropharyngeal cancer offers favorable functional and oncological outcomes.

Keywords: Robotic surgery, radiation therapy, oropharyngeal cancer.

PO-39

Track: *Others - Animal Biotechnology*

ASSOCIATION OF POLYMORPHISM IN OLR1 GENE WITH MILK PRODUCTION TRAITS IN IRANIAN HOLSTEIN DAIRY CATTLE

Masuod Soltani, Saeid Ansari Mahyari, Gholam Reza Ghorbani and Mohammad Ali Edriss

Department of Animal Science, Isfahan University of Technology, Isfahan, Iran;
E-mail: masoud_sol32@yahoo.com

The role of oxidized low-density lipoprotein receptor 1 (OLR1) in lipid metabolism and the results of previous QTL studies prompted the investigation of OLR1 as a candidate gene affecting milk production traits. The present study investigated the impact of single nucleotide polymorphism (SNP) in the untranslated region of OLR1 gene (OLR1g.8232 C >A) on milk production traits in Holstein Dairy Cattle. The analysis was conducted on 408 Iranian Holstein cows in five farms located in Isfahan province. Genotypes were identified using PCR-RFLP technique. Fragments with 270 bp indicated allele A and those with 250 bp and 20 bp represented allele C. Using SAS software (Proc GLM), the effect of the polymorphism on milk production traits was investigated. Results showed that individuals with genotype CC had significantly more fat percentage in comparison with genotypes AC and AA ($P<0.001$). Furthermore cows carrying genotype CC and AC showed significantly more milk fat yield compared to genotype AA ($P<0.01$). This SNP or another SNP that is in linkage disequilibrium, might influence the expression level of OLR1. Regarding the association between the polymorphism and these traits, the SNP has potential to be used as a marker in marker-assisted selection programs.

Keywords: OLR1 Gene, Polymorphism, Milk Fat.

PO-9

Track: *Medical Biotechnology*

PREPARATION OF PHOTO CURABLE NATURAL POLYMER DERIVATIVES FOR MEDICAL APPLICATION

Tae-Il Son, Yoshihiro Ito, Ju-Young Yun, Kwang-Il Kim, Ha-Na Na, Si-Yoong Seo, Shin-Hye Park, and Hyung-Jae Lee

Deptartment of Biotechnology, Chung-Ang University, Anseong, Gyeonggi-do 456-756, Korea;
E-mail: tisohn@cau.ac.kr

Principally, chemical and physical methods have been used for immobilizing bioactive materials. However, there are some of drawbacks with those methods. For example, not only chemical method may produce potential toxic by-product and, the cost is high but also in case of physical method shows low efficiency of immobilizing bioactive material and it is difficult to control the best condition of reaction. To solve these problems, recently, immobilizing bioactive materials by photo reaction has been researched widely. The advantages of photo-immobilizing are 1) high selectivity of chemical reactions or processes under mild conditions (ambient temperture of also much below), 2) typically no need for added catalysts or special solvents, 3) spatially addressable effects (2D and 3D structuring possible), 4) applicable to very small and (relatively) large scales and 5) simple procedures. To use for photo-immobilization, various natural polymers, such as gelatin, chitosan, hyaluronic acid are reacted by irradiation to UV or visible light. They could be applied for medical area widely. For example, coating agent for bioinnert devices such as stent and implant, anti-adhesive agent, wound dressing and bio-adhesive.

Keywords: Photo reaction, immobilizing, release, medical application.

PO-27

Track: *Industrial and Manufacturing*

EVALUATION OF THE FACTORS THAT AFFECT THE CARBON CONTENT IN THE REED CANARYGRASS *(PHALARIS ARUNDINACEA* L.)

Aleksandrs Adamovics, Liena Poisa and Silvija Strikauska

Latvia University of Agriculture, Liela iela 2, Jelgava, LV-3001, Latvia; E-mail: Silvija.strikauska@llu.lv

Energy crops are used widely in various sectors of the economy. They also have a positive effect on the environment, they reduce soil erosion and contamination with chemical substances, and they can be grown in soil which cannot be used for food crops [1, 2, 3, and 4]. Reed canary grass is used fuel briquettes and pellets production. The objective of this research was to evaluate the influencing factors of carbon content in reed canarygrass (*Phalaris arundinacea* L.) crop yield.

The field trials were organized with reed canary grass varieties 'Marathon' and 'Bamse' in the sod-podzolic loam soil. Reed canarygrass variety 'Marathon' was sown on the 12th August in 2008 and varieties "Marathon' and 'Bamse' - on 29th April in 2009. Nitrogen (N) supplementary fertilizer was given to 'Marathon' 08 on the 20th May 2009, and on the 22nd July 2009 for 'Marathon' 09 and 'Bamse' 09. On the 13th April 2010 the reed canarygrass plant growth was renewed. N fertiliser (ammonium nitrate) was applied at 21st April 2010. The reed canarygrass samples were taken on the 12th October 2009 and the 4th April, 6th October 2010. Carbon content presence in samples of the reed canary grass was determined using *Eltra CS-2000*.

The data of our investigation showed the following chemical content in reed canary grass: nitrogen (N) 3.28% - 4.31%, sulphur (S) - 0.22% to 0.26%, hydrogen (H) - 6.01% to 6.76%, carbon (C) - 41.31% to 45.93%, oxygen (O) - 40.85% to 47.46% in reed canarygrass in Latvia (4). In our study, the carbon content average 37%, indicating the older the plant, the more it is carbon. Of all the studied factors (variety, the nitrogen fertilizer rates of sowing time), the variety had the greatest impact on the reed canary grass yield and quality. The canary seed sowing time and the nitrogen fertilizer rates influenced the size of the resulting yield, ash and carbon content.

Keywords: *Phalaris arundinacea* L., chemical composition, carbon content.

Acknowledgment: The study was supported by ESF Project 'Attraction of human resources to the research of the renewable energy sources', Contract Nr. 2009/0225/1DP/1.1.1.2.0/09/APIA/VIAA/129.

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PO-128

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

PHYTOCHEMICAL EVALUATION OF TRADITIONAL MEDICINAL HERB, *EXACUM BICOLOR* ROXB. (*GENTIANACEAE*)

Paulsamy Subramaniam

Department of Botany, Bharathiar University, Coimabtore- 641 046, India; E-mail: paulsami@yahoo.com

Exacum bicolor Roxb. (Gentianaceae) is a phytochemically unexplored traditional medicinal herb, generally distributed in the grasslands of the northern Kerala during July-October. The present study through GC MS analysis revealed the presence of six phytochemicals of medicinal importance (two compounds of polyphenolic group viz., 7'-Chloro-3'-(2, 4 dichlorophenyl)-3',4'-dihydrospiro(1, 3- dioxolane- and a'-D- Galactopyranoside, methyl 2,6- bis-0-(trimethylsilyl) -, cyclic butylboronate two compounds of alkaloid group *viz*., 1, 16- Cyclocorynan-16-carboxylic acid, 17-(acetyloxy)-19,20-didehydro-10-methoxy-, methyl ester, (16.xi., 19E)- and 4 – (4 – Chlorophenyl)- 5 – morpholin - 4 - yl- thiophen

-2- carboxylic acid, ethyl ester; one compound of glycoside group, a'-D- Galactopyranoside, methyl 2,3- bis-0- (trimethylsilyl) -, cyclic phenylboronate and one compound of steroid group, 9,19 – Cycloergostan – 3 – ol – 7 – one, 4, 14 – dimethyl –) in addition to number of other compounds. In bioinformatics approach, by using the software, Prediction Activity Spectra for Substances (PASS), molecular formula, pharmacological effects and drug likeness were determined for all the six compounds scientifically which confirm the traditional usage of *Exacum bicolor.*

PO-5

Track: *Medical Biotechnology: : stem cells; gene therapy; tissue engineering; biopharmaceutical manufacturing; cell based therapy; cell cultivation; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers*

ASSESSMENT OF MUTANT GENOTYPES IN *PLASMODIUM FALCIPARUM* GAMETOCYTES FOLLOWING MALARIA TREATMENT REGIMENS IN SOUTH EASTERN TANZANIA

D. Sumari, K. Hosea, J. Mugasa, P. Kachur and S. Abdulla

Ifakara health Institute, Plot 463, Kiko Ave., Mikocheni, P. O. Box 78373, Dar es Salaam, Tanzania; E-mail: dsumari@ihi.or.tz

This study was undertaken to assess the prevalence of mutant genotypes in gametocytes of *P. falciparum* following the treatment of malaria using SP, SP+Artesunate and Coartem drugs in Rufiji and Ulanga districts. Children under fives with uncomplicated malaria were recruited and followed up for 28 days. The follow up included any day after treatment that has shown presence of parasitaemia and gametocytes microscopically. The analysis for the molecular markers was done by nested PCR followed by Sequence Specific Oligonucleotide Probing (SSOP). Results indicated that children who were treated with SP harbored significantly higher gametocyte numbers than those treated with ACT (P= 0.0001). Furthermore, it was established that younger children (1-24 months) harbored higher gametocyte density than older ones (25-59 months). Drug resistant genotypes of *Pfdhfr* and *Pfdhps* genes analyzed indicated that; frequency of *Pfdhfr* and *Pfdhps* haplotypes in gametocytes obtained after treatment with SP had significant (P= 0.000001) higher prevalence of triple *Pfdhfr* mutant genotypes (53.6%) than those in asexual stages. The prevalence of mutant genotypes on gametocytes indicated the possibility of spreading resistant parasite population in endemic areas. Therefore, these findings strongly support SP withdrawal as first line drug as well as part of combination therapy in Tanzania mainland.

PO-47

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology, Clinical Research/clinical trials; bioethics; nanobiotechnology*

IMPROVEMENT IN RECOVERY RATE OF GLYCOLIPIDS IN MEMBRANE MICRODOMAIN AFTER DETERGENT REMOVAL BY 1,2-DICHLOROETHANE EXTRACTION

Yusuke Suzuki, Kazuya Kabayama, Yujin Iwata, Saori Katsuta, Hisashi Kamimiya, Hisao Kojima, Akira Okamoto and Yasunori Kushi

College of Science and Technology, Nihon University, Japan; E-mail: suzuki.yuusuke@nihon-u.ac.jp

There has been tremendous interest in the ganglioside-rich membrane microdomains over the past two decades. To elucidate functional roles and signal transduction mechanisms, structural characterizations of gangliosides and other molecules existed in the membrane microdomain are required. In biochemically isolation of membrane microdomain, non-ionic detergents have been used in extraction of domain organization of membrane from cells. Although the presence of detergents is crucial in protocol, detergents are usually incompatible with further biochemical analysis. There are several methods for detergent removal, but the procedures are complicated or require expensive instruments and reagents. Previously, we demonstrated that our convenient and rapid method for detergent removal from gangliosides. It is based on selective detergent extraction by washing with organic solvent after drying sample on a glass tube. We investigated 18 species of organic solvents and we confirmed by thin-layer chromatography and matrix-assisted laser desorption/ionization quadrupole ion trap time-of-flight mass spectrometry (MALDI-QIT-TOF MS) that dichloroethane (DCE) is the most suitable solvent and can completely remove non-ionic detergent Triton X-100 without detectable ganglioside losses. Furthermore, the DCE extraction was also found to effectively remove interference of other non-ionic, zwitterionic, or ionic detergents in the MALDI-QIT-TOF MS analysis. In this study, we investigated an

optimal condition of coating with oligosaccharides or polymers on a glass surface for improvement of recovery rate of gangliosides in DCE extraction.

Keywords: Glycolipids, Detergent removal, Membrane microdomain.

PO-77

Track: *Other areas: Food Science*

THE APPLICATION OF *YARROWIA LIPOLYTICA* LIPASES FOR MILK FAT HYDROLYSIS AND PRODUCTION OF AROMA PREPARATIONS USEFUL IN CHEESE MAKING TECHNOLOGY

M. Szołtysik, J. Niedbalska, A. Dąbrowska, K. Babij, M. Pokora and J. Chrzanowska

Wrocław University of Environmental and Life Sciences, Department of Animal Products Technology and Quality Management. Chełmońskiego Str. 37/41, 51-630 Wrocław, Poland; Email: kort@o2.pl

The aim of the research was the application of noncommercial microbial lipases for milk fat degradation. The obtained hydrolysates may be applied as flavor preparations in food industry for intensification and mimicking cheese flavor. Natural substrates such as 36% cream and butter oil emulsion were used for the hydrolysis. The degradation was performed in basic pH with the use of (I) extracellular lipase isolated form yeast *Yarrowia lipolytica* and (II) mixture of extracellular lipase with intracellular lipases and esterases (enzyme cocktail). Enzymes were introduced at the dose of 35U/g of substrate and incubated for 72h in 37^{o}C. Substrates without enzymes were used as a control. The degradation of fat was analyzed after 48 and 72 hours of hydrolysis by the determination of free fatty acids (FFA) content by solid phase extraction combined with gas chromatography and mass spectrometry (SP/GC/MS). In obtained hydrolysates the volatile compounds were analyzed by solid phase microextraction combined with gas chromatography and mass spectrometry (SPME/GC/MS). It was showed that the use of enzyme cocktail (II) caused very intensive fat degradation. In cream and in butter oil samples incubated with this enzyme preparation the free fatty acid content was 4631 and 5370mg/kg respectively, what was two times higher in comparison to the FFA content in hydrolysates obtained only with extracellular *Yarrowia lipolytica* lipase (I). Also the concentration of volatile compounds in those hydrolysates were significantly higher. The main FFA identified after hydrolysis of cream and butter oil were butyric, caproic and caprylic acids. Their contribution in all detected volatile compounds content at the end of the hydrolysis were 80,49% for cream and 72% for butter oil.

Acknowledgment: This work was financially supported by the Ministry of Science and High Education. Project N N31221 3036.

PO-19

Track: *Plant and Environment*

ASSOCIATION OF RGA-SSCP MARKERS WITH RESISTANCE TO DOWNY MILDEW AND ANTHRACNOSE IN GRAPEVINE

Piyada Tantasawat, Atitaya Sorntip and Oythip Poolsawat

Suranaree University of Technology111 University Ave., Muang District, Nakhon Ratchasima 30000, Thailand; E-mail: piyada@sut.ac.th

Downy mildew (*Plasmoparaviticola*) and anthracnose (*Sphacelomaampelinum*) are two major diseases which severely affect most grapevine (*Vitisvinifera* L.) cultivars grown commercially in Thailand. The objective of this study was to evaluate the association between ten resistance gene analog (RGA)-single-strand conformation polymorphism (SSCP) markers with resistance to downy mildew and anthracnose in 63 segregating progenies of 6 cross combinations between susceptible cultivars and disease resistant lines. Three RGA-SSCP markers were found to be significantly correlated with anthracnose resistance, whereas significant correlation with downy mildew resistance was only observed in one RGA-SSCP marker. These results demonstrated the usefulness of RGA-SSCP markers,and identified a few candidate markers having significant associations with resistance to two major diseases of grapevine. Nevertheless, the putative associations between these markers andresistance need to be verified with larger segregating populations before they can be used for marker-assisted selection (MAS) in the future.

Keywords: Correlation, Resistance gene analog, Single-strand conformation polymorphism, *Vitis*.

PO-19

Track: *Plant and Environment*

IN VITRO INDUCTION OF EMBRYO-LIKE STRUCTURES IN CUCUMBER OVULE CULTURE

Piyada Tantasawat, Atitaya Sorntip and Oythip Poolsawat

Suranaree University of Technology111 University Ave., Muang District, Nakhon Ratchasima 30000, Thailand; E-mail: piyada@sut.ac.th

The effects of various factors including genotypes, induction media and thermal shock pretreatment were evaluated on embryo-like structure (ELS) and callus formation in unpollinated ovule culture of cucumber (Cucumis sativus L.). It was found that the ELS and callus formation abilities of five cucumber cultivars varied significantly. Addition of thidiazuron (TDZ) and 6-benzylaminopurine (BA) into the induction medium resulted in the highest percentage of ELS formation (60.4%), compared to the addition of TDZ alone (51.4%), a combination of BA, kinetin and 2,4-dicholorophenoxyacetic acid (2,4-D; 37.0%), a combination of kinetin and 4-chlorophenoxyacetic acid (4-CPA; 19.3%), and a combination of BA, indole-3-acetic acid (IAA) and gibberellic acid (GA3; 37.6%). However, the highest percentage of callus formation was observed in the BA, IAA and GA3-containing medium (70.8%). Thermal shock pretreatment reduced the percentage of ELS formation ca. 1.3-fold, but had no significant effect on callus formation. These results are useful for production of cucumber doubled haploids in the future.

Keywords: BA, 6-benzylaminopurine, Callus, Cucumis sativus, ELS, TDZ, Thidiazuron.

PO-57

Track: *Others: Clinical research/biomarkers*

SERUM PLATELET-ACTIVATING FACTOR ACETYLHYDROLASE ACTIVITY AND LIPID STATUS IN HORSES WITH SUBCLINICAL LEPTOSPIROSIS

R. Turk, J. Habus, Z. Flegar-Mestrić, V. Mojčec, A. Svetina, S. Perkov, M. Robić, M. Belić, V. Staresina and N. Turk

Department of Pathophysiology, Institution Faculty of Veterinary Medicine, University of Zagreb, Croatia; E-mail: rturk@vef.hr

Leptospirosis is a global zoonosis with major importance on public health. Horses can be host-adapted to particular serovars representing a potential hazard to human health.

PAF-AH is an anti-inflammatory enzyme degradeting platelet-activating factor. The aim of this study was to investigate the effect of subclinical infection with Leptospira spp. on PAF-AH activity and lipid status in horses.

A total of 63 horses' sera were divided into three groups according to antibody titre against Leptospira spp. Group 1 (n=21) comprised sera serologically negative on leptospirosis; Group 2 (n=23) had residual antibody titre (50-200) and group 3 (n=19) had high antibody titre (>1600). All horses were without clinical signs of leptospirosis.

Serum PAF-AH activity was not statistically different between serologically negative horses (740 U/L) and those with residual (735 U/L) and high antibody titre (790 U/L). Lipid status was not significantly different among study groups as well. However, significant positive correlations of PAF-AH activity with total cholesterol ($r=0.307$; $p<0.05$) and HDL-C ($r=0.434$, $p<0.001$) were found for all samples.

Results indicate low level of systemic inflammatory response in horses with subclinical leptospirosis. Further studies with clinically manifested disease are needed to elucidate the potential role of PAF-AH as an inflammatory marker of leptospirosis.

Keywords: Leptospirosis, platelet-activating factor acetylhydrolase, inflammation, horses.

PO-172

Track: *Plant & Environment*

CHARACTERISATION OF COMPOUNDS RESPONSIBLE FOR FLAVOUR OF TRADITIONAL FERMENTED *PENTACLETHRA MACROPHYLLA* FOR UGBA PRODUCTION

C.O. Nwaokeleme and J.O. Ugwuanyi

Department of Microbiology, University of Nigeria, Nsukka, Nigeria; E-mail: jerryugwuanyi@yahoo.com

Ugba is a popular condiment and delicacy made from traditional household solid substrate fermentation of African oil bean seed (*Pentaclethra macrophylla*). It is consumed by over 20 million people in Eastern and other parts of Nigeria. The method of production varies from one producer to another resulting in a non-uniform product. The beans that have been fermented for 2-3 days are taken as a delicacy. Well fermented beans are added to soup as flavoring and as meat substitute. Ugba flavor is a complex mixture of several aroma compounds which changes as the fermentation progresses as well as when different organisms are used as starter culture. In this study GC-MS technique was used to characterize the flavor volatiles generated during the fermentation. Flavor profiles of mixed culture, naturally fermented products and products fermented using pure cultures of *B.subtilis* as well as *B.meganterium* were identified. 36 aroma compounds made up of 12 hydrocarbons, 10 esters, 5 alcohols, 2 phenols, 2 ketones and one each of furan, amine, acid, thiophene and lactone were formed during natural fermentation. A total of 30 compounds comprising of 10 hydrocarbons, 8 esters, 3 alcohols, 2 sulfur compounds, 2 amines and one each of acid, aldehyde, phenol, ketone and furan were identified in products made with *B. subtilis*. Sample fermented with *B.meganterium*, on the other hand, produced 29 aroma compounds consisting of 9 hydrocarbons, 10 esters, 2 nitrogenous compounds, 2 ketones and each of alcohol, thiazole, lactone, aldehyde, furan and amine. The results suggest that 9-Octadecenoic acid methyl ester; Pentanoic acid methyl ester; Nonadecane, 1,14-Tetradecandiol, 11,14-Eicosadienoic acid; methyl ester, Methyl 12-methyl tetradecanoate, 1,10-Decanediol and Cyclododecane are the key aroma compounds responsible for the characteristic flavour of Ugba. Meaningful differences exist in the aromatic profiles of the samples produced. This could cause qualitative differences in product and may be manipulated to adjust product flavour as desired.

PO-26

Track: *Plant and Environment: Transgenic Plants and Crops; Bioremediation; Microbial Diversity; Bio-monitoring*

ELICITORS FROM *LEPTOSPHAERIA MACULANS* INDUCING RESISTENCE IN *BRASSICA NAPUS* PLANTS

K.P. Dinh, V.M. Sasek, L. Burketová and O. Valentová

Department of Biochemistry and Microbiology, Institute of Chemical Technology Prague, Technická 3, 166 28 Prague 6, Czech Republic; Email: olga.valentova@vscht.cz

Elicitors are important elements which activate defense mechamisms in plants after plant-pathogen interaction. *Leptosphaeria maculans* represents the most severe disease of oilseed rape causing blackleg stem canker. The aim of this study was to investigate the active substances secreted to the cultivation medium by *Leptosphaeria maculans* that are able to induce defense reresponse in oilseed rape plants against pathogens. The cultivation medium induced PR-1 gene expression and enhanced resistance of plants to *Leptosphaeria maculans*. Active components were further purified and characterized. Mass spectrometry analysis of the most active fraction revealed mainly enzymes which can be involved in cell wall polysaccharide degradation. Very effective elicitor fraction was also obtained from the mycelium of *Leptosphaeria maculans*.

Keywords: Oil seed rape, *Leptosphaeria maculans*, elicitor, induced resistance.

Acknowledgement:

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PO-162

Track: *Industrial and Manufacturing*

INFLUENCE OF CARBON AND NITROGEN SOURCES ON BACTERIAL CELLULOSE PRODUCTION BY GLUCONACETOBACTER XYLINUS CECT 7291

Carbajo Santos and Juan C. Villar

Forestry Research Center, INIA, Spain ; E-mail: villar@inia.es

The capacity of the Gram-negative bacterium Gluconacetobacter xylinus to produce cellulose is well known. Bacterial cellulose (BC) has high purity and crystallinity, elasticity and high water absorption. Although its production depends on the carbon and nitrogen sources used in the medium, there aren't studies on their effect on the BC properties.

The first aim of this study was to evaluate the effect of the carbon source on the cellulose production from Gluconacetobacter xylinus CECT 7291. On a Hestrin-Schramm base medium, five carbon sources were tested (glucose, sucrose, fructose, mannitol and glycerol). The initial pH (6,3) and the temperature (30ºC) were maintained with no agitation. Experiments were made at four different times (4, 7, 10 and 13 days) and the BC characterized in terms of pH, weight, thickness and optical and mechanical properties. With the best carbon source (fructose) in the HS medium, seven nitrogen sources were tested (peptone-yeast extract, peptone-(H4N)2SO4, peptone-K2NO3, peptone-asparagine, yeast extract-(H4N)2SO4, yeast extract- K2NO3, yeast extract-asparagine) and proceeded as in the carbon source study.

The results have showed that C and N sources determine the formation of BC and its physic-mechanical properties. Under optimum conditions, the quality of BC is adequate for its use in paper restoration.

Keywords: Gluconacetobacter, bacterial cellulose, paper restoration

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PO-136

Track: *Plant and Environment: Transgenic Plants and Crops; Bioremediation; Microbial Diversity; Bio-monitoring*

DETECTION OF CANDIDATUS LIBERIBACTER ASIATICUS IN DIFFERENT CITRUS ROOTSTOCKS AND INTERSTOCKS, ITS ULTRASTRUCTURE OF HUANGLONGBING

Shokrollah Haji Vand, Thohirah Lee Abdullah and Mehrzad Mostashari

Department of Crop Science, Faculty of Agriculture, University Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia; E-mail: shokrollah2006@gmail.com

Candidatus Liberibacter is the causal agent for the Asian Huanglongbing, as known as citrus greening disease. It spread in citruiculture area such as Asia, Africa, Europe and USA as well. There is little information pertaining to the effects of different citrus rootstocks and interstocks against HLB in Citrus. This research was carried out to evaluate the beneficial effects of different combinations of citrus rootstocks and interstocks against HLB disease. There were no symptoms of HLB when *C. grandis* was used as rootstock with *C. hystrix* as the interstock and vice versa six months after inoculation. However, Ca. Liberbribacter asiaticus was detected in the scion using second PCR amplification. A high rate of disease severity was observed when *C. aurantum* was used as rootstock and *C. aurantifolia* as the interstock and vice versa.This study showed that Ca. Liberibacter asiaticus can be detected by conventional PCR and characteristics of their detrimental effects include low rate of vegetative growth and reduction of dry matter, root dry matter, plant height and stem diameter. Infected samples were then examined under transmission electron microscope for the determination and identification of Ca. Liberibacter asiaticus. The spherical and rod shaped particles of this agent were found in phloem cells. Cell wall membranes were irregular in shape and were of different thickness. Damage was caused by Candidatus Liberibacter asiaticus penetrating through the cell wall and their movement between cells.

Keywords: HLB detection, Conventional PCR, Citrus rootstocks, Interstocks.

PO-32

Track: *Medical Biotechnology: stem cells; gene therapy; tissue engineering; biopharmaceutical manufacturing; cell based therapy; cell cultivation; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers*

NEW NANO COMPOSITE BONE TISSUE ENGINEERING SCAFFOLDS

M. Albu, L. Radev, G. Georgiev, C. Simionescu and T. Vladkova

University of Chemical Technology and Metallurgy, 8 "Kl. Ohridski Blvd., 1756 Sofia, Bulgaria; E-mail: tgv@uctm.edu

We present new nanocomposite scaffolds for bone tissue engineering based on fibrilar collagen mats, bioactive glass-ceramics and mimicing the natural ECM polymer gels. The fibriliar collagen mats were prepared by original freese-dry technology [1] .The bioactive glass-ceramics were synthesised via developed by us procedure [2]. Following the nature, double interpenetrating networks (gels) were prepared using natural polymer components, the same forming the complex network of the natural extra cellular matrix.

The fibriliar collagen/bioactive glass ceramic composites were prepared at varied collagen: glass-ceramic ratios at 20:80, 50:50 and 80:20 wt. % , after that they were transformed into sponged form by 48 hours freeze-dry procedure at different temperature gradients to control the pore size followed by polymer gel coating. The above described 3D scaffolds were characterized by FTIR, X-Ray Difracction, SEM, Inductive Coupled Plasma-Optical Emission Spectrometry (ICP-OES), enzimatic biodegradability, in vitro bioactivity (1.5 SFB test) and osteablasts growth. The presence of B-type carbonate, containing the characteristic for the natural bone tissue hydroxyapatite (CO_3HA), named bioappatite, on the new scaffolds was confirmed by FTIR, XRD and SEM and a possible explanation is hypotesied.

The authors are gratefully acknowledged for the financial support of the National Fund Scientific Investigations, Bulgaria (grant DVU 10-0024/2010. and DNTS 02-16/2010.)

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PO-106

Track: *Industrial and Manufacturing: bio-fuels; energy crops (cellulosic ethanol industry); industrial enzymes; bioprocess engineering and optimization*

COMPUTATIONAL MODEL AND CHARACTERIZATION OF THE GLYCOSIDE HYDROLASE3 ENZYME FROM *MICROBACTERIUM ESTERAROMATICUM* GS514 FOR BIOTRANSFORMATION OF GINSENOSIDES

Sathiyamoorthy Subramaniyam, Sathishkumar Natarajan, Lin-Hu Quan and Deok-Chun Yang

Ginseng genetic Resource Bank, Kyung Hee University, South Korea; E-mail: dcyang@khu.ac.kr

Ginsenosides are dammarane tri-terpeniod components in *Panax* family, well known as Ginseng in the world wide. Ginsenosides are grouped in to three, based on their carbohydrates position: the oleanane, protopanaxadiol (PPD), and protopanaxatriol (PPT) types. Those sugar molecules are deciding the activity on human diseases. In our study, we isolated recombinant *β*-glycosidase enzymes (Bgp1) from *Microbacterium esteraromaticum* GS514 found in ginseng field soil and over expressed through the *Escherichia coli* BL21 (DE3). Sequence and phylogenetic analyses shares the similarity between glycoside hydrolase (GH) family 3. Our sequence was subjected to homology modeling with *Thermotoga neapolitana* thermostable GH3. Enzyme were characterized for the bio-transformation of ginsenosides, Bgp1 enzyme involved in the hydrolysis of glucose attached to C-20 position of ginsenosides Rb1, Re and Rg1 at 20°C and pH 7.0 to attain the ginsenoside pathway: Rb1→Rd→20(*S)*-Rg3. Also, Bgp1 involved in conversion of ginsenoside Re→Rg2 and Rg1→Rh1. So this enzyme plays a more important role in the industrial application of ginsenosides conversion.

Keywords: Ginsenosides, *Panax ginseng.*

PO-3

Track: *Other areas: Food; Marine; Bio-safety; Systems Biology, Clinical Research/clinical trials; bioethics; nanobiotechnology*

THE BIO-PROTECTIVE EFFICACY OF *HIBISCUS SABDARIFFA, MORINGA OLEIFERA, ZINGIBER OFFICINALE,* TELFAIRIA OCCIDENTALIS ON THE LIVER AND KIDNEY OF ALBINO RATS EXPOSED TO CEMENT DUST

T. Yahaya, J. Okpuzor and O. Ajayi

Department of Cell Biology and Genetics, University of Lagos, Nigeria; E-mail: yahayatajudeen@aim.com

Hibiscus sabdariffa (Roselle), *Moringa oleifera* (Moringa)*, Zingiber officinale* (Ginger), *Telfairia occidentalis* (Ugwu) are plants used as food ingredients in most communities in Nigeria. The bio-protective efficacy of the individual extracts of Roselle, Moringa, Ginger, Ugwu and a mixture of the extracts were assessed in the liver and kidney of *Rattus norvegicus* exposed to cement dust around a polluted environment. Six groups of rats comprising ten rats each were exposed to cement dust 200 m from a cement factory in South West, Nigeria. The control group was administered distilled water, while the test groups were given ethanolic extracts of Roselle, Moringa, Ginger and Ugwu and the mixture of the extracts for 180 days. They were subsequently sacrificed for the histopathologic studies of the harvested liver and kidney. The organs of the control rat group presented abnormal cellular architecture, vascular congestion and inflammation whereas normal cellular pattern, slight inflammation and no vascular congestion were evident in the group that received the mixture.

However, the organs of the rats administered the extracts of Roselle, Moringa, Ginger and Ugwu respectively, presented normal and moderate to severe conditions of the histopathologic abnormalities observed in the control group.

Consequently, these results may suggest that these food plants could play a role in health care delivery, through bio protection of the kidneys and liver of inhabitants of polluted environments and may also be useful in ameliorating the effects of occupational hazards.

PO-87

Track: *Plant and Environment*

POSSIBLE ADVERSE EFFECTS OF GENETICALLY MODIFIED CROPS ON HUMAN HEALTH

Iraz Haspolat[1], Ege tuna[2], Nilufer Kocak[2], Mustafa Yildiz[2] and İlhan Yetkin[3]

[1]University of Ankara, Coordinator of Scientific Research Projects Coordination Unit, 06100 Tandogan, Ankara, Turkey ; [2]University of Ankara, Faculty of Agriculture, Department of Field Crops, 06110 Dışkapı, Ankara,Turkey ; Email: Mustafa.Yildiz@ankara.edu.tr

From one hand, current food production is insufficient against rapidly increasing world population, on the other hand cultivated areas covering 3% of the total world surface area are getting narrowed rapidly due to erosion, salinity, acidity, intensive agriculture and extreme grazing. It is estimated that cultivated area per capita will be decreased from 0.26 to 0.15 hectare in 2050. That is why, high-yielded new cultivars should be improved to meet food demand of increasing world population. However, conventional plant breeding has some disadvantages in respect to improve the new cultivars due to the fact that hybridzation is possible only among limited number of genus, transition undesired properties along with desired characters to the progeny cannot be prevented, elimination of undesired traits by means of back-crossing takes too much time. In order to provide yield increase, use of biotechnological methods which are complementary of conventional plant breeding programs is a necessity. Since an isolated gene can be directly transferred by these methods, hybridization will not be compulsory among genus and species; the most important obstacles which are sterility and incompatibility, in utilizing wild gene sources in conventional breeding will be eliminated.

The sowing area of genetically modified crops, which were firstly started to be cultivated as 2.8 million hectare in 1996, reached to 148.0 million hectare (4.63% of total world cultivated area) in 2010. Since genetically modified (transgenic) crops, different from conventional ones grown in nature, have genes which do not belong to their own species, potential risks of these crops have brought up in recent years. Nowadays majority of genes transferred to plants via biotechnological methods are bacteria and virus originated which forms the basis of several problems. Since transgenic crops include new transformed gene products and seconder metabolits, they have a potential risk on human health. The most important health risks of genetically modified crops are allergy, toxicity and cancer. Gene products (proteins)

formed by foreign genetic material can cause to several discomforts in humans who suffer from allergy. In addition, genetically modified crops have a potential risk with respect to toxicity. Toxins produced by transferred genes which are used for killing insects and in terminator technology, generates significant risks in case of accumulating in tissues. Since toxins are being produced continuously in plants having Bt genes, they are called as "Pesticidal Plants". Many researchers have reported that transgenic plants might have a carcinogenic effect directly or indirectly. It is known that chemicals such as "bromoxynil" and "glufosinate" used in herbicide resistant transgenic cotton, soybean, corn and canola cultivars have a direct carcinogenic effect. On the other hand, foreign DNA segments escaped from digestion can joined to normal genome and be effective on diseases. Plant biotechnology should focus on plants that will improve production stability; give nutritional benefits to the consumer; reduce the environmental impacts of intensive and extensive agriculture; and increase the availability of pharmaceuticals and vaccines. Public health regulatory systems need to be put in place in every country to identify and monitor potential adverse human health effects of transgenic crops.

Keywords: Genetically modified crops, possible adverse effects, human health.

PO-23

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring*

LIPID EXTRACTION FROM CONCENTRATED MICROALGAL CULTURE THROUGH CELL DISRUPTION USING OSMOTIC SHOCK

Gursong Yoo, Won-Kun Park, Yoon-E Choi, Chul-Woong Kim, and Ji-Won Yang

KAIST, Daejeon, Republic of Korea; E-mail: maljaebul@kaist.ac.kr

Lipid extraction step consumes highest amount of energy in the microalgal biodiesel process due to drying process requiring substantial amount of heat. Therefore, lipid extraction from wet biomass is necessary, but it suffers low lipid recovery due to inefficient contact of solvents such as organic solvents and microalgal cells in water phase. In this study, osmotic shock pretreatment was applied to concentrated Chlamydomonas reinhardtii culture (wildtype and cell wall-less mutant) in order to induce cell wall disruption and lipid body release. C.reinhardtii was cultivated and concentrated, and the osmotic shock was given with NaCl and sorbitol at concentration of 60g/L. As the result of the osmotic shock, lipid recovery of the mutant strain was enhanced by up to 142%, and the wildtype was enhanced by up to 48%. Cell wall-less mutant showed better extractability than the wildtype, but there is a trade-off between that and the mutant's lower lipid productivity and lower resistance to contamination. Different growth phase affected the osmotic shock because of cell wall changes during their lifespan. Later growth phase showed better extractability, but its lower lipid productivity should be taken in to practical consideration.

Keywords: Lipid extraction, microalgae, osmotic shock, cell wall-less mutant, growth phase.

PO-141

Track: *Plant and Environment*

STUDY OF SOME IRANIAN THYMUS SP. ACCESSIONS GENETIC DIVERSITY BASED ON RAPD AND ISSR MARKERS

Valiollah Yousefi, Abdollah Najaphy, Alireza Zebarjadi, Hooshmand Safari

Department of Plant Biotechnology, Razi University of Kermanshah, Iran; E-mail: vali_yousefi@yahoo.com

Thyme (Thymus vulgaris L.), one of the most important aromatic medicinal plants, is widely distributed in the Old World. The Mediterranean region can be described as the center of the genus. Thymus belongs to Lamiaceae family that has grown more extent by people of Spain and Turkey. Knowledge about genetic diversity among breeding materials could be an invaluable help in the strategies of improvement. The present study investigated genetic polymorphism in this herb using 14 Thymus sp. accessions collected from different geographic regions in Iran using inter simple sequence repeat (ISSR) and random amplification of polymorphic DNA (RAPD) markers. 12 ISSR primers chosen for analysis revealed 177 bands, and 12 RAPD primers selected for analysis exposed 179 bands, of which 164 (92.65%) and 264 (94.62%) were polymorphic, respectively. Specific groupings were revealed by each cluster analysis with slight variation between two different markers. Jaccard's similarity indices based on ISSR profiles were subjected to UPGMA cluster

analysis. The generated dendrogram revealed three major groups. The highest genetic similarity was observed between 6 and 8, with similarity coefficient 0.78 by ISSR primers and 0.72 by RAPD ones. ISSR primers revealed that 8 and 6 were the least similar accessions genetically, with coefficient 0.18, and similarly, 1 and 3 introduced least genetic similarity by RAPD primers, with similarity coefficient 0.08. These are the first comparative results for RAPD and ISSR reporting interrelationship among Thymus species.

Keywords: Genetic diversity, ISSR, Multivariate statistical analysis, RAPD, Thyme.

PO-163

Track: *Other Areas: Nanobiotechnology*

SURFACE ACOUSTIC WAVE COUPLED ON-CHIP BIOAEROSOL-TO-HYDROSOL SAMPLING

Pun To Yung

Department of Electronic Engineering, the Chinese University of Hong Kong, Shatin, N.T., Hong Kong; E-mail: ptyung@ee.cuhk.edu.hk

Bioaerosol is a topic of recent interest in medical diagnosis, which has seen an explosive growth in the past decade and studies of which underpin a lot of biomedical engineering designs. Bioaerosols are solid or liquid forms of microorganisms (viable or nonviable) and biogenic materials suspended in the air. Inhalation of bioaerosols has adverse and chronic effects on respiratory and immune systems. Traditional culture-based assessments make use of methods including impingement and impaction into solid or liquid substrates, dry filtration using membrane filters, and gravitational sedimentation. They present challenges to a high-throughput, real-time and quantitative viability assessment. This talk introduces new design paradiams on bioaersol sampling in the area of medical biotechnology. The intenst is to extract discrete microbial information from continuous and inherently heterogeneus air sampling in a real world setting.

The choice of topics include bioaerosol-to-hydrosol sampling, atomization, and the application of surface acoustic wave, dielectrophoresis and micro-electro-mechanical systems. This suite of new technologies provides an effective concentration, collection and handling platform to bridge sampling, detection and storage of bioaerosols.

PO-78

Track: *Pharmaceutical Biotechnology: biopharmaceuticals discovery (CNS, cancer, cardiovascular, endocrine, immune); vaccines; antibodies; protein engineering*

ESSENTIAL OIL AND CO2 EXTRACT OF CBS AROMATIC PLANTS MIXTURE

Zoran Zeković, Dusan Adamović, Marija Radojković and Senka Vidović

Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia;
E-mail: zzekovic@tf.uns.ac.rs

Two different methods of essential oil (EO) isolation, steam distillation and supercritical fluid extraction (SFE) by CO_2, were investigated. The equal mass mixture of three well-known aromatic/medicinal plants, coriander (Coriandrum sativum L.) - C, common basil (Ocimen basilicum L.) - B, and winter savory (Satureja montana L.) - S, was the started material for extraction. After milling, the mean particle size of plants mixture was obtained (0.476 mm). Content of CBS mixture essential oil (EO), determined by steam distillation, was 0.6 65% (V/w). SFE of investigated mixture by CO_2 was performed at pressure of 100 bar and temperature 40oC (i.e. solvent density of 0.630 g/cm3), and, after 3 hours of extraction, total extract (TE) yield was obtained (1.165%, w/w). GC-MS method was used to determine the qualitative and quantitative composition of EO and TE samples. Two predominant compounds detected in both of EO and TE, were linalool (characteristic for C and B "pure" EO) and carvacrol (characteristic for S "pure" EO). Contents of linalool and carvacrol were 36.0% in EO (26.2% in TE) and 35.7% in EO (33.0% in TE), respectively. The content of other extracted compounds (more than 15) in investigated samples was much lower (up to 7%). Qualitative and quantitative composition of EO (steam distillation) and TE (SFE extraction) of CBS aromatic plants mixture were different.

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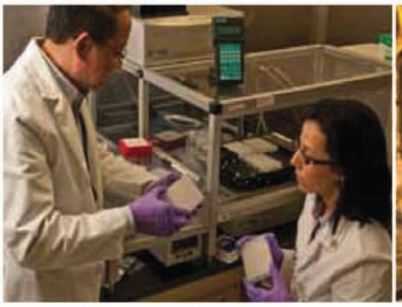


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CONTACT:

Rathnam Chaguturu, Ph.D.
Senior Director, Exploratory Research
rathnam.chaguturu@sri.com

Krishna Kodukula, Ph.D
Executive Director, Center for Advanced Drug Research
krishna.kodukula@sri.com

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