



2nd Biotechnology World Congress

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BIOTECHNOLOGY CONFERENCE A STEP AHEAD

Under the Patronage of
H.E. Sheikh Nahayan Mubarak Al Nahayan
Minister of Higher Education and Scientific Research
Chancellor, Higher Colleges of Technology

ABSTRACTS

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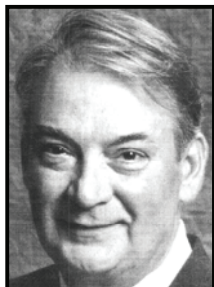
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WELCOME MESSAGE



It is our pleasure to extend a very warm welcome to the honourable scientists and young researchers participating in the two conferences --- the *2nd Biotechnology World Congress* and the *5th International Conference on Drug Discovery & Therapy* here in Dubai.

This series of conferences has attracted twenty six 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 Mabarak 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

PLENARY LECTURES

JL-2**GULF PHARMACEUTICAL INDUSTRIES (JULPHAR) FROM PHARMACEUTICS TO BIOPHARMACEUTICS****Talal Al Zaher**

Biotechnical Senior Manager, Julphar (Gulf Pharmaceutical Industries), PO Box 997, Ras Al Khaymah, UAE; E-mail: talal.alzaher@julphar.net

Julphar's market presence, already a major global pharmaceutical company. We have a firm commitment to keep up-to-date with the healthcare needs of the region by offering highly sophisticated solutions and technologies to a wide range of healthcare burdens, including diabetes.

With the establishment of our facilities, we are well poised to enter new therapeutic segments offering more complex and innovative products. Our look into the future has a strong emphasis on biotechnology. While we leverage technology to move into new therapeutic products to address serious healthcare threats, we are also eyeing expansion to continue providing solutions to healthcare providers in the region. The therapeutic trends worldwide are shifting to biotech upon the technical and scientific advances in this field. Many bioproducts are becoming off patent thus opening the way for more competition.

As a leading pharmaceutical company in the region and in order to keep our leading edge Julphar took a strategic decision to develop and expand its biotech portfolio. Building upon its successful experience in the first 2 projects we are expanding into 4 new projects with plans for 2020 to have a well-established biotech portfolio and expertise in place to lead the company to more advances.

PL-56**ROLE OF ADENOVIRUS 36 IN OBESITY, DIABETES, AND BREAST CANCER: POTENTIAL TREATMENT AND PREVENTION OPTIONS****Richard L. Atkinson**

Virginia Commonwealth University, Director, Obetech Obesity Research Center, Richmond, VA, USA; E-mail: ratkinson2@vcu.edu

The global prevalence of obesity began to increase dramatically starting about 1980. In the USA, the prevalence of obesity (BMI > 30 kg/m²) increased from 15% to 31% from 1980 to 2000. Across the world, obesity increased regardless of economic or educational status, but the geographic pattern was irregular. In developed countries fast foods, sugary beverages, and decreased activity were blamed for the epidemic of obesity, but these explanations are not tenable in less developed countries. Animals also have become heavier since 1980 with pets, lab animals, and wild animals all increasing in weight. The pattern of rapid spread cannot be due to genetic changes and fits best with an environmental agent such as an infectious disease or worldwide pollutants. Eight viruses have been shown to increase adiposity in animals. Human adenoviruses are of particular interest and human adenovirus-36 (Ad-36), Ad-37, and Ad-5 have been shown to cause obesity in animals. Ad-36 is best studied and experimental infection results in increased adiposity in chickens, mice, rats, and non-human primates. Visceral or total adipose tissue mass increased 50% to >100% in chickens and mice and about 70% in non-human primates. About 60% to 70% of infected chickens and mice became obese compared to control animals and 100% of monkeys, so the effect of this virus is powerful. Humans cannot be experimentally infected but the presence of antibodies to Ad-36 is specific evidence of past infection because the antibodies do not cross-react with other known human adenoviruses. Presence of Ad-36 DNA in tissues also is direct evidence of past infection. Adv36 is a "common cold" virus that is spread by droplet and fecal/oral routes. Multiple investigators across the world have tested people for Adv36 antibodies and the prevalence has ranged from 6% to 65% in obese humans and 4.5% to ~45% in non-obese people. In initial studies in the USA, about 30% of obese adults and 11% of non-obese adults were infected with Ad-36 and there was a very strong correlation of Adv36 infection and obesity. Later studies in adults have given mixed results on the correlation of Adv36 and body weight, but the prevalence may have increased. Children are much more consistent with six studies from four countries showing a prevalence of about 28% in obese children and 15%-20% in non-obese. A unique study has just been completed in military personnel. Adv36 infection predicts development of overweight/obesity over time. In addition to obesity, preliminary data shows that women with breast cancer have a 2-3 fold higher prevalence of Adv36 infection than non-



cancer patients. In tissue culture, benign human breast cells infected with Adv36 acquire malignant characteristics of increased growth, greater migration, and the appearance of multiple cancer markers. The effect of Adv36 on diabetes is paradoxical. Diabetics have a lower prevalence of Adv36 infection than normal weight or obese. Adv36 infection of mildly diabetic mice produces a decrease in hyperglycemia and increased glucose disappearance. The mechanism of Adv36 actions appears to be a direct effect on cells to alter intracellular metabolism, particularly glucose and lipid metabolism. Viral DNA is spread throughout the body during the initial viremia phase of acute infection. Viremia lasts about 2-8 weeks, then Adv36 can no longer be cultured. However, Adv36 DNA can be recovered in multiple organs months after the initial infection. Adv36 DNA assayed by polymerase chain reaction has been found in adipose tissue of humans and multiple animal species who have been naturally infected. Adv36 in tissue culture alters leptin, lipoprotein lipase, multiple lipogenic enzymes, and PPAR-gamma. Glucose transporters are increased in infected cells, particularly Glut 4, and non-insulin dependent glucose transport into cells is increased. Stimulation of the Ras pathway appears to be responsible for the increased glucose disappearance rate. The E4orf1 gene of Adv36 is responsible for most of the effects of the virus. When the E4orf1 gene was deleted or blocked with siRNA, the lipogenic effects were blocked. When the E4orf1 gene was cut from Adv36, inserted into a lentivirus, and transfected into preadipocytes or human breast cells *in vitro*, this reproduced the lipogenic and glucose transporting effects of the virus. A vaccine has been produced to Adv36 and given to animals. Serum from vaccinated rabbits inhibits growth of virus at 17 serial dilutions. Prior experience in the US military with adenovirus vaccines suggests that the Adv36 vaccine will be safe and effective. For individuals already infected, it will be necessary to find antiviral compounds that are capable of blocking Adv36 from stimulating lipid and glucose metabolic pathways. Simply blocking replication of the virus will not be effective as there is minimal or no viral replication noted in terminal cells. Two compounds have been identified that decrease Adv36 effects on cells *in vitro*. The combination of these two agents is greater than either alone. A protein has been derived from the E4orf1 gene of Adv36 that facilitates glucose transport *in vitro* and *in vivo*. This may represent a new method of treating diabetes. In summary, Adv36 causes obesity in animals and is associated with obesity in humans in multiple studies of populations across the world. It seems very likely that Adv36 infection has played a significant role in the worldwide epidemic of obesity since 1980. Adv36 infection is increased in women with breast cancer and *in vitro* causes or exacerbates malignant changes in cells. Antiviral agents may be useful for the treatment of obese individuals and women with breast cancer. Adv36 proteins may be useful for treating diabetes. A vaccine that could prevent or reduce the prevalence of obesity and/or breast cancer would be a major public health advance.

PL-70

DISCOVERY OF ANTI- INFECTION AND MULTI-DRUG RESISTANCE REVERSAL AGENTS

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, iqbal.choudhary@iccs.edu



Anti-infection drug discovery and development is unfortunately receiving lesser attention and funding in global healthcare R&D. Many pharmaceutical conglomerates have either closed down or down-sized their anti-infection drug discovery programs. Similarly fewer researchers are engaged in academic institutions in this field. This is created an alarming situation which needs urgent action to avoid the menace of pre-antibiotic era. In this lecture, examples of our studies, focusing on the discovery of potential anti-infective leads will be presented.

Multidrug resistance (MDR) is a challenging problem for the healthcare sector. It is very common in most important pathogens, such as vancomycin-resistant *Enterococci* and *Staphylococcus aureus*. Exposure and inappropriate use of the antibiotics is the major cause of MDR, both in developed and developing regions. We have been focusing our efforts on the discovery of natural and synthetic compounds, active against MDR bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* (resistant to over 20 antibiotics). About 1400 fully characterized natural and synthetic compounds were evaluated by high throughput screens against MDR *S. aureus* and *P. aeruginosa* (NCTC strains and various clinical isolates were used for comparative studies). We have discovered some potent, reproducible and highly active MDR inhibitors of flavonoids, monoterpenes, sesquiterpenes, quinolones, thiourea derivatives and organometallic. Mechanism-based studies on selected compounds of both synthetic and natural origin were also carried out to assess the compound-induced effect on membrane potential, efflux pump inhibition, etc. Biochemical and enzymatic alteration on ultra structure defects in cells also evaluated out by transmission and scanning electron microscopy. In addition to this, we also studied the cell damage by superoxide, produced by selective natural and

synthetic compounds, and the reversal of multidrug resistance by using the MDR inhibitors which boost the antimicrobial activity of antibiotics by improving their penetration into the bacterial cells.

Leishmaniasis is caused by protozoal parasites of the genus *Leishmania*, a biologically diverse group of flagellate parasites. Leishmaniasis is endemic in tropical and subtropical regions, such as south and east Africa, Afghanistan, Iran, China, Nepal, Brazil, Bangladesh, etc. Based on the high prevalence of leishmaniasis in our region and associated morbidity, we conducted a systemic study on folk medicines used against leishmaniasis in Pakistan. We have isolated antileishmanial agents of natural origin and conducted *in vitro* screening as well as animal toxicity assays. We also conducted human clinical trials on leishmaniasis patients by applying topical applications of new ointment based formulations. A total of 110 patients were recruited with clinical leishmaniasis, diagnosed by smear examination on lesions. The results of this clinical study unambiguously established the efficacy, safety and cost effectiveness of the *Physalis minima* extracts-based topical gel.

PL-71

MICRORNA TO STUDY PHYSIOLOGICAL REGULATION OF GENE PRODUCTS IMPLICATED IN CNS DISORDERS: DISCOVERY OF NOVEL APP AND BACE-SPECIFIC MicroRNAs IMPORTANT FOR ALZHEIMER'S DISEASE

D.K. Lahiri and J.M. Long

Laboratory of Molecular Neurogenetics, Department of Psychiatry, Institute of Psychiatric Research, Indiana University School of Medicine, 791 Union Drive. Indianapolis, Indiana, USA; E-mail: dlahiri@iupui.edu



The mammalian central nervous system (CNS) is a complex organ system, demanding an equally complex network of molecular pathways controlling the multitude of diverse, cellular activities.

Gene expression is a critical node at which regulatory control of molecular networks is executed. Thus, deciphering the various mechanisms employed in the physiological regulation of gene expression in the CNS is important for understanding the diseased state and for validating drug targets available for disease intervention. Our aim is to utilize the novel approach of studying the regulation of these gene products by microRNAs (miRNAs). MiRNAs are an abundant class of small RNAs that mediate potent inhibitory effects on global gene expression. Recent advances in molecular methods allow us to study the contribution of these miRNAs to gene expression in CNS disorders, such as Alzheimer's disease (AD) (Long and Lahiri, *Experimental Neurology*, 2012). Aberrations in AD are believed to result, in part, from the over-production of amyloid- β peptide (A β), a product of A β precursor protein (APP). Expression studies suggest that dysregulation of proteins involved in A β production, such as APP and beta-secretase, or BACE1, may contribute to excess A β deposition. Elucidating how expression of these proteins is regulated will ultimately reveal new drug targets. Here we present data demonstrating miRNA-mediated regulation of APP and BACE1.

Recently, by using multiple bioinformatic tools and a series of functional studies in neuronal and glial cultures, we reported specific microRNA species (miR-101 and miR-153) regulate APP levels (Long and Lahiri, *Biochem. Biophys. Res. Commun.*, 2011; Long, Ray and Lahiri, *J. Biol. Chem.*, 2012). We and others have also identified additional set of miRNAs predicted to target the APP mRNA 3'-UTR and regulate APP levels (reviewed Long and Lahiri, *Curr. Med. Chem.*, 2011). Here we report the discovery of novel BACE1-specific miRNAs. First, we prepared a chimeric BACE1 3'-UTR reporter construct (10.1kb) by inserting the 3.9 kb BACE1 3'-UTR downstream of a reporter *Renilla* luciferase gene and then delivered the reporter construct along with several miRNAs predicted to target the BACE1 3'-UTR into human astroglial U373 cells. Several "hits" (e.g. miR-339-5p) resulted in reduced reporter expression. We further validated the reporter expression data for miR-339-5p by Western analysis of native BACE1 levels, which were significantly reduced following miR-339-5p delivery, with a potential in reducing toxic A β levels. Our results reveal a novel regulatory interaction between two important AD-related genes (APP and BACE1) and specific endogenously expressed miRNA species. These regulatory interactions are likely to serve as novel therapeutic targets and should enable the development of treatment strategies that may prove beneficial in the fight against AD.

This work is supported by grants from Alzheimer's Association and NIH to Dr. D.K. Lahiri.

PL-24

Track: CNS Drug Discovery & Therapy

Category and Type: Plenary Speaker

FROM SUPRAMOLECULAR CHEMISTRY TOWARDS ADAPTIVE CHEMISTRY BIORGANIC AND DRUG DISCOVERY ASPECTS

Jean-Marie Lehn

ISIS, Université de Strasbourg, France

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 reversibly, 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 constitutional diversity to allow variation and selection so as to achieve *adaptation*.

In the process of reaching higher levels of complexity, CDC gives access to the generation of networks of dynamically interconverting constituents connected either structurally (molecular and supramolecular arrays) or reactionally (set of connected reactions) or both. They define a class of *constitutional dynamic networks* (CDNs), presenting *agonistic* and *antagonistic* relationships between their constituents, that may couple to thermodynamic or kinetic processes and respond to perturbations by physical stimuli or to chemical effectors.

Applications of these approaches to biological systems and to drug discovery will be described.

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PL-57

REVISITING NATURAL PRODUCTS CHEMISTRY: SYNERGY WITH SYNTHESIS TO ENHANCE NATURE FOR HUMAN HEALTH AND WELLBEING

Goverdhan Mehta

School of Chemistry, University of Hyderabad, Hyderabad 500046, India

Nature, the master craftsman of molecules, has created almost an inexhaustible array of molecular entities, replete with myriad combination of rings, functionalities and stereochemical diversity. These natural products, evolving through millions of years of evolutionary experience, exhibit wide ranging bioactivity profile and have served as lead platforms for exploring a range of interactions in biological systems and to build diversity around their structures. Indeed there have been very impressive



advances in employing natural product based platforms in drug discovery endeavors. With the pharma pipelines showing signs of decline in recent years, reverting back to natural products for inspiration seems obvious and necessary but in a newer context. This emerging scenario will be captured in a few snap-shots.

Our group has been interested in the total synthesis of a variety of natural products that are endowed with novel structural features and unusual biological activity. The lecture would highlight the intrinsic synergy, inherent between natural products, total synthesis and drug discovery through recent examples from the literature and research endeavors from my research group. In particular, the focus will be on the development of 'global' strategies for total synthesis of natural products that provide access to a whole family of natural products rather than a single entity for further biological evaluation and development. In this lecture, we will briefly cover our synthetic approaches to several natural product types, particularly those exhibiting neurotrophic activity, which are conceptually simple and diversity oriented.

PL-1

MEMBRANE PROTEINS: IMPORTANCE, STRUCTURES, FUNCTIONS

Hartmut Michel

Max Planck Institute of Biophysics, Max-von-Laue-Str. 3, D-60438 Frankfurt am Main, Germany

Compared to the membrane lipids the membrane proteins are the more active players in biological membranes. They catalyze:

- (i) transmembrane transport, e.g. the specific uptake of nutrients and substrates, the exchange of ions, and the excretion of waste products and extracellular proteins across the membrane.
- (ii) biological energy transfer and energy conservation in photosynthesis and respiration.
- (iii) signal reception, signal transduction across the membrane and signal amplification.
- (iv) reactions by enzymes with preferentially hydrophobic substrates.



Most drugs available to treat diseases act by inhibiting or activating a membrane protein making membrane protein structure determination extremely interesting for drug design and virtual screening. However, membrane proteins are difficult to study because of material limitations caused by insufficient availability of membrane proteins and their instability. At present the structures of around 370 membrane proteins are known compared to ten thousands of water soluble proteins. However, the structures of only 20 human membrane proteins (of about 6000 to 8000) could be determined.

The importance of membrane proteins is also evident from the fact that the 2012 Nobel Prize in Chemistry was awarded for functional and structural studies of a membrane protein.

The methods of membrane protein structure determination and several recent successes of the author's lab with membrane proteins of medical interest will be presented.

PL-23

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

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, tetrahydrobiopterin, 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 antacid, 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 formation 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 research 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 industries will also be discussed.

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INVITED LECTURES

IL-9

Track: Regenerative Medicine

REGENERATIVE MEDICINE APPROACHES TO NEURO DEGENERATIVE DISEASES OF THE GUT: BIOENGINEERED INTERNAL ANAL SPHINCTER: A BIOLOGIC PLAUSIBILITY

Khalil N. Bitar

Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Virginia Tech-Wake Forest School of Biomedical Engineering and Science, Medical Center Blvd., Winston-Salem, NC 27157, USA; E-mail: kbitar@wakehealth.edu



1-Intrinsically Innervated Human Internal Anal Sphincters constructs were bioengineered from autologous primary smooth muscle cells and enteric neural progenitor cells (stem cells). These cells were isolated and expanded in culture. They were then co-cultured around a scaffold. The smooth muscle cells formed into a concentric 3 dimensional layer. The neural stem cells differentiated and innervated the smooth muscle constructs resulting in functional intrinsically innervated constructs.

2-Bioengineered intrinsically innervated human IAS constructs, neovascularized upon implantation in situ into an athymic rat, and preserved physiological functionality.

Methods: Cells from enteric neurospheres were co-cultured with IAS circular smooth muscle cells using dual layered hydrogels and allowed to form circular constructs containing both cell types. These constructs were implanted in situ adjacent to the native IAS of an athymic rat. They were excised and tested for physiologic functionality after 32 days.

Results: Following implantation, the rodents moved freely, showed no signs of bowel obstruction and had normal bowel movements. Implanted constructs were neovascularized. When excised, these constructs generated spontaneous basal tone and relaxed in response to electrical field stimulation. Electrical field-induced relaxation was attenuated by treatment with L-NAME (nitric oxide synthase inhibitor).

Conclusions: These data represent the first demonstration of physiologically functional, intrinsically innervated bioengineered human IAS tissues successfully implanted in situ into an animal model. The bioengineered constructs survived implantation, was neovascularized and maintained both myogenic and neuronal functionality. These data present the platform for further approaches for successful therapies for treatment of neurodegenerative diseases of the gut using autologous smooth muscle and neural progenitor (stem) cells.

IL-13

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

INSIGHTS INTO MONITORING CHANGES IN THE VIABLE CELL DENSITY AND CELL PHYSIOLOGY USING SCANNING, MULTI-FREQUENCY DIELECTRIC SPECTROSCOPY

John Carvell

Aber Instruments Ltd., UK; E-mail: johnc@aberinstruments.com



Real-time bioprocess monitoring is fundamental for maximizing yield, improving efficiency and process reproducibility, minimizing costs, optimizing product quality, and full understanding of how a system works. The FDA's Process Analytical Technology initiative (PAT) encourages bioprocess workflows to operate under systems that provide timely, in-process results. At the same time the demand for ever increasing supplies of biological pharmaceuticals, such as antibodies and recombinant proteins, has fueled interest in streamlined manufacturing solutions. Bioreactors that are monitored continuously and in real-time offer the advantage of meeting current and future supply demands with biological product of the utmost quality and safety, achieved at the lowest overall cost and with least risk. . This paper will focus on how several research groups in 2012 have used scanning multi-frequency dielectric spectroscopy to comparatively profile multiple bioreactor runs and elucidate fine details concerning cell viability and mechanism of cell death. The cellular information observed has not been available through other technologies. The presentation will also focus on how the technology can also be applied to Single use Bioreactors in a cGMP environment and on samples down to 1ml volume.

IL-6

Track: Regenerative Medicine (cell based therapy)

TREATMENT WITH MESENCHYMAL STROMAL CELLS (MSC) OF LESIONS INDUCED BY ACCIDENTAL IRRADIATION AND RADIOTHERAPY. FIFTEEN YEARS OF PRECLINICAL AND CLINICAL EXPERIENCES

Alain Chapel

Institute of Radiation and Nuclear safety (IRSN), Department of man radioprotection, Laboratory of Radio-Pathology and Innovative Therapies, Fontenay aux roses, France; E-mail: alain.chapel@irsn.fr



We have developed and tested cell therapy for protection against radiation side effects (radiotherapy, radiation accident), in several animal models, and we are the first who explored new therapeutic approach. We established that MSC migrate to damaged tissues in immunotolerant mice model and in non-human primates (J Gen Med 2003, Blood 2004, Stem Cells 2006, Br J Radiol 2007). In immunotolerant mice, we showed that the intravenous injection of MSC (i) sustains haematopoiesis after total body irradiation (Blood 2004), (ii) improves wound healing after radiodermatitis (Annals of Hematology 2006) and (iii) protects gut function (Adv Exp Med Biol 2006). In rat, MSC restore gut functions after radiation (Cell Death Differ 2010), through regulation of endogenous epithelial cell homeostasis (Methods Mol Biol 2012). We reported haematopoiesis recovery in patients with Bone Marrow failure after MSC intravenous injection (Leukemia 2004, 2007). We demonstrated the efficiency of MSC therapy in 8 patients with acute cutaneous and muscle damages following accidental irradiation delivered at doses and to fields higher than initially planned (Regen Med 2007, Health Phys 2010). We treated over irradiated in Epinal with infusion of MSC, four patients were successfully treated for pelvic overdose exposure (IJROBP in press, Cytotherapy 2012).

IL-16

Track: Pharmaceutical Biotechnology

CREATING NOVEL CHEMICAL SPACE FROM EXISTING ONE- BIOTRANSFORMATION FOR NEW PHARMACOPHORES

M. Iqbal Choudhary, Atia-tul-Wahab 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



Biocatalysis or biotransformation is an important field of biotechnology, which utilizes biological systems, such as microorganisms, cells, and pure enzymes to catalyze the synthetic process. The use of biocatalysts offers a remarkable arsenal of highly chemo-, regio- and stereo-selective methods for chemical conversions, which are often difficult to achieve even from state-of-the-art synthetic procedures. Thus requires environment friendly reaction conditions. In last two decades, this methodology has become an indispensable tool for asymmetric synthesis, not only at the academic level but also at the industrial scale. There is a need to fully exploit the potential of biotransformation in creating new and novel chemical space for the discovery of lead molecules against prevalent diseases.

During this presentation, recent developments in biotransformation technologies and the potential of microbes and plant and animal cell cultures to create new molecular entities from the existing compounds will be presented, along with the results of our work in this existing field. This includes our efforts to use whole microbial and plant cell suspension cultures for the structural transformations of various classes of bioactive compounds, including anti-cancer, anti-inflammatory, oral contraceptive, etc. The main objective of the on-going research study is to discover new and effective lead molecules from existing one, for improved therapeutic activity against various biological targets.

IL-82

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

RAMAN SPECTROSCOPY APPLIED TO DETECTION OF PATHOGENIC MICRO-ORGANISMS: AN ALTERNATIVE TYPE OF BIOSENSOR

Philippe Hubert Daniel, F. Lagarde-Abribat, J.F. Pilard, G. Thouand and P. Phyniochep

Institut des Molecules et Materiaux du Mans - IMMM UMR CNRS 6283, Université du Maine - Le Mans France; E-mail: philippe.daniel@univ-lemans.fr



This presentation is mainly devoted to the new applications of Raman spectroscopy in biology for the detection of pathogenic micro-organisms and its use as alternative biosensor. Actually in food processing industry, detecting bacteria or viruses is crucial, nowadays, it can be achieved with microbiological tests but it requires several days. In this work we have synthesized new specific surfaces which are suitable for biomolecules immobilization in order to develop a biosensor for the detection of targeted pathogenic micro-organisms. A double detection signature is then developed thanks to the use of Quartz Crystal Microbalance and Raman Scattering.

Actually Raman spectroscopy appears as a very convenient method to chemically and structurally investigate all materials, of which bacteria or viruses now constitute a possible and interesting field of applications. An important advantage is that biochemical information of all cell components is present in the bacterial Raman spectra and that this technique is non-invasive, non-destructive, specific and with very fast measurements.

Our new procedure of surface functionalization and detection of bacteria using Raman techniques has been done considering 3 methods: i) direct functionalization of gold surfaces ii). polymeric polyethylene surfaces treated by plasma and iii) synthesis of photocrosslinkable monomer on every type of surface.

All these surfaces are finally used to immobilize biomolecules and bacteria and we will show that the functionalization of gold surface by monolayer deposition constitutes a very efficient and low cost technique which could be applied in food industry.

IL-27

Track: Industrial and Manufacturing: bio-fuels; energy crops (cellulosic ethanol industry); industrial enzymes; bioprocess engineering and optimization.

PERSPECTIVES OF APPLIED MICROBIOLOGY WITH PURPLE BACTERIA DRIVEN BY SYSTEMS BIOLOGY

Hartmut Grammel, Steffen Klamt and Robin Ghosh

Biberach University of Applied Science, Biberach, Germany ; E-mail: grammel@hochschule-bc.de



Anoxygenic photosynthetic purple bacteria are well-known to offer highly attractive opportunities for industrial applications. Potential products derived from intracytoplasmic photosynthetic membranes (ICM) include pigments, coenzymes, biohydrogen, biopolymers and recombinant membrane proteins. Since high levels of ICM are formed anaerobically at low-light intensities, most attempts to exploit purple bacteria were so far conducted phototrophically, using light as energy source. However, mass cultivation of photosynthetic microorganisms is generally inefficient due to the inevitable limitation of light when cell densities become very high. It is thus interesting that in *Rhodospirillum rubrum* the high-level production of ICM can be completely separated from light, when the bacteria are grown microaerobically in the dark with a two-carbon substrate growth medium. On the basis of this cultivation process, we applied a systems approach using a combination of bioreactor cultivations, metabolomics and computational modelling to develop *R. rubrum* for biotechnological applications.

This work is intended to open a new perspective for utilizing photosynthetic bacteria in biotechnology. The presented examples include the production of biohydrogen, industrially relevant carotenoids and the utilization of carbon dioxide as a feedstock for bioprocesses.

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IL-46

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

BIXIN ACTION IN MOUTH WOUND HEALING PROCESS

Ana Maria Trindade Grégio

Department of Pharmacology, Pontifícia Universidade Católica do Paraná, Brazil; E-mail: ana.gregio@pucpr.br

Mouth diseases that manifest as ulcer lesions are very common and cause discomfort to the patient. Searching for medicines to accelerate the healing of those lesions is constant. Bixin is a molecule found in annatto (urucum) seed, and it can be a therapeutic option to treat such lesions, because it has an inflaming, anti-oxidant and healing properties. Therefore, this study aimed to evaluate the effect of the bixin solution in the ulcer healing process in rats oral mucosa. Ulcers were introduced with punches of 0,5 cm in the very middle of the top of the tongue of 64 rats Wistar. The animals were randomly divided in 8 groups: 4 groups were treated with saline solution and the other 4 ones with bixin solution. The animals were killed in the periods 2, 7, 14 and 21 days after the beginning of the treatment. The species were histologically processed and colored with hematoxylin and eosin and picrosirius. It was observed fibroblasts, reepithelialization and wound contraction, besides quantifying neutrophils, macrophages, plasma cells, lymphocytes, mature and immature collagen. The experimental group showed bigger deposition of fibroblasts, reepithelialization and contraction in the wounds when compared to the control group. It was observed a reduction of the average number of neutrophils in the experimental group in all the periods compared to the control group ($p=0,000$). To two days, the total collagen area was bigger ($p=0,044$) in the experimental group ($4139,60 \pm 3047,51$) compared to the control group ($1564,81 \pm 918,47$). The deposition of mature collagen, to 14 days, was bigger ($p=0,048$) in the experimental group ($5802,40 \pm 3578,18$) compared to the control ($1737,26 \pm 1439,97$). The results found in the present study aim that the bixin inhibits the acute inflamed response with a minor average number of neutrophils and accelerates the reepithelialization, the wound contraction and the collagen maturation showing that it is an important adjuvant in ulcers treatment.

IL-28

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring; photosynthetic microorganisms, cyanobacteria and microalgae.*

UNRAVELING DEVELOPMENT: BASIC CONTROL OF BODY PATTERNS AND GROWTH IN PLANTS

Hilde-Gunn O. Hoen-Sorteberg, Zhe Liang and Jennifer C. Fletcher

Department of Plant and Environmental Sciences, Norwegian University of Life Sciences, Norway; Current address Department of Molecular, Cellular and Developmental Biology, Yale University, USA; E-mail: hilde-gunn.hoen-sorteberg@umb.no

The orientation of cell walls is a fundamental key to body patterning and possible creation of multi cellular organisms. In plants with fixed cell walls, cells cant relocate only expand and divide antiparallel (anticlinally) or parallel (periclinally)

to the outer surface. Periclinal divisions are needed to create new cell layers and new cell identities. Recent findings suggest that microtubule positioning/cellulose control this. Several genes with functions in division plane control and orientation have been identified, however not one initial actor with all components of the cytoskeleton present. We will do so by presenting *dek1* mutant phenotypes, cytoskeletal disorders, embryo and meristem marker changes and dexametazone induced mutations.

DEK1 is conserved as the single calpain of land plants, and calpain is found in most eukaryotes from unicellular via the moss *Physcomitrella patens* to angiosperms. Calpains regulate a multitude of physiological processes like cell motility, signal transduction, development, membrane fusion and remodeling of the cytoskeleton. This makes it possible that DEK1 allowed the transition from basal charophyte algae to land plants, since maintained through evolution.

Auxin and/or PLT2, MAP65 and CLASP have been shown to control formative cell division planes, periclinal cell wall orientations and finally epidermal (L1) cell identity. How is still only partly unraveled. MAP65-2 is sufficient to trigger cell division plane switches in epidermal root cells possibly through directing CLASP localization. Microarray data of a *dek1* calpain mutant, interestingly shows the expression level of MAP65 only is affected while PLT2 and CLASP are unchanged. This suggests that DEK1 is controlling MAP65 regulation specifically and possibly thereby cell division and identity. We will present a new hypothesis on how DEK1 might act as GPS signals in multi cellular organisms.

The L1 layer is also necessary to keep underlying meristem cells indetermined and removed L1 cells has been reported not to be replaced by underlying cells respecification, suggesting cell lineage dependency. The meristem has further been suggested controlling divisions and differentiation in the L1 by controlling hormone transport. Meristem size is further suggested linked to cell cycle progression, and whether the giant cells of the *dek1* shoot meristem are due to arrested cell cycle progression and/or the lack of L1 identity is not known. However, DEK1 has been shown to control giant sepal cell's epidermal identity upstream of cell cycle regulation. The regulation of ATML1, WUS, STM, REV, PIN4 depend on DEK1, and these results will be presented, discussed and possibly explained. Understanding basic control of cell identity and growth will have major impact on future plant applications for food, feed, energy and industrial purposes.

IL-79

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

NEW DRUG TARGET DISCOVERY WITH MAGNETIC NANOBEADS TECHNOLOGY-HIGH PERFORMANCES AFFINITY PURIFICATION FOR IDENTIFICATION OF 15-DEOXY-Δ^{12,14}-PGJ2 INTERACTING FACTORS USING MAGNETIC NANOBEADS.

Takeshi Imai

Department Head of Aging Intervention, National Center for Geriatrics and Gerontology, Bio Frontier Center, Tokyo Institute of Technology, Japan; E-mail: timai@ncgg.go.jp

Prostaglandin J2 (PGJ2) family have been reported to show various kinds of biological activities. Considerable progress has been made toward understanding the mechanism of adipogenesis, however, the mechanisms of other actions of PGJ2 family remain controversial. 15-deoxy-Δ^{12,14}-PGJ2 (15d-PGJ2) is one of the members of PGJ2 family, and is known as a ligand for peroxisome proliferator-activated receptor © (PPAR-©), which promotes the expression of the crucial genes for adipogenesis. In this study, we found that 15d-PGJ2 did not stimulate PPAR-©-mediated gene expression in HEK293 cells whereas 15d-PGJ2 transactivated PPAR-©-dependent transcription in other cell lines. Moreover, we confirmed that 15d-PGJ2 suppressed the growth of HEK293 cells. These observations suggest that 15d-PGJ2 shows another biological activity e.g. growth inhibition in HEK293 cells *via* unknown receptor for 15d-PGJ2. The aim of this study is to develop and validate effective purification system for PGJ2 interacting factors (PGJIFs). We have recently developed high performance magnetic nanobeads. In this study, we have newly developed 15d-PGJ2-immobilized beads by conjugating 15d-PGJ2 to the surface of these nanobeads. Firstly, we showed that PPAR-© specifically bound to 15d-PGJ2-immobilized beads. Secondly, we newly identified voltage dependent anionic channel 1 (VDAC1) as new PGJIF from crude extracts of HEK293 cells using this affinity purification system. These data presented here demonstrate that 15d-PGJ2-immobilized beads are effective tool for purification of PGJIFs directly from crude cell extracts.

IL-12**Track:** *Regenerative Medicine***POSSIBILITY TO PARTLY WIN THE WAR AGAINST****Xin-Yuan Liu***Laboratory of Molecular Cell Biology, Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200031, China*

We hope to partly win the war against cancer, because that we have two antitumor stars:

1. The Cancer Targeting Gene-Viro-Therapy (CTGVT) which was constructed by inserting an antitumor gene into an oncolytic virus (OV), it is actually OV-gene therapy. We are the funder of CTGVT (OV-gene) and will construct some Onco^{Pox}-gene with higher antitumor than that of Onco^{HSV}-GM-CSF which Amgen purchased it by 1 billion USD and also with higher antitumor than that of Onco^{Pox}-GM-CSF which has published a paper in Nature.

2. We discovered with others an interferon which has 1000 folds higher anti-HIV-1 effect than that of regular IFN α -2b. So it was named as Super Interferon-I (sIFN-I). The sIFN-I also has super antitumor effect. A lung cancer patient by only sIFN-I treatment, his pleural effusion was completely eliminated and his tumor mass reduced by 50%. Recently, we found a mechanism of sIFN-I action that sIFN-I has strong inhibitory effect on Wnt signal pathway, which is strongly related to cancer growth and metastasis, while the IFN α -2b not or very little, supporting its strong antitumor effect.

By combination of the above two excellent antitumor stars and other antitumor methods, we hope we can partly win the war against cancer.

**IL-42****Track:** *Medical Biotechnology: biopharmaceutical manufacturing; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers.***NEAR-INFRARED SPECTROSCOPY OF THE BLADDER: NEW PARAMETERS FOR DIAGNOSING VOIDING DYSFUNCTION****Andrew Macnab, Lynn Stothers, Kourosh Afshar and Babak Shadgan***Professor and Head of NIRS Research Study Group, Division of Urology, Faculty of Medicine, University of British Columbia, Vancouver, Canada ; E-mail: ajmacnab@gmail.com*

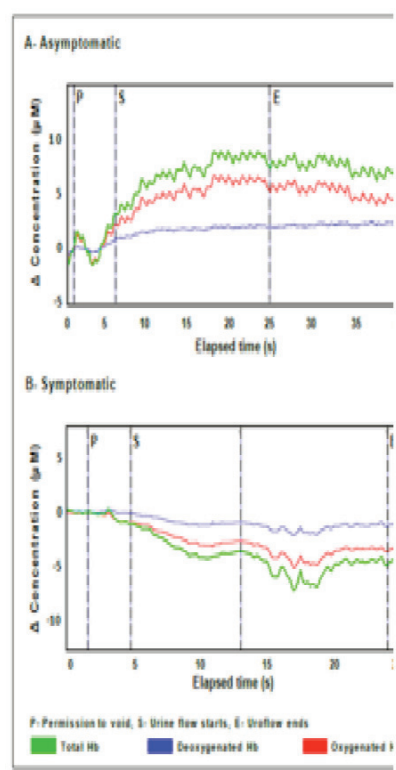
Introduction: Near-infrared spectroscopy (NIRS) is a non-invasive optical technique that monitors alterations in tissue oxygenation and haemodynamics *via* changes in chromophore concentration (oxygenated [O₂Hb] and deoxygenated [HHb] haemoglobin, and their sum, total haemoglobin [tHb]).

Purpose: To describe recent innovation using wireless NIRS to monitor bladder function.

Material: Subjects: (4-68 yrs) with and without lower urinary tract symptoms (LUTS). Monitoring instrument: Miniaturized wireless NIRS device (83 x 52 x 20 mm, 84 g) incorporating self-contained emitter/detector interface, light emitting diode light source (wavelengths 760/850 nanometers), silicon photodiode detector and 'Bluetooth'.

Methods: Data collected transcutaneously at 10 Hz during spontaneous voiding following natural filling with device tapped to abdominal skin over the bladder. Patterns of change in chromophore concentration were compared from permission to void, through uroflow start, to uroflow end.





Results: Asymptomatic subjects showed three consistent directional changes: $[O_2Hb]$ and $[tHb]$ increased following permission; rose further following uroflow start; and had a positive trend during voiding. Subjects with LUTS showed different chromophore patterns: $[O_2Hb]$ $[tHb]$ response absent or blunted after permission; fell following uroflow start; with negative trend during uroflow.

Conclusions: Non-invasive NIRS monitoring during natural bladder contraction (voiding) detects changes that suggest LUTS can be due to abnormal detrusor oxygenation or hemodynamics.

IL-3

Track: Regenerative Medicine

KGF RESCUES MICE FROM VAGINAL ATROPHY AND CONTRIBUTES TO NON-GENOMIC ER α PATHWAY

Simona Ceccarelli, Sirio D'Amici, Enrica Vescarelli, Antonio Angeloni and Cinzia Marchese

Department of Experimental Medicine, Sapienza University of Rome, Viale Regina Elena, 324 - Rome, Italy; E-mail: cinzia.marchese@uniroma1.it

Introduction: Reduction in circulating estrogen occurring in post-menopausal women results in atrophy of the vagina in over than 70% of patients. The treatment of choice is the oral hormone replacement therapy (HRT), even if it has been associated with increased risk of breast cancer, or estrogenic preparations administered vaginally. Keratinocyte growth factor (KGF/FGF7), a key paracrine mediator of epithelial/mesenchymal interactions, regulates epithelial proliferation of different organs. Recently, growth factors have been shown to interact with the estrogen pathway; however, the mechanisms of such interaction are not fully clarified.

Methods: We investigated, through Western blot analyses, Real Time PCR and luciferase assays, the crosstalk between estrogen and KGFR pathways, to clarify the mechanisms underlying the growth-promoting effect of estrogens and the potential role of growth factors in the development of novel therapeutic strategies for mucosal atrophy. Moreover, we compared efficacy of local estrogen treatment with topic administration of KGF *in vivo* in a murine model.



Results: We assessed KGF effect on cell proliferation in an *in vitro* reconstructed human vaginal mucosa, and its crosstalk with estrogen non-genomic pathways. Moreover, we demonstrated that the efficacy of KGF local administration in a murine model is comparable to that of intravaginal estrogenic preparations.

Conclusions: Therefore, we proposed KGF as a possible alternative therapy for postmenopausal vaginal atrophy or other dysfunctions, such as those occurring to patients subjected to radiotherapy after endometrial cancer surgery, devoid of the risks related to HRT treatment.

IL-59

Track: Medical Biotechnology: biopharmaceutical manufacturing; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers.

PROTEOMIC PROFILING OF PLASMA AND CSF FROM NEURODEGENERATIVE DISEASES UTILIZING ANTIGEN AND ANTIBODY MICROARRAYS

Peter Nilsson

Science for Life Laboratory, Department of Proteomics, School of Biotechnology, KTH - Royal Institute of Technology, Stockholm, Sweden ; E-mail: peter.nilsson@scilifelab.se



The Human Protein Atlas (www.proteinatlas.org) currently contains 17.298 validated antibodies targeting 14.079 proteins corresponding to approximately 70% of the encoded human proteins. The publicly available portal contains several million high-resolution images generated by immunohistochemistry on tissue microarrays and confocal microscopy for subcellular localization. The antibodies are antigen-purified and the long-term objective is to generate paired antibodies towards all human protein targets. A systematic biomarker discovery approach has been implemented, utilizing array-based platforms and the massive antigen and antibody production pipeline.

Proteomic profiling of serum, plasma and CSF in multi-disease cohorts are performed with large number of antigens on planar microarrays for the analysis of autoimmunity repertoires. Broad screenings and verifications with antigen-based profiling is performed within neurodegenerative related diseases, such as multiple sclerosis, ALS, Alzheimer as well as psychiatric disorders utilizing thousands of targets. Furthermore, large set of samples from the same diseases are also profiled with massive numbers of antibodies on highly multi-parallel suspension bead arrays which utilizes magnetic color-coded beads functionalized with antibodies to generate protein profiles from labeled samples for biomarker discovery. The results from both autoimmunity and antibody-based proteomic profiling within neurodegenerative related diseases utilizing thousands of targets in both platforms will be presented.

IL-78

Track: Medical Biotechnology: biopharmaceutical manufacturing; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers.

SYNTHESIS OF BIOCOMPATIBLE NANOMATERIALS BY THE PULSED PLASMA IN LIQUID

Emil Omurzak, Zhympargul Abdullaeva, Shintaro Takebe and Tsutomu Mashimo

Priority Organization for Innovation and Excellence, Kumamoto University, 2-39-1 Kurokami, Chuo-ku, Kumamoto City, Japan; E-mail: emil@kumamoto-u.ac.jp

One of the best biocompatible magnetic materials is iron (Fe) nanoparticles coated with carbon, where Fe provides magnetic response, while the carbon will serve as the biocompatible component. Carbon coated ZnO is also interesting materials because of its possibility to be applied for the bio-medical applications. Coating of ZnO with carbon can extend its current applications even to more fields, particularly, bio-medical applications, because, carbon is a biocompatible material and less affected in physiological conditions.

We achieved the production of carbon coated magnetic nanoparticles Fe@C (carbon coated iron) by the pulsed plasma in liquid method. Fe core nanoparticles with good crystalline structures of an average size between 20 and 30 nm were encapsulated in carbon coatings of 2-4 nm thickness. The synthesized samples showed good ferromagnetic properties at room temperature. In addition, our samples showed very high thermal stability (up to 700 °C). Produced by our method

magnetic nanoparticles showed the highest biocompatibility: more than 95 % of cells remained alive for carbon iron nanoparticles.

In addition, carbon coated ZnO nanorods with about 20 nm thickness and 150 nm length were synthesized by this method. Room temperature PL spectroscopy revealed that the sample exhibits UV (381 nm) and green (535 nm) emission peaks. When different surfactant materials (CTAB, SDS, OGM and SB) were used, ZnO nanorods with different morphology were produced. Closer look at the nanorod structure formed under SDS and CTAB conditions revealed that in both cases the surface of the nanorods were coated with carbon layers with different thickness: 2 nm in case of SDS and 5 nm for CTAB.

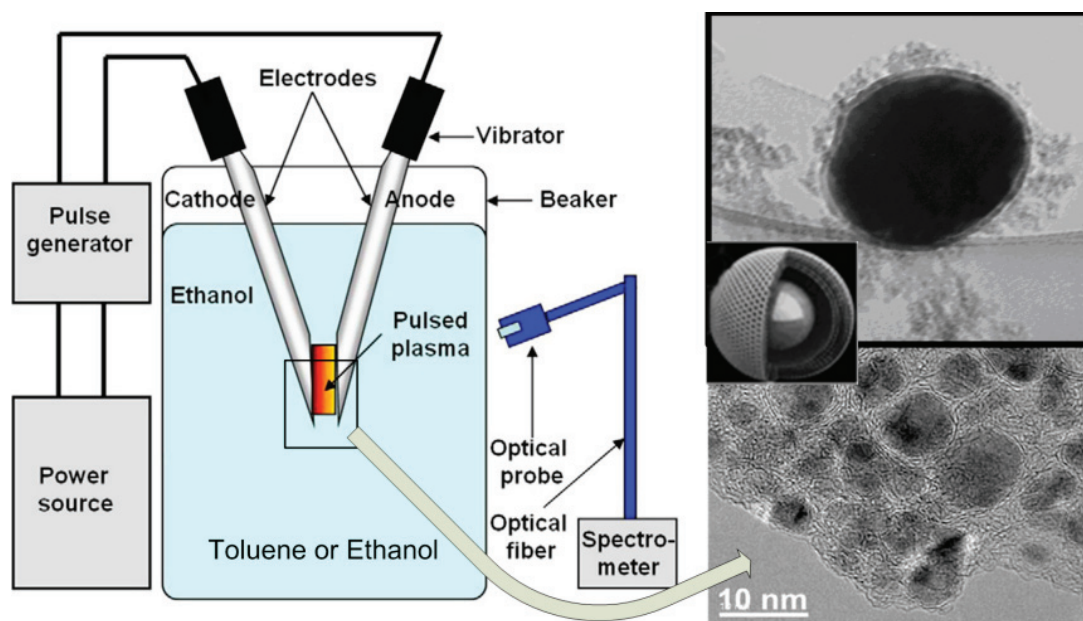


Fig. (1). Schematic illustration of the pulsed plasma in liquid method and the Transmission Electron Microscopy images of the synthesized iron nanoparticles by this method.

IL-5

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

LIFE EXPECTANCY STUDIES IN SPACE: CORRECTION OF MAGNESIUM DEFICITS

William J. Rowe

Medical University of Ohio, at Toledo, 1485 Bremerton La, Keswick, Virginia, 22947, USA; E-mail: rowerun@aol.com

It seems clear that we should take advantage of the microgravity of the International Space Station (ISS) to serve as a laboratory in the investigation of aging – both on Earth and in Space as this process is accelerated in Space. The unique environment of the ISS would facilitate experimental model systems that would otherwise not be possible and could be utilized in studying the aging process over an entire lifespan. Even life span studies of rats require a period of 3 years on Earth. How much shorter might these studies be in Space? One hypothesis proposes that the aging process is triggered by the shortening of telomeres, species specific, repetitive DNA sequences and associated proteins that cap and protect the ends of chromosomes from deteriorating. Telomeres function somewhat like the ends of shoe laces which slowly unravel with increasing age and also with stress. The preservation of natural chromosome ends and the rejoining of broken DNA ends rely on a set of proteins thought to decline with advancing age. Magnesium (Mg) levels have been shown to be significantly decreased with space flight which may be at least partially responsible for the accelerated aging process in Space. It has been postulated that reductions in Mg. levels might disrupt the DNA and/or the proteins associated with the telomere required for telomere capping and in turn accelerate erosion of the telomeres. The

mechanism of telomere shortening may be enhanced by Mg. - deficient induction of oxidative stress and inflammation as well as insulin resistance. Furthermore recent studies have also demonstrated a direct molecular link between telomeres and that telomeres play a major role in regulating mitochondrial dysfunction providing another link to aging. Identification and characterization of the underlying processes involved may require decades of research on the ISS. Until we have such information we will not be ready to spend long periods of times beyond low earth orbit.

IL-29

Track: Business Development

BIOECONOMY: FROM THEORY TO PRACTICE

George Sakellaris

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



The expansive growth of global population has led into a rapid depletion of many resources, including energy and food and to environmental pressures and climate change. The current economic crisis in Europe and the United States, partially due to the above reasons guided both parts to release official documents in which they consider bioeconomy as a key element for smart and green growth.

Advancements in bioeconomy research and innovation will allow to improve the management of the renewable biological resources and to open new and diversified markets in food and bio-based products. Establishing the bioeconomy concept, a great potential becomes achievable: it can maintain and create economic growth and jobs in rural, coastal and industrial areas, reduce fossil fuel dependence and improve the economic and environmental sustainability of primary production and processing industries.

Modern Biotechnology has become a key factor in modern bioeconomy worldwide. This potential is increasing since is touching a wider areas of applications within various domains, especially in the area of GMO's and their connection to Agrifood, Molecular Farming, Biomaterials, Bioenergy, Environment etc. As long as the potential of modern Biotechnology expands, lateral issues like regulatory frames, harmonized legislation, public perceptions and communications, ethical or moral issues are becoming more demanding and requiring. This perspective must be examined in the frame of the new emerging markets and the need of sustainable considerations for all applications.

A substantial research has been already done on Bioeconomy and its huge potential in modern societies and economies. In these researches is shown the way to a more innovative, resource efficient and competitive society that reconciles food security with the use of renewable resources for food, energy and industrial purposes, while ensuring environmental protection.

It is however imperative to carefully consider a number of parameters in order to make this model feasible:

- Sustainability in all levels, economic, environmental and social.
- Globalization and Universality consideration in all aspects (scientific and business), respecting the particularities on a case by case basis.
- Science and Technology Governance in the new conditions.
- Technology Transfer and the new poles of know-how creation.
- Social issues considering the benefits for the community, the diversification in public perceptions, the risk and the safety of products and practices.

In this whole new context, obtaining the full benefits of the bioeconomy will require purposive goal-oriented policy both by governments but also by leading firms, to establish goals for the application of biotechnology to primary production, industry and health; to put in place the structural conditions required to achieve success such as obtaining regional and international agreements; and to develop mechanisms to ensure that policy can flexibly adapt to new opportunities.

IL-7

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

COUNTING PCR: ABSOLUTE DNA COPY NUMBER WITHOUT STANDARDS**John SantaLucia**

DNA Software, Inc. 334 East Washington Street 48236, Ann Arbor, United States; E-mail: john@dnasoftware.com

The shape of a qPCR curve contains more information than previously appreciated. We have discovered how to analyze the shape of a PCR curve to reveal the absolute copy number of DNA at cycle zero. This discovery has led to the development of a method for absolute DNA quantification called Counting PCR (cPCR). In cPCR, each copy of DNA is literally counted for each cycle of PCR, the results of which are absolute DNA copy numbers. Because these results are instrument and fluorophore independent, qPCR results from different laboratories can be compared and metaanalysis studies of archived data sets can be performed.

DNA Software has incorporated the principles of cPCR in to qPCR CopyCount, which is a cloud-based service that automatically analyzes quantitative PCR (qPCR) data to derive the absolute DNA copy number of all qPCR reactions without any standards. qPCR CopyCount has been rigorously validated on more than one hundred thousand samples. The high quality of absolute quantification from qPCR CopyCount can be used for a variety of applications such as quantification of next generation sequencing fragment libraries, viral load, non-invasive detection methods, copy number variation, and gene expression. qPCR CopyCount is available as an online service at: <http://www.dnasoftware.com/product/copycount>.

IL-61

Track: *Medical Biotechnology: biopharmaceutical manufacturing; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers.*

AFFINITY-BASED DISCOVERY OF PLASMA PROTEIN PROFILING IN DISEASE BIOBANKS**Jochen M. Schwenk**

Science for Life Laboratory, Department of Proteomics, School of Biotechnology, KTH - Royal Institute of Technology, Stockholm, Sweden ; E-mail: jochen.schwenk@scilifelab.se



The growing numbers of large biobanks hosting categorized patient material opens new possibilities to screen for protein biomarkers in plasma. For such systematic exploration, suspension bead arrays have been developed with antibodies from the Human Protein Atlas (www.proteinatlas.org, [1]), where non-fractionated, biotinylated and heat-treated samples are analyzed in 384-plexed assays [2] and generate up to 150,000 immunoassays per day. This single-binder approach has yet revealed interesting profiles in the context of prostate cancer [3] and renal impairment [4], while there is a focus on larger scaled, hypothesis-free efforts. In a first pilot study 600 samples from 20 diseases (such as cancer, cardiovascular and neurodegenerative diseases) were profiled with 4,600 antibodies. This assay was then using 7,600 antibodies to identify the profiles' age and/or gender association in 384 samples from donors aged 5-85 and recently serum and plasma of patients suffering from cancer (384 samples, 5 cancer types) or cardiovascular disorders (384 samples, 4 categories) using 10,000 antibodies. The strategy around this discovery setting is to statistically identify candidate targets, replicate and verify these antibody-derived profiles in further experimental and biological investigations, also considering other body fluids. Experimental challenges such as the off-target binding susceptibility is addressed by developing sandwich assays, while making most use of the technological benefits to profile additional samples from independent sample cohorts and biobanks for to decrease the impact of sample-derived confounders. The presentation will give an overview of the conducted biobank profiling efforts and how to proceed with candidate verification when using antibodies and multiplexed technologies.

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IL-25

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring; photosynthetic microorganisms, cyanobacteria and microalgae.*

COMPREHENSIVE, NON-INVASIVE, AND QUANTITATIVE MONITORING OF THE HEALTH AND NUTRITION STATE OF CROP PLANTS BY MEANS OF HYPERSPECTRAL IMAGING AND COMPUTATIONAL INTELLIGENCE BASED ANALYSIS

Udo Seiffert

*Biosystems Engineering, Fraunhofer Institute for Factory Operation and Automation (IFF)
Magdeburg, Germany; E-mai: Udo.Seiffert@iff.fraunhofer.de*



Quantitative assessment of phenotypic properties of crop plants in relation to different genotypes, nutrition, stress tolerance, and fruit quality has become increasingly important in crop plant research, modern plant breeding, and particularly in precision agriculture / smart farming. The required assessment can generally be based either on morphological features, such as plant height, leaf shape, root structure etc., or on biomolecular/biochemical analyses. While the latter one is typically invasive and destroys the intact biological structure, morphological features are often not sufficient to unravel all relevant information at the required level of detail. Moreover, wet lab analyses typically assess only a more or less small number of samples and are not suitable to monitor crop plants in productive operation on the field or for large-scale (highthroughput) phenotyping of many genotypes in plant breeding. The results of wet lab analyses typically cannot be incorporated into on-line monitoring systems. There are several non-invasive systems currently available on the market. Typical applications are ground-based or airborne data acquisition for precision agriculture / smart farming as well as automated greenhouses in crop plant research and plant breeding. Technically these systems are based on broadband or selective colour imaging (ca. 400-800 nm) that is sometimes accompanied by a few selected spectral bands in IR (>800 nm) or UV (<400 nm) range. >From the application's perspective these systems can only monitor for example the level of green colour (chlorophyll) of the plants (leaves) as indicator of nitrogen nutrition (e.g. Yara N-Sensor), the water content, or some particular biochemical compounds. Monitoring of a comprehensive health and nutrition state of crop plants is currently not commercially available. Hyperspectral imaging linked to subsequent computational intelligence based analysis has proven its suitability to unravel complex information in a number of different application areas, such as geology, defence, etc. The extension of this approach to crop plant research, plant breeding, agriculture, and food processing has started just quite recently. Here, the image acquisition ranges from airborne sensing mainly for agricultural applications down to single leaf analysis in the context of precision and high-throughput plant phenotyping. All these applications have in common, that particular relevant compounds of the plant need to be determined by means of hyperspectral signatures as complement or substitute to extensive biochemical analyses.

2. Technical approach and functional characteristics Hyperspectral imaging has paved the way to obtain image data across wide wavelength ranges in selective spectral bins. Using state-of-the-art sensors typically leads up to several hundred spectral images of the same object or scenery. Every acquired image contains different information depending on the reflection/absorption properties of all objects within the field of view of the camera. In the case of plant or tree leaves these properties are mainly determined by biochemical compounds of tissues near to the leaf surface and by composition and morphology of the leaf surface itself. Each spatial entity, typically one pixel, is assigned with a vector containing the spectral data of this position – the spectral fingerprint. The length of this vector corresponds to the number of spectral bins of the used sensor. Compared to traditional images that cover a wavelength range in one single bin and basically correspond to the spectral sensitivity of the human eye (approx. 390-770 nm) the information contained in hyperspectral images is significantly higher. Since the biochemical composition of plant or tree leaves is a mixture of organic and inorganic molecules and elements, the relevant information is distributed over many spectral bins. Consequently, an analysis of just one or a few selected single wavelength images is usually not sufficient to get all relevant information. From the information processing point of view the complexity of the statistical model that contains the relationship between hyperspectral data and relevant biochemical compounds depends on the number of acquired wavelength bands and the number of considered biochemical compounds. This combinatorially increasing complexity is difficult to handle (Hughes Phenomenon; Curse of Dimensionality). This appears to be the reason why currently available systems cannot cope with such high-dimensional multivariate data spaces to make full advantage of the information acquired by hyperspectral imaging.

Implemented and potential applications range from:

- High-throughput plant phenotyping against the background of plant breeding under greenhouse and field conditions.
- Nutrition maps for typical field crops, such as wheat, rye, maize, etc. by airborne monitoring.

- Early stage detection of plant pathogen infection (e.g. mildew) and pest infestation of plants and trees (e.g. Red Palm Weevil in palm plantations), up to
- Quality control in harvesting/storing of crops as well as food processing.

This talk will briefly introduce the physical basis and demonstrate characteristic properties of a recently developed screening system along with extensive data from systematic field trials obtained between 2010 and 2012.

IL-4

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

NEW REGULATORS OF REPRODUCTIVE FUNCTIONS

Alexander Sirotkin

Research Centre of Animal Production, Luzianky, Slovakia; E-mail: sirotkin@cvzv.sk

Progress in animal biotechnology, assisted reproduction, human and veterinary medicine and animal production is promoted by discovery and application of the new extra- and intracellular regulators of reproductive functions. This is the review of original data concerning the role of some metabolic hormones (GH, leptin, ghrelin, obestatin), growth factors (IGF-I, IGFBPs, EGF, thrombopoietin), intracellular mediators of their action (cyclic nucleotides, protein kinases, transcription factors and related cDNA, siRNA and miRNA gene constructs) on basic ovarian functions (cell proliferation, apoptosis, secretion, oogenesis, ovulation, production and viability of pups) in different species (pig, rabbit, humans and chicken). Practical applications of some these molecules for characterisation, prediction and control of reproductive processes in these species was examined too.

It was shown that these hormonal and intracellular regulators are able to control apoptosis, proliferation and secretory activity in porcine, rabbit, human and chicken ovarian cells and maturation of porcine oocytes and cumulus oophorus in vivo and in vitro, as well as to suppress or promote the response of ovarian cells to other hormones (gonadotrophins, IGF-I, ghrelin). Immuno-blockade of these hormones prevented their effects. Effects of hormones on rabbit, human and chicken ovarian cells and on porcine and bovine oocytes were associated with changes in PKA, MAPK and CDK and transcription factors CREB, STAT-1 and p53 in such cells, whilst blockers of these kinases prevented or promoted hormones action. Transfection of porcine and rabbit granulosa cells with gene constructs for these transcription factors affected ovarian cell functions and prevented or reversed hormones action. Down-regulation of approx. 1./3 known protein kinases by specific siRNA constructs resulted not only decrease in accumulation of these kinases within human ovarian granulosa cells, but also changes in expression of kinase-dependent transcription factors, markers of cell proliferation, apoptosis and release of steroid hormones and IGF-I. Transfection of human granulosa cells with constructs up and down regulating expression of some miRNAs are able to increase or decrease ovarian cell proliferation, occurrence of apoptosis, as well as the release of progestagen, androgen, estrogen and IGF-I. *In-vivo* experiments demonstrated that leptin, IGF-I, steroid hormones and some regulators of PKA, MAPK and CDK could be used to predict reproductive efficiency, for direct *in-vitro* control of maturation of oocytes and for *in-vivo* stimulation of reproduction in pigs and rabbits.

These observations suggest, that metabolic hormones, growth factors and intracellular regulators and mediators of their action (protein kinases, transcription factors, siRNAs, miRNAs) can be used for characterization of state of ovarian cells, for identification signaling pathways (hormones-growth factors-protein kinases-transcription factors-genes regulating proliferation, apoptosis and secretory activity) controlling reproductive processes, as well as for prediction and control of basic ovarian cell functions (proliferation, apoptosis, secretory activity, maturation of oocyte-cumulus complex and fertility).

IL-43

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

ADVANCED BIOPROCESS MONITORING KEY TO PROCESS AND SYSTEMS UNDERSTANDING

Gerald Striedner, Markus Luchner, Rene Gutmann and Karl Bayer

ACIB GmbH, Graz, Austria; Department of Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria; E-mail: gerald.striedner@boku.ac.at

Biotechnology with its empirically driven process engineering approaches represents one of the fastest growing and successful industry branches of the last decades. However, if this success story should continue the general accepted rule that comprehensive process understanding represents the key for rational process design and successful implementation of economic processes must not be longer ignored.

The major challenges on the way to process understanding and rational bioprocess engineering are the complexity of biological systems and the limited capabilities of in situ and real-time monitoring. Therefore, improvements in bioprocess monitoring and diagnostic capabilities are imperative.

The expansion of off-line analytics to -omics techniques and advanced analysis provides a comprehensive data base down to the molecular level. The thus gained physiology relevant process information represents the first pillar of a rational bioprocessing approach.

Control and intervention during the process depend on real time access on relevant key variables but common bioprocess conditions demand a great deal on sensor/analyzer technology. In addition there is a mutual exclusivity of non-invasive signal acquisition and metabolic relevance of the signals. Consequently two different approaches are followed in parallel. The first one is the acquisition of non-specific signals and spectra indirectly related to either the cell population or compounds in the medium. The valuable but “hidden” cell physiology–relevant information is extracted by setting up correlations to off-line acquired bio-analytical data using statistics and chemometrics. Based on this information, data-driven soft-sensor systems have been successfully developed for on-line “monitoring” of complex variables such as cell density or product titer.

The alternative approach aims at analyzers for direct detection of physiologically relevant process variables. As the major part of metabolites is enclosed in the cell compartment direct access is very limited. Volatile organic compounds (VOCs) emitted by cells are directly connected to physiologically relevant information. They perfectly match the key requirements of analytes for real-time, non-invasive bioprocess monitoring as they are easily accessible via headspace sampling. Proton transfer reaction–mass spectrometry (PTR-MS) was selected as this technology allows for on-line measurement of VOCs with high sensitivity, down to concentrations in the pptv range. The thus accessible VOC spectrum represents a host- and process-specific metabolite panel for host cell characterization, efficient process operation, and automation.

IL-80

Track: *Other areas: Nanobiotechnology*

SCANNING TUNNELING MICROSCOPY STUDIES OF CONDUCTING PROTEIN NANOWIRES

Stuart Tessmer

Michigan State University, Biomedical Physical Sciences, 567 Wilson Road, Rm 4237, East Lansing, MI 48824, USA; E-mail: tessmer@msu.edu

Geobacter sulfurreducens is a bacterium that expresses protein filaments or pili that act as electrically conductive nanowires. The pili have dimensions similar to carbon nanotubes: a few nanometers in diameter, yet up to microns in length. These microbial nanowires transport metabolically generated electrons outside the cell body to electron acceptors in the organism's environment. Here I will present scanning tunneling microscopy images of these nanowires with molecular-scale resolution, and point spectroscopy curves to elucidate the mechanism of conductivity.



IL-45

Track: Medical Biotechnology: biopharmaceutical manufacturing; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers.

DNA DAMAGE REPAIR GENES AND HEART FAILURE: FOCUS ON BRCA1 AND BRCA2

Subodh Verma, Krishna K. Singh, Praphulla C. Shukla, Adrian Quan, Amie Creighton, Yi Pan, Fina Lovren, Hwee Teoh and Mohammed Al-Omran

Division of Cardiac Surgery, St. Michael's Hospital, 30 Bond Street, Toronto, Ontario, M5B 1W8, Canada; E-mail: vermasu@smh.ca



The tumor suppressor breast cancer susceptibility genes 1 and 2 (BRCA 1 and 2) play important roles in DNA damage repair. We have demonstrated that BRCA1/2 have previously unrecognized roles in heart failure. In mice, loss of BRCA1 in cardiomyocytes results in adverse cardiac remodeling, poor ventricular function and higher mortality in response to ischemic or genotoxic stress. In human adult and fetal cardiac tissues, ischemia induces DNA double-stranded breaks and upregulates BRCA1 expression. Similarly, deletion of BRCA2 in mouse cardiomyocytes results in greater susceptibility to cardiac failure induced by doxorubicin due to increased apoptosis and DNA double-stranded breaks. The notion that BRCA1/2 mutations may have significant implications outside of cancer etiologies is one that has gained considerable momentum in recent years. Consequently, we investigated the role of BRCA1 in sepsis. In mice that were subjected to cecal ligation and perforation (CLP), it was found that BRCA1 gene therapy significantly increased survival. BRCA1 gene therapy also blunted CLP-associated cardiac, pulmonary, hepatic and renal dysfunction and reduced CLP-elicited DNA double-stranded breaks and apoptosis in the liver. These findings present intriguing insights into the multifaceted role of the DNA damage repair genes BRCA1/2 in the setting of cardiac and inflammatory disorders.

IL-19

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

SINGLE-USE SYSTEMS SUPPORT CONTINUOUS PROCESSING IN BIOPRODUCTION

William George Whitford

Thermo Fisher Scientific, 925 West 1800 South Logan, Utah 84321 United States;
E-mail: bill.whitford@thermofisher.com



It's been over eight years since the FDA articulated the goal of "facilitating continuous processing to improve efficiency and manage variability" in pharmaceutical manufacturing. Since that time there has been a steady development of the concept in both literature and in practice. From individual programs to consortiums and government backed initiatives, many have acknowledged the value and invested in establishing CP in the pharmaceutical Industry. The most popular modes of animal cell culture in bioproduction today remain the severely discrete or discontinuous batch and fed-batch suspension culture approaches. However the recent development of disposable-based processing in single-use bioreactors to the 2000L scale are inspiring many companies to re-look at the newer implementations of CP available. The modularity and flexibility of SUS can aid in reducing process steps and facilitate adaptability in a CP flow and layout. Inexpensive SUS support hybrid re-configurations where required, and easily accommodate novelty in process design. Quite a number of culture modes supporting continuous or semi-continuous manufacturing approaches have existed for decades, and we see single use technology supporting their recent re-examination and development.

IL-26

Track: Business development

OPEN INNOVATION IN TRANSLATIONAL MEDICINE AND DIVERSIFIED HEALTHCARE BUSINESS**Mingde Xia**

Senior Director, Johnson & Johnson Corporate Office of Science and Technology, Cheng Kai International Tower, 355 Hong Qiao Road, Shanghai 200030, China; E-mail: mxia@its.jnj.com

Drug discovery and development is a high risk, high cost and high return business. It may take 10-12 years with \$1.4 billion to develop a new molecule entity. Global pharmaceutical market is expected to reach to \$1065-1095 billion in year 2015 with emerging market percentage of 28%. Current blockbuster revenues are seriously threatened by patent expiration, regulatory hurdles, increasing competition, reduced R&D efficiency, and drug price pressure. The presentation will give an overview of global pharmaceutical business and discuss about new business models in biotechnology focusing on innovation and collaboration with case studies.



SESSION LECTURES

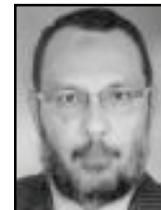
SL-20

Track: Other areas: Clinical Research / clinical trials

AMELIORATION OF DOXORUBICIN-INDUCED GENOTOXICITY IN ISOLATED CULTURED HUMAN LYMPHOCYTES BY THYMOQUINONE, CURCUMIN AND L-CARNOSINE

Mahmoud A. Naga, Mohamed A. Abd ElAziz, Mohamad-Hesham Y. Daba, Nadia K. El-Gamal and Sabry M. Zeid

Department of Clinical Pharmacy, College of Pharmacy, Salman Bin Abdulaziz University, Alkharij, Saudi Arabia; Emails: drabd_aziz729@yahoo.com; m.abdelmotaal@sau.edu.sa



In spite of the successful use of Thymoquinone (TQ) as antioxidant antimutagenic in many studies, it was not tried yet against the genotoxic effect of Doxorubicin (DOX) in isolated cultured human lymphocytes. Hence, we conducted the present study to investigate the possible antioxidant genoprotective effects of TQ 1 μ M on the chromosomal injury induced by DOX 0.15 μ g/ml in isolated cultured human lymphocytes in comparison with the well-known antioxidants L-Carnosine (L-Car) 20 mM and Curcumin (CMN) 15 μ M. After the end of culture period (72 hr), the levels of chromosomal aberrations (CAs); mitotic index (MI); reduced glutathione (GSH); malondialdehyde (MDA); and 8-hydroxydeoxyguanosine (8-OH-dG) were measured. DOX caused oxidative genotoxic effect as shown by significant increase in the levels of structural CAs, MDA and 8-OH-dG together with significant decrease in the levels of MI and GSH when compared to non treated group. DOX-induced toxic changes were significantly ameliorated when combined with TQ, L-Car or CMN with the highest protection for TQ > L-Car > CMN. The genoprotective effects of TQ, L-Car and CMN were mainly due to antioxidant activity as shown by decreased MDA and 8-OH-dG together with increased GSH. The higher activity of TQ over L-Car and CMN might be explained by the difference in structure where TQ has least molecular weight that allow it to penetrate cell wall and act efficiently on the level of cytosole and DNA. However, CMN has the highest molecular weight that limits its action to the level of lipid membranes.

SL-69

Track: Medical Biotechnology: biopharmaceutical manufacturing; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers.

PRELIMINARY STUDY ON ELEMENTAL DISTRIBUTION OF SEPIA OFFICINALIS BONES USING INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS

Ibrahim O. Abugassa and Khaled K. Doubali

Tajoura Nuclear Research Center, Department of Neutron Activation Analysis, Tajoura, Libya; P.O. Box 84518, Tripoli, Libya; E-mail: ibabugassa@yahoo.com

Hydroxyapatite has been utilized in manufacturing a suitable scaffold. But due to the shortcoming of the artificially proposed scaffold, studies are devoted on using natural products. Skeletons of marine species turned out to be a better substitute. Recent studies pointed out that Sepia officinalis bone is the most important marine species in the field of scaffold manufacture. Little information is available about the elemental constituent of this species. The present study focuses on the determination of major and trace elements in sepia officinalis bones that collected from the west coast of Libya and provided by local fisheries. The collected samples were treated at the Marine Biology Research Center and further preparations were performed at Tajoura Nuclear Research Center. Instrumental neutron activation analysis technique has been applied in this study. A comparison on the obtained results between the treated and sintered samples were conducted. 15 elements were investigated on both types of samples. The distribution of these elements according to the sample treatment is discussed.

SL-73

Track: Medical Biotechnology: biopharmaceutical manufacturing; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers.

MUC1-C ONCOPROTEIN ACTIVATES THE ZEB1/miR-200c REGULATORY LOOP AND EPITHELIAL-MESENCHYMAL TRANSITION

Rehan Ahmad, Hasan Rajabi, Maroof Alam, Hidekazu Takahashi, Akriti Kharbanda, Minakshi Guha and Donald Kufe

College of Medicine, King Saud University, Riyadh, Saudi Arabia;
E-mail: rehan_ahmad@outlook.com



The epithelial-mesenchymal transition (EMT) is activated in cancer cells by ZEB1, a member of the zinc finger/homeodomain family of transcriptional repressors. The mucin 1 (MUC1) heterodimeric protein is aberrantly overexpressed in human carcinoma cells. The present studies in breast cancer cells demonstrate that the oncogenic MUC1-C subunit induces expression of ZEB1 by a NF- κ B p65-dependent mechanism. MUC1-C occupies the ZEB1 promoter with NF- κ B p65 and thereby promotes ZEB1 transcription. In turn, ZEB1 associates with MUC1-C and the ZEB1/MUC1-C complex contributes to the transcriptional suppression of miR-200c, an inducer of epithelial differentiation. The coordinate upregulation of ZEB1 and suppression of miR-200c has been linked to the induction of EMT. In concert with the effects of MUC1-C on ZEB1 and miR-200c, we show that MUC1-C induces EMT and cellular invasion by a ZEB1-mediated mechanism. These findings indicate that (i) MUC1-C activates ZEB1 and suppresses miR-200c with the induction of EMT, and (ii) targeting MUC1-C could be an effective approach for the treatment of breast and possibly other types of cancers that develop EMT properties.

SL-66

Track: Other areas: Clinical Research

A METHOD FOR CONTINUOUS INTRACRANIAL PRESSURE REGISTRATION IN THE FREELY MOVING RAT

Mohamed Al-Olama, Stefan Lange, Kliment Gatzinsky and Eva Jennische

Department of Neurosurgery, Sahlgrenska University Hospital, SE 41345 Göteborg, Sweden, KG. Rashid Hospital, Dubai Health Authority, Dubai, UAE; E-mail: kliment.gatzinsky@neuro.gu.se/ dralolama@gmail.com

Background: Rise in intracranial pressure (ICP) induces increased morbidity and mortality calling for new techniques for ICP surveillance. Described models commonly use anesthetized animals, in which the anesthetic drugs influence ICP. Consequently, a method for ICP registration without anesthesia needs to be elaborated in order to provide an optimal surveillance.

Aim: Development of continuous ICP registration in the freely moving rat.

Methods: A pressure transducer catheter (Data Sciences Int.) was inserted epidurally via a hole in the right parietal bone. After fixing the catheter to the bone with dental cement, surgical closure followed. The rats recovered in a cage placed on a receiver registering ICP.

In a group of experimental rats a right-sided, cryogenic brain damage was performed, immediately followed by catheter implantation.

Results: ICP values between 2-5 mm Hg were found in 10 control rats up to seven days after operation. Increased ICP values, starting some 24 hours after the cryogenic damage, were registered in 10 experimental rats.

Conclusion: Continuous monitoring of ICP in freely moving rats is easy to perform and well tolerated by the rat. Our model can bring new information about the dynamics of pathological increase in ICP in different conditions involving brain injury/infection.

SL-60

Track: Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring; photosynthetic microorganisms, cyanobacteria and microalgae.

INDUCTION OF GLUTATHIONE S-TRANSFERASE AND GLUTATHIONE CONTENT BY HERBICIDE AND SAFENER TREATMENT IN BARLEY (*HORDEUM MURIANUM* L.) CELLS IN CULTURE

Renata Andrzejewska

Faculty of Biology and Environmental Sciences, Cardinal Stefan Wyszyński University in Warsaw, Poland; E-mail: r.andrzejewska@uksw.edu.pl

Treatment of barley suspension cells acclimatized and non-acclimatized with 0,2mM and 0,3 mM 2,4-D increased glutathione S-transferase activity by 1,35 - 2,35-fold. Total glutathione pool in acclimatized suspension cells was increased by the toxic compound during the entire experiment. The maximum glutathione concentration was determined after 72 hours and it amounted to 154% concentration with respect to control. Barley thus has a strong potential to neutralize toxic compounds, which may be further enhanced by safeners.

SL-72

Track: Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring; photosynthetic microorganisms, cyanobacteria and microalgae

AN IMPORTANT ROLE OF CARETNOIDS IN PROTECTION OF PHOTOSYNTHETIC APPARATUS UNDER VAM INOCULATION ON *MOMORDICA CHARANTIA*

Rafia Azmat

Department of Chemistry, University of Karachi 75270, Pakistan;
E-mail: rafiasaeed200@yahoo.com

The effect of mixed inoculums of VAM mycorrhiza on seed growth and photosynthetic apparatus in green house was monitored. The plants were watered daily with 200 ml of Hoagland solution in each pot in the month of April 2010 and cultivated in natural environment. The experimental treatments were arranged in parts based on a complete randomized block design with three replications. The positive effects of mycorrhizal as already mentioned in the literature were not reported on growth and chloroplast pigments. Results showed that initially increased in chlorophyll a & b in first phase (15 d) analysis of pigments were found to be decrease in the second phase (30 d) of growth whereas reverse results were observed with caretenoid pigment as compared to chlorophyll. The decrease in chlorophyll contents (30 d) may be directly related with the leaf area of plants which possibly attributed with absorption of solar radiation for the protection of plants. It was also supported by the higher concentration of carotenoids (30 d), that may have an additional function of regulation of certain developmental responses and screening of light to save the plants from stress conditions.

**SL-18**

Track: Regenerative Medicine: stem cells, gene therapy; tissue engineering; cell based therapy; cell cultivation.

NEO-INNervation OF BIOENGINEERED COLONIC SMOOTH MUSCLE CONSTRUCTS

Khalil N. Bitar

Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Virginia Tech-Wake Forest School of Biomedical Engineering and Science, Medical Center Blvd., Winston-Salem, NC 27157, USA; E-mail: kbitar@wakehealth.edu

1-We bioengineered a neuromuscular patch to restore motility.

2-Neo-innervation of bioengineered smooth muscle constructs was established.

Methods: 1) We bioengineered innervated and non-innervated concentrically aligned smooth muscle constructs. 2) The constructs were placed around biodegradable chitosan-based tubular scaffolds. The innervated



construct was placed attached to the non-innervated muscle construct around the same scaffold. (3) Real time force generation was performed on the constructs *in vitro*.

Results: At days 18-20, the constructs were taken off the scaffold and their physiological functionality was tested. **Initially non-innervated colonic smooth muscle construct:** (i) KCl induced a rapid and robust contraction. (ii) Ach induced an increase in force generation. Pre-treatment of the constructs with TTX attenuated the contraction by (40%). (iii) VIP induced a rapid relaxation, 50% of which was inhibited when the constructs were pre-incubated with TTX. This indicates the emergence of a new neuronal component in addition to the myogenic component. (iv) EFS induced a relaxation, which was totally inhibited by TTX, indicating the emergence of newly formed inhibitory motor neurons.

Conclusions: Neo-innervation of a bioengineered colonic smooth muscle construct occurred around a chitosan based scaffold. Our results indicate the emergence of new functional neuronal network in the initially non-innervated constructs. This is a successful demonstration of bioengineering neuromuscular patch as a way of innervating muscle tissues. This study provides a potential for regenerative medicine therapeutics to treat motility disorders of the gut.

SL-81

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

IMPLEMENTING CONTINUOUS BIOPROCESSING IN A SINGLE USE FACILITY OF THE FUTURE

John Bonham-Carter

Refine Technology, LLC, 26 Chapin Road, Unit 1107, PO Box 691, Pine Brook, NJ, 07058, USA;
E-mail: jbonhamcarter@refinotech.com

Manufacturing biological substances is a complex and expensive business. While previously approved drugs have been able to count on generating high revenues, this is no longer always true. Orphan drugs, competitive drugs for the same indication, biosimilars and health care costs are all challenging the status quo.

This talk will look at the impacts of new technology on biomanufacturing facilities. In particular, it will cover:

- Regulation, standardization and science: Fact or fiction.
- Upgrading facilities to handle continuous processing versus build new facilities Integrating continuous upstream and downstream processes.
- Overcoming operational issues for materials and staff.



SL-32

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

NOVEL LARGE SCALE BIOREACTOR SUPPORTS CONTINUOUS MANUFACTURING

John Cadwell

FiberCell Systems Inc. 905 W. 7th Street # 334 Frederick, MD 21701, Frederick, United States;
E-mail: jjcadwell@comcast.net

The most popular modes of animal cell culture in bioproduction today are the batch and fed-batch suspension culture approaches. A number of culture modes supporting continuous manufacturing approaches have existed for quite some time; however recent regulatory imperatives as well as the development of novel manufacturing platforms are inspiring companies to re-consider at the newer implementations of CP available. The flexibility of single-use hollow fiber bioreactors (HFBR) aids in reducing process steps and facilitating a CP flow and layout. Hollow fiber bioreactors offer a unique and more *in vivo* like cell culture environment than classical batch culture, with many



advantages for the production of secreted proteins. The high cell density facilitates the use of simplified, protein free medium and may also result in more complete and uniform post-translational modifications. Production can be maintained for several months, and the harvested secreted products can be 100 times more concentrated. FiberCell Systems has developed a completely closed and disposable large-scale hollow fiber bioreactor based upon a novel method for generating medium flow and gas delivery. Harvesting is performed on a continuous basis and is especially well suited for labile, toxic or difficult-to-express products. It is the first production bioreactor whose culture conditions approach those of the in vivo ideal and represents a potential paradigm shift for biomanufacturing.

SL-10

Track: Pharmaceutical Biotechnology

COMPARATIVE ANALYSIS OF THE EFFECTS OF IGF-1 AND ITS SPLICING VARIANT (MGF) ON HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM CELLS (HBMSCS) *IN VITRO*

Ziwei Luo, Haibin Li, Yu Can, Yonggang Lv and Li Yang

Key Laboratory of Biorheological Science and Technology (Chongqing University), Ministry of Education, Bioengineering College, Chongqing University, Chongqing 400044, P. R. China; '111' Project Laboratory of Biomechanics and Tissue Repair, Bioengineering College, Chongqing University, Chongqing 400044, P.R. China; E-mail: yanglibme@cqu.edu.cn



Mechano growth factor (MGF) is a novel splice variant of the insulin growth factor-1 (IGF-1), also known as IGF-1Ec in humans and IGF -1Eb in rodents. It is actually originally named MGF because of its character of mechanical-induced response, such as overload or damage in skeletal muscle. MGF has been shown to boost muscle mass by improving the ability of wasted tissue to grow and improve itself by activating muscle satellites (muscle stem cells) and increasing the upregulation of protein synthesis. However, little is known about its effect on human bone marrow-derived mesenchymal stem cells (hBMSCs). Comparing with IGF-1, the effect of MGF on hBMSCs growth and migration was investigated in this study. The results showed that hMSCs treated by MGF grew significantly slowly than IGF-1. This suggested that MGF may play a different role in hBMSCs growth from IGF-1. Further, both wound healing assay and transwell migration assay proved that MGF enhanced hMSCs migration during the first 4 hours and the roles of MGF and IGF-1 were different. Our results suggested that MGF could preferentially active hBMSCs homing to the injury site and promote wounds healing while the effect of MGF on hBMSCs growth is smaller than that of IGF-1. Further studies are still needed to be done to test these results.

Acknowledgements

This work was supported in part by grants from the National Natural Science Foundation of China (11032012) and the Program for New Century Excellent Talents in University (NCET-10-0879).

SL-83

Track: Pharmaceutical Biotechnology

mi-RNA-150 AND 7 REGULATORS OF MESENCHYMAL STROMAL CELLS ACTION ON COLON CANCER INFLAMMATORY TUMOR MICROENVIRONMENT

Sabine Francois, Bruno Lhomme, Marc Benderitter, Norbert-Claude Gorin, Luc Douay, Annette Larsen, Marie-Elisabeth Forgue-Lafitte and Alain Chapel

Institute of Radiation and Nuclear safety (IRSN), Department of man radioprotection, Laboratory of Radio-Pathology and Innovative Therapies, Fontenay aux roses, France; E-mail: alain.chapel@irsn.fr



This study is the first step toward the use of MSC therapy to reverse pelvic radiotherapy complications on healthy tissue without secondary effect on residual cancer. A rat model of colorectal carcinogenesis close to human cancer was used as preclinical model. MSC treatment reduces cancer significantly lowering the number of adenomas and adeno-carcinomas and extends the life of animals. Anti-cancer effect of MSC was mediated by their immunologic properties. In adenocarcinoma of MSC-treated rats, monocytes/ macrophages CD68+ infiltration was lowered and lymphocytes CD3+

increased. MSC induce macrophage to turn into regulatory cells involved in phagocytosis, inhibiting the production of pro-inflammatory cytokines. MSC decrease NK cells and rTh17 cell activities, Treg recruitment, CD8 + lymphocytes and endothelial cells number, restore Th17 cell activity. MiRNA mi-150 and miRNA-7 are the key effectors. Mi-150 inhibits tumor invasion and mi-RNA-7 regulates negatively the pathway EGFR / AKT promoting cell death. MSC infusion have a durable action on colon cancer development by modulating the immune component of the tumor microenvironment. For the first time, two mi-RNA were identified as responsible of MSC anti-cancer effect. This study is the first step toward use of MSC in therapy to alleviate the effects of radiation exposure in patients.

SL-67

Track: Medical Biotechnology: biopharmaceutical manufacturing; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers.

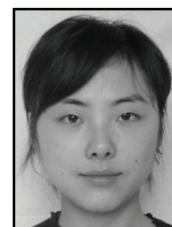
IDENTIFYING RARE CANCER CELLS IN BLOOD USING WORLD'S FASTEST REAL-TIME CAMERA**Claire L. Chen**

University of California, Los Angeles, 420 Westwood Plaza 63-128 Engineering IV, Los Angeles, CA 90095, USA; E-mail: lchen@seas.ucla.edu

Harvesting and analyzing circulating cancer cells in blood is intensively studied due to its great importance for early detection of cancer and its metastasis and for monitoring the efficacy of treatments. However, most of the widely used diagnostic methods are based on conventional optical microscope, which is useful for detailed examination of a small amount (<10,000) of microscopic entities, but incapable of statistically relevant screening of large populations (>100,000,000) with high precision.

Based on the world's fastest camera developed in our group previously, we present an automated imaging-based flow cytometry that can not only overcome the limitation of conventional microscope by acquiring sensitive blur-free images during high-speed flow, but also perform nonstop real-time recording and classification of microparticles based on their unique footprints, including cell size, EpCAM affinity, protein content and so on. This is made possible by integrating ultrafast optical imaging technology, self-focusing microfluidic technology, optoelectronic communication technology, and information technology.

Preliminary results indicate that this new technology has the potential to enable detection of rare circulating tumor cells from a large volume of whole blood in a short time, which leads the way to statistically accurate early detection of cancer, making timely clinical decision, and monitoring efficacy of drug and radiation therapy.



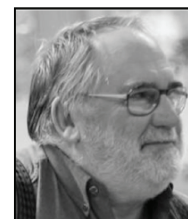
SL-65

Track: Medical Biotechnology: biopharmaceutical manufacturing; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers.

DATA ACQUISITION WITH MEDICAL LOW RADIATION SCANNERS: THE MANUFACTURERS' RELIABILITY VERSUS CLINICAL APPLICATION RELIABILITY**J.P. Clarys, A. Scafoglieri, J. Tresignie, O. Louis, S. Provyn, E. Cattrysse and J. De Mey**

Experimental Anatomy and Radiology, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussel, Belgium; E-mail: jclarys@vub.ac.be

The quality control of medical data acquisition using low radiation scanners based on mathematical algorithms introduced by the manufacturer needs an accuracy and precision validation with directly obtained and absolute data. Dual energy X-ray absorptiometry scanners (DXA) have become popular in radiology and public health e.g. nutrition and osteoporosis studies, just because of its low radiation. The DXA equipment is designed for human and animal research and clinical diagnoses. Controversial impression concerning its use is dictated by various critical appraisals and clinical reports. This study cross-validates and compares DXA fan beam scanings with direct dissection of the related variables.



Twelve porcine carcasses, were measured with DXA and CT before dissection into its related components. Tissue samples were analysed for their chemical and hydration composition. The skeleton was ashed. There are three main assumptions in determining the mathematical algorithms for body composition using DXA: (i) hydration of bone-free lean tissue is constant. (ii) antero-posterior diameter of the body is constant and (iii) tissue distribution is homogeneous throughout the whole body. This users-quality evaluation confirms that part of the existing problem results from erroneous terminology suggested by the manufacturer. The predictive values of DXA are good. The precision capacity of DXA variables resulted into significant differences and none of the assumptions made by the manufacturer are valid. This study presents indications that clinical precision for the individual patient is at risk in particular for bone and density data.

SL-37

Track: Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring; photosynthetic microorganisms, cyanobacteria and microalgae

STUDIES ON THE BIOCONTROL OF PHYTOPATHOGENS OF *VIGNA RADIATA* USING *PSEUDOMONAS FLUORESCENS* IN SUSTAINABLE AGRICULTURE

S. Cynthia Adeline and R. P. Dhanya

Department of Microbiology, Noorul Islam College of Arts & Science, Kanyakumari District, Tamil Nadu, India; E-mail: sldagu@yahoo.com

Legumes for human consumption constitute about 5% of the cultivated crops. Most leguminous vegetables are rich in phosphorus, calcium, iron, and a number of essential vitamins. Mungbean is one among the leguminous plants that is nutritious and economically important in India. India accounts for about 60% of the world's mungbean area and harvests 47% of the world production. The bacterial and fungal pathogens cause serious damage in agriculture, resulting in critical losses of yield, quality and profit. Chemical insecticides are normally used against phytopathogens. Due to serious demerits of using chemical insecticides, bioinsecticides are used as alternatives. Particular bacterial strains in certain natural environments prevent infectious diseases of plant. The *Pseudomonas fluorescens* in addition to their ability to aid plant growth promotion is also good biocontrol agent. In the present study, *Pseudomonas fluorescens* was isolated from soil and it was confirmed as *Pseudomonas fluorescens* A506 by performing 16S rDNA sequencing in Sanger's method. *Pseudomonas fluorescens* was checked for its antibiosis activity against the phytopathogens *in vitro*. The formation of biofilm of *Pseudomonas fluorescens* with PGPRs like *Rhizobium* and *Bacillus subtilis* was analyzed to confirm that they can coexist. Thirty alginate beads entrapping *Pseudomonas fluorescens* and other PGPRs were able to inhibit the bacterial and fungal growth in broth. Inoculation of *Vigna radiata* plants with *Pseudomonas fluorescens* and other PGPRs induced a significant increase in root and shoot length, nodules, fresh weight and protein content in *Vigna radiata* as compared to uninoculated control. Diseases like mosaic caused by mungbean mosaic virus, anthracnose disease caused by *Colletotrichum truncatum* and bacterial halo blight caused by *Pseudomonas syringae* was found in uninoculated control plants. Further investigations on the type of antimicrobial components and *in vivo* experiments will make *Pseudomonas fluorescens* as one of the most suitable candidate biocontrol agent in suppressing the phytopathogens and replace chemical pesticides.

SL-53

Track: Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring; photosynthetic microorganisms, cyanobacteria and microalgae.

USE OF BIOTECHNOLOGY IN *ARGANIA SPINOSA* (L.) SKEELS DIVERSITY ASSESSMENT

Yasmina EL Bahloul, Fatima Gaboun and Ikrame Machtoune

Plant Breeding Unit Conservation and Valorisation of Plant Genetic Resources. Regional Centre for Agricultural Research in Rabat. B.P 6570 Rabat-Instituts 10101. INRA-MOROCCO; E-mail: yas_ba@yahoo.fr

Argania spinosa (L.) Skeels is a tree native to Morocco. Besides the countless virtues of Argane oil, It contributes at economic, environmental, historical and cultural levels and has a great interest for local populations. Unfortunately, it is a very threatened species. Efforts are focusing on regeneration



and biodiversity assessment to understand the evolution to protect the forest.

This study is using biotechnological tools to contribute to the preservation of *Argania spinosa* (L.) Skeels forest, by developing new patterns for the genetic researches.

Microsatellite markers were developed by a gDNA enriched library. Firstly a set of 100 probes were generated. Further analysis by sequencing showed that 98 % were containing tandem repeats. After primers design and genotyping of a random sample of Argane trees, a high polymorphism has been revealed. The number of alleles reached 15 alleles per locus, with an average diversity ranging from 0.59 to 0.75.

The developed molecular markers showed their high ability for molecular biodiversity evaluation and phylogenetics studies, to better predict the evolution of *Argania spinosa* forest and related species. Results highlighted the benefit of biotechnology contribution at a large scale, to the development.

SL-38

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

PROMISING THERAPY OF ALZHEIMER'S DISEASE TARGETING ANGIOTENSIN - CONVERTING ENZYME AND THE CYCLOOXYGENASE -2 ISOFORM

Nesrine S. El Sayed, Lobna Abdel Aal Kassem and Ola A. Heikal

Pharmacology and Toxicology, German University in Cairo, Egypt; E-mail: nesrine_salah2002@yahoo.com

Deposition of beta-amyloid in brain is one of the pathological hallmarks of Alzheimer's disease (AD) that is often associated with inflammatory response. Much evidence also points to a link between renin-angiotensin system, hypertension and dementia. Accordingly, the potential use of anti-inflammatory and antihypertensives might be beneficial agents in AD therapy. In this study, we investigated the possible mechanisms of celecoxib (cyclooxygenase-2 (COX-2) inhibitor), perindopril (angiotensin converting enzyme (ACE) inhibitor) and their combination in lipopolysaccharide (LPS) model of AD. Mice were injected with LPS (0.8mg/kg, i.p.) for 7 days and treated with celecoxib (30mg/kg/day, i.p), perindopril (0.5mg/kg/day, i.p) and combination of both drugs. Learning and memory function were tested using Y-maze and locomotor activity was assessed using open-field test. Cerebral specimens were subjected to histopathological studies. Rate of energized and de-energized mitochondrial swelling and brain tumor necrosis factor-alpha (TNF- α), interleukin-1beta (IL-1 β) levels were measured. LPS decreased locomotor activity and percentage of correct choices in Y-maze test. It also produced significant increase in area percentage of vascular angiopathy, area of lamellated plaques, apoptotic index and induced mitochondrial dysfunction. These were associated with increased TNF- α and IL-1 β . Administration of either celecoxib or perindopril partially improved the cognitive impairment, mitochondrial dysfunction and decreased inflammatory cytokines and amyloid deposition. Combined therapy of both drugs completely prevented LPS-induced neurodegenerative and cognitive changes. These findings establish a link between COX-2, ACE activity and cognitive impairment in AD and provide a novel promising strategy for the complete cure of AD.

SL-31

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring; photosynthetic microorganisms, cyanobacteria and microalgae.*

ANALYSIS OF ORGANOGENETIC APTITUDE OF CITRUS VARIETIES FOLLOWING AN *IN VITRO* MULTIPLICATION OF THE REJECTIONS OF OLD PLANTS OF TEN YEARS

Maiza-Benabdesselam Fadila and Maddi Taklit

Laboratory of Plant Biotechnology and Ethnobotany- Bejaia University- 06000- Algeria; E-mail: fadilamaiza@yahoo.fr

In the present study, different media and various combination of growth regulators are used to determinate the organogenetic aptitude of three species of *Citrus* (*Citrus Sinensis*, *Citrus Limon* and *Citrus Deliciosa*) from 10 years old. For this, we used four types of media. We notice that Murashige and Skoog medium supplemented with 2,4-dichlorophenoxy acetic acid (2,4-D) and BAP provides the best callogene for the three species but the combination of

the naphthalen acetic acid (NAA) and benzylaminopurine (BAP) at the respective concentrations of 0.1 and 1 mg/l, offers the best caulogenesis with a rate of 30%. However the multiplication of axillary buds gives a percentage of 100% in two media: MS medium supplemented with BAP (1mg/l) without auxin and on MS medium BAP 1mg/l added by indolylacetic acid (IAA) at concentration of 0.1 mg/l, although all other media answer favorably but with less significant rates especially in the case of the Mandarin Carvalhal.

Whole plants were obtained starting from leaf shoots. To this end the experiments which we undertook on the rhizogenesis, showed that the Murashige and Skoog medium added with indol-butyric acid AIB (0.1 mg/l) and the activated charcoal with 0.5 g/l proved particularly favorable to the rooting of the buds of Lemon tree and Orange tree with rates of 42% and 43% respectively.

SL-11

Track: Other Areas

HIGH-RESOLUTION LINKAGE MAPPING FOR HEAT TOLERANCE BY WHOLE-GENOME RESEQUENCING IN CHINESE CABBAGE

Hui Shen, Xiang Yu, Pinglin Liu and Yuke He

National Key Laboratory of Plant Molecular Genetics, Shanghai Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 300 Fenglin Road, Shanghai 200032, China



Chinese cabbage is an important vegetable crop, and heat stress usually causes great losses in yields and quality. Under heat stress, the leaves become etiolated due to the disruption and disassembly of chloroplasts. The varieties of non-heading Chinese cabbage (*B. rapa* ssp. *chinensis*) are significantly distinct in heat tolerance. This work employed high-resolution linkage mapping of quantitative trait loci (QTL) based on recombination maps for a population of 150 recombinant inbred lines (RILs) derived from the above two subspecies that showed distinct heat resistance. Using approximately $40 \times$ coverage sequencing, 0.7 million and 0.9 million SNPs were detected between the reference and two parents, respectively. About 80 thousand SNPs in the gene region caused nonsynonymous mutations, and INDEL-caused frameshift mutations were considered to be important factors associated with genetic variance. About 300 genes were found to be premature or abnormally terminal, and six target genes of the miRNAs exhibited SNP mutation in the miRNA complementary site. We combined and simplified two sequencing-based genotyping methods developed in rice for optimization of genotyping and linkage mapping. All of the recombination breakpoints in RILs shuffled 10 chromosomes of *B. rapa* into 1803 bins. After filtering out recombination hotspots, a total of 1726 bins served as genetic markers, and 13 QTLs of heat tolerance with $\text{LOD} \geq 3$ and phenotypic effect (R^2) $\geq 5\%$ could be detected by high-resolution linkage mapping. QTLs, clustered with the two economic traits, were observed in Chr. 3 and Chr. 6, respectively. The peak signals at several leaf trait loci were closely tied to the identified homologous genes in Arabidopsis. The present work constructs the first whole genome-wide SNP and INDEL annotation and mapping of multiple leaf trait loci between two varieties in *B. rapa*, and can help to accelerate the research on molecular mechanisms underlying variance of heat tolerance.

SL-17

Track: (Other Areas)

ALL FROM STEM CELL IDENTITY TO GROWTH LAID DOWN BY CELL WALL CONTROL

Hilde-Gunn O. Hoen-Sorteberg

Department of Molecular, Cellular and Developmental Biology, Yale University, USA; E-mail: hilde-gunn.hoen-sorteberg@umb.no

Understanding development is important to systems biology/modeling, and applications. Meristem/specialized cell types might be used to develop future food, feed and energy crops by GM technology. RNA sequencing and laser-microdissection of specialized cells can give a complete overview of genetic networks and true gene-banks. Recent know-how and options will be presented.

Understanding how a new organism develops from fertilization and control of cell identity, body patterns and growth is fundamental for optimal functions and applications. Full genome sequences give us increasing number of new receipts

or say spices alphabets, acquiring needs to learn and make sense of these biological languages. To do so takes resources and high quality results demand accurate assumptions. Systems biology and modeling depend on high quality experimentation, accurate understanding and constantly updating current know-how of biology.

Despite vast global efforts to understand life through history, we are still not there yet. All multicellular organisms are organized in L1 (ectoderm; epidermal and nervous systems), L2 (mesophyll/muscle) and L3 (gut/vasculature) cell layers, and even obvious differences between animals and plants, there are similarities. RNAi was first discovered in plants, while we have profited from other findings done within medical science. Cellularization of the cereal seed endosperm development, follow the same pattern as *Drosophila* embryo cell divisions by alveolation. Recently accumulating evidence point to the cell wall when it comes to guiding developmental cues, cell identity and growth. Plant architecture depend on indetermined stem cell pools in the shoot and root. Results from microscopy, immunostaining, cytoskeleton monitoring of cell divisions, gene regulation of embryonic and meristematic markers, induced genetic up and down-regulation and microarray hybridization will be presented to show how a new understanding of basic deveopmental cues is revealed. Final suggestions to generate results for some relationships between hormone effects and signal transduction pathways during development will be suggested including laser micro-dissection of specific cells and RNA sequencing.

Understanding cell differentiation and development, opens options to modify cell types e.g. increase number of cells interesting to increase harvestable products from plants in the field or cell cultures. It can further be applied to increase usability for biofuel harvesting potentials by making cell walls more accessible in certain species, while increasing food and feed potentials in other specialized crops. When possible certain metabolic pathways can be optimized, while other combined goals might have to be accepted not possible due to unbreakable correlations or conflicts of interests. All such progress will depend on carefully controlled bioethical considerations, must be harmonized with international economic trades agreement, economic sustainability, natural sustainability, diplomatic relationships, cultural interests and finally individual and public moral.

SL-63

Track: *Medical Biotechnology: biopharmaceutical manufacturing; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers.*

RISKS OF BIOLOGICAL OXIDATIVE STRESS AND THE ROLE OF ANTIOXIDANTS

M. Ibrahim

Northern Border University, Faculty of Science, Physics Department, Arar, KSA; E-mail: mahmoudbio@yahoo.com

Many abnormal metabolic reactions inside the biological system might be due to oxidative stress. Oxidative processes give rise to free radicals or reactive oxygen species which, in turn, produce the toxic hydrogen peroxide.

Redox (reduction-oxidation) potential may be defined as a quantitative expression of the tendency that a molecule has to give or receive electron. Redox potentials would correlate with the observed biological effects since living organisms function at an optimum redox potential. Disturbance in redox reactions which results in an oxidative stress, might lead the biological system to undergo abnormal metabolic reactions giving rise to diseases or even cancer.

In this work, Control eye lenses and lenses from patients with **Cataract** (clouding of the eye's clear lens) have been studied. Blood samples of control persons and different patients with **Leukemia** (blood or bone marrow cancer), **Melanoma** (a malignant tumor of melanocytes or skin cancer), **Renal Failure** (the failure of kidney to filter toxins and waste products from the blood), **Diabetes** (high blood sugar) and **Hepatitis** (liver inflammation) have been also investigated spectroscopically by the use of double beam UV-Vis spectrophotometer and spectrofluorometer. Some antioxidants, accordingly, have been used.

The results showed that hydrogen peroxide (H_2O_2) is produced during the progress of the investigated diseases as a result of reactive oxygen species. On the other hand, nicotinamide adenine dinucleotide (NADH), glutathione (GSH), ascorbic acid (Vitamin C), Vitamin E, Vitamin A and ginseng extract showed good and outstanding effects on both scavenging the reactive oxygen species and decreasing lipid peroxides.

The results, therefore suggest that: 1) Spectroscopy is a good, if not the best, tool to differentiate between control and diseased cells "diagnosis", 2) Cataract, leukemia, melanoma, renal failure, diabetes, hepatitis are attributed as extensive oxidative stresses which result in a disturbance in the redox system "the biological balance", 3) Antioxidants are highly recommended for treatment of most diseases and cancers.

SL-74**Track: Plant and Environment****SALINITY AND DROUGHT INDUCED ANTIOXIDANT RESPONSES IN DIFFERENT CULTIVARS OF SAFFLOWER (*CARTHAMUS TINCTORIUS*, L).****Sadia Javed, Iftikhar Hussain Bukhari, Munazzah Meraj and Saqib Mahmood***Department of Chemistry, Government College University, Faisalabad, Pakistan; E-mails: Sadiaamer11@gmail.com, diyajav1@yahoo.com*

Six cultivars of safflower which were (PI-387820, PI-251978, PI-170274, PI-387821, PI-386174 and Thori-78) grown in net house of NIAB under salinity (10 ds m⁻¹) and drought (60% field capacity) conditions and compared for their oxidative damage and antioxidative responses. Plant samples (leaves) were collected for the determination of malonaldehyde (MDA), antioxidative enzymes (catalase, ascorbate peroxidase, glutathione reductase, and peroxidase), proline, and photosynthetic pigments. Salinity and drought decreased the chlorophyll a and b contents but a decrease in chlorophyll a and b was less in safflower variety (THORI-78) which could be a useful marker for selecting a stress tolerant variety. Both stresses considerable increases the accumulation of proline in PI-251978, PI-170274, PI-387821, PI-386174 and THORI-78 varieties of safflower whereas the proline accumulation did not appear to be an essential part of the protection mechanism against salinity and drought in variety PI-387820. Enzyme activity measurements revealed that THORI-78 can tolerate salinity and drought stress well by increasing the activity of catalase and APX enzymes whereas variety PI-386174 showed increased activity of glutathione reductase enzyme under salinity and drought and appear to be very crucial antioxidative defenses during intense stress conditions. The results indicate that the photosynthetic pigments, proline and activities of the enzymes are important mechanism for the stress tolerance in safflower plant and can be considered as genetic improvement for the plant in salinity and drought soil conditions.

SL-49**Track: Pharmaceutical Biotechnology****SCREENING OF INDIGENOUSLY ISOLATED FUNGI FOR THE PRODUCTION AND OPTIMIZATION OF CHOLESTEROL LOWERING DRUG LOVASTATIN AND ITS IN VIVO EVALUATION****Sadia Javed, Munazzah Meraj and Shazia Anwer Bukhari***Department of Chemistry, Government College University, Faisalabad, Pakistan; E-mails: Sadiaamer11@gmail.com, diyajav1@yahoo.com*

Hypercholesterolemia is an important risk factor in cardiovascular diseases and represents the most important cause of death. Only one-third of the total body cholesterol is derived from diet while two-thirds of is being synthesized directly from intracellular precursors by various organs of the body. Lovastatin is a potent drug for lowering blood cholesterol and competitive inhibitor of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) which catalyzes the rate limiting step of cholesterol biosynthesis. In the present study seven indigenously isolated fungal strains (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Penicillium citrinum*, *Penicillium notatum*, *Penicillium ostreatus* and *Trichoderma viridae*) were tested for their potential to produce lovastatin biologically by using different agro-industrial wastes (Corn cobs, corn stover, banana stalk, wheat straw, wheat bran, bagasse) in submerged and solid state fermentation. *Aspergillus terreus* showed maximum production of 18.74 mg/L by wheat bran in solid state fermentation. The fermentation parameters (pH, temperature, Inoculum size, moisture contents and fermentation time) were also optimized for better production of lovastatin. The optimized lovastatin was extracted from fermented broth and orally administered to rats. The hypocholesterolemic effect of fermented lovastatin was evaluated on serum ALT, AST, HDL-C, LDL-C, TG and TC level of rats.

SL-50***Track: Plant & Environment*****‘FETUS TEA’ AND SELECTION OF ITS CULTIVATION PROGRAM****Jinzhong Jiang, Yanfen Huang, Chaoying Wang, Cuijuan Ren and Cong Pan***Guizhou Normal College, Guizhou Bioresource Development Key Laboratory, Guiyang (550018), China; E-mail: jjz9911@163.com*

Background and Aims: Tea has been a most widespread natural drink with highest drink value among three kind of main drinks without alcohol in the world. There are 58 countries in which tea tree are grown and 150 countries in which tea are used as drink in the global. The tea tree seed output of a tea garden amounts to two-thirds of its tea leaf output according to which more than 10 million Kg of tea seed are produced per year in China. Although it was known early that tea seed can be used to extract tea seed oil, up to date, most of them were discarded or burned as trash except small amount mixed into *Camellia oleifera* seed used to produce oil; which undoubtedly is a huge waste of resource of tea seed. Therefore, the research is trying to produce a kind of new and unique tea with tea seeds so to set up a new way to develop and use resource of tea seeds. Our primary idea is to produce tea with tea seedlings (from tea seeds) that are growing in different states. Because the new and unique tea are produced from very young tea seedlings those similar to fetus, we called the tea as ‘fetus tea’ compared with the traditional tea produced with tea leaves from adult tea tree.

Methods: 1) Aggregate culture: Pine needle mulch was spread with 10-15 cm of thickness in the bottom of wood breeding box (50×30×20cm); 3cm tea seeds were sowed uniformly on pine needle mulch and then the seeds were covered pine needle mulch. The thickness of pine needle mulch was different according to different types of tea seedling. If the seedlings were to be used for ‘fetus needle’ (a type of ‘fetus tea’) production, the thickness was approximately 8 cm. And if for other types of ‘fetus tea’, the thickness was approximately 2 cm. 2) Aquiculture culture: The bottom of wood breeding box (50×30×20cm) was distributed on many small holes (D: 0.5cm) so that redundant water was discharged. Tow layers of waste newspaper were unfolded on the bottom and on the waste newspaper a layer of tea seeds were sowed uniformly. The tea seeds were covered with waste newspaper and water was sprinkled full over it per day. Then the wood breeding boxes were put (monolayer) in greenhouses in which temperature kept 15-25 and air humidity maintained over 95%.

Key Results: ‘Fetus tea’ was named firstly by us; it was a new type of tea made with tea tree seedling which was growing before its 2 big euphylla arise. In the period in which tea seed germinated and grew with 2 big euphylla, tea seedling varied in morphology and ratio between leaves and stem. According to the variation of morphology and ratio between leaves and stem, five production schemes were formulated which were ‘fetus crow’, ‘fetus pearl’, ‘fetus needle’, ‘fetus butterfly’, white seedling, respectively. The fetus teas produced with the five production schemes mentioned above were tasted and analyzed in morphology and biochemical components, the results indicated that ‘fetus needle’ and white seedling schemes had obviously developmental potential. The theanine, caffeine and tea polyphenol content of ‘Fetus needle’ was 175% higher, 4% lower, and 30% lower than control, respectively. The development of ‘fetus tea’ opened up a new way by which tea tree seed was transformed to a new product with high additional value.

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SL-44

Track: Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring; photosynthetic microorganisms, cyanobacteria and microalgae.

CO-EXPRESSION OF A VACUOLAR Na^+/H^+ ANTI-PORTER AND AN H^+ -PYROPHOSPHATASE WITH AN IRES-MEDIATED DICISTRONIC VECTOR IMPROVES SALINITY TOLERANCE IN TOMATO**Habib Khoudi**

Laboratory of Plant Protection and Improvement, Center of Biotechnology of Sfax (CBS), Route Sidi Mansour Km 6, B.P'1177', 3018 Sfax, Tunisia; E-mail: hkhoudi@hotmail.com



Tomato (*Solanum lycopersicum* L.) is the second most important vegetable crop in the world after potato (*Solanum tuberosum* L.). Tomato is consumed in many forms and is a valuable source of health promoting compounds such as the antioxidant lycopene whose consumption is known to reduce the incidence of many types of cancer. Although cultivated tomato is moderately tolerant to various abiotic stresses, the crop losses due to salinity can be severe. One key mechanism of salinity tolerance in plants is the ability to remove Na^+ ions from the cytosol and its sequestration into the vacuole to limit cell damage. This sequestration is mediated by Na^+/H^+ antiporters using electrochemical gradient of proton provided by H^+ -PPase and H^+ -ATPase pumps. In the present study, we generated transgenic tomato plants expressing an antiporter gene *TNHXS1* alone or in combination with H^+ -PPase gene. Results have shown that both types of transgenic tomato lines have significantly higher salt tolerance than the wild type. Interestingly, the transgenic lines co-expressing both genes exhibited higher salinity tolerance than those expressing singly the *TNHXS1* gene. This study demonstrates the effectiveness of bicistronic constructs as novel tool for multigene stacking in crop biotechnology.

SL-35

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

EXPRESSION AND GLYCOMODIFICATION OF THERAPEUTIC MONOCLONAL ANTIBODIES IN TRANSGENIC PLANT**Kisung Ko**

Department of Medicine, Chung-Ang University, 84 Heukseok-Ro, Dongjak-Gu, 156756 Seoul, South Korea; E-mail: ksko@cau.ac.kr

Transgenic plants were generated to express anti-colorectal cancer mAb CO17-1A fused with KDEL retaining it in ER. Expression of mAb CO17-1A was compared between mAbs fused with and without KDEL (mAbK and mAb). Expression of the plant-derived mAbK (mAbPK) CO17-1A was significantly enhanced compared to mAbPCO17-1A. Cell ELISA with human colorectal carcinoma cells confirmed higher expression of mAbPK compared to mAbP. mAbP had plant-specific glycans whereas mAbPK had mainly oligomannose glycans. The Fc domains of both mAbPK and mammalian-derived mAb (mAbM) had similar binding activity to the FcγRI receptor. However, the Fc domain of mAbP had lower binding activity to the FcγRI receptor than mAbM. ADCC of mAbPK against human colorectal cancer cells was as efficient as mAbM. These results suggest that KDEL accumulated mAbP in ER eventually enhancing its expression with similar anti-cancer biological activities to mAbM.

SL-39

Track: Industrial and Manufacturing: bio-fuels; energy crops (cellulosic ethanol industry); industrial enzymes; bioprocess engineering and optimization.

HAIRY ROOTS AS AN INNOVATIVE PRODUCTION SYSTEM FOR PLANT CELL DERIVED ACTIVE AGENTS – CURRENT CHALLENGES AND SOLUTIONS

F. Lenk, T. Bley and J. Steingroewer

*Institute of Food Technology and Bioprocess Engineering, TU Dresden, Dresden, Germany;
E-mail: felix.lenk@tu-dresden.de*



Secondary metabolites produced by plant *in vitro* cultures such as the pharmaceutical relevant Oleanolic and Ursolic acids 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 [1]. Furthermore Hairy roots allow for a significant speed-up of the downstream process.

However several challenges in establishing a production with Hairy roots still exist. Researchers at the chair of Bioprocess Engineering at the Dresden University of Technology address several bottle-necks in the screening and cultivation stage of differentiated plant *in vitro* cultures. In order to find productive and promising candidate samples an automatic image recognition method for non-invasive screening of Hairy root cultures has been developed. With the presented innovative, customized solution it is possible to quantitatively track a morphological growth process over the cultivation period on an Agar plate [2]. To improve the cultivation process (higher yield, shorter cultivation time) and the bioreactor design (bubble column vs. stirred) a structured growth model for Hairy Root tissue on agar plates with consequent simulations and a visualization engine is presented as until now no theoretical description of the growth processes exist [3].

Facing the need of a standardized cultivation system a lab-scale STR has been modified to minimize shear-stress to the Hairy root cultures with separating the aeration of the medium from the compartment containing the cultures using a membrane for separation.

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SL-47

Track: Plant & Environment

THE CHALLENGES AND DIRECTION OF RESEARCH ON MAIZE TOLERANCE TO THE ABIOTIC STRESSES

You-Zhi Li

State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources /Key Laboratory of Microbial and Plant Genetic Engineering, Ministry of Education; College of Life Science and Technology, Guangxi University, Guangxi 530005, P.R. China



Maize is one of the main food crops. Its production has encountered great challenges from more and more abnormal changes in the environment. The factors that limit maize production include abiotic stresses such as drought, high salinity, low temperature, and aluminium toxicity. Enhancing resistance to such sources of the stresses has been one of breeding major goals sought by maize breeders. It is believed that although many desirable traits related to stress tolerance may be present in historical germplasm they have been lost in modern varieties partly due to excessive emphasis on high-yield traits by breeders during a long-term breeding action. Additionally, some germplasms with better stress tolerance traits are not suitable as parent materials for breeding owing to poor agronomic traits and/or weak combining ability. Therefore, the

novel varieties with ideal stress-tolerant traits still need to depend on depth analysis of tolerant mechanisms of the existing parent materials. Defects of the traditional conventional breeding strategies are apparent, time-consuming, laborious, empirical, and non-directional. Thus, development of the ideal maize varieties pins hopes on molecular aided design breeding through engineering because they are faster and can afford greater control over agronomically useful traits.

However, in-depth understanding of the molecular mechanisms controlling response to abiotic stresses is needed. The living organisms rarely live in unchanging environments. Post-genomics such as transcriptomics, metabolomics, and proteomics can generate knowledge that is closer to the biological processes. In practice, the stresses more or less present a law of appearing and subsiding. Therefore, growth recovery of maize following withdrawal of the stresses is an integral part of maize abiotic stress tolerance. However, most of the existing researches are very interested in gene expression under the stresses, but they only care about stress-responsive mechanisms of the part of the tissues and organs in the processes of the stress, and have not paid great attention to the promoter, a decisive self-control elements for gene expression. According to the literature and combining with our work, we believe that future research on maize stress tolerance in the era of post-genomics should focus on metabolomics and proteomics; stress tolerance of whole plant rather than individual tissues or organs; coordination of expression of genes among tissues; characterization of promoters of stress-responsive genes; interrelation between mechanisms for tolerance to, and growth recovery from the stress; gene promoter analysis and foundation genotypes as major research targets.

SL-8

Track: Plant & Environment

BIODIVERSITY OF MARINE BACTERIA IN DEEP SEDIMENT ENVIRONMENT OF INDIAN OCEAN

Xin Peng Tian, Jun De Dong and Li Juan Long

Key laboratory of Marine Bio-Resources Sustainable Utilization, CAS; RNAME Center for Marine Microbiology, CAS; Guangdong Key Laboratory of Marine Material Medica; South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, 510301, PR China; E-mail: longlj@scsio.ac.cn



In recent years, microbial resources in marine extremely environments were focused on increasingly by oceanologists, microbiologists, chemists, ecologists and so on. Marine bacteria were being dug out more and more from the deep sea environments, and many of them have the novel characteristics including the adaptability to the abysmal deep, producing novel compounds with special functions, which were very different from terrestrial microorganisms.

Indian Ocean is the third largest of the world's oceanic divisions, bounded by Asia, Africa, Australia, and Antarctica. The average depth of the ocean is 3,890 m and the deepest point is 8,047 m deep. In this study, one sediment sample was collected during May 2010 from north-east Indian Ocean at the depth of 4301m. Five modified media of ISP medium 2, R2 medium, ISP medium 4, Nutrition agar, Marine agar were used for the isolation of marine bacteria by using a standard dilution plating method within 2 hours. Colonies were picked after incubation at 25 °C for 1 week. The result showed that total 2.6×10^4 cells could be recovered from one gram fresh sediment.

After purification, total 274 strains were selected for further research. Genomic DNA was extracted and 16S rRNA gene was sequenced. BLAST analysis of the partial 16S rRNA gene sequences revealed that these strains have the highest similarity with 91 described species in 31 genera, and 28 strains were selected as for the new candidates because of the lower (less 97 %) similarities of 16S rRNA gene sequences. 148 strains in 42 species were grouped in genus *Bacillus*, which was the most abundant culturable members inhabiting this sediment environment. Secondly, it was the *Microbacterium* group in phylum *Actinobacteria*, which includes 47 strains of 8 species. Another 32 strains were also distributed into 10 genera in phylum *Actinobacteria*.

These preliminary results showed that, in the sediment environment at a depth of 4300 meters, aerobic or facultative anaerobic *Bacillaceae* with endospores was the dominant group as the 58% ratio of pure cultural microorganisms. 29 % was the actinobacterial members. The new microbial resources were very abundant. In conclusion, deep marine habitats constitute a relatively untapped resource for the discovery of rare microorganism.

SL-75

Track: Medical Biotechnology: biopharmaceutical manufacturing; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers.

THE EVOLUTION OF WIRELESS CONTINUOUS WAVE NEAR INFRARED SPECTROSCOPY (NIRS) DEVICES FOR BIOMEDICAL APPLICATIONS

Andrew Macnab and Babak Shadgan

Professor and Head of NIRS Research Study Group, Division of Urology, Faculty of Medicine, University of British Columbia, Vancouver, Canada; E-mail: ajmacnab@gmail.com



Progress in research applications of near infrared spectroscopy (NIRS) and growing clinical interest have led to significant improvements in hardware and software. Amongst continuous wave systems the use of light emitting diodes, incorporation of spatially resolved optical geometry, algorithm refinement, development of portable systems, and wireless telemetry led first to portable NIRS instruments, then wearable systems, and now miniaturized self-contained devices. Measurement of absolute tissue oxygen saturation in both muscle and brain, and mapping of event related cortical hemodynamic responses using functional NIRS (fNIRS) have added specific measurement modalities. Wireless wearable systems and self-contained devices capable of measuring such modalities in addition to providing conventional monitoring of trends in oxygenated and deoxygenated haemoglobin concentration from baseline have increased the scope of research, expanded the populations it is possible to monitor, and opened new clinical avenues for applications involving NIRS. This presentation will provide an overview of the range of biomedical applications reported to date using wireless continuous wave (CW) NIRS and fNIRS systems, summarize key elements in the specification of devices now available, and explore potential future directions for clinical and research use of wireless NIRS technologies.

SL-40

Track: Industrial and Manufacturing: bio-fuels; energy crops (cellulosic ethanol industry); industrial enzymes; bioprocess engineering and optimization.

ENHANCED PRODUCTION AND PURIFICATION OF URICASE BY *BACILLUS SUBTILIS* BSM-2 MUTANT STRAIN

Munazzah Meraj, Rao Irfan and Sadia Javed

Peoples University of Health and Medical Sciences, Nawab Shah, Pakistan; E-mail: munazzahmeraj@yahoo.com

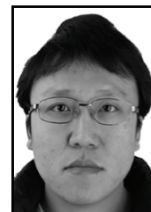
Uricase catalyses the oxidation of uric acid to allantoin, plays an important role in the purine degradation pathway. In this project hyperproduction of urate oxidase was studied by *Bacillus subtilis* mutant in submerged fermentation. The organism was subjected to ultra violet irradiation and chemical mutagenesis. Ethyl methan treated *B. subtilis* (180 minutes) was proved to be the best for optimum production of urate oxidase by 3 log kill/survival curve. On optimization of fermentation medium, it was found that substrate concentration (0.5%), fermentation period (36 h), pH (8.5), temperature (35 °C), yeast extract (0.3%) and sucrose (2%) enhanced the activity of the parent and mutant derived enzyme. The enzyme was purified by adopting different techniques i.e ammonium sulfate precipitation, ion exchange and gel filtration chromatography. It was observed that mutated enzyme exhibited 97.56 U/mg specific activities with 256.73 fold improvement. The purified urate oxidase was run on SDS-PAGE which determined a single band with molecular weight of 34 kDa. The purified BSM-2 possessed K_m and V_{max} value of 0.067 M and 133.3 IU $mg^{-1} min^{-1}$ respectively.

SL-22

Track: Medical Biotechnology: Diagnostics

SEVERAL NOVEL LONG-ACTING GLP-1 ANALOGS EXHIBITED IMPROVED PHYSIOLOGICAL ACTIVITIES IN PRE-CLINICAL DEVELOPMENT**Gong Min**

Tianjin Institute of Pharmaceutical Research, Tianjin, China; E-mail: gongm@tjipr.com



The multiple physiological characterizations of glucagon-like peptide-1 (GLP-1) make it a promising drug candidate for the therapy of type 2 diabetes. However, the biological half-life of GLP-1 is short *in vivo* due to degradation by dipeptidyl peptidase-IV (DPP-IV) and renal clearance. The stabilization of GLP-1 is critical for its utility in drug development. The aim of our laboratory is seek long-acting GLP-1 analogs by conformational alteration or novel biomaterial formulations: 1) We found formation of homodimer of GLP-1 by disulfide bond increased the half-life of GLP-1 to 50 hours significantly and possessed the long-term blood glucose regulatory; 2) construction a cyclic-like structured GLP-1 contributed to extended half-life of GLP-1 in animals (72 hours approximately); 3) self-assembled peptides is capable to form stable complex with human GLP-1, and induced the sustained release of GLP-1 consequently (half-life ~4 days). Thereafter, the results from cellular receptor binding assay, insulin secretion stimulation activity, single-dose/multiple-dose glucose tolerance tests suggested our GLP-1 analogs and new GLP-1 formulation are potent therapeutics in the treatment of type 2 diabetes. More importantly, our study suggested the possibility of self-assembled peptides in therapeutic peptide formulation to achieve improved stabilization, oral available, and solubility in future.

SL-36

Track: Industrial and Manufacturing: bio-fuels; energy crops (cellulosic ethanol industry); industrial enzymes; bioprocess engineering and optimization.

COST EFFICIENT LOW GHG EMISSION BIOFUEL PRODUCTION**Dan Nilsson**

Scale Biofuel ApS, Niels Jernes Vej 10, DK-9220 Aalborg, Denmark;
E-mail: dn@scalebiofuel.com



Scale Biofuel ApS has developed a patent pending technology platform of solar driven fermentation processes for producing biofuels like bioethanol, biobutanol and other volatile organic compounds, which also can form building blocks for industrial chemicals. The benefit of the technology is a 5-10 fold lower capital expenditure to construct new biofuel production plants, low to no fossil energy consumption, full flexibility of production scale and with significantly lower green house gas emissions than conventional production technologies. Scale Biofuel's technology platform was developed with the vision of making biofuels easy accessible to everybody by low cost and low GHG emission production processes requiring a minimum of investment. The basic principle of the technology consists of a flat tank fermentor heated by solar energy, where the accumulated heat is used for continuous evaporation of volatile organic compounds from the fermentate into a gas flow circulated between the headspace of the flat tank fermentor and a condensation unit. The fermentation and the initial evaporation are in our processes combined into one process step getting the external energy requirements to drive the process from the solar insolation. The energy used to circulate the gas is only 0.05 % of the evaporation energy and heating energy originating from the solar insolation. The solar derived heat is further recovered and will be used for final product purification and raw material processing e.g. pretreatment of lignocellulosic materials. The technology will be particularly beneficial in tropical and subtropical regions of the world where both biomass and solar influx is available. Our present focus is on India, Central and Latin America, South East Asia, and the southern regions of the USA and China. The technology is further suitable for use in developing countries with limited infrastructures due to the low CAPEX, simplicity, and robustness of the production concept, and also allowing the opening of new markets currently excluded from producing biofuels caused by high investment barriers and infrastructure requirements in form of large agricultural land supplying raw materials, which is required by conventional technology.

SL-55

Track: Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring; photosynthetic microorganisms, cyanobacteria and microalgae

DNA BARCODING: AN ESSENTIAL TOOL FOR BIODIVERSITY CONSERVATION IN THE CONTEXT OF CLIMATE CHANGE IN AFRICA

Christopher Didigwu Nwani, Ogbonna Stella and Isa Mohammed Chado

University of Nigeria Nsukka, Division of Molecular Biology and Biotechnology, Department of Zoology & Environmental Sciences, Nigeria; E-mail: didigwunwani@yahoo.com

Plant and animal species in tropical Africa face threats both by stress from climate change and over exploitation. Species are going extinct and populations decline at an alarming but poorly understood rate. For sustainable exploitation of our flora and fauna resources, a crucial management tool is to have a comprehensive understanding of the species composition. Many species may face extinction before they can be identified or described. This presents a problem for conservation planning and prioritization, obviously because species that have not been identified cannot be protected effectively. The limitations inherent in morphology-based identification systems and the limited pool of taxonomists paved way for the introduction of new molecular diagnostic tools (DNA barcoding) for effective species identification. Hitherto, a wide variety of protein- and DNA-based methods have been evaluated for the molecular identification of species in Africa but these studies, however, are not comparable for the purposes of species identification because they lack standardization (e.g. different regions of the mitochondrial genome like cytochrome b and 16S-rDNA) were used. To address this situation, DNA barcoding which uses a single gene sequence (a 650-bp fragment of the 5' end of the mitochondrial cytochrome c oxidase-subunit I (COI) gene) to discriminate the vast majority of species was proposed as a global bioidentification sequence for animals. This technology (DNA barcoding) relies on the observation that the 'barcode' sequence divergence within species is typically much lower than the divergence exhibited between species and may arise from selective sweeps and the intricacies of mito-nuclear co-adaptation. A sufficient accumulation of DNA barcodes will help conservation managers to identify interim priority areas for conservation efforts in the face of continuous threat arising from climate change. This paper examines the potentials, progress, possible challenges of DNA barcoding and its possible utility in enhancing biodiversity conservation and climate change mitigation in Africa.

SL-76

Track: Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring; photosynthetic microorganisms, cyanobacteria and microalgae

EFFICACY OF DIFFERENT AGRICULTURAL WASTES (BIOSTIMULANTS) ON MICROBIAL DEGRADATION OF HYDROCARBON IN CRUDE OIL POLLUTED SOIL IN NIGERIA AND ITS RESIDUAL EFFECT ON PLANT GROWTH

A.F. Ogundola and M.O. Aremu

Department of Pure/Applied Biology, Ladoke Akintola University of Technology, P.M.B. 4000 Ogbomoso, Nigeria; E-mail: adfogundola@yahoo.com, afogundola@lautech.edu.ng

Worldwide, industrial and agricultural activities have released large quantities of natural and synthetic hazardous compounds into the environment. More frequently this is due to careless waste disposal, illegal waste dumping and accidental spills. Hence various sites in the world require cleanup of soils and sludges. To restore these polluted sites, bioremediation as a safe, efficient, cost effective and environment friendly is acknowledged among other technologies. This study was designed to investigate the effects of agricultural wastes as stimulants on the indigenous microbial biodegradation of hydrocarbon in crude oil polluted soil in Nigeria.

The experiment was carried out using plastic buckets as bioreactors while agricultural wastes were pig dung and food material waste (beans shell and cassava peel) used as biostimulation agents.

The results showed that there was a significant reduction in the crude oil concentration by 93.85% under stimulation with the waste nutrients amendments moreover; a maximum of 58.39% total petroleum hydrocarbon removal was obtained in natural attenuation. The decreasing orders of performance in the enhancement of crude oil biodegradation were as follows: pig dung > (pig dung + cassava peel) > cassava peel > (pig dung + beans shell) > Mixture all biostimulants > beans shell > (beans shell + cassava peel) > control.

In conclusion agricultural wastes used as biostimulation nutrient enhanced the bioremediation of soil contaminated with crude oil through increased activity of the indigenous microorganisms in the soil.

SL-84

Track: Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring; photosynthetic microorganisms, cyanobacteria and microalgae

LARVICIDAL AND DEVELOPMENTAL REGULATORY ACTIVITIES OF METHANOLIC LEAF-EXTRACT OF *CARICA PAPAYA* (CARICACEAE) AGAINST *CULEX PIPIENS PIPIENS* MOSQUITOES (DIPTERA: CULICIDAE)

Kayode Isreal Olayemi, H. Yakubu and I.C.J. Omalu

Department of Biological Sciences, Federal University of Technology, Minna, Nigeria; E-mail: kaylatiyemi@yahoo.com

This study evaluated the potentials of leaf-extract of *Carica papaya* as a larvicide and Immature development regulator against *Culex pipiens pipiens* mosquitoes. Methanolic extract of the plant material was obtained by solvent extraction and bio-assayed against 4th instar larvae of the mosquito species, using extract-test concentrations ranging from 5 – 60 mg/ml, and following World Health Organisation's protocols. The results indicated larvicidal activity 24 hours post-exposure, with LC50 and LC90 of 25.49 and 49.68 mg/ml, respectively. Sub-lethal concentration of the extract elicited significant ($P < 0.05$) larval development regulatory effects against the mosquitoes; as the duration of immature stages was more than doubled (9.97 ± 0.74 days in the untreated, as against 21.78 ± 7.72 days in the test mosquitoes); and survival rate was reduced by more than 80%. The treated mosquitoes were significantly ($P < 0.05$) smaller (wing length = 3.12 ± 0.40 mm) than their untreated counterparts (wing length = 3.81 ± 0.17 mm). Likewise, daily survival rate and longevity of the adult mosquitoes were significantly reduced in the treated group. The sub-lethal concentration of the extract, however, had no significant ($P > 0.05$) effect on wing symmetry. The findings of this study suggest that *C. papaya* is a promising source of lead compounds for sustainable mosquito vector control.

SL-15

Track: Regenerative Medicine

DEVELOPMENT OF A HIGHLY ELASTIC BIOENGINEERED CORNEA: FROM RESEARCH TO COMMERCIALIZATION

Mehrdad Rafat, Neil Lagali, May Griffith, Marina Koulikovska and Per Fagerholm

Assistant Professor in Biomaterials Engineering, Department of Biomedical Engineering & Dept. of Clinical and Experimental Medicine, Linköping University, 581 85 Linköping, Sweden; E-mail: Mehrdad.rafat@liu.se

Background: Despite the promising clinical results that we previously reported on biosynthetic corneas, more elastic materials are required for surgical manipulation and withstanding the adverse host conditions faced by high risk corneal transplants.

Purpose: The overall objective was to develop novel bioengineered materials that can replace the damaged corneal tissue. Another objective was to evaluate the *in vivo* integration of the materials in rabbit models using a femtosecond laser intrastromal surgical technique

Methods: Bioengineered corneas were prepared using porcine collagen cross-linked by carbodiimide at various compositions and pH. Promising formulations were tested for their mechanical, optical, and enzymatic and thermal degradation properties as well as for interactions with corneal cells, and *in vivo* implantation in rabbit's eyes. A femtosecond laser was used to cut 100 micron thick discs of mid-stromal tissue from corneas of 15 rabbits and replaced with the bioengineered materials.

Results: The new material demonstrated improved mechanical properties while maintaining its clarity and biocompatibility. The bioengineered implant retained its shape, thickness, and clarity 8 weeks post-surgery in rabbits.

Conclusions: The bioengineered cornea developed in this work has the potential to be used and commercialized as corneal implants to replace the damaged tissue or for corrective surgery applications.



SL-58

Track: *Plant and Environment: transgenic plants and crops; bioremediation; microbial diversity; bio-monitoring; photosynthetic microorganisms, cyanobacteria and microalgae.*

A STUDY ON PETROLEUM DEGRADING GORDONIA GPCVC1 ISOLATED FROM PONDICHERRY COAST

Chandraja Rathish and G. Immanuel

Department of Microbiology, Noorul Islam College of Arts and Science, Kumaracoil 629180, Kanyakumari District, Tamilnadu, India; E-mail: chandrajarathish@gmail.com

Three actinomycetes strains of excellent hydrocarbon degrading activity were isolated from the coastal region of Pondicherry, with high contamination of crude oil and byproducts. These organisms were subjected to biochemical and phylogenetic study, among the three strains, CVC1, CVC4 and CVC5, the latter showed differentiating degrading activity by utilizing majority of hydrocarbon substrate, especially petroleum and a variety of other hydrocarbon provided, at different pH and salinity. Strains provided good growth between pH 5-6, and salinity 35ppm. Partial 16S r DNA sequencing showed *Gordonia* GPCVC1(HM352835) are related distantly to *Gordonia* aarii, *Gordonia* defluvii, *Gordonia* hirsute and showed 97% similarity to *Gordonia* rubripentincta. The other two organisms are also identified as *Rhodococcus* SRNICAS(HM246707), *Gordonia* SPARC2(JN003575) which showed greater degrading activity towards pesticides and polynuclear hydrocarbon.

**SL-33**

Track: *Industrial and Manufacturing: bio-fuels; energy crops (cellulosic ethanol industry); industrial enzymes; bioprocess engineering and optimization.*

MICROBIAL BIOSYNTHESIS OF AMINO ACIDS, DEVELOPMENTS AND ACHIEVEMENTS AT THE INSTITUTE OF MICROBIOLOGY - BAS

Alexander Ratkov, Ignat Dimov, Julia Kristeva and Filip Filipov

Institute of Microbiology, Bulgarian Academy of Sciences, Acad. G. Bonchev str., Block 26, 1113 Sofia, Bulgaria; E-mail: ratkovaal@microbio.bas.bg

Objectives: At present the processes for microbial production of amino acids are among the most important in terms of tonnage and economical value. Market development has been particularly dynamic for the flavor-enhancer glutamic acid and the animal feed amino acids L-lysine, L-threonine, and L-tryptophan. Significant increase of the production of branched chain amino acids and all other amino acid is observed during the past decade. The growing market for amino acid led to significant improvements in bioprocess and downstream technology as well as in molecular biology. During the last decade big efforts were made to increase the productivity and to decrease the production cost.

Methods: Classical breeding methods and mutagenesis have been applied to channel the metabolic pathway in the biosynthesis of different amino acids followed by screening for mutants with different characteristics. Comparative studies of efficiency of the applied different methods for cultivation of the chosen producers have been done.

Results and Conclusions: The presentation gives a short overview of the world market for amino acids. Attempt and achievements in investigations and developments of the technologies for some amino acids production at the Institute of Microbiology, Bulgarian Academy of Sciences are summarized. Improvements in selection of different microbial strain-producers for microbial production of L-lysine, L-valine, L-leucine as well as for simultaneous production of L-lysine plus L-threonine are briefly analyzed. Developments and achievements in bioprocess technology, i.e. development of lab scale, semi-industrial scale and production scale technologies for production of some of the above mentioned amino acids are summarized too. Specificity and efficiency of applying of fed-batch and repeated fed-batch (fed-batch with droppings) methods for cultivation of lysine producers as well as the industrial attempt of applying and regular production of the technology for microbial production of L-lysine from one side and for cultivation of the producers and technology specificities for production of branched chain amino acid (L-valine and L-leucine) from the other side are shortly discussed.



Keywords: Amino acids, microbial production, repeated fed-batch cultivation.

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SL-68

Track: *Regenerative Medicine: Gene Therapy*

LONG SPACE MISSIONS AND GENE THERAPY

William J. Rowe

Medical University of Ohio, at Toledo, 1485 Bremerton La., Keswick, Virginia, 22947, USA; E-mail: rowerun@aol.com

With microgravity (M) there is invariable malabsorption necessitating subcutaneous (SC) pharmaceuticals (P). With no replenishable SC silicon device available to administer them a therapeutic dilemma exists. Also SC P are limited in number and several P shown to deteriorate with space flight (SF) even in low earth orbit possibly radiation-induced. Liver, kidney functions deteriorate possibly complicating endothelial dysfunction stemming from invariable serum magnesium (Mg) deficits despite low sensitivity ($p < 0.0001$) and in turn adrenaline elevations with vicious cycles between the two with in turn inflammation, oxidative stress, mitochondrial injuries. Other than simulating exactly 1 G. thereby entirely avoiding M complications, there appears to be no alternative except gene therapy (GT) on a limited basis. Suggest beginning with the correction of as many as 4 angiogenesis – related gene deficiencies (1) atrial natriuretic peptide (ANP) reduced by $> 40\%$ after only 7-12 SF days stemming partially from Mg deficits reducing synthesis and release of ANP; (2) nitric oxide (NO); c GMP is a 2nd. messenger of both NO and ANP and is not detectable after 5 months in M (3) vascular endothelial growth factor (VEGF) triggered by SF- thrombocytopenia. Platelets are the primary source of VEGF. (4) erythropoietin reduced by about 10 % along with 10 % SF- reductions of plasma volumes (PV). Using PV substitute and a SC device to administer Mg may reduce complexity of GT. If unsuccessful in correcting liver, kidney dysfunction in order to resume P therapy, additional serial GT will be required.

SL-48

Track: *Medical Biotechnology: biopharmaceutical manufacturing; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers.*

CONCEPT OF MICROBIAL FUEL CELL BASED ANTIBIOTIC RESISTANCE DETERMINATION

György Schneider, Viktor Rostás, Tamás Kovács, Miklós Czeller and Béla Kocsis

University of Pécs, Department of Medical Microbiology and Immunology, Pécs, Szigeti str. 12., 7624, Hungary; E-mail: gyorgy.schneider@aok.pte.hu

Microbial Fuel Cells (MFCs) are devices that generate electricity by utilising microbial metabolism. Recently they started to gain increasing research attention because they offer promising alternatives in renewable energy generation. Since microbial activity assures the driving force of MFCs, this technology not only have to be outlined as possible alternative energy production devices, but also as good candidates to diagnostic tools where monitoring the microbial activity is crucial. From this point of view the most important target field is microbial diagnostic, where establishment of the antibiotic resistance pattern of a certain pathogen is the key issue. In the frame of the “MFCDiagn” consortium we have adapted the MFC technology to diagnostic purposes. We have constructed a complete system that is proper to reveal the antibiogram of a certain pathogenic bacterium in 6-8 hours. The major components of the system are: (i) a disposable panel system; (ii) an instrumental background and (iii) a software background. In our presentation we draft the major concept of this new system by introducing the major parts and by sharing the first experiences. We also will focus on those difficulties that raised during the development process and outline possible future prospects of the system.



SL-21

Track: Regenerative Medicine

DEVICES FOR BIOMEDICINE, PRODUCED BY NATURAL BIODEGRADABLE POLYMERS

Ekaterina Igorevna Shishatskaya

Department Institute of Fundamental Biology and Biotechnology, Siberian Federal University, 79 Svobodnyi Avenue, Krasnoyarsk 660041, Russian Federation; E-mail: shishatskaya@inbox.ru

Tissue restoration after traumas, surgical operations and other damaging situations is very actual medical problem and needs new materials. Polymers of various origins are under the focus of researchers in cross-emerging disciplines.

We are synthesizing highly purified polyhydroxyalcanoates, PHAs-samples, BioplastotanTM, synthesized by bacteria *Ralstonia eutropha* B5786 under specific grow conditions for the developing and production of devices for reconstructive medicine, as a scaffolds for tissue engineering and other implantable constructions. Basing on high biocompatibility and having techniques of varying polymer properties and rates of biodegradability of implants, we can made practically whole human body components of one natural material. Biocompatibility of polyhydroxyalcanoates of various compositions with trademark BioplastotanTM was proved in the numerous *in vitro* and *in vivo* works. Wide range of physical properties, causing by various copolymer compound make possible different processing techniques for the developing and production of various biomedical devices. Gel-spun technology and melt extrusion were used to produce solid monofilaments which were proved to be suitable as a bioresorbable surgical suture material. Electrospinning is in use for the production of nonwoven tissue and 3D devices. Various ways of molding and scanning are approved for the imitation of extracellular matrix of hard tissues so as fixing implantable devices. Experiments are performed *in vitro*, *ex vivo*, *in vivo*. The first stage of clinical trials was performed for some devices – bile stents, surgical meshes, nonwoven. Fabrics, which were assigned for the tissue reconstruction as a artificial ECM-scaffolds, shown as biocompatible towards cultures of cells of different origins. This type of natural microbial polyesters is very perspective for the restoration of soft tissues - skin, mucosa, ducts, ligaments, so as hard one, as bone and cartilage.

SL-30

Track: Industrial and Manufacturing: bio-fuels; energy crops (cellulosic ethanol industry); industrial enzymes; bioprocess engineering and optimization.

BIOCHEMICAL STUDY OF A NEUTRAL ALPHA AMYLASE PURIFIED FROM THE SONICATES OF THE CYANOBACTERIUM SPIRULINA PLATENSIS

S. Sivakami

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



Spirulina platensis is a multicellular, filamentous photosynthetic Cyanobacterium, which grows under a variety of habitats at a rapid growth rate. Despite the widespread use of this organism in food and pharmaceutical industries and its several other biotechnological applications, there has been limited systematic efforts in understanding the biochemical properties of the enzymic machinery of *Spirulina*. This knowledge is essential for finding appropriate commercial uses for the constituent enzymes. Understanding the enzymic machinery needed for metabolism and the molecular mechanisms of enzyme action may reveal the mechanism of adaptation of this organism to diverse environments. These studies may also help in understanding evolutionary aspects of the amylase gene.

In this work, neutral α -amylase was purified to homogeneity from the cell free extracts of the Cyanobacterium *Spirulina platensis*. Purification was achieved using conventional purification methods like ion exchange chromatography and gel filtration. This enzyme is starch inducible and calcium dependent. It acts with maximum catalytic efficiency on starch and releases maltose and other maltooligosaccharides as the major products. It does not produce free glucose and is devoid of pullulanase and xylanase activities. It has a molecular weight of 57kDa. Though the enzyme was stable over a wide range of pH from 7-11, it was maximally active between pH 6.5-7.5.

The amino acids essential for catalysis have been identified by group specific modification. Molecular cloning of the α -amylase yielded the amino acid sequence. With this sequence it became possible to study the conservation of critical amino acids involved in catalysis, calcium binding and substrate specificity by the method of Multiple Sequence

Alignment. Based on this analysis, the alpha amylases from *Spirulina platensis* can be said to belong to amylase family 13.

SL-41

Track: Plant & Environment

STUDIES ON NON-TISSUE-CULTURE PLANT GENETIC TRANSFORMATION

Y. Sun, L.Y. Yang, G.M. Cui, H.M. Zhang, J.Z. Du, Y.S. Hao, Y.X. Wang, C.B. Wang, M. Wang, Z. Chen, Y.H. Dong and J. Zhao

Biotechnology Research Center, Shanxi Academy of Agricultural Sciences, 030031 China;
E-mail: sunyi692003@yahoo.com.cn



Introduction

Genetic transformation is a powerful tool for plant breeding and genetical, physiological or biochemical research. Two methods are currently widely used for producing transgenic plants, namely *Agrobacterium* mediation and particle bombardment. However, both of them need tissue culture procedures, which are labor- and time-consuming, tedious and expensive.

We have been attempting non-tissue-culture or *in planta* plant transformation methods for more than one decade and successfully obtained transgenic plants with following approaches.

1. Pollen-Mediated Plant Transformation

Pollen with certain treatments can be an ideal exogenous DNA carrier; exotic DNA can get into ovule along with the growth of pollen tube, and integrated into the recipient genome; ultrasonication is one of the ways to make the pollen competent and introduction of foreign DNA.

Procedures: Collect fresh pollen suspend it in sucrose solution → 1st sonication → mixing foreign DNA with pollen → 2nd sonication → collect pollen from the treatment and pollination → harvest seeds from pollinated ears → sow seeds, make molecular analysis and bioassay → self putative transgenic plants and do further investigations. The genetic transformation was achieved by pollen-mediated approach with more than 10 maize (*Zea mays* L.) inbred lines. Transformants were confirmed by PCR amplification and Southern blot hybridization.

2. Agrobacterium Transformation of Germinating Seeds

Using germinating seeds as recipients, and *Agrobacterium* strains with exotic genes as gene donors. Steps are as follows:

1. Soaking mature seeds for 12-24 h;
2. Making wound in the meristematic region of germinating seeds with a scalpel in a hood;
3. Wounded seeds are co-cultivated with the *Agrobacterium* strain for 24-48 h;
4. Co-cultivated seeds are washed and sown in seed beds;
5. Seedlings at 3 ~5 leaf stage are screened with herbicides or antibiotics according to the selection gene in the construct;
6. Survived seedlings are transplanted into pots and assayed with PCR at ~10 leaf stage to determine putative transformants which are selfed at florescence;
7. Harvested seeds are sown for further molecular and biological assays to confirm transformants.

Successful experiment: Germinating seeds of maize (*Zea mays*) were co-cultivated with an *Agrobacterium tumefaciens* strain harbouring a Ti plasmid. Seedlings produced from the treatment were screened by an antibiotic or herbicide selection. Fertile transgenic T0 and T1 plants were obtained. PCR amplification and Southern-blot analysis showed that the foreign gene had been introduced into the inbreds of maize. About 29% of T0 seedlings examined were confirmed to be transgenic.

3. In situ Transformation of Woody Plants

For some tree plants with vegetative propagation, we have developed *in situ* transformation method, using buds of plants as recipients, and an *Agrobacterium* strain with exotic genes as the gene donor.

In situ transformation of poplar: Transgenic poplar shoots were obtained using *Agrobacterium*-mediated *in situ* bud transformation. The method is demonstrated for the first time for poplar. Buds of *Populus cathayana* Rehd were co-cultivated with an *Agrobacterium* strain LBA4404 harbouring the binary vector pBI101-Bmk-chi. With the procedure described, transformation efficiencies of 1% and 2.24% were achieved in 2006 and 2007, respectively. Stable integration of the transgene sequence was confirmed by PCR and Southern hybridization. The transgenes could be inherited by vegetative propagation. The novel approaches especially useful for species or cultivars not responding to tissue culture.

Conclusions

Advantages of *in planta* transformation are: obviate tedious tissue culture procedures; rapid, thus time-saving; economical; labor-efficient; genotype independent; simple and ready to be integrated into conventional breeding programs; and easily to be adopted in marker free transformation. Transformation efficiencies of the methods vary between 1-40%. Studies for further enhancing transformation efficiency are under way.

SL-51

Track: Medical Biotechnology: biopharmaceutical manufacturing; diagnostics; imaging; pharmacogenomics (personalized medicine); microarray technology; biomarkers.

APPLYING SCANNING PROBE MICROSCOPY TO IMAGE ELECTRONIC STRUCTURE AT THE NANOSCALE

Stuart Tessmer

Michigan State University, Biomedical Physical Sciences, 567 Wilson Road, Rm 4237, East Lansing, MI 48824, USA; E-mail: tessmer@pa.msu.edu

A transformation is occurring in electronic and biological technologies – based on the application of tools capable of imaging and manipulating charges at the atomic scale. This talk will discuss and introduce two such tools: Scanning Tunneling Microscopy and Subsurface Charge Accumulation Imaging. Example applications will be presented in the fields of semiconductor defects and conducting microbial nanowires.



SL-52

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

MONOLITHIC COLUMNS FOR THE DOWNSTREAM PROCESSING AND IN-PROCESS CONTROL OF LARGE BIOMOLECULES

Lidija Urbas, Miloš Barut, Matjaž Peterka and Aleš Štrancar

BIA Separations d.o.o., Mirce 21, Ajdovscina, Slovenia; E-mail: lidija.urbas@monoliths.com

Monolith is a stationary phase made of a single piece of porous material. Unlike conventional particle-shaped chromatographic supports, the pores in the monolith are interconnected and form a network of channels with the diameters of 1300 up to 6000 nm. The binding sites in these channels are highly accessible for target molecules and since the predominant mass transfer depends on convection rather than diffusion, the dynamic binding capacity is flow independent. These characteristics make the monolithic supports suitable for fast separation and purification of large biomolecules such as large proteins (IgM), viruses, virus-like particles, DNA, which sometimes exceed 200 nm in size and thus have very low diffusion constants. Monolithic supports are not only suitable for the downstream processing of large biomolecules but can be applied for the continuous monitoring during their processing (in-process control) and for the quality control of the final product as well.

In this presentation the difference between conventional and monolithic chromatographic supports will be presented. The characteristics and overview of CIM monolithic columns and their applicability for the DSP of molecules like influenza and adenoviruses will be shown. Examples of in-process control (PAT) of various process stream fractions of large biomolecules will be discussed.

SL-62

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

A COMPREHENSIVE BIOINFORMATICS ANALYSIS OF THE LIPOXYGENASES SUPERFAMILY IN SHEWANELLA WOODYI STRAIN (STRAIN ATCC 51908 / MS32)

Silvana Pompeia Val-Moraes and Eliana Gertrudes Macedo Lemos

Department of Technology, Universidade Estadual Paulista UNESP/FCAV, Brazil; E-mail: valmoraes.silvana@gmail.com



Lipoxygenases are a family of iron-containing enzymes that catalyse the dioxygenation of polyunsaturated fatty acids in lipids. We used I-Tasser models based on Lipoxygenases sequences from the PFAM and PROSITE databases to identify Lipoxygenases encoded by the *Shewanella Woodyi*. Three Lipoxygenases were identified and classified into individual families by pairwise sequence alignments. (strain ATCC 51908 / MS32) was originally isolated from the squid, sediment and water of the Alboran Sea (mixture of Atlantic and Mediterranean Sea). We suppose that a Lipoxygenase like enzyme is responsible for this production so we have looked for the Lipoxygenase gene in the Gene Bank and we have found three probable LOX gene in the Swo_2318 *Shewanella woodyi* ATCC 51908: Proteins Code (ACA86597.1) hypothetical protein, (ACA87192.1) arachidonate 15-lipoxygenase and (ACA87683.1) arachidonate 15-lipoxygenase precursor. 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 NCBI, PROSITE, CDART, CDD, InterProScan, SWISS-PROT, TOPPRED 0.01 and ProtParam program, which provides the biochemical characteristics of proteins and Blast-P (Basic Local Alignment Search Tool), which allows the verification of the similarity between the protein of interest and its orthologs. The analyses carried out using the tool NCBI-GeneBank, PROSITE, CDART and CDD the three lipoxygenases have the same profile. However, using the tool InterProScan, although it has been classified as Lipoxygenase-3, there was a significant difference between the sequences: the hypothetical protein has only 2 sites predicted features and 4 sites are considered absent features, the arachidonate 15-lipoxygenase has 5 sites predicted features and arachidonate 15-lipoxygenase precursor has 3 sites predicted features and 2 sites are considered absent features. The TOPPRED 0.01 analyzed hydrophobicity considered when the value exceeds the threshold of 1. In hypothetical protein found 2 segments putative candidate membrane-spanning segments, in arachidonate 15-lipoxygenase found 3 segments putative and 1 certain candidate membrane-spanning segments and arachidonate 15-lipoxygenase precursor found 3 segments putative and 2 certain candidate membrane-spanning segments. The ProtParam found for hypothetical protein has the characteristic: Number of amino acids: 714. Molecular weight: 81948.5. Theoretical pI: 5.04. The instability index (II) is computed to be 44.57. Aliphatic index: 77.58. Grand average of hydropathicity (GRAVY): -0.481. The arachidonate 15-lipoxygenase has the characteristic: Number of amino acids: 725. Molecular weight: 82757.4. Theoretical pI: 5.18. The instability index (II) is computed to be 43.10. Aliphatic index: 88.52. GRAVY: -0.285. The arachidonate 15-lipoxygenase precursor has the characteristic: Number of amino acids: 756. Molecular weight: 85788.7. Theoretical pI: 4.93. The instability index (II) is computed to be 44.32. Aliphatic index: 76.92. GRAVY: -0.433. The I-Tasser simulated model the quaternary structure for hypothetical protein and arachidonate 15-lipoxygenase and arachidonate 15-lipoxygenase precursor, which suggests that are lipoxygenase with special features.

SL-64

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

BIOTECHNOLOGY - THE WAY TO THE FUTURE

Tatiana Grigorievna Volova

Institute of Fundamental Biology and Biotechnology, Siberian Federal University, 79 Svobodnyi Avenue, Krasnoyarsk 660041, Russian Federation; E-mail: volova45@mai.ru

Nowadays in a world growing rates of consumption of food and energy resources, population increase, and human-induced impacts have reached the limit beyond which uncoordinated economic activity can lead to irreversible changes in the biosphere. The only way to achieve harmonic coexistence between the growing population of our planet and the biosphere is to pursue coordinated development of science and technology. Development of novel technologies for

complete treatment of natural resources and production of environmentally friendly energy sources and materials, that can be involved in the biospheric cycling, are the objectives of conception of the "Agenda of the Twenty-first Century". Biotechnology, as well as nanotechnology, is among the currently most popular words and areas of study in the world. It has been discovered that properties of individual molecules are quite different from those of their large aggregates and that microorganisms are actually chemical reactors. If we have a great number of these reactors and successfully control them, we'll be able to synthesize the substances we wish to produce. Hydrogen bacteria proper, bacteria oxidizing hydrogen, or "detonating gas" bacteria, may synthesize wide spectrum of molecules, including single cell protein, SCP, and reserved macromolecules - polyhydroxyalkanoates, PHAs. Practical interest in hydrogen bacteria is caused with their potential use as a regenerative component in closed biotechnological life support systems. More recently, hydrogen bacteria, which grow much faster than other chemoautotrophic organisms, attracted the attention of researchers as a potential source of feed, or even food, protein, SCP. The late 1980s and the early 1990s saw an upsurge of interest in hydrogen bacteria as very promising producers of polyhydroxyalkanoates - polymers, similar to polypropylene but degradable in the natural environment and biocompatible. Our group established three Pilot Production Facilities (PPF) exploiting hydrogen bacteria, with different volumes of cultivating vessels, having data of determination of mineral and biochemical composition of biomass, evaluation of parametric growth dependencies of bacterial cells and their nutritional requirements, and designing of laboratory equipment for continuous bacterial cultures. Two targets are in the focus of investigation - PHAs and SCP. The facilities produced sufficient amounts of biomass for experiments with agricultural animals, aimed at assessing its biological value, so as up to 50 kg of PHAs per year, for tests of biological safety and producing of biomedical devices.

SL-14

Track: Other Areas: Marine

THE ECONOMIC SEAWEED AQUACULTURE IN CHINA

Guang-Ce Wang

*Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China;
E-mail: gcwang@qdio.ac.cn*



The aquaculture of seaweeds in China and the reduction of marine eutrophication with seaweed aquaculture was introduced in this paper. The history of seaweed cultivation in China was also included. The two types of the most important economic seaweeds, *Lamilaria japonica* and *Undaria pinnatifide*, both of which were introduced into China in 20 century, triggered both the seaweed aquaculture and the marine aquaculture in China, so the techniques for the two seaweeds were described in detail, which include 5 parts: spore collection and indoor cultivation of sporeling, sporeling transplantation, setting up cultivation raft in the field, cultivation management in the field, and harvesting. The second algal aquaculture industry which was developed in China was *Porphyra* cultivation. *Porphyra* is different from *Laminaria* since *Porphyra* has a very special character, being survived during desiccation. Thus, there 3 ways for *Porphyra* aquaculture, fixed pillars, semi floating method and fully floating. The third algal cultivation industry is the *Gracilaria* which has been appreciated as a food and feed for culturing marine animals. The most important use of *Gracilaria*, however, is the production of agar. It is known that the finfish aquaculture waste production include solid wastes (Uneaten Food, Feces) and dissolved metabolic wastes (CO_2 , NH_4 , PO_4), the heavy eutrophication will greatly decrease the production of marine animal such as finfish and scallop, so at present time the measures for the integrated cultivation of animal and seaweeds was strongly suggested.

Keywords: *Lamilaria japonica*, *Undaria pinnatifide*, *Porphyra*, *Gracilaria*, Seaweed aquaculture.

SL-34**Track: Plant & Environment****CO₂ SEQUESTRATION COUPLED WITH LARGE-SCALE *SPIRULINA*(*ARTHROSPIRA*) CULTIVATION IN FULL SEAWATER-BASED MEDIUM****Wenzhou Xiang, Hualian Wu, Feng Zhang and He Hui***South China Sea Institute of Oceanology Chinese Academy of Sciences, Guangzhou 510301, China*

China has become one of the biggest countries on *Spirulina* (*Arthrospira*) industry since the mid-90s. At present its annual production capacity of *Spirulina* has reached 20000 tons, and annual actual production for thousands of tons. Techniques of large-scale *Spirulina* cultivation in full seawater were established by South China Sea Institute of Oceanology (SCSIO), Chinese Academy of Sciences in the early 90s. Study shows that seawater not only improves the nutritional composition of *Spirulina*, also effectively prevent problems from happening including excess levels of heavy metals and microcystins which are common occurrences when cultured in fresh water medium. By now, SCSIO is the only one which possesses techniques of *Spirulina* cultivation in full seawater not only in China, but also in the world. While problem of seawater cultivation is the high level of calcium and magnesium ions which precipitate out the low level of phosphorus and carbonate ion in sea water, resulting in waste of a large amount of phosphorus and carbonate. Moreover, the quality of *Spirulina* is the most concerned problem by consumers. In this study, a technology based theoretically on the feature of rapid pH drift and high pH adaptability of *Spirulina platensis* was established to combine CO₂ sequestration with large-scale *spirulina* culture in seawater. A simple structure, CO₂ leakage prevention covering-box, was designed to collect CO₂ escaped from culture medium when CO₂ gas was injected into the culture. The results from the pilot-scale cultivation of seawater *S. platensis* (HS 331) combined with the CO₂ addition technology showed the cost of carbon source was remarkably reduced and deposition of CaCO₃ and MgCO₃ was effectively avoided. The toxic heavy metal contents (Pb, As, Cd and Cr) in the biomass of *S. platensis* were all below the legal limits, indicating that CO₂ supplement has no undesirable effects on the quality of *S. platensis*. In addition, the average productivity was rather high. The technology was successfully applied in industrial production of seawater *S. platensis* and the cost of producing seawater *S. platensis* was dramatically reduced. Therefore, this study would not only provide a stagey for large-scale *Spirulina* culture in seawater keeping low-cost, but also an important theoretic and technical baseline for the establishment of the industrial technology of carbon dioxide sequestration.

**Keywords:** *Spirulina*(*Arthrospira*), seawater-based medium, CO₂ sequestration, pH drift.**SL-54****Track: Plant & Environment****PREDICTION MODEL OF HYBRID PERFORMANCE USING MOLECULAR MARKER BASED ON ADDITIVE-DOMINANT EFFECT****Lu Xiao-Ping, Liu Dan-Dan, Mi Fu-Gui, Wang Shu-Yan and Xue Chun-Lei***College of Agronomy of Inner Mongolia Agricultural University, Huhhot 010019, China*

Based on molecular markers to construct the heterosis prediction model could provide some advice for *Sorghum* × *Sudan* grass breeding. 90 hybrid combinations were formed according to the incomplete diallel cross design (NCII) with 5 sorghum sterile lines as maternal parents and 18 Sudan grass lines as paternal parents. The trials were carried in the farms of Huhhot and Baotou to evaluate environmental effects. The 8 trait phenotypic values of hybrid F₁ were investigated and selected marker loci with SSR, AFLP and SRAP, set up the evaluation system of marker effect and tag value. Using the specific loci to evaluate the trait effects and hybrid tag value, and analyze the correlation between hybrid tag value and heterosis. The prediction models of 8 traits for the hybrid were constructed with the stepwise regression analysis. The Jackknife sampling method was used to test the accuracy and steadiness of the model. The result showed, considering dominance and additive effect separately, 8 traits showed the average correlation index is 0.65 between tag value and phenotypic value. The coefficient of determination range is 0.51-0.88 in the 8 traits. The results in two places are coherent. The model could be instructive for hybrid and parents selection.

**Keywords:** *Sorghum* × *Sudan* grass, Genetic effect, AFLP, SSR, SRAP, heterosis, prediction model.

POSTERS

PO-81**Track:** Plant & Environment**INFLUENCE OF *ASPERGILLUS FLAVUS* AND *A. TERREUS* ON THE PROTEIN CONTENTS CONTAMINATED WITH AFLATOXINS IN PEANUT SEEDS AT AL-BAYDA GOVERNORATE, LIBYA****Suliman S. Abdelrawaf***Department of Microbiology, Faculty of Science, Omar Al-Mukhatr University, Box 919, Al-Bayda, Libya; E-mail: sulimanbio4@yahoo.com*

Protein contents showed a big difference in peanut grains assessed by Kjeldahl's and Bailey's methods, the overall mean of protein content of the two peanut grains varieties are not differ significantly at $P < 0.05$. The protein content of peanut grains affected significantly ($P > 0.01$) by the time after contamination with *Aspergillus* species (*A.f* Vs *A.t*), we suggested that it seems that the protein content of peanut increased from 0 day to 4 days after contamination, then decreased as the time after contamination increase until 20 days, the result from effect of contamination with *Aspergillus* that produce aflatoxin .The grains had been examined for aflatoxins's existence inside it shown that English peanut (*Arachis hypogea* L.), had been produced aflatoxins. The isolated fungi had been investigated for their capabilities to produce aflatoxins shown that only *Aspergillus flavus* had been produced aflatoxins.

PO-77**Track:** Medical Biotechnology**PREVALENCE AND MOLECULAR CHARACTERIZATION OF SARCOCYSTIS SPECIES IN****Karam I. Ashmawy, Mohamed Y. Sokkary, Somaia S. Abu-Akkada and Mohamed Bn Ghashir***Department of Parasitology, Faculty of Veterinary Medicine, Alexandria University, Egypt; Email: somaia_abuakkada@yahoo.com*

A study was planned to investigate the prevalence of Sarcocystis spp. among slaughtered water buffaloes (*Bubalus bubalus*) at Alexandria province, Egypt. Three hundred blood samples were collected from slaughtered buffaloes (5-7 years old) at El-Amria and Abbes slaughter houses, Alexandria. Two techniques were used to evaluate the seroprevalence of Sarcocystis spp., Enzyme linked immunosorbent assay (ELISA) and indirect haemagglutination assay (IHA). Bradyzoites (ZC) antigen was prepared from large-sized Sarcocystis cysts and their protein concentration was estimated to be used in ELISA and IHA assays. The comparative diagnostic tests of evaluation revealed that 203 (67.6%) of the tested serum samples were seropositive to Sarcocystis spp. by ELISA. While, there were 191 (63.6%) positive samples by IHA among naturally infected water buffaloes. ELISA test detected relatively higher percentage of infection with Sarcocystis spp. (67.6%) than those detected by IHA test (63.6 %). For molecular characterization of inter and intra species genetic polymorphism within Egyptian isolates of Sarcocystis spp, PCR and PCR-RFLPs were performed on four macroscopic isolates. The isolates represent two different geographical regions, Alexandria and Assiut provinces. Molecular differentiation between isolates was performed by using PCR and PCR-RFLPs. The PCR yielded an amplicon of approximate length of (1200 bp) for Large sized-cysts of Alexandria and Assiut isolates, while they were different from the small- sized isolates of the same provinces (1300 bp). From the obtained cysts, the 18S rDNA of samples of macroscopic cysts were amplified using PCR and characterized, in tandem, by 4 restriction endonucleases. *Rsa*I and *Mbo*I enzymes did not show any restriction sites for all isolates leaving the amplified fragments without cutting. *Ssp*I showed two fragments in Alexandria and Assiut small-sized isolates cut by the enzyme at 600 bp - 700 bp fragments, while Alexandria and Assiut large-sized cysts amplicons were not digested by this enzyme. The fourth enzyme, *Dra*I cut PCR products of Alexandria large-sized cysts into two fragment (420 bp - 780 bp), while Assiut large-sized amplicon was not cut. It could be concluded that there was a far distance between the two local isolates (small and large sized) but there was no differences between the large size isolates.

Keywords: Sarcocystis- water bubbaloes, ELISA- P.

PO-19**Track:** Industrial and Manufacturing**UTILIZATION OF POULTRY WASTE FOR THE LABORATORY CULTIVATION OF CHLORELLA FOR BIOMASS AND LIPID PRODUCTION****O.K. Agwa, S.N. Ibe and G.O. Abu***Department of Microbiology, University of Port Harcourt, P.M.B.5323, Rivers State, Nigeria; E-mail: o_agwa@yahoo.com*

Chlorella sp. is a potential source of value added biochemicals and renewable material for a variety of applications in biotechnology. The investigation of using poultry waste for the cultivation of this microalga for biomass and lipid production was evaluated. About 927 ml poultry waste extract was obtained from 30g of poultry waste suspended in a litre of distilled water. Extracts from poultry waste on average contains principally PO_4^{2-} 9ppm, NO_3^{2-} 4ppm, NH_4^+ 24ppm and SO_4^{2-} 6ppm, pH 7.0-8.5 and a conductivity of 830 $\mu\text{MHOS}/\text{cm}$ was used as nutrient for the cultivation of *Chlorella*. The isolate was cultured in 300ml of poultry waste extract, maintained under (i) sunlight (ii) aerated and (iii) unaerated conditions and finally monitored at a temperature of $28 \pm 2^\circ\text{C}$, pH 7.5 within a retention period of 21 days. The best growth and highest biomass of 2.50mg/ml (dry matter) was realized after culturing in the sunlight; 1.68mg/ml in the aerated and 1.58mg/ml in the unaerated conditions. *Chlorella* showed potential for lipid production in the poultry medium with about 18.32%(w/w) lipid in the wet cells in the sunlight, 11.19%(w/w) in the aerated and the unaerated condition gave 7.17%(w/w). The investigation revealed that poultry waste can be used as a suitable renewable medium for the growth of this oilgae (*Chlorella*). There is therefore a potential of algal biotechnology in the area of renewable, alternative green energy (bioresource/bioenergy) production using inexpensive growth media formulations such as poultry wastes which support the growth of *Chlorella* sp.

Keywords: Biomass, *Chlorella*, growth conditions, lipid production, poultry waste, renewable energy.**PO-47****Track:** Medical Biotechnology**PREVALENCE OF BACKACHE AMONG SCHOOL GOING CHILDREN OF HYDERABAD, SINDH****Shireen Rahat Khanzada, Muhammad Akram, Fatima Siddiqui, Raheel Mehran, Sania Kanwal Khanzada, Naveed Khanzada, Zubair Khan, Jamshed Husnain, Zain Ali, Faisal Qureshi and Rahim Ali***Isra School of Rehab Sciences, Hyderabad, Pakistan and Dow University of Health Sciences, Karachi, Pakistan***Objective:** The objective of this study project was to find out the prevalence of back pain among the school going children of Hyderabad, which is the second largest city of Sindh Province of Pakistan.

Introduction: Back pain is felt in the back that usually originates from the muscles, nerves, bones, joint and the spine. It may have sudden onset or could be a chronic one. It may be persistent or intermittent, and may also have the capability to radiate as per the dermatomes. Its character may be justified as dull, sharp, piercing, and burning sort of pain. The major regions of the body that are equally affected by the nerve-wrecking back pain can be divided into neck pain, upper back pain, lower back pain or tail back pain. Statistically, back pain presents in childhood and its prevalence vary from 13 to 51%, prevalence of recurrent lower back pain ranges from 7 to 27% (Salminen *et al.*, 1992 ; Vikat *et al.*, 2000).

Methodology: The methodology used in this particular study was Cluster Sampling technique. The sample size of our study was calculated through pre-designed formula and found out to be 240. Each cluster consisted of a school. The subjects aged between 7-14 years, including all those present on the day of Performa filling and excluding the absentees. The collected data was entered and analyzed using SPSS (Statistical Packages for Social Sciences) version 16.0.

Results: After all the analysis was done, the results were surprising. That is, a huge number of school age children were already suffering from bad postures and an alarming number of them had symptoms of backache. The prevalence of back pain was 46.7% among the total 240 subjects studied. Out of which 14.4% boys and 32.3% girls were affected. The



majority of affected children were age group of 10-12 years old. In our study 61% children had school bags weighing around 5 kg, which is a point to be considered by high officials of Primary Education System in Pakistan.

Conclusion: To conclude this study, we came to know that a significant number of students of school going age were already the victims of bad posture and a good enough number of them had symptoms of backache. Backache is slowly and gradually becoming a menace for our society and we need to address the issues at a preliminary stage, delay in which will result in a society with enough back problems, resulting in less productivity in all walks of life. Parents and school teachers are hereby advised to make their children familiar with ideal posture and sitting positions.

PO-62

Track: Plant and Environment

COMPARATIVE STUDIES OF THE EFFICACY OF SOME SELECTED FUNGICIDAL PLANT EXTRACTS

Taiga Akpovughave

Department of Biological Sciences, Kogi State University, Anyigba, Nigeria; Email: akpotaiga@gmail.com

Studies were carried out to isolate and identify fungal pathogens associated with dry rot disease of yam in storage in Ibaji Local Government Area of Kogi State, Nigeria; and to screen leaf extracts from some selected plants for their fungicidal properties against dry rot disease pathogens of yam. Both *in vitro* and *in vivo* assay of the fungicidal plant extracts were carried out. Yam, *Dioscorea rotundata* tuber (Ekpe line) and plant leaves of (*Azadirachta indica* (neem), (*Nicotiana tabacum* (Tobacco) and *A. barbadensis* (Aloe vera) were collected for the study, based on folkloric traditions and previous biological activities. Using Yam Dextrose Agar (YDA), four fungal pathogens - *Fusarium oxysporium*, *Aspergillus niger*, *Rhizopus stolonifer*, *Penicillium oxalicum* were isolated from the rotted *D. rotundata* and identified. Pathogenicity test was carried out with the fungal isolates on healthy yam tubers and all the fungal isolates were found pathogenic. Hot and cold extracts of the plant leaves were prepared into 10, 20, 30 and 40% concentrations and each concentration was screened *in vitro* for fungitoxic activities. The efficacy of three plants extracts (*Azadirachta indica*, *Nicotiana tabacum* and *Aloe barbadensis*) were tested *in vivo* on the fungal pathogens, using four different concentrations of cold and hot aqueous extracts. The 30 and 40% concentrations of cold and hot extracts of *N. tabacum*, completely prevented rot-depth caused by the four isolated pathogens (*F. oxysporium*, *R. stolonifer*, *P. oxalicum* and *A. niger*) in the yam tubers tested. Only the hot extract of *A. barbadensis* at 20, 30 and 40% concentrations completely prevented rot caused by *F. oxysporium*, *R. stolonifer* and *P. oxalicum*, while only the 40% concentration of its cold extract had similar effect on only the three pathogens like the hot extract. Also, only 30 and 40% concentrations of hot extract of *A. indica* prevented rot lesions caused by three of the pathogens (*F. oxysporium*, *R. stolonifer* and *P. oxalicum*). The hot extract of *N. tabacum* was most fungitoxic, followed by hot extracts of *A. barbadensis* and *A. indica* respectively. The results of comparisons showed clearly that there were variations in fungitoxicity of each plant extracts at various concentrations compared to their control tests.

Keywords: Comparative, Fungicidal studies, selected plant, Extracts concentrations.

PO-17

Track: Medical Biotechnology

HIGHLIGHTING THE DARKNESS: DESIGN AND SYNTHESIS OF A PEPTIDYL-FRET SUBSTRATE FOR TUMOR MARKER ENZYME HUMAN MATRIX METALLOPROTEASE-2 (hMMP-2)

Mehtab Alam, Hubert Kalbacher, Wolfgang Voelter, Atiya Abbasi, and Syed Abid Ali

*Department of Biochemistry, Dow University of Health Sciences, Karachi, Pakistan;
E-mail: promet213@yahoo.com*

Objective: Matrix metalloproteases (MMPs) in particular MMP-2, have been associated with several pathological conditions such as ovarian, urothelial, cutaneous, gastric, breast, and cervical cancers, etc. Successful treatment of these pathological conditions requires sensitive, reliable, quick and effective diagnostic tools such as fluorescence resonance energy transfer (FRET) based



assays in early stage of the disease. The objective of this research was to design and synthesize FRET peptidyl substrate for the detection of very low amount of tumor marker enzyme MMP-2.

Material and Methods: A peptidyl-FRET substrate having seven amino acid residues (PLGLKAR) with methoxycoumarin (Mca)/dinitrophenyl (Dnp) as fluorophore/quencher group has been synthesized using solid-phase fluorenylmethoxycarbonyl (Fmoc) peptide chemistry. The peptide was purified on RP-HPLC equipped with fluorescence detector and monitored using wavelength $\lambda_{\text{ex}} = 340$ and $\lambda_{\text{em}} = 405\text{nm}$.

Results: The newly designed substrate is stable and shows a K_m value of $15\ \mu\text{M}$ for hMMP-2. This K_m value is the lowest compared with all other known hMMP-2 substrates having Mca/Dnp. Validation of the new FRET substrate in presence/absence of scorpion venom chlorotoxin, a known hMMP-2 inhibitor, shows an increase in detection efficiency of 6,250 times as compared to commonly used gelatin zymography.

Conclusion: The new FRET substrate is much more cost effective and can be used for the detection of slight change in MMP-2 level and high throughput screening of hMMP-2 inhibitors in the laboratory for research and diagnostic purposes.

PO-54

Track: Others - Nanotechnology

GREEN SYNTHESIS OF GOLD NANOPARTICLES USING *GALAXAURA ELONGATA* AND CHARACTERIZATION OF THEIR ANTIBACTERIAL ACTIVITY

Nouf Alenazi, N. Abdel-Raouf and I.B.M. Ibraheem

*Department of Botany and Microbiology, Faculty of Science, Al-kharj University, Saudi Arabia;
E-mail: no_sa2007@hotmail.com*

The development of a reliable green chemistry process for the biogenic synthesis of nanomaterials is an important aspect of current nanotechnology research. Gold nanoparticles (Au NPs) have been known for their inhibitory and bactericidal effect. In the present investigation the use of marine alga *Galaxaura elongata* for powder and extracellular biosynthesis of Au NPs from HAuCL₄ solution is reported. It was observed that the aqueous gold (Au⁺) ions, when exposed to the powder particles or filtrate of *Galaxaura elongata*, were reduced in solution, thereby leading to formation of extremely stable Au NPs. These Au NPs were characterized by TEM; UV-Visible spectroscopy; FTIR and Zeta potential measurement for the average size, morphology and structure of particles. In addition, the elemental analyses using HPLC system for amino acid analyses; GC-MS system for fatty acid and terpenes analyses were done. The reduction of the metal ions resulted in the formation of high density, extremely stable Au NPs. The nanoparticles were also evaluated for their antibacterial activities against 2 gram-positive and 2 gram-negative pathogenic bacteria. The results showed better antibacterial effects.

Keywords: Gold nanoparticles, Green biosynthesis, Marine alga *Galaxaura elongata*, Antibacterial activity.

PO-55

Track: Regenerative Medicine

BEHAVIOR OF VASCULAR SMOOTH MUSCLE CELLS ON POLYMERS FUNCTIONALIZED WITH BIOACTIVE MOLECULES AND NANOPARTICLES

Lucie Bacakova, Katarina Novotna, Marketa Bacakova, Martin Parizek, Nikola Slepickova Kasalkova, Petr Slepicka and Vaclav Svorcik

Institute of Physiology, Academy of Sciences of the Czech Republic, Videnska 1083, 142 20 Prague 4 -Krc, Czech Republic; E-mail: lucy@biomed.cas.cz

The attractiveness of synthetic polymers for cell colonization can be effectively modulated by changing physical and chemical properties of the polymer surface. Polyethylene of a low density (LDPE) or a high density (HDPE), i.e., materials used for construction of body implants, were activated by exposure to Ar⁺ plasma and then grafted with glycine (Gly), polyethylene glycol (PEG), fibronectin (Fn), bovine serum albumin (BSA), colloidal carbon nanoparticles (C) and with



a combination of BSA+C. The changes in the physicochemical surface properties were in general more pronounced on HDPE than on LDPE. Also the improvement of the adhesion, growth and phenotypic maturation of vascular smooth muscle cells was more apparent in cultures on the modified HDPE than on LDPE in comparison with the pristine polymers. On the other hand, the cell numbers obtained on LDPE were generally higher than on HDPE. All bioactive molecules grafted to both polymers usually further improved the cell performance compared to the only plasma-treated polymers. An exception was BSA, which supported the cell adhesion in a serum-supplemented medium, but inhibited it in a serum-free medium. Thus, the cell performance on synthetic polymers for potential tissue engineering can be manipulated by the polymer type, its modification with plasma, subsequent biomolecule grafting, and also the composition of the cell culture media.

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Keywords: Synthetic polymers, plasma treatment, biomolecule grafting, vascular cells, bioartificial vascular replacements, tissue engineering.

PO-6

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

THE ANTI-OBESITY EFFECT IN MICE DEPENDING ON THE COMBINATION OF MONASCUS PIGMENT DERIVATIVES

Deokyeong Choe, Heeyoung Jang, Soomin Song, Hyun Ho Jung and Chul Soo Shin

Department of Biotechnology, College of Life Science and Biotechnology, Korea;

E-mail: cd02@yonsei.ac.kr

Monascus pigments as a GRAS compound are one of the representative microbial colorants and have been produced by fermentation. There are many reports about their biological activities such as cholesterol-lowering, lipase-inhibitory, anti-inflammatory, and anti-cancer activities. Among various derivatives, it was shown by our group that amino acid and amine derivatives have in vitro and in vivo anti-obesity effect. Threonine (Thr), tryptophan (Trp), and 2-(p-toyly)-ethylamine (TEA) derivatives had high cholesterol-lowering, lipase-inhibitory, and adipogenic differentiation-inhibitory activities, respectively. In this study, the anti-obesity effects depending on the combinations of these 3 derivatives were evaluated in mice. C57BL/6 mice were fed with a high-fat diet (HFD) including the combinations of L-Thr, L-Trp, and TEA derivatives. After 10 weeks, the weight gain of mice fed with the combinations of derivatives decreased by 10-33%, compared to the mice fed with HFD. The combination of L-Thr and L-Trp derivatives was the most effective on the reduction of weight gain by 33%. The epididymal adipose tissue (EAT) weight of mice fed with the combinations of derivatives decreased by 40-60%. The combination of L-Thr and L-Trp derivatives greatly reduced the EAT weight by 60%. The EAT size of mice fed with the combinations of derivatives decreased, too. Besides, micro-CT images of the abdomen of mice were taken for evaluating the size of adipose tissue layers. As a result, the adipose tissue layers of mice fed with the combinations of derivatives were remarkably decreased compared to the mice fed with HFD. The combinations of three derivatives showed high in vivo anti-obesity effect. Among them, the combination of L-Thr and L-Trp derivatives was the most effective, and this combination is expected to be one of functional food ingredients with anti-obesity effect.

Keywords: Monascus pigment, Anti-obesity, Monascus derivative, Monascus fermentation.



PO-33**Track:** *Pharmaceutical Biotechnology***EXTRACTION OF VOLATILE OIL FROM *ANGELICA SINENSIS* AND APPRAISAL OF ITS BIOLOGICAL ACTIVITY****Li Chongbi, Duan Zhifang, Li Sainan, Huang Lihua, Huang Zehuang and Wu Xufang***Zhaoqing University, Center of Biopharmaceutical Engineering, China; E-mail: chongbili@163.com*

Angelica sinensis which could be enrich the blood, smooth cataminia and moist intestines and so on is one of traditional chinese medicines (TCM) from of old. *Angelica sinensis* Volatile Oil is main component in its' functions. *Angelica sinensis* Volatile Oil was extracted by Petroleum, Chloroform—Hexyl hydride; The results showed that the best extracting solvent was Chloroform—Hexyl hydride, and the oil extracting rate is highest up to 3.258% using the process uniform design. Additioned, it is known that *Angelica sinensis* Volatile Oil contains Ligustilide and Butylidene-phthalide by thin-layer chromatography (TLC). Volatile oil could inhibit *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* proved by anti-bacteriostatic test. Pharmacology test showed *Angelica sinensis* Volatile Oil is not the principal component what could protect the mice liver injury. The analgesia test indicated that *Angelica sinensis* Volatile Oil had an analgesia effect on the experimental mice.

**Keywords:** *Angelica sinensis*, Volatile Oil, Extraction, Property Detection.**PO-4****Track:** *Others - Clinical Research***ELEVATED NEOPTERIN LEVEL IN PATIENTS WITH PRIMARY ARTERIAL HYPERTENSION****Artur Cieřlewicz, Paweł Bogdański, Iwona Smolarek and Anna Jabłeczka***Department of Clinical Pharmacology, Poznań University of Medical Sciences, Poland; E-mail: artcies@gmail.com*

Introduction: Neopterin is a pteridine derivative synthesized by macrophages. Numerous studies have confirmed the usefulness of neopterin as a prognostic marker in patients with coronary artery disease, diabetes and angina pectoris. There was also a correlation observed between elevated levels of neopterin and the occurrence of cardiovascular events in patients with hypertension, but without coronary artery disease. However, there is a lack of researches studying neopterin in patients with hypertension. Therefore, the aim of the study was to assess neopterin level in patients with primary arterial hypertension.

Materials and Methods: 46 patients with primary arterial hypertension were enrolled to the study. The control group consisted of 11 healthy individuals. 2 ml of blood were collected from elbow vein. Neopterin level was measured with ELISA immunoassay (DRG International Inc., USA).

Results and Conclusion: Statistically significant difference in neopterin level was observed between patients and controls ($p = 0.0378$). Obtained result supports the role of inflammation in progression of arterial hypertension.

Keywords: Hypertension, neopterin.

PO-61*Track: Pharmaceutical Biotechnology***ANTICANCER ACTIVITY OF METHANOL EXTRACT AND ITS FRACTIONS FROM HALURUS EQUISETIFOLIUS****Afef Dellai, Valérie Le Morvan, Jacques Robert and Abderrahman Bouraoui***Laboratoire de Pharmacologie Marine, Faculté de Pharmacie, 5000 Monastir, Tunisia;
E-mail: afef.dellai@hotmail.com*

Marine macrophytes contain a variety of biologically active compounds, some reported to have anticancer activity in vitro. Thus in the present study we investigated in vitro the efficacy of methanol extract and its semi-purified fractions (F2-F3) of *Halurus equisetifolius* for their antiproliferative effects by their potential cytotoxic activity using the MTT colorimetric method and clonogenic inhibition against three human cancer cell lines (A549, lung cell carcinoma, HCT15, colon cell carcinoma and MCF7, breast adenocarcinoma). Among the series F3 exhibited interesting growth and colony inhibitory effects against the three cell lines in a concentration-related manner which was correlated with its total phenolic content. These findings suggest that the polar active fraction F3 could contain a new antiproliferative compound(s). The purification and the determination of chemical structure of compound(s) of this active fraction are under investigation.

Keywords: Cytotoxic activity, MTT colorimetric method, clonogenic inhibition assay, lung cell carcinoma, colon cell carcinoma, breast adenocarcinoma.

PO-58*Track: Plant and Environment***AN EXPERIMENTAL ASSESSMENT OF THE FACTORS INFLUENCING AGROBACTERIUM-MEDIATED GENETIC TRANSFORMATION IN VALERIA (*VALERIANA OFFICINALIS* L.)****Sarwan Dhir, Adriana Mejía, Katy Sanon and Tomeka Howard***Department of Plant Science, Center for Biotechnology, Fort Valley State University, USA; Email: dhirs0@fvsu.edu*

Valeria (*Valeriana officinalis* L.) is a hardy, perennial, flowering plant used as a herbal medicine. The roots contain a compound, Valerian, an excellent remedy for anxiety, nervous tension and insomnia. Tissue culture and molecular engineering have provided rapid methods to develop desirable varieties of cultivated plant species. Transient expression has a wide range of applications in molecular biology. The goal of this work was to establish an optimal transient expression system using *Agrobacterium* for T-DNA gene delivery into different explants from which the whole plantlets can be regenerated. A reproducible multiple shoots regeneration system using node as an explants while studying the effect of benzyl amino purine (BAP) and kinetin (KN) was developed. Similarly, leaf explants cultured on MS media supplemented with 0.1- 3.0 mg/l 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 derived from one-month-old seedlings of in-vitro-grown Valeria plants were infected by *A. tumefaciens* carrying a binary vector that harbors a 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 GUS expression per responsive leaf explant. Parameters tested in this study included - different acetosyringone, Silver Nitrate (AgNO_3) and Calcium Chloride (CaCl_2) concentrations used during the incubation period, the length of the pre-culture period of explants prior to infection, different bacterial density (OD) and duration of immersion periods. 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, 60 μm AgNO_3 , and 0.25 μm CaCl_2 showed 80-90 % transformation efficiency. Therefore, the investigation of factors that influence T-DNA delivery is an important first step in the utilization of *Agrobacterium* in the transformation of Valeria tissue.

Keywords: Valeria, *Valeriana officinalis* L., *Agrobacterium*, Genetic Transformation.

PO-56**Track:** Plant and Environment**IDENTIFICATION, DNA SEQUENCING AND MOLECULAR GENETIC VARIATION IN ARUNDO DONAX POPULATIONS****Seema Dhira, Shawn Harrison, Michael Melvin and Srinivasa Chaluvadi***Department of Biology, Fort Valley State University, USA; Email: dhira@fvsu.edu*

Arundo donax (giant reed) is a potential biofuel feedstock crop which is distributed throughout the southern half of the United States, from California to Maryland. *A. donax* is a native to Asia and was initially introduced into North America from the Mediterranean region, although subsequent introductions were from multiple regions. *A. donax* is hypothesized to displace native plants and associated wildlife species as a consequence of the massive stands it forms, and it subsequently becomes a dominant component of the flora of that region. Invasive species such as *A. donax* are interesting for geneticists because these species often evolve rapidly in response to novel abiotic and biotic conditions, which further influence evolution of native species in response to invasion. DNA sequences are important sources of data for phylogenetic analysis. Nowadays, DNA sequencing is a routine technique in molecular biology laboratories. However, there are specific questions associated with project design and sequencing of plant samples for phylogenetic analysis, which may not be familiar to researchers starting in the field. In the current project, we are interested in understanding local genetic diversity of *A. donax* stands in Houston and Peach counties of Georgia, with the goals of 1) identifying superior genotypes for biofuel production, and 2) understanding the potential for colonization and establishment, geographic patterns of invasion and range expansion, and the potential for evolutionary responses to novel environments. In the current study, we analyzed *A. donax* individuals from 12 distinct populations in and around Peach and Houston counties. The current study provide the overview of methods and protocols involved in the sequencing of plant samples, including general recommendations on the selection of species/taxa and DNA regions to be sequenced, and field collection of plant samples. Protocols of plant sample preparation, DNA extraction, PCR and cloning, which are critical to the success of molecular phylogenetic projects, are described in detail. Further analysis of *A. donax* genome is underway to understand the genetic basis of invasiveness and identification of superior genotypes to be promoted as superior biofuel feedstock.

Keywords: DNA Sequencing, Molecular Genetic Variation, *Arundo donax*.**PO-36****Track:** Plant & Environment**CLONING AND ANALYSIS OF SERK1 GENE FROM TRIPLOID PLANT PINGYI TIANCHA AND TETRAPLOID STRAIN 33 IN MALUS****Wenxuan Dong, Lijie Zhang, Yating Gao, Yuxia Wang and Aide Wang***College of Horticulture, Shenyang Agricultural University, Shenyang 110866, China*

Using the ovaries of triploid plant *Pingyi Tiancha* (*Malus hupehensis* (Pamp.) Rehd. var. *pingyiensis* Jiang) and tetraploid strain 33 (the offspring strain derived from the cross of *Pingyi Tiancha* and Zha'ai Shandingzi (*M. baccata*)) during the full bloom stage as the experimental materials, the cDNA sequences of SERK1 (somatic embryogenesis receptor-like kinase1) gene in *Malus* were finally obtained by the method of homo-cloning strategy.



By the comparative analysis, the homology between the gene sequences was 99.95%, and the most important difference was as follows. The sequence from triploid plant *Pingyi Tiancha* (*MhSERK1*) was longer than that from the tetraploid strain 33 (*MhdSERK1*), in which the numbers of nucleotides were 1899 and 1881 respectively. And the different part was located from 696 to 714 and the sequence was “**ggc att gca tct tta gtt**”, from which the related amino acid sequence was “**GIASLV**” which located from number 233 to 238.

The origin CDS sequence of *MhSERK1* (from 1 to 1899) (accession number in Genbank is JQ231272): (1)atggagagaa aggttgggaa ttcatgtgt ctatgttca tttgtgtgc tcatccacta tggatgacaa tgggtcctgc taatatggaa ggtgatgcct tgcatactct aaggaccaac (121)ttggaggacc ccaacaatgt cctgcaaagt tgggataccta ctctgtgaa ccctgttacg tggtttcacg ttacatgcaa caatgaaaat agcgtcatag gattgatct tggaaatgca (241)gcctgtctg gtcaactgt tccacagctt ggcccttca agaattaca atattggag ctctacagta ataacatgag tggaccaatt ctagtgaac tggggaacct aaccagcttg (361)gtgagcttg atctttatt gaatagtttt tcaggtctaa tccaggcacc ctgggcagag

ctgtcaaaac tgcgattcct ccggcttaac aacaacagct tggcgggtcc gattcccatg (481)tcttgacta atatctcttc acttcaagta ctggatctat caaataatcg tctctcagga gtagttccag acaatggctc cttttctta ttactccca taagttttgc taacaacatg (601)gatctgtgtg gccagtaac tggtcgcccc tgcccaggat ctctccatt ttacctccc ccacttttg tcccaccacc cccaattca acaccaggca ttgcattctt agttggaggt (721)aatagtcca ctggggctat tgctgggtga gttgccgctg gtgctgcttt actatttgct gctcctgcaa tgcatttgc atgtggcgcg cggaggaagc cgcaagaatt tttcttgat (841)gtacctgagg agggagatcc tgaagtacat ctggggcagc tcaagaggtt ttcttgcca gaattacaag ttgcaacgga tagttttagt acaaaaaa tcttggggag aggtggattt (961)ggtaaggtct acaaggggag cctgcagat ggctcgctag tcgctgtgaa aagactgaaa gaagagcgca cccctggtgg ggagttgcag ttcaaaactg aagtagagat gatcagcatg (1081)gctgtgcac gaaatcttct tcggttacgt gggttctgta tgacaccaac tgaacgggta cttgtttatc ctacatggc taatgggagt gttgcctcat gttaagaga acggccgcca (1201)aaccaaccac ctcttgattg gccaactcgg aagcgaattg cactgggagc tgcaaggggt ctttctatt tgcagatca ctgtgaccg aagattattc accgtgatgt gaaagctgca (1321)aacatttgc tggatagga gtttggagct gttgtggag actttggtt ggctaaactt atggactaca aagacacca cgtcactact gctgtacgtg gcacaattgg acatatagct (1441)ccagagtacc tgcctaccg gaagtcact gagaaaactg atgttttcgg ttatggtatc atgcttctgg agcttattac tggtcagagg gcttttgatc ttgctgcct tgcacatgat (1561)gatgatgca tttgcttga ttgggtgaaa ggactactga aggagaaaaa gctagaaatg ctggttgatc ctgatctcca gactaattat gtagaagctg aggttagacca gctaattcaa (1681)gttgactgc tctgcacaca aggtcccca atggaccggc ctaagatgc agaagttgta aggatgctg aaggtgatgg cttggcagaa agatgggatg agtgccaaaa ggtcgaagtg.

Acknowledgement

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PO-25

Track: Medical Biotechnology

THE DEVELOPMENT OF NANOTECHNOLOGY-BASED DETECTION SYSTEMS FOR DIAGNOSIS OF BREAST CANCER

Mustafa Drah, Paul Mushonga, Francois Taute, Martin Onani, Bongani Ndimba and Mervin Meyer

Department of Biotechnology, DST/Mintek Nanotechnology Innovation Centre Biolabels Platform, University of the Western Cape; E-mail: m11al2drah@gmail.com



Molecular techniques such as qRT-PCR, Fluorescent In Situ Hybridization (FISH) and Immunohistochemistry are often used to evaluate the presence of cancer biomarkers in biopsy samples. The objective of this study is to develop alternative detection systems for cancer biomarkers based on the use of molecular beacons and quantum dots. Molecular beacons are oligonucleotide hybridization probes that can be used assess the presence of specific DNA or RNA molecules representing cDNA or mRNA of genes encoding biomarkers. We selected 19 genes that are known breast cancer biomarkers. The expression levels of these biomarkers were investigated in two human breast cell lines (a cancer cell line, MCF7 and a non-cancerous cell line, MCF12) using qRT-PCR. We developed molecular beacons for the detection of some of these biomarkers in cancer cells and show that the results obtained using the molecular beacons correlated very well with the qRT-PCR results. The application of quantum dots in molecular beacons can facilitate the development of multiplex detection systems, which can significantly reduce the cost and the time of doing the diagnostic test, while improving the accuracy of the diagnosis.

PO-64

Track: Medical Biotechnology

DEVELOPING COUNTRIES AND EMPHASIS ON INTRODUCTION OF DNA VACCINES

Adel Tari Farrokhru and Hajie Lotfi

Plants Technology Group, Institute of Technical and Vocation Higher Education Jahad – e – Agriculture, Tabriz, Iran; Email: tarifarrokhi@yahoo.com

Introduction: DNA vaccination is a technique for protecting an organism against disease by injecting it with genetically engineered DNA to produce an immunological response. DNA vaccines have been successfully directed against a wide variety of tumor. DNA vaccine is stable and can be stored and delivered without requiring a cold chain, which is much

preferred to distribute vaccines efficiently in rural areas. Because of many advantages of DNA vaccine, it has attracted much interest in the developing world.

Important point to mention is the time and costs involved in bringing these vaccines to the developing countries, for their populations, especially who live under economically disadvantaged conditions. Why introduction and production of DNA vaccine should be considered an additional 15–20 years and neglected by the modern biotechnology industry into developing countries?

We would like to make interest our industry to protect the production of DNA vaccines. But, for developing DNA vaccines in developing countries, several additional issues are to be considered: The immunogenicity of the gene(s) of interest, the delivery system and route of delivery, Preventive and therapeutic DNA vaccines. Some developing-country DNA vaccine manufacturers have made major strides in technology but in our country, technology does not sweep development off its feet. Also, the lack of capability in developing countries until recently has resulted in stagnation of effort either for DNA vaccine improvement in these facilities. On the other hand, because of these recent developments and initiatives, there are increasing opportunities for developing countries to advance in applied vaccine research and development. The application of political, social, and economic pressure in support of introducing new vaccines to developing countries requires vigorous effort and innovative thought. It seems important and appropriate to set up joint research groups of scientists from developed and developing countries to study these problems and to develop the vaccines. Joint research groups can select genes of interest from the microbial isolates of developing countries, and local scientists can provide information to choose the first few DNA vaccines to be developed. In addition, large animal studies in DNA immunization experiments will be less expensive in the developing countries, and the fund for field experiments can be significantly decreased. Since ultimately, these DNA vaccines will be mainly used in people of the developing countries, an early involvement of local workers will build up mutual understanding and confidence, which may bypass some barriers in future vaccine production and clinical trials. Besides, early involvement would also shorten the time for the transfer of technology know-how in DNA vaccine production, which will bring DNA vaccines more rapidly to those who need these vaccines the most.

Finally, we mentioned that accelerating the time from first research of new DNA vaccines to actual use in public sector immunization programs in the developing world must be accomplished. We have described a set of elements that we believe must be addressed to accelerate the process.

Keywords: DNA vaccine, developing countries, manufacture, technology.

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PO-35

Track: Plant & Environment

CLONING AND ANALYSIS OF THE KEY ENZYME GENES IN ANTHOCYANIN SYNTHESIS FROM PURPLE-FLESHED SWEET POTATO [*IPOMOEA ATATAS* (L.) LAM]

Gao Feng

School of Life Sciences, South China Normal University, Shipai, Tianhe District, Guangzhou, 510631, Guangdong, China

Flavonoid 3'-hydroxylase (F3'H) and dihydroflavonol 4-reductase (DFR) are involved in the biosynthetic pathway of anthocyanin and might be the committed step of this pathway. To isolate and understand the gene structure and expression characteristics in purple-fleshed sweet potato [*Ipomoea atatas* (L.) Lam], F3'H and DFR genes were isolated by using the RACE methods, and bio-informatical analysis, Real-Time Quantitative PCR and western blot were also made. The full-length cDNA of F3'H and DFR genes were isolated (No. EU113299 and No. EU402466). The F3'H and DFR genes were expressed in all tested tissues, but most abundantly in storage roots and also strongly associated with anthocyanin



accumulation in five different sweet potato cultivars. It suggesting that F3'H and DFR genes were the key genes in anthocyanin biosynthesis in purple-fleshed sweet potato. The results would supply a basis of theory and technology for molecular breeding in purple-flesh sweet potato.

PO-9

Track: Plant and Environment

THE REGULATORY FRAMEWORKS OF NOVEL MOLECULAR WATER TESTING BIOTECHNOLOGIES IN NORTH AMERICA

I. Ngueng Feze, N. Prystajecy, J. Isaac-Renton, C. Cook, Y. Joly, G. Dunn, E. Kirby, B.M. Knoppers and V. Ozdemir

McGill University, Faculty of Medicine, Centre of Genomics and Policy, 470 Avenue Dr Penfield, Montreal, H3A 1A5 Canada; Email: ida.nguengfeze@mcgill.ca



Microbial water quality assessment is one of the key tenets of public and environmental health risks management associated with contaminated water. As it would be impractical, if not impossible, to test for all possible microbiological parameters, water quality assessment instead tests for organisms that are indicative of recent fecal contamination. Molecular tests, such as Nucleic Acid Amplification Tests ("NAAT"), could precipitate a paradigm shift in water quality assessment, circumventing the challenges of currently available tools and developing a water quality testing approach that is faster, more accurate and more sensitive. These same techniques and tools have revolutionized clinical diagnostic microbiology providing testing results in matter of minutes where it used to take days or weeks. While NAAT-based tests have been successfully applied to water quality testing, targeting both traditional and novel microbial indicators, their application has been limited in scope and largely research-based. To make the transition from research to routine use, a rigorous and standardized translation of these approaches requires that the associated regulatory frameworks facilitate their accessibility as practical tools for all stakeholders.

We reviewed current legislation, guidelines and protocols to assess how new biotechnologies for water quality assessment are developed, validated and introduced into testing laboratories, and thereby integrated within the Canadian and American legal and policy frameworks.

We found that molecular tools, such as NAAT-based tests, are not currently systematically used for the routine analysis of microbial water quality in either country. Within the current Source-to-Tap model, the regulation of traditional bacterial indicators, more so in Canada than the United States, has mainly been centered on microbial water quality assessment at the tap, often integrating testing at the source to a lesser extent. These gaps attest to an overall need for technological improvement in the validation and standardization of practices, but also to a need to ensure that the legal and policy frameworks are conducive to the development, integration and dissemination of new biotechnologies for the assessment of health both for the public (at the tap) and the ecosystem (at the source). Addressing these gaps efficiently is vital to prevent, prohibit and remediate potential health threats, identify public health and environmental risks, implement effective preventive strategies, as well as provide real-time useful information to water managers, public health officials, governmental agencies, the water industry and the public at large.

This research is part of the Genome Canada-funded "Applied Metagenomics of the Watershed Microbiome" Project. See <http://www.genomebc.ca/portfolio/projects/environment-projects/applied-metagenomics-of-the-watershed-microbiome/>.

Keywords: Biotechnologies, Canada, method validation, microbial water quality, molecular test, North America, regulatory framework, research translation, source-to-tap model.

PO-65*Track: Plant and Environment***NEW PRODUCTS BASED ON COLLAGEN HYDROLYSATES FOR CEREAL SEEDS TREATMENT AND SUSTAINABLE AGRICULTURE****C. Gaidau, M. Niculescu, E. Stepan, D.G. Epure and M. Gidea***Leather Research Department, Leather and Footwear Research Institute Division, Romania; E-mail: carmen_gaidau@hotmail.com*

The world's population is on ongoing increasing trend, the areas intended for the production of bio-mass and bio-fuels are continuously growing, while the food demand of mankind is on a continuous ascending trend. In this context, an increased efficiency in the use of agricultural areas and in obtaining large and steady productions, in compliance with the requirements of sustainable development of the agricultural eco-system, is a priority; this priority requires adequate technological measures, in particular a good protection of crops starting with the seed phase. To be effective, the seed treatment shall answer to the following requirements: shall disinfect and protect the seeds on their surface as well as on the inside, against the pests and pathogen agents found in the soil, shall ensure the system protection, shall not pollute the soil, water and environment, shall have no remnant effect onto the environment and onto the crops and shall be bio-degradable, easy to transport and to use.

This paper aims at presenting new collagen hydrolysate based materials for cereal seeds treatment, which generates an increase of the quality indicators for treated seeds, guarantees an increased percentage of sprung plants, an early and effective protection of plantlets against pathogen and harmful agents through a disinfection of the seeds surface, by destroying the pathogen agents present on the grain, ensuring the protection of roots and hypocotyls against the attack of pests and pathogen agents - ensuring a rapid springing of the crops, with an optimum density - as a mandatory requirement for obtaining high productions. Creation of a new and advanced technology for treatment of cereal seeds, by using pesticide-collagen hydrolysate mixes has as targets the seed quality indexes increase; reduce pesticide consumption, which will in turn decrease environment pollution and the cost of treatment for cereal seeds; reduce costs for obtaining the seeds; achieve a better management of resources; reduce production expenses while preserving the environment, soil and bio-diversity. The technologies developed for protein raw materials processing and characteristics of collagen hydrolysates with bioactive properties are presented. The second route for ecological treatment of seeds is the use of microencapsulated plant extracts with insect repellent and fungitoxic properties in a shell made by using collagen hydrolysate. The method for collagen hydrolysates modification and encapsulation of rosemary and cinnamon essential oils in a coacervate structure with controlled delivery properties are presented as potential alternative for reduction of pesticide overuse and sustainable agriculture.

Keywords: Seeds treatment, collagen hydrolysates, microencapsulation.

PO-43*Track: Pharmaceutical Biotechnology***THE NIACIN/BUTYRATE RECEPTOR GPR109A IS A TUMOR SUPPRESSOR IN COLON****Nagendra Singh and Vadivel Ganapathy***Biochemistry and Molecular Biology, Georgia Health Sciences University, Augusta, GA, USA; E-mail: vganapat@georgiahealth.edu*

GPR109A is a G-protein-coupled receptor for the B-complex vitamin niacin. This receptor is expressed in immune cells and in adipocytes. We have shown recently that the receptor is also expressed in the intestinal tract and that the expression is maximal in the colon. Butyrate, a bacterial fermentation product in colon, serves as the physiologic agonist for the receptor in colon. Since butyrate is known to elicit protective effects in colon against inflammation and cancer, we hypothesized that GPR109A, by serving as the cell-surface receptor for butyrate, is responsible for these beneficial effects of the bacterial metabolite butyrate. We tested this hypothesis using Gpr109a^{-/-} mice. Here we show that Gpr109a is essential for butyrate/niacin-mediated induction of IL-18, a cytokine that prevents intestinal inflammation and carcinogenesis. In addition, Gpr109a^{-/-} mice have reduced levels of regulatory T cells (Tregs). Consequently, Gpr109a^{-/-} mice are highly susceptible for development of colonic inflammation in an experimental model. Further, Gpr109a^{-/-} mice develop highly increased number of polyps in inflammation-associated colon cancer as well as in ApcMin-driven intestinal cancer. Depletion of microbiota increases the risk for colonic inflammation and cancer, which

is effectively suppressed by niacin in a Gpr109a-dependent manner. Collectively, these data show that Gpr109a serves as a molecular link between gut microbiota and colonic health and suggest that maintenance of optimal Gpr109a signaling in intestine is an effective therapeutic strategy for prevention of intestinal inflammation and colon cancer.

Keywords: GPR109A, niacin receptor, butyrate, colon cancer, colitis, cancer prevention.

PO-24

Track: Medical Biotechnology

LOXL4 MONOCLONAL ANTIBODY HAS HIGH ANTIPROLIFERATIVE EFFECT AGAINST HUMAN HEAD AND NECK CARCINOMA CELLS

Tibor Gorogh and Markus Hoffmann

Otorhinolaryngology- Head and Neck Surgery, Section of Experimental Oncology, University of Kiel Schleswig-Holstein 24105 Kiel, Germany; E-mail: gorogh@hno.uni-kiel.de

Lysyl oxidase (LOX) is known as a secreted copper-dependent amine oxidase that catalyses the oxidation of peptidyl lysine to δ -aminoadipic β -semialdehyde, the intermediate precursor during the formation of covalent cross-linkages that stabilize fibres of elastin and collagen, and contributes to the development and maintenance of the extracellular matrix. LOXL4, a new member of the LOX family, has been noted in both extracellular and intracellular locations, is strongly up-regulated in head and neck squamous cell carcinomas. Using the hybridoma technology a monoclonal antibody has been developed against LOXL4 and investigated for its potency in a series of experiments conducted in head and neck squamous cell carcinoma (HNSCC) cell lines (UKHN-2, -3, -6, -7 and -9). A significant antiproliferative effect has been observed at a concentration as low as 5 micrograms per milliliter cell culture with a density of 1×10^5 cells. Complete destruction of the tumor cells has already been reached at 10 to 15 micrograms over a period of 48 hours, in contrast to normal epithelial cells- and fibroblasts. Among the carcinoma cells tested, the UKHN-9 tonsillar squamous cell carcinoma cells showed the highest sensitivity, suggesting some differences of HNSCC cells to the potency of the LOXL4 antibody. Collectively, the in-vitro therapeutic studies indicate that the new LOXL4 monoclonal antibody has preferential effect on HNSCC cells without causing any damage to both healthy epithelial cells- and connective tissue cells under similar treatment conditions. Ongoing animal experiments should provide further data in respect of the in-vivo usefulness of the antibody.



Keywords: LOXL4, monoclonal antibody, tumor therapy.

PO-20

Track: Industrial and Manufacturing

LINEAR AND NONLINEAR PROPERTIES OF BIOPOLYMERS AND THEIR BINDING CHROMOPHORES

Piotr Hanczyc, Bjorn Akerman, Marek Samoc and Bengt Norden

Department of Chemical and Biological Engineering, Chalmers University of Technology, SE-41296 Gothenburg, Sweden; E-mail: piotr.hanczyc@chalmers.se

Biomolecules such as amyloid fibrils and deoxyribonucleic acid (DNA) are widely investigated because of unique molecular properties which makes them interesting candidates for many applications including photonics, material science, advanced microscopy or nanobiotechnology.

Polarized-light spectroscopy methods [1] were used to investigate structural properties of amyloid fibrils and DNA including their interactions with nonspecifically binding chromophores. Study in poly (vinyl alcohol) (PVA) show that secondary structure of short synthetic nucleic acids depends on oligonucleotides length and water content in polymer matrix [2]. Highly dense gel-like environment affects also the DNA-drug interactions. The complex is destabilized, which leads to time dependent dissociation into PVA matrix from intercalation sites [3] if the binding constant is lower than 10^{10} M^{-1} . In this context, chromophores with high affinity to DNA duplex, such as the YOYO dimer are extremely interesting molecular probes in media different than just water solutions. Dimeric chromophores that interact electrostatically with DNA or amyloid fibrils might be also promising for implementing



highly advanced two-photon based technologies [4] because of their strong nonlinear response. Especially dimers of metal-organic intercalating drugs with ruthenium [5] that are substituted in either *orto*- or *para*- position are candidates of potential interest because the combination of large two-photon cross section and strong luminescence quantum yields for these molecules when intercalated makes the compounds uniquely bright and photo-stable probes for imaging and also promising as enhanced photosensitizers in two-photon sensitizing applications (e.g. photo dynamic therapy).

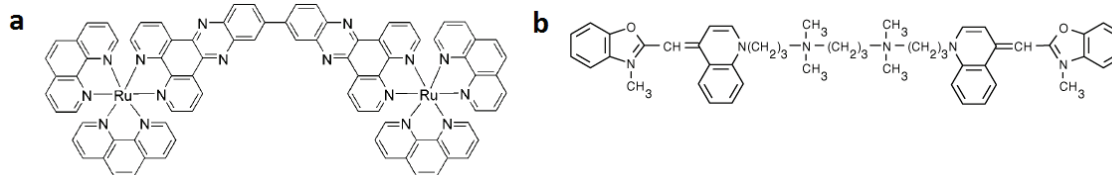


Fig. Structure of dimers: a) ruthenium complex; b) YOYO-1.

Acknowledgement

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PO-39

Track: Other areas: Marine Biotechnology

cDNA SEQUENCE AND TISSUE EXPRESSION ANALYSIS OF GLUCOKINASE FROM LIVER OF GRASS CARP (*CTENOPHARYNGODON IDELLA*)

Cheng Han-Liang, JI Nan-Jing, Peng Yong-Xing, Shen Xin, Xu Jian-He, Dong Zhi-Guo and Wu Chen-Chen

Jiangsu Key Laboratory of Marine Biotechnology, Huaihai Institute of Technology, Lianyungang 222005, P.R.China; Email: CHL3139@163.com

A full-length cDNA coding glucokinase (GK) was cloned from liver of grass carp (*Ctenopharyngodon idella*) by RT-PCR and rapid amplification of cDNA ends methods. The cDNA obtained is 2066 bp exclusive of poly (A) residues with a 1431 bp open reading frame encoding 476 amino acids. The GK protein has a calculated molecular weight of 53.7 kDa and isoelectric point of 5.11. Some conserved functional sites were found, including one conserved hexokinase signature sequence Leu¹⁵⁶-Phe¹⁸¹; two N-linked glycosylation sites Asn¹⁷⁶ and Asn²¹⁴; one cell attachment sequence Arg²⁰²-Asp²⁰⁴; one glycosaminoglycan attachment site Ser⁴⁵⁵-Gly⁴⁵⁸. The amino acid sequence has a high similarity to GK of other species, the percent identity compared with topmouth culter, common carp, human and rat are 98.1%, 96.8%, 80.3% and 79.8%, respectively. Tissue distribution of GK mRNA in brain, mesenteric adipose tissue, spleen, white muscle and liver of grass carp was analyzed by SYBR real-time fluorescence quantitative RT-PCR method using β -actin as an internal control for cDNA normalization. The result showed that the expression level of GK mRNA in liver was significantly higher than in mesenteric adipose tissue, spleen and brain ($p < 0.05$). Relative expression profile of GK mRNA in liver normalized with β -actin level was 31, 454 and 649-fold compared with the levels in mesenteric adipose tissue, spleen and brain, respectively. Meanwhile, GK mRNA was not detected in white muscle.



Keywords: *Ctenopharyngodon idella*, glucokinase, full-length cDNA, tissue distribution, real-time PCR.

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PO-41*Track: Plant & Environment***EXPRESSION PATTERNS AND FUNCTION ANALYSIS OF SERKS IN SOMATIC EMBRYOGENESIS IN PINEAPPLE****Yehua He, Jun Ma, Zhongyi Hu, Chengming Liu, Wentian Xu, Jingxian Xia, Cuihong Guo, Li Cao, Chengjie Chen, Chenghou Wu and Junli Zhang***Horticultural Biotechnology College of South China Agricultural University, Guangzhou, Guangdong 510642, China*

Pineapple is a perennial tropical herbal fruit-produced crop. Study on the molecular mechanism of somatic embryogenesis (SE) is meaningful for the genetic improvement and industrial seed culture of pineapple. As a somatic embryogenesis-specific gene, *SERK* (somatic embryogenesis receptor-like kinase) played an important role in the competence acquisition of somatic cells. On the basis of the study on somatic embryogenesis, genetic transformation and isolation of *AcSERK1* partial sequence, we isolated the DNA and cDNA full-length sequences of *AcSERK1* by RACE technique. In addition, we isolated other two *AcSERKs*. It confirmed that *AcSERK* was a gene family consisted of three gene members, which were designated as *AcSERK1*, *AcSERK2* and *AcSERK3*. The analyze results of RNA in situ hybridization and Real time qRT-PCR indicated that among the three *AcSERKs*, only *AcSERK1* was expressed in high level during the formation of embryogenic cells to pro-embryo (up to 25 times), and only *AcSERK1* expression was elevated apparently in ovule after fertilization and lasted to pro-embryo. The transformation results of sense expression and RNAi constructs of *AcSERK* indicated that the over-expression of *AcSERK1* presented an increased somatic embryo occurrence rate of 97.5%, and the expression level of *AcSERK1* under the effect of RNAi was only 9.6% of wide type. The surface of the callus transformed with RNAi-*AcSERK1* was smooth and similar to non-embryogenic callus, and it could not originate somatic embryo. Therefore, among the three members of *SERK* family, only *AcSERK1* was closely related to the formation of embryo (included somatic embryo and zygotic embryo), played an important role in the induction of somatic embryo and the transition of non-embryogenic cells to embryogenic cells. The great increase of *AcSERK1* expression level during in vitro culture marked the transition of non-embryogenic cell to embryogenic cell, which indicated that *AcSERK1* could be used as a marker gene of cell differentiation during somatic embryogenesis in pineapple. These results provided molecular basis to the further study of the regulation mechanism of *AcSERK1* expression and the enhancement of somatic embryogenesis competence.

Keywords: *Ananas comosus*, somatic embryo induction, AcSERK gene, expression; function.

Acknowledgments

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PO-34*Track: Medical Biotechnology***SELECTION AND FUNCTION OF MICRORNA TARGETING MEPE****Baocheng Hu, Wei Luo, Jipo Sheng and Fang Yu***Beijing Institute of Biotechnology, Beijing, China; Email: baochengh@hotmail.com*

Matrix Extracellular Phosphoglycoprotein (MEPE) had been limited to regulation of bone metabolism, phosphate homeostasis. We previously reported an important function of MEPE in protecting cells from DNA damage induced killing, which would provide MEPE as a new target for sensitizing tumor cells to radiotherapy or chemotherapy. In this study, we will focus on selection of miRNAs targeting *Mepe* and its function in DNA damage response.

Here Targetscan was used to predict the candidate miRNAs targeting *Mepe*, 36 candidate miRNAs targeting *Mepe* were found, and only 6 predicated miRNAs targeting *Mepe* were selected and were identified by using dual luciferase assay, Luciferase analysis showed that the activity of wild type 3'UTR reporter was significantly suppressed by miR-376a, suppression by miR-376a depends on the wild type miR-376a complementary



sites, so miR-376a directly targets *Mepe* and miR-376a can repress the translation of *Mepe* gene in HeLa cells by Western blotting. The results show that introduction of miR-376a expression in human cells resulted in decrease of G2 phase arrest following ionizing radiation (IR) by the flow cytometry, and overexpression of miR-376a sensitized the cells to DNA damage inducers. All these results proved that miR-376a sensitized the cells to DNA damage by repressing the expression of MEPE, it will be basis to study potential drugs for radiotherapy or chemotherapy.

Keywords: MEPE, microRNA, target, DNA damage.

PO-40

Track: Plant & Environment

CONCURRENT CHANGES IN METHYL JASMONATE EMISSION AND ITS BIOSYNTHESIS GENES' EXPRESSION IN *CYMBIDIUM ENSIFOLIUM* FLOWER

Mingkun Huang, Cuiping Ma, Jia Hou, Rangcai Yu, Hongxun Fu and Yanping Fan

College of Horticulture, Center of Flower Research, South China Agricultural University, NO.483 Wushan Road, Tianhe District, Guangzhou, China

Methyl jasmonate (MeJA) was the one of most abundant scent compounds and its emission was under developmentally and rhythmically controlled in *Cymbidium ensifolium* flowers. Moreover, MeJA emission occurred in a tissue-specific manner, with maximum emission in sepals and petals. In this study, a number of key genes involved in the MeJA biosynthesis via the octadecanoid pathway were isolated from *C. ensifolium* flowers, including *CeLOX*, *CeAOS*, *CeAOC*, and *CeJMT*. Our real-time quantitative PCR (Q-PCR) data showed that the expression of these genes followed a similar pattern, changing during the anthesis, oscillating in a diurnal cycle, and coinciding with MeJA emission. Our results suggest that MeJA emission in *C. ensifolium* is regulated directly at the transcript level. In addition, the expression of these genes showed tissue-specific manner. The expression of *CeAOC* and *CeJMT* was floral tissue-specific, while *CeLOX* and *CeAOS* were also detected in leaves. To identify the function of *CeJMT* in the final step of MeJA biosynthesis, *CeJMT* was expressed in *E.coli* and the recombinant protein was tested on several substrates such as jasmonic acid (JA), benzoic acid, and salicylic acid (SA). Result showed that *CeJMT* specifically catalyzed the JA to form the corresponding ester MeJA.

Keywords: *Cymbidium ensifolium*, floral scent, methyl jasmonate, octadecanoid pathway.

PO-38

Track: Plant & Environment

EFFECTS OF DIFFERENT NACL CONCENTRATION ON SEED TUBE GERMINATION, SEEDLINGS GROWTH OF *HALOCNEMUM STROBILACEUM*

Wu Yu-Ling, Gong Hui-Fang, Hu Ling-Zhi, Jin Xin-Xin and Chen Hui

College of Life Science, Shan Xi Normal University, LinFen 041004, China

Halocnerrum strobilaceum, with the adaptability to saline habitats, is a dominant species in saline desert in Xinjiang province. Because of its small seeds, using waterish filter paper culture method or using sand culture in petri dishes method to study the seed germination rate, may affects the results accuracy, for small seeds were easily affected by water flooding, bacterial infection or mechanical resistance by sand buried. To investigate the effect of NaCl on seed germination (germination rate, relative germination rate, germination vigor and germination index) and seedling growth and development, including the determination of relative salt damage rate and surveying the high and length of shoots and roots, the seeds of *Halocnerrum strobilaceum* were cultured on MS medium plus with different concentrations of NaCl (0,100, 200, 300, 400, 500, 600 and 700mmol/L) respectively. For white medium background with grown seeds having contrast color were observable and statistics clearly, our result indicated that the optimal salt concentration of seeds germination and seedlings growth and development were determined: 100 mM and 200 mM NaCl respectively, and this salt concentration added in medium also promoted seeds germination for 2days, but when NaCl added in medium over 400mM, seeds germination and seedling's growth were strongly inhibited. This investigation laid a good foundation for further study on the salt resistance mechanism of *Halocnerrum strobilaceum*.

Keywords: *Halocnerrum strobilaceum*, NaCl concentrition, seed germination, seedling growth, tissue culture.



PO-7**Track:** Medical Biotechnology**EVOLUTIONARY RELATIONSHIP OF LOW DENSITY LIPOPROTEIN RECEPTOR (LDLR) GENE****Fauzia Imtiaz, Nusrat Jabeen and Mushtaq Hussain**

Department of Molecular Pathology, DDRRL, Dow University of Health Sciences, Karachi, Pakistan;
Email: f.imtiaz@duhs.edu.pk



Introduction: Low-density lipoprotein is essential constituent part of cholesterol metabolism. Normally LDL enters into the cell via specific receptor ascribed as Low Density Lipoprotein Receptor (LDLR). Truncation of the said gene(s) may result in the impairment of the LDLR receptor that entails the increased concentration of cholesterol in blood (hypercholesterolemia). The condition may render premature heart attacks, atherosclerosis, xanthomata and xanthomas formation etc.

Objective: The present study is designed to unravel the evolutionary history of the said genes among different phylogenetic groups of life forms.

Study Design: The study is based on *in-silico* analysis of gene variations.

Place and Period of Study: Department of Molecular Pathology, DDRRL, Dow University of Health Sciences, Karachi-Pakistan. (March- September 2011)

Methods: The gene sequence of LDLR was retrieved from NCBI database and subjected to non-redundant blasting of 1000 hits. Multiple sequence alignment of the selected sequences was conducted and phylogenetic tree was constructed by UPGMA and/or Neighbor joining methods. Boot strapping was exploited to calculate lineage distance.

Results: The cladogram indicates the ancestral presence of the LDLR genes among different taxonomic groups of animals. Important structural and functional variations have been noticed among primates, chordates and invertebrates. The sequential variations have been found increased according to the evolutionary placement of the group to which the organism(s) belong.

Conclusion: The findings suggest that phylogenetic tree based on LDLR may be explored for the classification of animals as earlier based on 23sRNA tree. Additionally, it may also provide more insights regarding the ancestral root of the anomalies associated with LDLR.

Keywords: LDLR, Cholesterol, Evolution, Hypercholesterolemia.

PO-74**Track:** Regenerative Medicine**THE INFLUENCE ON THE EXPRESSION OF VEGF/ VEGFR-2 AND $\alpha\beta 5$ INTEGRIN IN THE VITRIFICATION OF MOUSE OVARY INTERVENTION BY FSH****Chen Jie, Wang Yanrong, Wu Hao, Pei Xiuying, Ma Wenzhi, Chang Qing, Ma Huiming, Hei Changchun, Yu Jia and Sun Miao**

Key Laboratory of Fertility Preservation and Maintenance, Ningxia Medical University, Ministry of Education, Department of Histology & Embryology in Ningxia Medical University, Yinchuan, People's Republic of China 750004

Objective: To investigate the best way of FSH interposed in vitrification process via observation the expression of VEGF /VEGFR-2 and $\alpha\beta 5$ integrin.

Method: 4-week-old C57BL/6J mouse were divided into three groups: the first group is fresh control group (FCG), the ovaries come from normal mice; the second group is vitrification control group (VCG), there was no FSH in the medium and the third group is the vitrification group intervention by 0.3IU/mL FSH, including the group of FSH interposed in the overall process of vitrification group (OG-FSH), FSH interposed in the before process of vitrification group (BG-FSH) and FSH interposed in the after process of vitrification group (AG-FSH). Through the morphology, immunohistochemical technique and western-blot to observe and analysis the expression of VEGF/VEGFR-2 and $\alpha\beta 5$ integrin in every group.

Result: The percentage of normal follicles in VCG is the lowest($P<0.05$), it had a significant statistical difference from other groups. The results of immunohistochemical technique and western-blot shown that the expression of VEGF and VEGFR-2 protein from high to low in turn is in the OG-FSH,BG-FSH,AG-FSH,FCG and VCG,($P<0.05$). The results of immunohistochemical technique revealed that the highest expression of $\alpha\beta 5$ integrin was upregulation in the OG-FSH compared with VCG,which also correlated with increased VEGF.

Conclusion: FSH interposed in the overall process of vitrification group(OG-FSH) is favourable to kept the ovary morphological structure and also favour the expression of VEGF/VEGFR-2 protein and $\alpha\beta 5$ integrin.

Acknowledgement

Supported by the National Natural Science Foundation of China, No. 81160085 , and Natural Science Foundation of Ningxia of China(NZ1084).

Keywords: Mouse;Ovary;FSH;Vitrification;VEGF;VEGFR-2; $\alpha\beta 5$ integrin.

PO-57

Track: Medical Biotechnology

BIOEQUIVALENCE STUDY OF TWO ORALLY DISINTEGRATING RISPERIDONE FORMULATIONS IN HEALTHY KOREAN VOLUNTEERS

Gaeun Kang, Kyung-Yeol Bae, Hee-Young Shin, Seong-Wook Jeong, Jin-Sang Yoon and Jong-Keun Kim

Department of Pharmacology, Chonnam National University Medical School, 5 Hak-dong, Dong-Gu, Gwangju, 501-746, Republic of Korea; E-mail: gekang@chonnam.ac.kr

Background: Risperidone is one of the atypical antipsychotic drugs that have effectiveness in the management of a range of psychiatric illnesses. Orally disintegrating (OD) formulations of risperidone rapidly dissolve in the mouth, prior to swallowing without water. The goal of this study was to evaluate the bioequivalence of newly developed 2 mg OD risperidone tablet (test) and Risperdal Quicklet® tablet of 2mg (reference).

Methods: This randomized, open-label, 2-way crossover trial was conducted in 36 healthy male volunteers that received OD risperidone tablet, either the reference formulation, or the test formulation, each in a single administration. Blood samples were obtained during a 24-hour period after dosing. Plasma was analyzed for risperidone by a validated LCMS/MS. Adverse events were monitored by safety assessments including clinical interview by clinician. Pharmacokinetics were calculated by noncompartmental analysis and compared between two formulations.

Results: The ANOVA showed no significant effect of sequence of $\ln(AUC_{last})$ and $\ln(C_{max})$. The 90% confidence intervals for the mean treatment ratios of the $\ln(AUC_{last})$ and $\ln(C_{max})$ were $\ln 0.97\sim\ln 1.09$, $\ln 1.02\sim\ln 1.21$, respectively. No serious adverse events were caused by both formulations.

Conclusion: In this study, a newly developed 2 mg OD risperidone formulation was bioequivalent to Risperdal Quicklet® tablet 2mg.

Acknowledgement

This study was supported by grants (A070001) from the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea.

PO-10*Track: Medical Biotechnology***ANALYSIS OF FILAGGRIN EXPRESSION IN THE SKIN OF NORMAL AND ATOPIC DERMATITIS SUBJECTS****In Sik Kim and Ji-Sook Lee***Biomedical Laboratory Science, School of Medicine, Eulji University, Korea; E-mail: orientree@eulji.ac.kr*

Atopic dermatitis (AD) is characterized by chronically pruritic and inflammatory dermatitis. AD is caused by a variety of pathogenic factors, which include environmental components, such as allergen and genetic factors. Filaggrin is critical for the regulation of epidermal homeostasis. Filaggrin monomers can become incorporated into the lipid envelope, which is responsible for the skin barrier function. In this study, we investigated the expression of filaggrin in the skin of normal and atopic dermatitis subjects by non-invasive method. We examined the usefulness of a new patch to obtain protein sample from the skin of healthy subjects, and the patch is effective on protein acquirement from the skin. We detected the expression of filaggrin in lysates of normal skin by Western blotting. The expression of filaggrin in normal skin is stronger than that of AD skin.

Keywords: Filaggrin, atopic dermatitis, diagnosis.**PO-46***Track: Others - Molecular biology***ROLE OF ATP IN PROTEIN FOLDING ACTIVITY OF HYPERTHERMOPHILIC PYROCOCOCCUS HORIKOSHII CHAPERONIN****Se Won Kim, Soo-Wan Nam and Jeong-Hwan Kim***Department of Biomaterial Control, Dong-Eui University, Busan 614-714, Korea; Email: arbatross2@nate.com*

Group II chaperonin-mediated protein folding is critically dependent on the closure of a built-in lid. ATP drives the conformational change of the group II chaperonin from the open lid substrate-binding conformation to the closed lid conformation to encapsulate an unfolded protein in the central cavity. It is thought that the folding activity is strongly correlated with the ATP-dependent conformational change ability. In this study, we investigated that the group II chaperonin (PhCpn) from *Pyrococcus horikoshii* OT3 can ATP-dependently induce improvement of ATPase activity, thermal protection and inactivation of foreign proteins, such as alcohol dehydrogenase (ADH) from *Saccharomyces cerevisiae* and citrate synthase (CS) from porcine heart. To identify ATP dependency in ATPase and thermal activity, Phcpn, ATP-binding enhanced mutants (D64G and D393G) and ATP-binding impaired mutants (D64A, G65C, D393A, D64A/D393A) were constructed through positional change in ATP binding site by site-directed mutagenesis, respectively. The ATPase activities of enhanced mutants were the highest because of their high ATP-binding efficiency. Also, PhCpn prevented the thermal aggregation and inactivation of ADH and CS. Especially, the addition of ATP with PhCpn more extensively prevented thermal aggregation and inactivation. Therefore, these results reveal that ATPase activity of chaperonin is mediated through ATP dependent action.

Keywords: ATP-Dependence, *Pyrococcus horikoshii* OT3, site-directed mutagenesis.

PO-82*Track: Plant and Environment***CHARACTERIZATION AND FUNCTION OF GA733-FC COMPLEX PROTEIN AS A VACCINE CANDIDATE FOR COLORECTAL CANCER IN PLANT****Jeong-Hwan Lee, Zhe Lu, Kyung-Jin Lee, Yingxue Shao, Junsik Ahn, Se-Ra Park, Youngtae Byun, Qiau Lu, Young-Kug Choo, Doo-Byoung Oh, Young-Sun Kang, Seung Han Oh, Yeon Soo Han, and Kisung Ko***Department of Medicine, Medical Research Institute, College of Medicine, Chung-Ang University, Seoul 156-756, Korea; Email: ksko@cau.ac.kr*

The tumor-associated antigen GA733 is a cell-surface glycoprotein highly expressed in colorectal carcinomas. In this study, 3 recombinant genes were constructed as follows: GA733 tagged to the ER retention sequence KDEL (GA733K), GA733 fused to the immunoglobulin Fc fragment (GA733-Fc), and GA733-Fc fused to the ER retention sequence (GA733-FcK). *Agrobacterium*-mediated transformation was used to generate transgenic plants expressing recombinant genes. The presence of transgenes was confirmed by genomic PCR. Western blot, confocal immunofluorescence, and sandwich ELISA showed the expression of recombinant proteins. The stability, flexibility, and bioactivity of recombinant proteins were analyzed and demonstrated through N-glycosylation analysis, animal trials, and sera ELISA. Our results suggest that the KDEL retained proteins in ER with oligomannose glycan structure and enhanced protein accumulation level. The sera of mice immunized with GA733-FcK purified from plants contained immunoglobulins which were at least as efficient as the mammalian-derived GA733-Fc at recognizing human colorectal cancer cell lines. Thus, a plant system can be used to express the KDEL fusion protein with oligomannose glycosylation, and this protein induces an immune response which is comparable to non-KDEL-tagged, mammalian-derived proteins.

Acknowledgements

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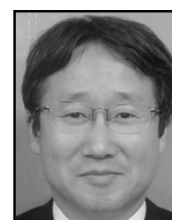
PO-16*Track: Regenerative Medicine***OPTIMAL MOUNT OF BASIC FIBROBLAST GROWTH FACTOR WITH GELATIN SPONGES****Makoto Komura, Hiroko Komura, Yushi Otani and Yasuhiko Tabata***Department of pediatric surgery, Tokyo University, Japan; E-mail: komura-tyk@umin.ac.jp*

Background: A gelatin sponge slowly releasing basic fibroblast growth factor (bFGF) enhances chondrogenesis. This study investigated the optimal mount of basic fibroblast growth factor with gelatin sponges to fabricate engineered cartilage.

Material and Methods: Chondrocytes were isolated and expanded from the auricular cartilage of C57B6J mice. bFGF (0, 10, 100, 500, 1000 and 2000 $\mu\text{g}/\text{cm}^3$)-impregnated gelatin sponges incorporating beta-TCP were produced before seeding auricular chondrocytes on the sponges. Expanded chondrocytes (10×10^6 cells/ cm^3) were seeded onto scaffolds. The construct assembly was implanted in the subcutaneous space of mice through a syngeneic fashion. Thereafter, constructs were retrieved at 2, 4 and 6 weeks.

Results: (1) Histological examination: Extracellular matrix in the center of the constructs was observed using gelatin sponges impregnated with more than 100 $\mu\text{g}/\text{cm}^3$ bFGF after 4 weeks of implantation. (2) DNA content: After 2 weeks of implantation, the DNA content peaked in all mounts of bFGF-impregnated constructs. (3) Protein assay: Glycosaminoglycan and collagen type 2 expression were significantly increased by more than 100 $\mu\text{g}/\text{cm}^3$ bFGF after 4 and 6 weeks of implantation.

Conclusion: More than 100 $\mu\text{g}/\text{cm}^3$ bFGF-impregnated gelatin sponges incorporating beta-TCP with chondrocytes (10×10^6 cells/ cm^3) can fabricate engineered cartilage after 4 weeks of implantation.



PO-23

Track: Other areas: Food

PHAGE THERAPY BASE PESTICIDES: PRACTICAL EXPERIENCES

Tamás Kovács, Nikolett Ágnes Várnai, Dóra Dömötör, Gábor Rákhely and György Schneider

Enviroinvest Corp., Nanophagetherapy Center, 7632 Pécs, Kertváros str. 1. Hungary; E-mail: kovacst@enviroinvest.hu

Bacteriophages (phages) are viruses that are obligate intracellular parasites of bacteria. Application of these agents can be suitable to control plant pathogenic bacteria causing severe diseases, which could be hardly treated with other pesticides, as our group demonstrated it in case of fire blight. *Erwinia amylovora*, member of *Enterobacteriaceae*, is the causative agent of this disease. Streptomycin was the conventional drug used to control fire blight, however, its extensive use has resulted in the emergence of streptomycin-resistant *E. amylovora* strains.



39 phage strains were isolated against *E. amylovora*, and tested under laboratory conditions. Two phage strains (PhiEaH1 and PhiEaH2) were chosen for field experiments. A phage cocktail containing PhiEaH1, PhiEaH2, UV-protectant and alginate was applied for spraying 66 apple trees, whereas 70 trees remained untreated control. A significant difference could be detected in the appearance of new fire blight cases among the treated and untreated trees. We not only will present the complete genome of the applied *E. amylovora* phage PhiEaH2, but also our preliminary results will be demonstrated against other plant pathogenic bacteria. Our results confirm that bacteriophage therapy may provide an effective solution for controlling fire blight and other bacterial plant diseases.

PO-78

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

DIETARY VALINE REQUIREMENT OF RED SEABREAM (*PAGRUS MAJOR*)

Samad Rahimnejad, Dae-Han Oh and Kyeong-Jun Lee

Faculty of Marine Biomedical Sciences, Jeju National University, Jeju 690-756, South Korea; email: kjlee@jejunu.ac.kr

A twelve-week feeding trial was conducted for determination of dietary valine requirement of red seabream. Six isonitrogenous (45% crude protein) and isocaloric (4.38 kcal g⁻¹ gross energy) experimental diets containing fish meal and L-crystalline amino acids were formulated with graded levels of valine (0.4, 0.8, 1.2, 1.6, 2.0 and 2.4%). Triplicate groups of fish (initial mean body weight, 32.04 ± 0.2 g) were fed one of the test diets to apparent satiation twice daily (08:00 and 17:00 h). At the end of the feeding trial, significantly ($P < 0.05$) higher weight gain (131.67%), protein efficiency ratio (1.29) and lower feed conversion ratio (1.58) were obtained in fish fed 2.0% dietary valine. Fish survival varied from 86 to 95% without significant differences among treatments. Respiratory burst and myeloperoxidase activities in fish fed the diet containing 2.0% valine were significantly increased. Significantly ($P < 0.05$) lower ammonia excretion levels were obtained in the groups fed dietary valine levels of 1.6 and 2.0% signifying a better protein utilization efficiency. The optimum dietary valine requirement level was estimated by a second-order polynomial regression analysis on the basis of fish weight gain and determined at 1.73% in diets.

PO-79

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

VITAMIN C REPLACEMENT BY CITRUS BY-PRODUCT IN DIETS FOR KOREAN ROCKFISH *SEBASTES SCHLEGELI*

Jin-Woo Song and Kyeong-Jun Lee

Faculty of Marine Biomedical Sciences, Jeju National University, Jeju 690-756, Korea; Email: kjlee@jejunu.ac.kr

The study was investigated to examine the effects of replacement of dietary vitamin C source (L-ascorbyl-2-polyphosphate, LAPP) by citrus by-product (CBP) on growth performance, feed utilization, innate immunity and disease

resistance of juvenile Korean rockfish. A basal diet without vitamin C was regarded as a control and four other diets were formulated to contain either LAPP or CBP at levels of 90 or 360 mg ascorbic acid/kg diet (designated as Con, LAPP-90, LAPP-360, CBP-90 and CBP-360, respectively). Juvenile Korean rockfish (body weight 11.0 g) were fed the five experimental diets to apparent satiation for 13 weeks. At the end of the feeding trial, growth performance, feed utilization and survival were significantly lower in the fish fed the control diet compared to those of fish fed other diets. Fish fed the control diet had significantly lower total immunoglobulin activity than fish fed the LAPP and CBP supplemented diets. Cumulative mortality in the control group recorded to approximately 61% by 18 days after a disease challenge with *Streptococcus iniae*. The mortalities of fish fed LAPP and CBP supplemented diets were significantly lower (24%, 9%, 40% and 16% for LAPP-90, LAPP-360, CBP-90 and CBP360, respectively) than that of fish fed the control diet. The results showed that CBP has great potential as an alternative vitamin C source in diets for Korean rockfish.

PO-12

Track: Others – Immunology

FUNCTIONAL ANALYSIS OF FIBROBLASTIC RETICULAR CELL ISOLATED FROM LYMPH NODE VIA TNFR SUPER FAMILY STIMULATION

Jae Seol Lee, Sang Bong Lee and Jong-Hwan Lee

Department of Biotechnology & Bioengineering, Dong-Eui University, Busan 614-714, Korea;
Email: conver100@naver.com



The heterotrimeric transmembrane lymphotoxin α 1 β 2 (LT α 1 β 2), member of TNF family cytokines including soluble homotrimeric LT α , plays an important role for lymphoid tissue architecture and organogenesis. We found that lymphotoxin β receptor (LT β R) stimulation using agonistic anti-LT β R antibody induced the changes in actin stress fiber and morphology of cells. To address the possibility that LT β R stimulation is involved in RhoA/ROCK signaling, we checked the level of Rho-GDP/GTP exchange activity associated with fibroblastic reticular cell (FRC) lysate. When LT β R was stimulated with agonistic anti-LT β R antibody, the exchange activity associated with LT β R stimulation was markedly reduced. Inhibition of Rho-associated protein kinase (ROCK) activity in FRCs induces changes in actin cytoskeleton organization and cell morphology similar to those observed in other types of cells with inhibited RhoA/ROCK signaling pathway. We show that phosphorylation of myosin light chains (MLC) reduced by LT β R stimulation in cells. DNA gene chip demonstrated that agonistic anti-LT β R antibody in FRC affected down regulation of actin filament and myosin component transcripts. Presented results indicate that RhoA-ROCK is responsible for the observed phosphorylation of MLC. Collectively, these results suggest that LT β R stimulation is involved in change of stress fiber via RhoA-ROCK signaling in FRC.

Keywords: Fibroblastic reticular cell, lymphotoxin β receptor, lymphotoxin α 1 β 2, ROCK.

PO-31

Track: Plant & Environment

STUDY ON THE FUNCTIONAL OF *AHAREB1* FROM *ARACHIS HYPOGAEA* L.

Yao Yao, Xiaoyun Li, Chaoyong He, Lan Hong, Xu Liu and Ling Li

Guangdong Key Lab of Biotechnology for Plant Development, College of Life Sciences, South China Normal University, Guangzhou 510631, Guangdong, People's Republic of China

AhAREB1 gene was cloned from *Arachis hypogaea* L. *AhAREB1* possesses the same features with the family of bZIP transcriptional factor, yeast activity expression experiment proves it has transcriptional activity. The N terminal of *AhAREB1* is transcriptional activity region, the C terminal can inhibit its own transcriptional activity, the C₂ conserved domain is the key transcriptional activity region. *AhAREB1* protein expresses in nucleolus in transgenic *Arabidopsis* plants transformed with pBI121-*AhAREB1*-GFP construct. The excessive expression of *AhAREB1* in transgenic *Arabidopsis* plant has obviously enhanced the tolerance to the drought and high osmotic stress, in addition the tolerance ability getting stronger with the higher expression of *AhAREB1*.



AhAREBI transcriptional factor plays an important role of regulating the response to stress in plants, its excessive expression notably enhances the tolerance to drought and high osmotic stress in transgenic plants. These results provide theoretical basis to use the genetic engineering means to improve the drought resistance of peanut.

Keywords: Peanut, *AhAREBI*, transcriptional activity domain, drought stress.

Acknowledgement

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PO-63

Track: Plant & Environment

INTRODUCTION OF OILSEED RAPE AS PLANT GENETIC ENGINEERING MODEL

Vahide Gugerchi and Hajie Lotfi

Department of Plant biotechnology, Azad University of Tehran, Iran; Email: lotfi.hajie@yahoo.com

The Brassicaceae is a large plant family and it is special interest as it include many crop plants (*Brassica napus*, *Armoracia rusticana*), ornamentals (Aubrieta, Iberis, Lunaria, Arabis, Draba) as well as model organisms in the plant sciences. Among these plants, oilseed rape (*Brassica napus*) is the most important oil plant in temperate regions of the world and ranks second amongst oilseed crops produced worldwide. The use of oilseed rape oils as industrial lubricants has considerable environmental benefits, because they are inherently biodegradable, low ecotoxicity, toxicity toward humans, derived from renewable resources and furthermore, rapeseed oil has a high viscosity index, and the oil structure endures mechanical stresses well. By the use of molecular techniques it is now possible to produce transgenic plants containing a variety of different genes. Oilseed rape is particularly amenable to *Agrobacterium tumefaciens*-mediated transformation, and during the last two decades, considerable progress has been achieved in the development of transgenic varieties. Soybean, cotton, corn, and canola are the four principal crops in which transgenic technology is utilized. After herbicide tolerant soybean and insect-tolerant Bt-corn and Bt-cotton, the fourth most dominant GM crop worldwide is herbicide-tolerant canola. The first generation of transgenic canola varieties showed a strong emphasis on herbicide tolerance and hybrid breeding systems; however, efforts are increasing in the areas of genetically modified fatty acid biosynthesis and, to a certain extent, in the introgression of transgenic pest and disease resistance. Genetic engineering of plant lipid biosynthesis in rapeseed has already led to commercialization, with transgenic varieties expressing genetically modified fatty acid patterns available since 1995.

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Keywords: Transgenic plant, oilseed rape, genetic engineering.

PO-45

Track: Other Areas: Molecular Biology

CANDIDA ALBICANS STIMULATES TRANSGLUTAMINASE ACTIVITY IN HUMAN HEPATIC CELLS

Atsushi Matsuo, Akiko Nakamura, Takahiro Oura, Soichi Kojima and Susumu Kajiwara

Graduate School of Biosciences and Biotechnology, Tokyo Institute of Technology, Nagatsuta-cho, Midori-ku, Yokohama-shi, Kanagawa-ken, 226-8501, Japan

Candida albicans is one of the most important opportunistic fungal pathogens found in the human organs. At early stages of the infection to human, the liver plays a role as the first barrier against spreading invasive fungus. However, little is known about effect of *C. albicans* on the liver cells. In this study, we investigated it using a human hepatic cell line, HC cells.

HC cells grown in microtiter plates were incubated with or without *C. albicans* in the presence of 5BAPA (a biotin containing amine substrate) and then were triple stained with TRITC-conjugated streptavidin, anti-transglutaminase 2 (TG2) polyclonal antibody, and Hoechst 33258. After digital images of the cells were obtained, TG2 activity and expression in both cytoplasm and nucleus were determined from their respective fluorescent intensities by using an imaging cell analyzer.

TG2 activity in hepatic cells, especially at nucleus region, was enhanced about 3.5-folds following incubation with living *C. albicans* but not with dead *C. albicans*, whereas TG2 expression levels in the nuclear enhanced only about 1.3-fold.

This result suggested that the TG2 activity in HC cells was stimulated without a significant induction of TG2 expression after contacting with living *C. albicans*.

Keywords: *Candida albicans*, Transglutaminase 2, Human hepatocyte.

**PO-22**

Track: Medical Biotechnology

THE INFLUENCE OF UNIAXIAL STRETCHING ON THE MRNA EXPRESSION OF CONNEXIN 43 IN VASCULAR SMOOTH MUSCLE CELLS

Jana Musílková, Tomáš Riedel, Eduard Brynda and Lucie Bačáková

Department of Biomaterials and Tissue Engineering, Institute of Physiology AC CR, Prague 4, Czech Republic; E-mail: musilkova@biomed.cas.cz

We evaluated the influence of uniaxial stretching on the mRNA expression of connexin 43 (GJA1), i.e., a calcium channel component, and beta-actin (ACTB) in vascular smooth muscle cells (VSMC). ACTB was used as housekeeping. The experiment was performed using the equipment Strex (B Bridge International, Ltd). Flexible silicone chambers were coated with type I collagen and fibronectin. VSMC (passage 7) were seeded in a density of 45 000 cells/cm² for qRT PCR experiments. After a 2-day static culture, the VSMC were subjected to stretching at frequency of 0.5Hz and amplitude of 5% for 30, 60, 120 min and 2 days. Moreover after 2 days, the periodical stretching frequency was changed to 1Hz and measured again in intervals 30, 60, 90 a 120 min and 24 hours. At the same time, the cells were cultivated under static conditions.

The expression changes were tested by qRT-PCR. We have found that the expression of GJA 1 under conditions of periodical stretching increased, when compared to HSB cells not exposed to periodic stretching, where the maximal increase occurred at 60 min / 30 min for excitation at 0.5 Hz / 1Hz. For longer time periods the expression of GJA 1 decreased later again. It can be concluded that dynamic stimulation supports the intercellular communication of VSMC.

Acknowledgement

Supported by the Grant agency of the Czech Republic (grants No. P108/11/1857 and P108/10/1106).

Keywords: Connexin 43, uniaxial stretching, vascular smooth muscle cells.



PO-75

Track: Pharmaceutical Biotechnology

ANTI-INFLAMMATORY AND MEMBRANE STABILIZING PROPERTIES OF TWO OLEANOLIC ACID DERIVATIVES

Benedicta N. Nkeh-Chungag, Eugene J. Ndebi, Opeoluwa O. Oyedeji and Adebola O. Oyedeji

Zoology Department, Walter Sisulu University, Mthatha 5117, South Africa; Email: bnkehchungag@wsu.ac.za

Acetylation and methylation semi-synthesis of oleanolic acid isolated from *Syzygium aromaticum* L. afforded 3-acetoxyoleanolic acid and 3-acetoxy 28-methylester oleanolic acid. Structural elucidation of the semi-synthesis was achieved using 1D and 2D, NMR, FT-IRT and MS. Using albumin inflammatory model, we evaluated the anti-inflammatory properties of compounds in male Wistar rats weighing 170 – 225g. Initial right paw volumes were measure followed by pre-treatment of animals with drugs. 30 minutes later right paws were injected with fresh egg albumin and right paw volumes measured at predetermined times. Furthermore, the membrane stabilizing effect of compounds was investigated using heat- and hypotonicity-induced haemolysis test models. Compounds significantly ($p < 0.05$) inhibited albumin induced inflammation better than oleanolic acid and indomethacin from 1- 5 hours post administration. Both compounds were membrane stabilizing in heat while only 3-acetoxyoleanolic was membrane stabilizing in hypotonicity. Membrane stabilizing property is a desirable attribute for an anti-inflammatory drug as this affects the release of early phase mediators.

PO-68

Track: Industrial and Manufacturing

IMPROVED YIELD AND STABILITY OF AMYLASE BY MULTIPOINT COVALENT BINDING ON POLYGLUTARALDEHYDE ACTIVATED CHITOSAN BEADS; ACTIVATION OF DENATURED ENZYME MOLECULES BY CALCIUM IONS

Bartholomew N. Okolo, Hideki Aoyagi, Anene Muoneke and Tochukwu Nwamaka Nwagu

Department of Microbiology, University of Nigeria Nsukka, Nigeria; Email: tochukwu.nwagu@unn.edu.ng

In this study raw starch digesting amylase (RSDA) from *Aspergillus carbonarius* (Bainier) Thom 171 IMI 366159 was stabilized by covalent binding on polyglutaraldehyde (PG), glutaraldehyde (G) activated chitosan beads or post immobilization cross linking of enzyme adsorbed on chitosan. Presence of Ca^{+2} ions (0.5-1.5 mM) activated the PG and G derivatives but repressed the cross linked enzyme. Optimum pH for cross linked derivative shifted from 5 to 7 but was unaltered for PG and G derivatives. Optimum temperature for soluble and immobilized derivatives was 30 °C. At 80 °C soluble enzyme retained only 42% while PG derivative had 95% and cross linked 90% of their residual activity. PG derivative lost only 6% activity after 12 h storage at 60 °C in 0.2 M citrate phosphate buffer, pH 5. All derivatives maintained 100% activity after storage at 4 °C for 30 days and retained above 90% activity after 10 batch reactions of 60 min each. Immobilization successfully stabilized RSDA and immobilized enzyme from *A. carbonarius* can be applied in numerous industries for cheap, cost effective and environmentally friendly starch hydrolytic processes to simple sugars.

Keywords: Stabilization, amylases, immobilization, chitosan, activity.

PO-76**Track:** Industrial and Manufacturing**ACTIVITY AND STABILITY OF ASPERGILLUS CARBONARIUS AMYLASE UPON INTERACTION WITH MONOVALENT AND DIVALENT CATIONS****Bartholomew N. Okolo, Hideki Aoyagi, Anene Muoneke and Tochukwu Nwamaka Nwagu***Department of Microbiology, University Of Nigeria Nsukka, Nigeria; Email: tochukwu.nwagu@unn.edu.ng*

Though raw starch hydrolyzing amylases (RSHA) can be utilized in numerous bioprocesses, poor activity and stability remains a limiting factor. In this study, the effect of metal ion substitution on the RSHA of *Aspergillus carbonarius* was investigated. The amylase was inactivated using the chelating agent EDTA. The effect of different metal ions on the reactivation of the amylase was examined. Most efficient was 5 mM concentration of Co^{2+} with 94.6% activity recovery. Others included 5 mM Zn^{2+} (77.7%) and 5 mM Ca^{2+} (68.7%). Incubating the Co^{2+} activated amylase in 5 mM and 10 mM Mn^{2+} further stimulated the activity of the amylase to 127.5% and 136.7%. Compared to Zn^{2+} , Na^{2+} , Ca^{2+} , Mg^{2+} and Fe^{2+} ions tested, Mn^{2+} had the most stabilizing effect on the amylase; the amylase exhibited 126.4%, 148.2% and 136.5% activity at 10 oC, 70 oC and 80 oC respectively in the presence of 5 mM Mn^{2+} . Ca^{2+} inhibited the amylase activity and inhibition rate increased with increasing concentration of Ca^{2+} ; $99.7\% > 93.5\% > 91.5\% > 91.3\%$ at 2.5, 5, 7.5 and 10 mM concentrations, respectively. K_m of the reactivated amylase was 0.18 mg/ml while that of native amylase was 0.3 mg/ml.

Keywords: Amylase, metal ions, reactivation, activity, stability.**PO-2****Track:** Others: - Plant Biotechnology**MITOCHONDRIAL REGULATION STUDIES THROUGH SPECIFIC RNA KNOCKDOWN IN THE ORGANELLES****Romain Val, Etienne Delannoy, Clara Chicois, Marta Gabryelska, Eliza Wyszko, Frederique Weber-Lotfi, Maciej Szymanski, Jan Barciszewski And Andre Dietrich and Adnan Khan Niazi***Department Institut de Biologie Moleculaire des Plantes (IBMP), Institution CNRS, France; E-mail adnan-khan.niazi@ibmp-cnrs.unistra.fr*

The complex genetic system of higher plant mitochondria could not be studied by transgenic approaches because conventional methods do not permit genetic transformation of these organelles. An alternative approach has been developed in the laboratory, thanks to the existence of a natural process of transfer RNA (tRNA) import from the cytosol into mitochondria. It was shown that a tRNA mimic can be used *in vivo* as a shuttle for importing into plant mitochondria passenger RNA sequences expressed from nuclear transgenes. Taking a trans-cleaving ribozyme as a passenger sequence allowed to obtain the specific knockdown of a major messenger RNA (mRNA) in the mitochondria of transformed plant cells. This strategy has been used to develop mitochondrial regulation studies. Five mitochondrial mRNAs were chosen as targets for specific trans-ribozymes in transgenic *Arabidopsis thaliana* and *Nicotiana tabacum*. Specific *in vivo* ribozyme-mediated knockdown of the targeted mitochondrial RNAs was established. The regulation response to the knockdown of the individual targets was analyzed at the whole transcriptome level. Whereas it has been generally considered so far that mitochondrial regulation processes in plants essentially occur at the post-transcriptional stage, our results strongly support mRNA coordination mechanisms within the organelles and between the organelles and the nucleus.

Keywords: Genetic regulation, mitochondria, ribozyme, RNA trafficking.

PO-49*Track: Other Areas: Bioscience***DEVELOPMENT OF SIMPLE MOLECULAR DIAGNOSIS SYSTEM FOR MALASSEZIA SPECIES****Eriko Noda, Takahiro Oura, Nuvee Prapasarakul, Ariya Chindamporn and Susumu Kajiwara***Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuda-cho, Midori-ku, Yokohama-shi, Kanagawa, Japan; E-mail: enoda@bio.titech.ac.jp*

Malassezia yeast is a commensal organism of human and animal skin. This microorganism is associated with a number of different diseases in both humans and animals. Recently, several researchers reported that *M. globosa* and *M. restricta* were mainly detected from several human skin disorders. On the other hand, *M. pachydermatis* is known to act as a facultative pathogen on the skin of animals. The morphological diagnosis by using microscopy is usually used for *Malassezia*-associated diseases. However, this method has only limited capabilities in distinguishing the different species of *Malassezia* yeast in detail. We tried to develop simple molecular detection system for three *Malassezia* species by using Loop-Mediated Isothermal Amplification (LAMP) method. As the LAMP method is simple, rapid, specific and cost-effective for nucleic acid amplification, the development of this system can contribute appropriate and prompt treatment to a lot of *Malassezia*-associated patients. We succeeded in developing new LAMP primers to detect *M. globosa*, *M. restricta* and *M. pachydermatis* and confirmed that this system can be used for both of lab strains and clinical strains of three *Malassezia* species.

Keywords: *Malassezia*, LAMP, molecular diagnosis system.

PO-21*Track: Plant and Environment***IMPACT OF PETROLEUM REFINING ACTIVITIES ON NITRATE AND NITRITE CONTENT OF EDIBLE VEGETABLES AND ON THEIR *IN VIVO* KINETICS IN ALBINO RATS****Gerald Otti and Polycarp Nnacheta Okafor***Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Nigeria; E-mail: pnokafor@yahoo.com*

The influence of pollution from petroleum refining activities on the levels of nitrates and nitrites in five edible vegetable species was investigated. Besides, the kinetics of nitrite and nitrate was studied *in vivo* using albino rats with focus on the possible influence of concentration difference on kinetics and implications to toxicity. Leaf samples of the five vegetable species were collected randomly from various locations within Eleme, a host community of Port Harcourt Refinery Company and the Indorama Petrochemical Company. Also, samples were collected from Umuahia, which served as pollution-free control. The leaf samples were analyzed for their nitrite and nitrate contents. Nitrite was determined spectrophotometrically while nitrate was determined after cadmium column reduction. Results showed that samples from Eleme had higher mean nitrate (349.20mg/100g dry leaf mass; $P < 0.05$) and nitrite (63.12mg/100g dry leaf mass; $P > 0.05$) as compared to the same samples from Umuahia. Solutions of nitrate and nitrite, equivalent in concentration to mean nitrate and nitrite content of the vegetable samples from the two locations were administered enterally to four groups of albino rats. Analysis of their blood levels were monitored five times at 30 minutes intervals following administration. Rates of change of blood nitrites and nitrates were found to be fairly constant in absorption as well as in the elimination phase. Their peak blood concentrations varied proportionately with their concentrations in administered solutions. However, peak blood nitrate was attained later in group of animals receiving higher amount of nitrate solution. Refining activities may pre-dispose people living within Eleme community to health hazards through contamination of edible vegetables.

Keywords: Pollution, Nitrate, Nitrite, albino rats, edible vegetables.

PO-60***Track: Industrial & Manufacturing*****HYDROLYSIS OF CELLULOSIC MATERIALS AND DETECTION OF HYDROLYTIC PRODUCTS USING *ASPERGILLUS FLAVUS* CELLULASE****I. F. Okonkwo, F. J. C. Odibo and J. C. Okonkwo***Department of Agricultural and Bioresources Engineering, Nnamdi Azikiwe University, PMB 5025 Awka, Anambra State, Nigeria; Email: drjcokonkwo@yahoo.com*

Hydrolysis of various agricultural wastes cum cellulosic materials including saw dust, corn cob, cotton wool, filter paper, carboxyl methylcellulose and crystalline cellulose (sigma 20) were carried out using both crude and purified enzyme of *Aspergillus flavus*, objective being to determine the relative rate of hydrolysis of these materials by *Aspergillus flavus* cellulase. One percent preparation of each of the substrate in 0.2 M citrate phosphate buffer pH 5.0 was incubated with the crude and the purified enzyme solutions (1:1), that is, 0.5 ml amounts at 70 % for 1 h. The reducing sugar released was determined by Dinitrosalicylic acid method (DNS) method. Both crude and purified cellulases showed considerable hydrolysis on all the cellulosic materials, however, the purified enzyme gave the highest activity, while cotton wool showed the highest affinity to the enzyme than other substrates degraded. The end product of hydrolysis was glucose and an unidentified oligomer as given by the paper chromatography using the solvent system of butanol-glacial acetic acid- water (4:1:1). Consequently, both the crude and purified enzyme system of *Aspergillus flavus* is recommended for use in industries for biodegradation of agricultural wastes and other cellulosic materials.

Keywords: *Aspergillus flavus*, biodegradation, hydrolysis, cellulosic materials, crude and purified cellulase.

PO-59***Track: Other Areas*****GENETIC CORRELATION BETWEEN EGG QUALITY TRAITS****J. C. Okonkwo***Department of Agricultural and Bioresources Engineering, Nnamdi Azikiwe University, PMB 5025 Awka, Anambra State, Nigeria; Email: drjcokonkwo@yahoo.com*

Genetic correlation coefficients between egg quality traits were studied using fifty- six harco pullets raised under standard management practices. The estimates were carried out at 22-week, 26-week, 30-week and 32-week of age. Egg quality traits studied include egg weight (EW), shell thickness (ST), haugh unit (HU), yolk index (YI) and shape index (SI). Zero to slight negative correlation coefficients was observed between EW and HU, EW and YI, EW and SI, ST and SI, and HU and YI. This implies that selection for any of the character entails minimal retrogressive response on corresponding trait. Conversely, zero to slight positive correlation coefficients was recorded between EW and SI, SI and YI, and HU and SI. Again, selection for any of this character will bring about a slight genetic gain on the corresponding trait. Furthermore, the study maintained that the genetic correlation coefficients between egg quality traits are independent of the laying age, and for faster genetic response in egg quality traits, independent culling method with some modifications was advocated.

Keywords: Egg quality, pullets, correlation, genetic gain.

PO-8

Track: Industrial and Manufacturing

MULTIVARIABLE CONTROL FOR ANAEROBIC DIGESTION PROCESSES: A SIMULATION STUDY

Grace Oppong, Mathew McEwan, Gary Montague and Elaine Martin OBE

Chemical Engineering and Advanced Materials, Newcastle University, UK; E-mail: grace.oppoing@newcastle.ac.uk

Anaerobic digestion with biogas production has both economic and environmental benefits. 25% of all bioenergy in the future could potentially be sourced from biogas. Although anaerobic digesters have seen wide applicability, they typically perform below their optimum performance as a consequence of the complexity of the underlying process. Various reviews have concluded that in order to achieve optimum performance, advanced control systems are required. Advanced control strategies can offer an opportunity for optimisation, for processes such as anaerobic digestion which operate under strict regulatory constraints. The complex nature of the process dynamics provides sufficient motivation for the use of a model based control strategy. With the use of mathematical simulation models; the application of model based control is investigated for the anaerobic digestion process. This paper presents and discusses the results of this investigation.

Keywords: Anaerobic digestion, optimisation, control, modelling, simulation.

**PO-30**

Track: Plant and Environment

DE NOVO SEQUENCING, ASSEMBLY AND GENE ANNOTATION OF AN ARACHIDONIC ACID-RICH GREEN MICROALGA, MYRMECIA INCISA REISIGL H4301, BY 454 PYROSEQUENCING

Long-Ling Ouyang, Zhi-Gang Zhou, Si-Hong Chen and Yan Li

College of Aqua-life Sciences and Technology, Shanghai Ocean University, China; Email: llouyang1988@163.com

To understand the global metabolic pathways in an arachidonic (ArA)-rich oleaginous green microalga, *Myrmecia incisa* H4301, 454 pyrosequencing was applied to generate 371, 740 high-quality reads, which were assembled into 51,908 unique sequences consisting of 22,749 contigs and 29,159 singletons. A total of 11,873 unique sequences were annotated by homology searches, and 3,733 among them assigned with Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways uncovered a C4-like photosynthesis pathway in *M. incisa*, suggesting that this alga was able to survive under a low CO₂ ambient. Biosynthesis pathways of fatty acids, especially of ArA, and triacylglycerol (TAG) were portrayed in detail, and the TAG was proposed to be accumulated in the form of oil bodies in cytosol with the help of caleosin or oil globule associated protein. In addition, biosynthesis pathways of carotenoids were discussed to provide a foundation for the integrated exploitation of *M. incisa* besides ArA production.

Keywords: *Myrmecia incisa* Reisigl H4301, 454 pyrosequencing, C4 pathway, arachidonic acid, oil body.



PO-71*Track: Regenerative Medicine***POLYCAPROLACTONE SCAFFOLDS TRIGGER GROWTH AND DIFFERENTIATION OF GASTRIC STEM CELLS****Sunitha Pulikkot, Yaser E. Greish and Sherif M. Karam***Department of Anatomy, Faculty of Medicine and Health Sciences, UAE University, UAE; Email: sunitha.pulikkott@gmail.com*

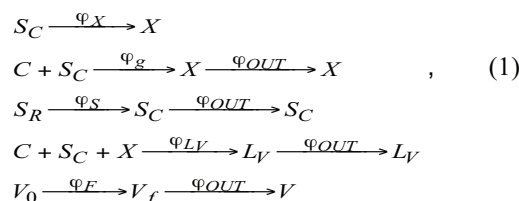
Recent therapeutic trends for some diseases include stem cell therapy. This is an interdisciplinary field that combines the technology of cell culture with material sciences to generate artificial tissues that can be used for organ replacement. In this study, the aim is to fabricate three dimensional polycaprolactone (PCL) scaffolds for the cultivation of our immortalized mouse gastric stem (mGS) cells for 12 days to test whether PCL will support their growth and induce their differentiation. Cell proliferation and differentiation were monitored using PicoGreen assay of DNA quantification, scanning electron microscopy and lectin-/immuno-histochemistry. Results revealed the attachment and growth of mGS cells on the microfibrinous scaffold in the standard culture conditions. Confocal microscopic analysis of cryosections indicated that, within 9 days, more than 50% of the mGS cells differentiated into secretory cells rich in N-acetyl-D-glucosamine characteristic of mucus-secreting neck cells of the gastric epithelium. Therefore, PCL scaffolds support not only adhesion and growth of mGS cells, but also their differentiation into gastric mucus-secreting cells. These promising results provide the basis for future plans of studies using animal models of gastric cancer (2nd leading cause of cancer deaths worldwide) to produce a new modality that overcomes the high morbidity/mortality rates following gastrectomy.

Keywords: Stem cells, Regeneration.**PO-5***Track: Industrial and Manufacturing***MODELLING AND OPTIMIZATION OF THE PROCESS FOR INTENSIVE MICROBIAL PRODUCTION OF BRANCHED CHAINED AMINO ACIDS****Tzanko Georgiev, Ignat Dimov, Julia Kristeva, Filip Filipov and Alexander Ratkov***Institute of Microbiology, Bulgarian Academy of Sciences, Acad. G. Bonchev str., Block 26, 1113 Sofia, Bulgaria; E-mail: ratkovaal@microbio.bas.bg*

Introduction: The aim of the article is synthesis of model of fed-batch fermentation process with intensive droppings for L-valine production. The presented approach includes: determination of generalized stoichiometric equations; calculation of specific rates for kinetic variables; identification of the specific rates takes into account the dissolved oxygen tension; parametric optimisation of dynamic model.

Methods: The fed-batch fermentation process is carried out at laboratory scale fermenter with 7 litres total volume. *Corynebacterium glutamicum* sp. – B-023 is used as a producer. Analytical methods used for the characterisation of the process are: biomass is measured as dry cell mass; sugar concentration as reducible compounds; L-valine by chromatographic method. On-line measurements of physical-chemical variables are done by proper sensors.

Results: The fermentation processes is described by the scheme of generalized stoichiometric equations:



where: φ_X , φ_g , φ_S , φ_L , φ_F , φ_{OUT} are rates of the reactions; V_0 – initial and V_f – final volume; concentrations: X – biomass, S_r – sugar remain, S_c – sugar consumed, L_V – L-valine, C – dissolved oxygen (DO).

The hypotheses concerning the specific rates are:

$$\hat{i} = \hat{i}(S_c, C), \hat{i} = \hat{i}(\hat{i}, X), \hat{n} = \hat{n}(\hat{i}, X), \hat{a} = \hat{a}(\hat{i}, X, C) \quad (2)$$

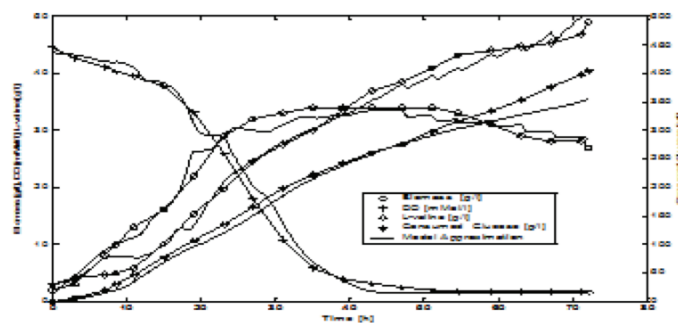
The dynamic model is

$$\frac{d\hat{i}}{dt} = \mathbf{K}\varphi(\hat{i}) - D_L^N \hat{i} + F - D_L^{OUT} \hat{i} \quad (3)$$

$$y = \mathbf{P}\hat{i},$$

where ξ^T – state-space vector; F^T – vector of input streams; P – matrix; y – measurable output from sensors; K – matrix of yield coefficients.

The results after optimization are presented as follows



Conclusion: The trend of simulation results and the trend of the experimental data, shown in figure above, conforms the adequacy of the mathematical model.

Keywords: Modelling, optimisation branched chained amino acids, fed-batch process with droppings, L-Valine.

PO-67

Track: Industrial and Manufacturing

CULTIVATION OF OYSTER MUSHROOM (PLEUROTUS HK-37) ON SOLID SISAL WASTE FRACTIONS SUPPLEMENTED WITH COW DUNG MANURE

Prosper Raymond, Anthony M. Mshandete and Amelia K. Kivaisi

Molecular Biology and Biotechnology, University of Dar Es Salaam, Tanzania ; Email: promosha@yahoo.com

Solid sisal waste fractions which included sisal boles and sisal leaves decortication residues supplemented with cow dung manure at various rates used singly and/or in combination as substrates were investigated for cultivation of Oyster mushroom (Pleurotus HK-37). The effect of the test sisal waste substrates and cow dung manure of various supplementation rates were evaluated by mushroom yield, biological efficiency and mushroom size. Pinheads occurred in all substrates within 3 to 5 weeks of transfer of bags to the cropping room. The overall best results of mushroom production were obtained in a substrate combination of 50 % sisal leaves + 50 % sisal boles supplemented by 30 % cow dung manure with the mushroom yield of 184.64 g fresh mushrooms/kg moist substrate weight and percentage biological efficiency (B.E) of about 63 %. Mushroom size of 6.10 was obtained in sisal boles substrate supplemented by 20 % cow dung manure. This study concluded that, supplementation using cow dung manure may play an important role on increasing the yield and productivity of Pleurotus HK-37 on solid sisal waste fractions under the conditions investigated.

Keywords: Solid sisal wastes, Pleurotus HK-37, cow dung manure.

PO-1**Track:** Pharmaceutical Biotechnology**TO-2 HAMSTER: UNIQUE ANIMAL MODEL FOR SEVERE CARDIOMYOPATHY****Aiji Sakamoto and Kageyoshi Ono***Laboratory of Vascular Biology, National Cerebral and Cardiovascular Center, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan ; E-mail: aiji@ri.ncvc.go.jp*

Background and Objectives: Animal disease models are indispensable to assess the effectiveness of novel therapy against intractable diseases. TO-2, natural mutant hamster, develops cardiomyopathy (CM) and cardiac failure. However, its pathogenetic cause has not been well understood, which has limited its biomedical application. TO-2 derives from BIO14.6 manifesting hypertrophic CM and exhibits severe dilated CM with shorter life-expectancy. Both hamster strains share genetic loss of delta-sarcoglycan, a member of dystrophin-associated proteins. In this study, we searched for another genetic alteration aggravating CM in TO-2.

Methods: Male hamsters were used: normal Golden, BIO14.6 and TO-2. We performed transmission electron microscopic analysis of hamster left ventricles. We analyzed the content of Z-disc-associated proteins by immunoblot. We cloned and sequenced cDNAs for desmin which is an intermediate filament (IF) protein, consisting of four distinct domains: amino-terminal head, coil-1, coil-2 and carboxy-terminal tail. The nucleotide polymorphism of desmin gene was examined. We analyzed the potential phosphorylated state of TO-2 desmin *in vitro* and *in vivo*. We performed *in vitro* pull down binding assay for the coil-1 domain of desmin. We observed fiber formation of TO-2 desmin by transfecting desmin cDNA into SW13 cells which lack any IF proteins.

Results: In 14 weeks old TO-2 ventricles, Z-discs were significantly thinner compared with Golden and BIO14.6. In TO-2 ventricles, desmin, intermediate filament protein, was drastically reduced whereas alpha-actinin was conserved. Golden desmin cDNA had an open reading frame (ORF) of 1,407 nucleotides, encoding a polypeptide of 469 amino acids. BIO14.6 had the same ORF as Golden, whereas TO-2 had G to A substitution at position 571 (G571A). This caused amino acid substitution from alanine to threonine at position 191 (Ala191Thr) in the coil-1, which binds to itself to form a parallel homodimer. Genomic DNA analysis showed that only TO-2 was homozygous for 571A, in contrast to homozygous 571G in Golden and BIO14.6. *In vitro* pull-down assay revealed that the coil-1 with 191Thr has self-binding affinity similar to that with 191Ala. Nevertheless, the filament formation of 191Thr desmin appeared less robust and distributed sparsely when expressed in SW13 cells. From those findings, we consider Ala191Thr a deleterious mutation rather than a silent polymorphism.

Conclusions: The present study elucidated the genetic cause of severe CM in TO-2: coincidental mutations in two cytoskeletal proteins, delta-sarcoglycan and desmin. With this pathogenetic knowledge, TO-2 hamster would be widely utilized for the assessment and development of novel therapeutics for severe CM and cardiac failure.

Keywords: Cardiomyopathy, cardiac failure, animal model, dystrophin, desmin.

PO-70**Track:** Regenerative Medicine**GROWTH OF GASTRIC STEM CELLS ON CHITOSAN SCAFFOLD****Prashanth Saseedharan, Yaser E. Greish, Abdel-Hamid I. Mourad and Sherif M. Karam***Department of Anatomy, UAE University, UAE; Email: prashanth.s@uaeu.ac.ae*

Stomach diseases are approached with various treatments with surgery being the final option. Recent therapeutic trends include tissue engineering, which combines the techniques of cell culture with material sciences to generate artificial tissues that could be utilised to repair or regenerate the stomach. This study utilizes our recently established and immortalized gastric stem (mGS) cells and various chitosan scaffolds to test and determine whether these scaffolds could support the adherence and proliferation of cells and induce their differentiation. The seeded mGS cells on chitosan were maintained under normal culture conditions for 3, 6, 9 and 12 days. These samples were processed for DNA quantification using Pico Green assay, morphological analysis by light/electron microscopy, and differentiation follow up using lectin/immuno-cytochemistry. The results revealed the attachment and progressive proliferation of mGS cells onto the chitosan scaffolds up to 6 days. On days 9 and 12, lectin/immune-cytochemistry provided evidences for the expression of N-acetyl-D-glucosamine specific for gastric mucous cells. This study provides a new platform for the

possible utilization of chitosan in gastric tissue engineering following gastrectomy. However, further studies should be conducted to enhance the differentiation potential of mGS cells on chitosan scaffold.

Keywords: Chitosan, stem cell, scaffold, tissue engineering.

PO-80

Track: Others - Medicinal chemistry

BIOLOGICAL EVALUATION OF TRANS-[RU(DMB)2CL(ETOH)]PF6; DNA-BINDING, DNA CLEAVAGE AND CELL CYTOTOXICITY

Nafiseh Serri

Department of Chemistry, Isfahan University of Technology, Iran, Email: n.serry@ch.iut.ac.ir

Ru(II)-polypyridyl complexes are important in biotechnology and pharmacology for their anticancer effect and ability to form a probe in structure-function studies. The Ru(II) forms substitution-inert complexes with polypyridyl ligands such as phen, bpy, dppz and tppz. In this study, we have been synthesized a new mononuclear Ru(II) complex, trans-[Ru(dmb)2Cl(EtOH)]PF₆, (where dmb is 4,4"-dimethyl-2,2"-bipyridine).

The complex has been characterized by elemental analysis and spectroscopic methods. The solid state structure of the complex has been determined by single-crystal X-ray crystallography. The DNA binding properties of trans-[Ru(dmb)2Cl(EtOH)]PF₆ have been investigated by spectrophotometric (fluorescence and UV/vis), electrochemical (DPV and CV) and gel electrophoresis assay methods. Information obtained from this research provides evidence for the nature of the binding of the Ru(II) complex to DNA which is expected to offer potential therapeutic agents that are directed at DNA.

Keywords: Ru(II) complex, x-ray structure, DNA cleavage, cell cytotoxicity.

PO-27

Track: Plant & Environment

STUDY ON MOLECULE IDENTIFICATION AND RHIZOMANIA RESISTANCE OF TRANSGENIC SUGAR BEET WITH *RIPS* GENE

Sun Yaqing, Ma Jingjing, Zhang Shaoying and Zhang Yongfeng

College of Agronomy, Inner Mongolia Agricultural University Hohhot 010018, China; Email: syzh36@yahoo.com.cn

Sugar beet (*Beta Vulgaris* L.) is an endemic sugar crop and play an important role in agriculture production in north China. Yields of sugar beet is not high and the total output is instable, particularly sugar content showed a downward trend in the national. All had become the obstacle factors to the development of sugar beet. All sorts of proprietary diseases of sugar beet are the major causes leading to the problems mentioned above. Selecting disease-resistant varieties combined with good traits is the most economical and efficient method to prevent and control sugar beet diseases.



It was concluded that the ribosome inactivating protein (RIP) have wide-spectrum resistance to viruses, bacteria and fungi, so we introduced the *RIPs* gene into sugar beet to research its role in disease resistance, carried PCR and RT-PCR screening identification on the obtained T₁ and T₂ transgenic plants to study their genetic stability, then Western blot analysis were done to research the transgenic plants resistance to Rhizomania. The main results are as follows:

1. The PCR screening identification on the T₁ and T₂ transgenic plants with *RIPs* gene showed that the target genes had been integrated into the genome of the sugar beet plants and had been a genetically stability breed. The *RIPs* gene expression of the transgenic sugar beet which has been detected by RT-PCR showed that *RIPs* gene can be expressed at the level of transcription. The research results can provide reliable materials for sugar beet molecular breeding with disease resistance.
2. The root yield and sugar content of transgenic sugar beet were studied under different soil conditions (sick soil, carrying Rhizomania pathogen; sick-free soil), Compared with control group (wild type). The results in sick-free soil showed that, transgenic sugar beets were rather similar in size and appearance with the wild type plants, but showed

lower mean weight than the wild type and higher average sugar content; When growled in the sick soil, the transgenic beets were different from the wild types with regular morphology manifestation, while higher average weight and lower average percentage of sugar than the wild type ones.

3. Western blotting showed that the transgenic beet roots had lower beet necrotic yellow vein virus (BNYVV) content in sick soil. It has turned out that the transformed *RIPs* gene can significantly suppress viral reproduction in sugar beet plants, thus provided resistance to Rhizomania.

Keywords: Transgenic beet, RIP, disease resistance, molecular breeding.

Acknowledgement

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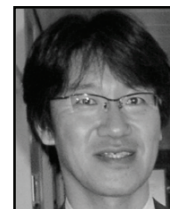
PO-50

Track: Others - Bioethanol

CADMIUM REMOVAL AND BIOETHANOL PRODUCTION FROM PHYTOREMEDIATION PLANT BY SIMULTANEOUS SACCHARIFICATION AND FERMENTATION (SSF)

Sho Shindo, Shoko Masuda, Hiroki Raib and Hiroyuki Hattorib

Department of Biorefinery, Akita Research Institute of Food and Brewing, Japan; E-mail: shindo@arif.pref.akita.jp



Natural processes such as volcanic eruptions, continental dusts and metal working industries lead to emission of heavy metals. These heavy metals are toxic because they cause DNA damage and their carcinogenic effects in animals and humans are probably caused by their mutagenic ability. Various engineering such as soil excavation, soil washing, or burning or pump and treat system are already being used to remediate metal contaminated soils. However, these techniques are not fully acceptable as they destroy the biotic components of soil and are technically difficult and expensive to implement. Phytoremediation is an emerging technology, which uses plants to remove pollutants from contaminated sites.

In this study, bioethanol production and cadmium removal from phytoremediation plant was investigated. We used the rice straw (*Oryza sativa* L.) for the removal of cadmium from contaminated sites. *Oryza sativa* L. was contained 80ppm of cadmium after phytoremediation treatment. When *Oryza sativa* L. was treated by 2% of sulfuric acid and enzymes, 75% of sugar yield and 90% of cadmium release were achieved. Furthermore, production of bioethanol from rice straw by simultaneous saccharification and fermentation (SSF) using *Schizosaccharomyces japonicus* was investigated. 15 (g/L) of bioethanol was produced after 30 hr.

Keywords: Cadmium, Bioethanol, phytoremediation.

PO-69

Track: Plant and Environment

CHEMOTHERAPEUTIC ACTION OF METHANOLIC EXTRACT OF THYMUS VULGARIS LEAVE IN *T. BRUCEI* INFECTED RATS

Oluwatosin K. Shittu

Trypanosomiasis Research Unit, Department of Biochemistry, Federal University of Technology, PMB 65, Minna, Nigeria; E-mail: toscue@yahoo.com

Current chemotherapeutic of African trypanosomiasis are beset with different problems including cost, toxicity and increasing resistance. Therefore, the search for new drugs and formulations that are safe, affordable and effective against both early and late stages of the disease is highly recommended. In this study, the efficacy of locally used medicinal spice *T. vulgaris* in the treatment of trypanosomiasis was investigated. Two different stages of infection were investigated and administered 500mg/kg body weight. The two groups were observed to show low rate of replication of parasite and extension of surviving days (8 days) than the infected not treated (6 days). Also, there was increased in the haemoglobin (Hb) concentration, packed cell volume (PCV), red blood cell (RBC), and white blood cell (WBC) of the

two groups when compared also to infected not treated group. Therefore, this investigation showed that *T. vulgaris* has antitrypanosomal potentials by ameliorating the disease condition.

Keywords: *Thymus vulgaris*, Antitrypanosomal, haematological parameters.

PO-66

Track: Others - Biosensor

ELECTROCHEMICAL DOSIMETRY OF GAMMA RAY EMITTED FROM TALLIUM-201 BY AMPEROMETRIC SUPEROXIDE ANION BIOSENSOR

Mostafa Shourian

Department of Biophysics, Institute of Biochemistry and Biophysics (IBB), Iran; Email: shorian@ibb.ut.ac.ir

Exposure of aqueous media to ionizing radiation (radiolysis) such as γ -rays produces several reactive oxygen species such as superoxide, hydrogen peroxide, and so on that damage bio-molecules. In recent years; there has been renewal interest in the determination of these species including superoxide. In this work, at the first step an aqueous media was exposed to γ -rays radiation resulting in superoxide production. According to this fact, we prepared a cysteine modified electrode and applied it for detection of superoxide. For this purpose, cysteine self assembled monolayer-modified gold electrode (Cys/Au) is used to detect of superoxide as a γ -ray produced reactive oxygen species from Thallium-201 (201Tl). The superoxide samples were produced by direct exposing the γ -ray source in phosphate buffer solution. The results showed that through measurement of superoxide produced by γ -ray source, the activity of ionizing radiation source can be estimated. In the presence of radioisotope, the amperometric detection of superoxide was designated as sensor response. At the applied potential of +250 mV (vs. Ag/AgCl), the developed sensor was able to detect the γ -ray in a linear range from 90 μ Sv/h to 1.25 mSv/h and a detection limit of 53 μ Sv/h. This approach could be useful to detect and dosimetry of gamma ray emitted from Thallium-201 to determination of superoxide.

Keywords: Thallium-201, Cysteine/Gold electrode, Gamma ray, Dosimetry, Electrochemistry.

PO-48

Track: Medical Biotechnology

OPTIMIZATION OF HUMAN INSULIN PRODUCTION PROCESS USING NEW IMPURITY BLOCKING METHOD

Young-Jin Son

College of Pharmacy, Suncheon National University, 413 Jungangno, Suncheon, Jeonnam 540-742, Korea; E-mail: sony@sunchon.ac.kr

Insulin is a peptide hormone which controls the glucose concentration in blood. The aim of this research is to improve the human insulin production by developing a commercially viable process. *E. coli* JM109/pPT-H27Rpi was cultured by three-step temperature shift methods. It gave the final dry cell weight of 45 g/L and expression content of 69%. The purification steps were homogenization, inclusion body collection, refolding, and the enzyme reactions. The converted insulin was purified by cation-exchange and reverse-phase chromatography followed by crystallization. To block the formation of insulin derivatives and des-threonine insulin, hydrogen peroxide and citraconylation were used during the enzyme reaction. Combination of H₂O₂ and citraconylation improved the insulin production yield to 50%. The overall yield of insulin from the enzyme reaction to final pharmaceutical ingredient was 67%. In 1 L fermentation broth, 0.48 g insulin was produced at cell concentration of 45 g dry cell weight/L. The purity of purified insulin was higher than 98.5%. Finally, the expression cell for human insulin was constructed. The fermentation and purification processes were optimized. The impurity blocking method, such as, hydrogen peroxide and citraconylation method were invented to allow the simple and cost-effective downstream process for production of human insulin.



PO-15**Track:** Others - Marine Biotechnology**INNATE IMMUNE RESPONSE OF COMMON CARP, CYPRINUS CARPIO L****Mahmoud Tanekhy**

Faculty of Veterinary Medicine, Alexandria University, Faculty of Veterinary Medicine, Alexandria University, Egypt; E-mail: tanekhyvet2020@yahoo.com



Carp is susceptible to variety of viral, bacterial, fungal and parasitic infections other than environmental stressors. These lead to great losses in carp production. Immunostimulants can increase the resistance to infectious disease by enhancing innate immune responses. However, in fish, the innate immune system is still unclear. In this study, the innate immune system which relate to Toll like receptor (TLR) was investigated in carp.

TLR7 cDNA was cloned and identified in common carp. The cDNA of carp TLR7 has 3427 bp contained a complete open reading frame (ORF) encoding 1049 amino acids. Carp TLR7 contains an ectodomain including 13 LRRs followed by an additional leucine-rich repeat C-terminal (LRR-CT) motif. The carp TLR7 has a 22 amino acid transmembrane segment and a cytoplasmic tail that contains the TIR domain. The expression analysis of carp TLR7 in the healthy tissues was manifested in intestine, heart, head kidney, skin, muscles, liver, spleen, brain and gills.

The different ligands of TLRs, LPS (TLR4 ligand), poly I:C (TLR3 ligand), and imiquimod (TLR7 ligand) were used to stimulate carp head kidney leukocytes for different time range, and the expression of related cytokine genes were examined.

Constitutive expression of pro-inflammatory cytokines (IL-1 β , TNF- α , and CXC-chemokine) genes in carp HK cells was observed in respect to LPS stimulation. The lack of a TLR4 ortholog in some fish species and the lack of the essential co-stimulatory molecules for LPS activation *via* TLR4 (i.e., myeloid differentiation protein 2 (MD-2) and CD14) in all available fish genomes led to hypothesize that the mechanism of LPS recognition in fish may be different from that of mammals.

In regard to poly I:C stimulation, type1 IFN and Mx protein were obviously up-regulated immediately after 1 h. The same effect has been occurred to carp tissues after imiquimod treatment with subsequent increase in type1 interferon and other pro-inflammatory cytokines.

Upon imiquimod treatment, pro-inflammatory genes expression is enhanced in carp HK leukocytes. IL-1 β , TNF- α , IL-10 and CXC up-regulated within 24 h. The up-regulation of Type-1 IFN in HK cells treated with imiquimod is not prominent as that seen after treatment with poly I:C. The difference in induction of IFN by imiquimod and poly I:C may result from a difference in the targets cell of the two stimulants; imiquimod primarily induces IFNs through immune cells whereas poly I:C induces IFNs in most nucleated cells. The cytokine genes responses were investigated in carp infected by *Aeromonas hydrophila*. Pro-inflammatory cytokine expression including IL-1 β and TNF- α was up-regulated after infection. On the other hand, these cytokine expressions were not expressed after treatment with formalin-killed *A. hydrophila*. The expression pattern of cytokine genes may be attributed to cytotoxic enterotoxin activated arachidonic acid metabolism in proliferating *A. hydrophila*, and an increased presence of bacterial components that trigger immune cell response.

Therefore, in order to prevent disease transmission from fish to human, we have to prevent its occurrence in fish by potentiating the immune responses against the specific pathogens.

PO-18*Track: Others - Nanobiotechnology***DEVELOPMENT AND APPLICATION OF NANOSTRUCTURAL AND MONOCRYSTALS MATERIALS IN MEDICAL X-RAY TECHNIQUE****S.V. Alekseev, A.A. Pavlov, M.L. Taubin, D.V. Chesnokov and A.A. Yaskolko***FSUE "SRISIA "LUCH", 24, Zheleznodorozhnaya St., Podolsk, Moscow Region, Russia, 142100;**E-mail: taubin@sialuch.ru*

In the report the concept of increase of characteristics x-ray tubes by use nano-and monocrystal materials is offered. Results of own work of the authors in creation nanocomposite of refractory metals with increased strength characteristics for cathodes and anodes are submitted. It is shown, that though monocrystals have shown the better characteristics in comparison with traditional polycrystals both as anodes and as cathodes, more radical way of increase of operational characteristics of the x-ray tubes is application refractory nanostructural materials.

Some results of investigations in which the **work function electrons** of nanocrystal tungsten is lower by 0.8 eV than that of traditional tungsten are given Fig. (1). The authors carried out some experiments to determine the **creep** of tungsten alloyed by potassium on samples as foils 100 μm thick, used when manufacturing X-ray tube cathodes. The results of these experiments showed that high-temperature sample annealing resulted in a considerable increase (by a factor of more than 3) in material creep resistance at a temperature of 2200°C Fig. (2). Investigations in the structure of this material samples after annealings (Fig. 4) allowed finding out nano dimensional (≤ 200 nm) inclusions along the grain boundaries of tungsten.

Proceeding from the above-mentioned, we think that producing a cathode with a flat emitter made of single-crystal and nanocomposite tungsten is one of the promising trends in the area under considerations.

Investigations in the structure of this material samples after annealings (Fig. 4) allowed finding out nanodimensional (≤ 200 nm) inclusions along the grain boundaries of tungsten subjected preliminarily to heat treatment, resulting in a sharp increase (by a factor of more than 3) in material creep resistance at a temperature of 2200°C (Fig. 3).

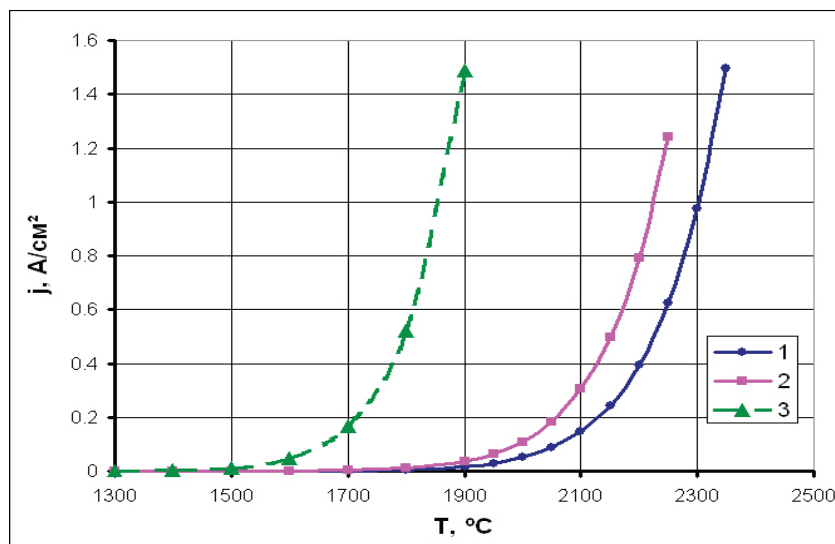


Fig. (1). Dependence of emission current density of temperature for: polycrystalline tungsten; single-crystal tungsten from plane [111]; polycrystalline tantalum.

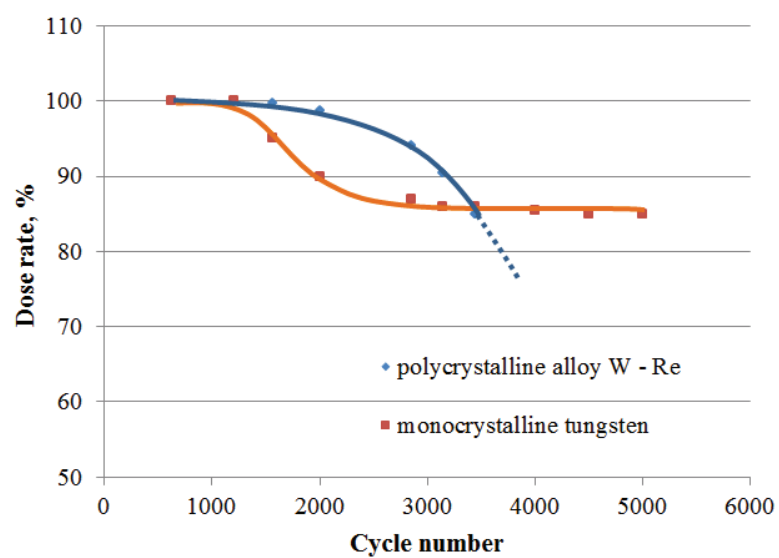


Fig. (2). Dose power dependence on the number of cycles.

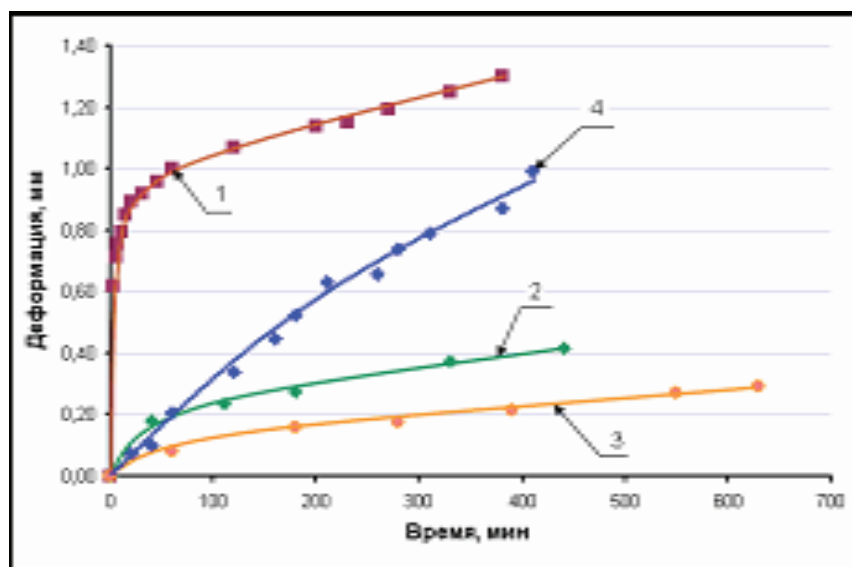


Fig. (3). Deformation dependence on time at creep tests of W.

1 - polycrystalline W without heat treatment; 4. monocrystal W.

2, 3 - polycrystalline tungsten preannealed at a temperature of 2500°C for 5 and 7 hours.

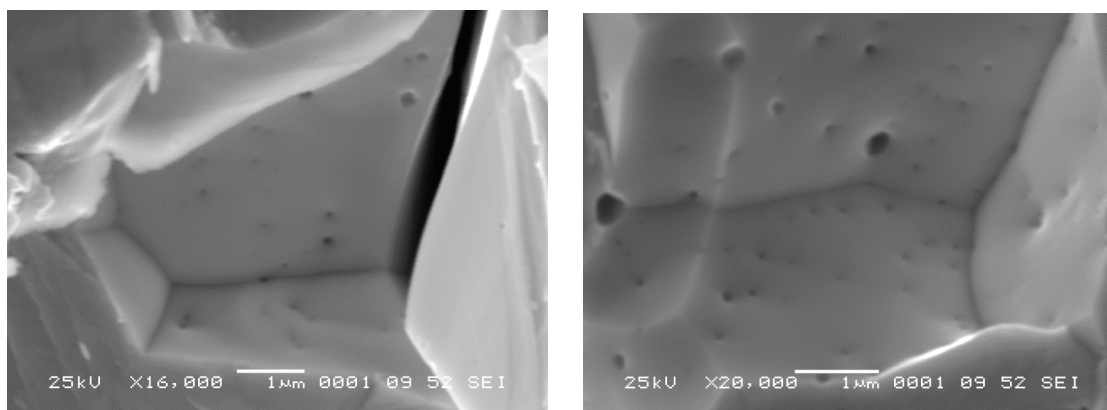


Fig. (4). Microstructure of polycrystal tungsten subjected to heat treatment at a temperature of 2500°C for 5 hours.

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PO-37

Track: Plant & Environment

EFFECT OF LOPNUR REGION *TAMARIX* L. THE CULTURABLE RHIZOPHERE SOIL ARCHAEA DIVERSITY AND ANTIBIOTIC

Chen Jin and Dilbar Tohty

College of Life Sciences, Xinjiang Normal University, No. 102, Xinyi Road, Urumqi, 830054 Xinjiang, China; E-mail: dilbar.th@163.com

The biodiversity is the result of the life evolves over long time, and which is the substantial base for the human being to exist on the earth. However it is now faced with the threatener from the increasing population and economic action. In order to build biology data of Lop Nur and offer the data for the further research, biodiversity of cultured microbial resources isolated from extreme plant rhizosphere were investigated, in Lop Nur Region of Xin-Jiang, China.



The region of Lop Nur is an arid ecosystem. *Tamarix* L. community is the dominant in this area. For the protection and utilize of microbial species and genetic resources within the rhizosphere and non-rhizosphere soil of *Tamarix* L. community, the quantity of microorganisms and important functional groups were surveyed in October 2007. The results indicate that in *Tamarix* L. community, the total numbers of microorganisms of different species in soil are all small. All species over ground and underground are correlative in ecosystem function and clear rhizosphere effect is shown.

A totally of one hundred and sixty-six halophilic archeal strains, 111 strains from rhizosphere soil and 55 strains from non-rhizosphere soil respectively were isolated using complete medium and their upstream partial 16S rRNA gene sequences were determined, which is 51 rhizosphere isolates and 55 non-rhizosphere isolates, in the basis of their morphology, growth and 69 physiochemical characteristics. Phylogenetic analysis based on 16S rRNA genes of all these strains indicated that the isolates from rhizosphere belong to 11 different species of genera *Natrialba*, *Natronorubrum*, *Haloterrigena*, *Natronococcus*, *Natrinema*, and that the isolates from non-rhizosphere belong to 8 different species of genera *Haloarcula*, *Halobiforma*, *Halorubrum*, *Haloterrigena*, *Natrinema*, *Natronorubrum* and *Natrialba*. Most of the 16S rRNA gene sequences related to the genera *Natrialba* and *Haloterrigena* were detected in rhizosphere soil. In contrast, sequences related to the genera *Natrinema* were obtained from non-rhizosphere soil. halophilic archaea LPN74, LPN119- am was used as an antagonist for postharvest biological control of blue mold(*Penicillium italicum*) on app les. Each treatment of strain LPN119 had the inhibitory action in the app le and on the PDA culture medium to

the apple blue mold. In addition, the isolates' diversities were analyzed and compared using different diversity indices, richness indices, evenness indices, and species abundance models.

There were certain correlations among these indices, and they indicated that halophilic archaea diversity of rhizosphere is higher than that of non-rhizosphere. The discovery of novel species, in a relatively small number of sites from two representative soil, indicated that there are some special microbial resources in Lop Nur region that should be protected and utilized.

In this study, the halophilic archaeal diversity of rhizosphere and non-rhizosphere in Lop Nur was determined, some new species were described, and the relationship between plants and halophilic archaea were explored, which may enlarge the boundary of halophilic archaeal diversity and provide halophilic archaea resources for further theoretical and applied researches.

Keywords: Biodiversity, *Tamarix L.*, community, Rhizosphere, Halophilic Bacteria, antibiosis.

Acknowledgement¹

Supported by the National Science Foundation of China No: 31260002; Natural Resource Platform of Institute of Microbiology Chinese Academy of Sciences, Project of the Microbiology Xinjiang Normal University.

PO-11

Track: Pharmaceutical Biotechnology

SCREENING OF ALPHA-AMYLASE INHIBITS OR PRODUCED BY MARINE MICROORGANISMS BY MODIFIED TLC METHOD

Jyothi Vanama, Nagamani Bolla, Anusha Alla, G. Girija Sankar and T. Prabhakar

Pharmaceutical Biotechnology Division, Andhra University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, Andhra Pradesh, 530003, India;
E-mail: jpk1305@gmail.com

Amylase inhibitors are useful tools for the determination of activities of amylase isozyme and purification of amylases. Inhibiting alpha- amylases could be a beneficial treatment for insulin-dependent Diabetes mellitus, obesity and hyperlipidemia. The objective of the present work was to develop effective and rapid screening of the alpha - amylase inhibitor producing marine actinomycetes by modified TLC (Thin Layer Chromatography) method.

Out of 200 marine isolates collected from the coast of Bay of Bengal, Tamilnadu, India, one promising isolate was identified which exhibited alpha-amylase inhibition activity. The isolate was characterized by conventional methods and identified as *Streptomyces lateritius* var. TP4/6 with accession number MTCC 10474. Various methods were employed to screen alpha-amylase inhibitor enzyme producing microorganisms like starch plate method, starch agar plate method, TLC plate method. To overcome the shortcomings of the conventional screening methods we developed modified TLC plate method, which is rapid and effective screening method and detection of proteinaceous alpha- amylase inhibitor was carried by novel RPTLC method. Quantitative estimation was carried out by modified Blue Value method.

Keywords: Marine actinomycetes, alpha-amylase inhibitor, TLC plate method, modified Blue Value method.



PO-44*Track: Medical Biotechnology***ASSOCIATION OF CAPN-10 GENETIC POLYMORPHISM WITH TYPE 2 DIABETES MELLITUS AND METABOLIC SYNDROME AMONG EGYPTIANS****Shaymaa Wagdy, Heba Kasem, Amira M. Embaby, Abir Adel, Medhat Haroun and Nader Mowafy***Institute of Graduate Studies and Research, Biotechnology Department, University of Alexandria, Egypt; E-mail: shwagdy_2009@yahoo.com*

This study defines three CAPN-10 variants [SNP-43 (rs#3792267), SN P-19 (rs#3842570) and SNP-63 (rs#5030952)] localized on chromosome 2q37 among diabetic patients with and without metabolic syndrome (MS) along with healthy individuals (a case-control study). Genomic DNA was isolated from whole blood for CAPN10 genotyping. Three separate PCRs were carried out to amplify three partial fragments of CAPN10 containing the aforementioned SNPs. PCR-RFLP was carried out to genotype SNP-43 and -63. However, SNP -19 (Ins /Del) was genotyped directly through determining the number of obtained repeats in PCR products after visualization on 3% agarose gels. Statistical analysis of data reveal that there are no significant differences ($p > 0.05$) in both allelic and genotypic frequencies either among diabetic and healthy individuals or among patients with and without MS. Interestingly, the present study suggested that the haplotype combination 111/112 confers a high risk for type 2 diabetes mellitus (OR= 10.15, 95% CI 1.2-225, $p=0.011$). Patients with the 122 as a haplotype and homozygous haplotype combination 122/122 are less susceptible to MS and obesity when compared to those with other haplotype combinations. In addition, the haplotype combination 111/121 demonstrate a protective role against obesity ($p= 0.009$). Conversely, patients with the haplotype combination 111/111 display a significant risk for high levels of total cholesterol ($p=0.047$). The present findings address that these haplotype combinations 111/112, 111/121 and 122/122 of CAPN-10 SNP-43, -19 and -63 constitute unique DNA biomarker fingerprints toward susceptibility and risk for type 2 diabetes mellitus and metabolic syndrome among Egyptians when compared to other haplotype combinations reported in other populations of different ethnicity.

**Keywords:** CAPN-10, Calpain 10, Type 2 Diabetes Mellitus, Metabolic Syndrome.**PO-53***Track: Medical Biotechnology***STUDY OF PLASMA MEMBRANE PROTEINS EXPRESSION IN MODIFIED YEAST STRAINS****Wittawan Watanasrisin, Mizuki Nachi, Takahiro Oura and Susumu Kajiware***Department of Life Science, Graduated School of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta-cho, Midori-ku, Yokohama, 226-8501, Japan;
E-mail: secret_garden_1@hotmail.com*

One of the major bottlenecks in studying the function of pharmaceutically important, eukaryotic membrane proteins is obtaining sufficient pure and functional protein. Yeasts are commonly used for the heterologous production of various recombinant proteins. However yeasts have different lipid membrane components from mammals and, especially, their major sterol is ergosterol not cholesterol known to influence the structure, function and activity of wide-range of mammalian membrane proteins. Previous reports showed that majority of human ATP-binding cassette (ABC) transporter ABCB1 (*HsABCB1*) expressed in *Saccharomyces cerevisiae* was accumulated inside the cell than transported to its plasma membrane and the activity of *HsABC1* was very low. In addition, it was shown that cholesterol in human cell membrane is related with the activity of *HsABC1*. It is suggested that ergosterol, may altered the activity of *HsABC1* and recruiting for *HsABCB1* to yeast plasma membrane.

In this study, by using a *S. cerevisiae* strain suitable for expression of other fungal ABC transporters, we succeed in constructing of gene manipulating strains that replacing two genes encoding enzymes required for ergosterol biosynthesis (*ERG5* and *ERG6*) with the genes encoding human dehydrocholesterol reductases (*HsDHCR7* and *HsDHCR24*). Sterol composition was determined following by expression of *HsABCB1* in this sterol modified strains.



Drugs susceptibility test will performs to observe the relationship between sterol and functional expression of *HsABCB1*.

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Keywords: *S. cerevisiae*, sterol manipulation, plasma membrane expression, *HsABCB1*.

PO-13

Track: Plant & Environment

NATURAL PRODUCTS FROM CYANOBACTERIA WITH ANTITUMOR ACTIVITY

Maria Estela Silva-Stenico, Ramon Kaneno; Fabiana Albani Zambuzi, Marcelo G.M.V. Vaz, Danilo O. Alvarenga and Marli de Fátima Fiore

Universidade de São Paulo, CENA, Lab. Ecologia Molecular de Cianobactérias, C.P. 96, 13400-970 Piracicaba-SP; E-mail: estela@cena.usp.br

Cyanobacteria are an important source of structurally bioactive secondary metabolites, with cytotoxic, antifungal, antiviral, anticancer, antimitotic, antimicrobial, antitumor, specific enzyme inhibition and immunosuppressive activities. This study reports on the evaluation of intra and extracellular cyanobacterial extracts for their antitumor and antimicrobial activity. A total of 436 cyanobacterial strains were screened for antimicrobial activity using pathogenic bacteria. For the *in vitro* antitumor activity, 19 strains were tested against human cancer cell lines (murine colon carcinoma CT-26 and murine lung cancer 3LL). Antimicrobial activity assays performed using intracellular extracts inhibited 31 and 26% of Gram-negative and Gram-positive pathogenic bacterial growth, respectively. The methanolic intracellular extract of *Cylindrospermopsis raciborskii* CYP011K and *Nostoc* sp. CENA69 showed inhibitory activity against human cancer cell lines, while strains *Synechococcus* sp. CENA154, *Chlorogloea* sp. CENA170, *Microcystis aeruginosa* NPJB-1 and *M. aeruginosa* NPLJ-4 showed stimulatory effect. The extracellular extract from *Fischerella* sp. CENA213 and *M. aeruginosa* NPJB-1 showed inhibition activity against 3LL lung cancer cells at 0.8 µg ml⁻¹ and *Phormidium* sp. CENA135, *Synechococcus* sp. CENA154, *M. aeruginosa* NPJB-1 and *M. aeruginosa* NPLJ-4 presented inhibition activity against CT26 colon cancer cells at 0.8 µg ml⁻¹. Other strains were able to inhibit 3LL cell-growth at higher concentrations (20 µg ml⁻¹) such as *Nostoc* sp. CENA67, *Synechococcus* sp. CENA154 and *M. aeruginosa* NPLJ-4, while CT26 cells were inhibited at the same concentration by *Nostoc* sp. CENA67 and *Fischerella* sp. CENA213. Such extracts has very low inhibitory activity on peripheral blood lymphocytes. Cyanobacterial strains that presented stimulatory effect on cancer cells were subjected to mass spectrometry, which revealed the presence of microcystin, molecule known as tumor promoter. This study showed that the cyanobacterial isolates are a rich source of natural products with potential for pharmacological and biotechnological applications.



PO-29*Track: Plant and Environment***TISSUE CULTURE-INDUCED SOMACLONAL VARIATION OF DECREASED POLLEN VIABILITY IN TORENIA (*TORENIA FOURNIERI* LIND.)****Shulan Sun, Jianqiang Zhong, Shuhua Li and Xiaojing Wang***Guangdong Key Lab of Biotechnology for Plant Development, College of Life Sciences, South China Normal University, Guangzhou 510631, Guangdong, People's Republic of China*

Tissue culture is a powerful tool in plant gene transformation and molecular breeding. Phenotypic and genotypic variations, collectively called somaclonal variations, are induced during tissue culture. Here, we studied the phenotypic variation in pollen viability of regenerants of torenia after subculturing for one to nine generations. We found that pollen viability of regenerants continuously decreased with increasing subculture time. High concentrations of plant growth regulators applied to the Murashige and Skoog (MS) medium also resulted in diminished pollen viability. Furthermore, antibiotic application during gene transformation also decreased pollen viability of the transformants. However, the process of long-term culture did not significantly change pollen viability. The mean methylation level of regenerants showed a 0.28% to 3.95% decrease in seedlings subcultured *in vitro* for nine generations. moreover, when the ninth subcultured regenerants with reduced pollen viability were recovered in soil to get seeds, the pollen viability of seed-derive plants was similar to that of the wild type. These results indicate the epigenetic nature of somaclonal variations in torenia.

**Keywords:** DNA methylation, Plant growth regulators, Pollen viability, Somaclonal variation, Torenia.**Acknowledgement**

This work was supported by grants from the Guangdong Province Natural Science Foundation (5300074 and 9251063101000002), the Youth Foundation of the National Natural Science Foundation of China (30900107) and Research Fund of MOE Doctoral Program (20104407110005).

PO-32*Track: Plant & Environment***PREDICTION MODEL OF HYBRID PERFORMANCE USING MOLECULAR MARKER BASED ON ADDITIVE-DOMINANT EFFECT****Lu Xiao-Ping, Liu Dan-Dan, Mi Fu-Gui, Wang Shu-Yan and Xue Chun-Lei***College of Agronomy of Inner Mongolia Agricultural University, Huhhot 010019, China*

Based on molecular markers to construct the heterosis prediction model could provide some advice for *Sorghum* × *Sudan* grass breeding. 90 hybrid combinations were formed according to the incomplete diallel cross design (NCII) with 5 sorghum sterile lines as maternal parents and 18 Sudan grass lines as paternal parents. The trials were carried in the farms of Huhhot and Baotou to evaluate environmental effects. The 8 trait phenotypic values of hybrid F₁ were investigated and selected marker loci with SSR, AFLP and SRAP, set up the evaluation system of marker effect and tag value. Using the specific loci to evaluate the trait effects and hybrid tag value, and analyze the correlation between hybrid tag value and heterosis. The prediction models of 8 traits for the hybrid were constructed with the stepwise regression analysis. The Jackknife sampling method was used to test the accuracy and steadiness of the model. The result showed, considering dominance and additive effect separately, 8 traits showed the average correlation index is 0.65 between tag value and phenotypic value. The coefficient of determination range is 0.51-0.88 in the 8 traits. The results in two places are coherent. The model could be instructive for hybrid and parents selection.

**Keywords:** *Sorghum* × *Sudan* grass, Genetic effect, AFLP, SSR, SRAP, heterosis, prediction model.

PO-73**Track:** *Regenerative Medicine***ERS PLAYS A IMPORTANT ROLE IN OVARIAN TRANSPLANTATION AFTER CRYOPRESERVATION****Pei Xiuying, Wang Yanrong, Ding Xiao, Chen Jie, Yan Bei, Ma Huiming, Sun Miao and Yang Yanzhou***Key Laboratory of Fertility Preservation and Maintenance, Ningxia Medical University, Ministry of Education, Medical Science and Technology Research Center of Ningxia Medical University, Department of Biochemistry & Molecular Biology in Ningxia Medical University, Yinchuan, People's Republic of China. 750004*

Transplantation of ovarian cryopreservation treatment strategy is effective but low success rate to improve the quality of life of young cancer patients who urgent need to retain fertility. To improve large ovarian cortex block frozen and no the vascular anastomosis transplantation reduce apoptosis faced with challenges, including frozen and transplanted follicle apoptosis due to restrictions transplants success rate. And the limited function of ovarian transplantation can not be long-term protective effect of the reproductive endocrinology of women's health. Therefore, It is becoming a research hotspot to explore the causes of the damage of ovarian cryopreservation and transplantation and find effective solution to save the female fertility *in vitro*. Frozen and transplanted follicles increased apoptosis, we found suitable vitrification can reduce the apoptosis of ovarian cells, including increased Bcl-2/Bax ratio and lowering active Caspase-3 expression. The most important reason of follicle loss and non-normal follicle activation is follicles lost due to ischemic injury give a greater blow compared with cryopreserved. Endoplasmic reticulum stress (ERS), following the death receptor and mitochondrial-mediated apoptosis pathway, is newly concerned about the important apoptosis-inducing way. ERS plays an important role for decision the outcome of the stress cells, such as, adaptation, injury or apoptosis. Our study shows that: the vitrification launched the ERS of the unfolded protein response. GRP78 protein and the CHOP protein of apoptotic pathway expression increased, suggesting that the vitrification process of the ERS. And that their expression decreased in frozen in FSH intervention group prompted FSH can reduce the occurrence of ERS.

Acknowledgement

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Keywords: Vitrification, ERS, Ovarian, FSH, apoptosis.

PO-28**Track:** *Other Areas: Food***iTRAQ-BASED QUANTITATIVE PROTEOMIC ANALYSIS REVEALS NEW METABOLIC PATHWAYS OF WHEAT SEEDLING GROWTH UNDER HYDROGEN PEROXIDE STRESS****Pei Ge, Pengchao Hao, Min Cao¹, Guangfang Guo, Dongwen LV, Saminathan Subburaj, Xiaohui Li, Wujun Ma and Yueming Yan**

College of Life Sciences, Capital Normal University, Beijing 100048, China; Email: yanyem@mail.cnu.edu.cn

As an abundant ROS, H₂O₂ plays pivotal roles in plant growth and development. In this work, we conducted for the first time an iTRAQ-based quantitative proteomic analysis of wheat seedling growth under different exogenous H₂O₂ treatments. The growth of seedlings and roots was significantly restrained by increased H₂O₂ concentration stress. Malondialdehyde, soluble sugar and proline contents as well as peroxidase activity increased with increasing H₂O₂ levels. A total of 3,425 proteins were identified by iTRAQ, of which 157 showed differential expression and 44 were newly identified H₂O₂-responsive proteins. H₂O₂-responsive proteins were mainly involved in stress/defense/detoxification, signal transduction and carbohydrate metabolism. Obviously up-regulated expression of signal transduction and stress/defense/detoxification related proteins under H₂O₂ stress, such as plasma membrane intrinsic protein 1, fasciclin-like arabinogalactan protein (FLA) and superoxide dismutase (SOD) could contribute to H₂O₂ tolerance of wheat seedlings. Increased gluconeogenesis (PEPCK) and decreased pyruvate kinase proteins might



be related to the higher H₂O₂ tolerance of wheat seedlings. A metabolic pathway of wheat seedling growth under H₂O₂ stress is presented.

Keywords: Wheat, seedlings, H₂O₂, proteome, iTRAQ, metabolic network.

PO-72

Track: Regenerative Medicine

THE INFLUENCE ON THE EXPRESSION OF CONNEXIN AND RESISTANCE APOPTOSIS ABOUT FSH INTERPOSE IN THE DIFFERENT STAGE OF VITRIFICATION OF MOUSE OVARY

Wang Yanrong, Chen Jie, Wu Hao, Pei Xiuying, Ma Wenzhi, Chang Qing, Ma Huiming and Sun Miao

Key Laboratory of Fertility Preservation and Maintenance, Ningxia Medical University, Ministry of Education, Department of Histology & Embryology in Ningxia Medical University, Yinchuan, People's Republic of China 750004

Objective : To investigate the best follicle stimulating hormone(FSH) intervention way for improving the vitrification effect.

Materials and Methods: Ovaries of 4 week-old mice were vitrification used interposed with 0.30IU/mL FSH in three ways. The first way is 0.30IU/mL FSH interpose in the overall process of vitrification (OG-FSH), the second way is 0.30IU/mL FSH interpose in the before process of vitrification(BG-FSH), the third way is 0.30IU/mL FSH interpose in the after process of vitrification(AG-FSH).

Main Outcome Measures: The percentage of normal follicles was investigated by count follicles in slides stained by HE. The expression of Cx43、Cx37 and active caspase-3 was investigated in ovarian tissues, which were fresh ovaries(FCG)、non- FSH intervention during vitrification process((VCG)) and interposed with 0.30IU/mL FSH in three ways during vitrification process by Immunohistochemistry、Western Bolt and Real-time PCR.

Results:The percentage of normal follicles comparison among groups show that the highest percentage group is theOG-FSH ,and the VCG is the lowest($P<0.05$).In each group, the expression of protein and mRNA of Cx43 and Cx37 from high to low in turn is OG-FSH、BG-FSH、AG-FSH、FCG and VCG. Moreover, the level of apoptosis detection show that it is complete opposite compared with connexin($P<0.05$).

Conclusion: The 0.3IU/mL FSH interpose in overall process of vitrification group (OG-FSH) could more conductive to keep the morphological structure of the ovarian tissues, more conductive to the expression of Cx43、Cx37 protein and mRNA, at the same time, could have the best antiapoptotic effect.

Acknowledgement

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Keywords: Mouse; ovary, FSH, vitrification, Cx43, Cx37, active caspase-3.

PO-14**Track:** *Pharmaceutical Biotechnology***UTILIZATION OF PHAGE DISPLAY LIBRARIES FOR THE IDENTIFICATION OF POLYPEPTIDE MOTIFS THAT BIND TO HSP90 PROTEIN FAMILY****Magdy M. Youssef***Department of Chemistry, Faculty of Science King Faisal University, P.O.Box 380, Hofuf 31982, Saudi Arabia; Email: mmm_youssef@yahoo.com*

Proteins as aspragenase and some of heat shock proteins (Hsps) are used as an antitumor's. The mechanism of action of many antitumor proteins is still obscure *in vivo*. We used genetic approaches to identify and characterize *in vivo* the active catalytic polypeptide chains partners of high temperature protein G (HtpG), as a member of Hsp90 family of proteins, by the aide of phage display biotechnology system. In the present work, the *htpG* gene from *E. coli* AB1157 was cloned in frame into the pGEX-2T DNA vector. The recombinant plasmid used to overexpress the GST-HtpG fusion protein. The GST-HtpG fusion and GST proteins were purified by chromatographic technique and applied with 12 mer and 15 mer phage libraries to identify polypeptides which bind with the HtpG protein. Identification of such polypeptides helped us to identify partner proteins that bind with the Hsp90 protein family *in vivo*. Nine of the 12 mer amino acids sequences were identified to bind tightly with the HtpG protein. In addition, thirteen of the 15 mer amino acids sequences were also identified to bind with HtpG protein. Proteins that have homology sequences with any of both the 12 mer and the 15 mer amino acids sequences were identified from protein data base. The binding domain of the Hsp90 family of proteins with client proteins as thyroid hormone receptor, protein kinase, nitric oxide synthase, SMC protein family, DNA polymerase, DNA topoisomerase, 50S ribosomal protein L2, ATP synthase, P53 and a quite number of proteins were determined. These data indicate that the HtpG and Hsp90 family of proteins bind with many proteins *in vivo* to facilitate their protein folding.

Keywords: Hsp90, Cloning, Overexpression, Purification, Glutathione S-transferase.**PO-42****Track:** *Plant & Environment***ANALYSIS OF CAROTENOIDS ACCUMULATION AND CAROTENOGENIC GENES EXPRESSION DURING FRUIT DEVELOPMENT IN LOQUAT****Zhang Ling, Lin Shunquan and Yang Xianghui***College of Horticulture, South China Agricultural University, Guangzhou 510642, China; E-mail: gzyxh@scau.edu.cn*

Loquat (*Eriobotrya japonica* Lindl.) which belongs to the Rosaceae family is a subtropical evergreen fruit tree originated in China. Carotenoids are the main pigments accumulated in mature loquat fruit. It is well known that the composition and content of carotenoids influences the appearance and internal quality of loquat. In this paper, the red-fleshed loquat, 'Zaozhong 6' and the white-fleshed loquat, 'Baiyu' were used as materials to determine the law of carotenoids' accumulation in peel and pulp during different fruit development stages; and some genes in carotenoid biosynthesis were cloned and their expressions were analyzed. The main results were as follows: 1. During loquat fruit ripening, the content of chlorophyll a and chlorophyll b declined quickly in the peel and pulp of 'Zaozhong 6' and 'Baiyu'; the content of β -carotene increased in the peel and pulp of 'Zaozhong 6' and the peel of 'Baiyu', while decreased in the pulp of 'Baiyu'; β -cryptoxanthin content in the peel and pulp of 'Zaozhong 6' and 'Baiyu' both rised steadily and gradually; lutein content decreased in the peel of 'Zaozhong 6' and the peel and pulp of 'Baiyu', while increased in the pulp of 'Zaozhong 6'. In ripe fruit β -carotene was the most abundant composition in the peel and pulp of 'Zaozhong 6' and the peel of 'Baiyu', however, lutein was the major carotenoid in the pulp of 'Baiyu'. In addition, β -carotene content in the peel and pulp of 'Zaozhong 6' was higher than it in 'Baiyu' respectively.

Developmental expression patterns of carotenogenic genes, including *DXS*, *DXR*, *IDI*, *PSY*, *LCYb*, *LCYe*, *PDS*, *ZDS*, *CRTISO*, *CYCB*, *BCH*, *ECH*, *ZEP*, *VDE*, *CCD*, were analyzed in this study using the *actin* gene as an internal standard. The results showed that the expression level of *PSY*, *LCYb*, *LCYe*, *CYCB*, *BCH*, *ECH* and *CCD* was likely to cooperatively regulate the accumulation of carotenoids. While the expression of *DXS*, *DXR*, *PDS*, *ZDS* and *CRTISO* did

not parallel with the carotenoids accumulation both in 'Zaozhong 6' and 'Baiyu' during fruit development. And further study was needed to show the relationships between carotenoids accumulation and the expression of *IDI*, *ZEP* and *VDE*.

Keywords: Loquat (*Eriobotrya japonica* Lindl.), carotenoids, gene cloning, expression.

PO-26

Track: Medical Biotechnology

ELEVATED EXPRESSION OF EPHA3 CONTRIBUTES TO MALIGNANT PROGRESSION OF PROSTATE CANCER BY PROMOTING NEUROENDOCRINE DIFFERENTIATION OF TUMOR CELLS

Rui-qin Wu, Hong-Tao Wang, Shan-Hu Li, Ya-Li Zhao, Jian Wang and Jian-Guang Zhou

Beijing Institute of Biotechnology, 27# Taiping Road, Haidian, Beijing 100850, P.R. China;
Email: zhou.jianguang@yahoo.com.cn



EphA3 gene has been identified to be associated with multiple tumor progression. Previous studies have revealed that EphA3 possesses transforming activity and confers malignant phenotypes to NIH3T3 cells. However, the functional relevance of EphA3 expression changes during prostate cancer (PCa) development and progression remains to be evaluated. In this study, the functional significance and mechanisms by which EphA3 contributes to PCa was investigated using *in vitro* cell lines, *in vivo* mice model and clinical specimens. Experimental data showed that EphA3 was overexpressed in the androgen-dependent and -independent PCa cell lines, LNCaP and C4-2B, respectively. EphA3 expression was in positive correlation with prostate cancer cell growth and anchorage-independent colony formation and cell migration, as well as tumorigenicity in athymic mice. EphA3 protein expression was found in 59/73 (80.8%) clinical prostate tumor specimens, and in 8/27 (29.6%) normal prostate tissues. A positive correlation between EphA3 expression and Gleason grades of prostate cancer was also observed. Moreover, EphA3 promotes androgen-independent growth and androgen antagonist Casodex resistance of PCa cells. Further, we found that EphA3 enhanced neuroendocrine differentiation (NED) of tumor cells, suppressing AR signaling, activating Akt pathway, which was essential for NED of PCa cells. Overall, these findings establish EphA3 as an important therapeutic target in the treatment of PCa.

Keywords: EphA3, prostate cancer, neuroendocrine differentiation, Akt pathway.

PO-3

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

GENETIC DIVERSITY IN CUCURBITA PEPO LANDRACES FROM NORTHERN KWAZULU-NATAL, SOUTH AFRICA, REVEALED BY RAPD MARKERS

Nontuthuko R. Ntuli, Pangirayi B. Tongoona, Nokuthula W. Kunene and Alpheus M. Zobolo

Department of Botany, University of Zululand, South Africa; Email: azobolo@pan.uzulu.ac.za



Genetic variation in seven selfed and unselfed *Cucurbita pepo* landraces from districts in KZN Province was investigated using the random amplified polymorphic DNA (RAPD). Out of 36 primers screened nine were selected, which gave 100 clear and bright fragments, out of which 94 (94%) fragments were considered polymorphic. The sizes of bands ranged from 75 to 1800 bp. The number of bands per primer ranged between nine (CB12) and 14 (CB19). Primer CB9 had the lowest: effective number of alleles (N_e), Nei's gene diversity index (H), genetic diversity index within populations (H_s), while primer CB23 had the highest. The genetic differentiation coefficient between populations (G_{ST}) ranged between 0.0022 (CB13) and 0.0100 (CB23), while the gene flow ranged between 49.4545 (CB23) and 223.7226 (CB23). The effective number of alleles, Nei's gene diversity index and Shannon's information index were the highest in ZS population ($N_e = 1.2046$; $H = 0.1677$; $I = 0.3060$) and the lowest in TNS population ($N_e = 1.1512$; $H = 0.1301$; $I = 0.2518$). The production of specific RAPD markers by different primers indicated gene diversity between: selfed and unselfed populations from the same geographic origin; populations that changed fruit colour to yellow/orange at maturity from a population that maintained green colour; and also among different populations in general. The TS and TNS populations, both from uThungulu district, were the highest in genetic identity

(IN = 0.9996) and the closest in the genetic distance ($D = 0.0004$). The CPSP and MNS populations (from Umkhanyakude district) as well as CPSP and TNS populations were the lowest in genetic identity (IN = 0.9985) and the furthest in genetic distance ($D = 0.0015$). The dendrogram grouped the populations into two main clusters which were discriminated according to fruit colour change at maturity, and then the second cluster had sub-clusters that grouped populations according to their geographical origin, where populations from Umkhanyakude district grouped together while those from uThungulu and Zululand districts made their own sub-cluster. All genetic parameters indicated that there was plentiful genetic diversity in *Cucurbita pepo* landraces of northern KwaZulu-Natal, South Africa.

Keywords: *Cucurbita pepo* landraces, genetic variation, self-pollination, RAPD markers, northern KwaZulu-Natal.

PO-??

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

A PILOT STUDY OF SMALL DOSAGE OF CHEMOTHERAPY AND TRADITIONAL MEDICINE IN ADVANCED CANCERS: REPORT OF TWENTY-FOUR CASES

George Zhu

The Institute of Oncology of George Zhu, 422407, China; E-mail: sansan4240732@163.com

Background Traditional systems of medicine all over the world even traditional medicine and cancer have been using plants and plants products for therapeutic purposes. In search for effective strategies of cancer therapeutics, I had summarized in my group the respective study of cancers under remission, with small dosage of chemotherapy in conjunction with plant medicine traditional medicine. Methods. 24 patients with available cancers were entered in the study during 1993-2011. The sex ratio of male:female was 12:12 respectively. The mean age at onset was 41.5 years(range 14-79 years).The other benign neoplasias were not statistically included.The criteria of complete remission(CR) and/or partial remission(PR) is according to the rules where physician have in common with in clinics. Results. In 24 cases, the complete remission(CR) was obtained in 17 advanced cancers. As to approach to the schedule of drug administration, fourteen patients with cancers were through a major plan of traditional herbs and the addition of small dosage of chemotherapy, and eight cancers with traditional medicine alone. All patients were survival over 12 or 18 months. In details, the survival time in three lung cancer was 5, 10 and 17 years, 1 gallbladder cancer 9 years, 2 lymphoma each 7 and 10 years, 1 gastric cancer 7 years, and 1 thyroid cancer 5 years respectively. During follow up, two patients the breast was inflamed redness, swelling, and a mega-enlarged and firm mass palpable. Cures were achieved by the primary use of traditional herbs with antibiotics regimen. A 62-year-old female multiple myeloma was in CR after low-dose thalidomide and plant medicine *Vinca rosea*(*Catharanthus roseus*). Traditional medicine consisted of *Vinca rosea*, *astragalus membranaceus* Bunge, *ophiopogon japonicas*, *asparagus cochinchinensis*, *angelica sinensis*, *poria cocos*, *coix lacryma jobi* L. var *ma-yuen*, *solanum nigrum* L, *houltuynia cordata*, *scutellaria barbata* d.don and *oldenlandia diffusa* roxb. During the follow up of four years, She was remained CR. CR was once obtained after all-trans retinoic acid (RA) and low-dose (1mg) plant medicine homoharringtonine intravenously in one case of acute promyelocytic leukemia(APL). Conclusion. In this study, I experienced that a CR was a pivotal influencing factor in those longest survival patients, and traditional medicine was also recommended. As a novel approach to APL treatment, one possible the action of RA, A consense sequence (5'-TCAGGTCATGACCTGA-3') has been postulated for the thyroid hormone (TRE) and retinoic acid responsive element(RARE) containing half palindromes, which located in the promoter region of target genes. High dose (100-fold) of RA-RARE-PML/RARa complex in intracellular localization appears to relieve repressor from DNA binding, including corepressors N-CoR, SMRT and HDACs, release PML/RARa-mediated transcriptional repression(Lallemand- Breitenbach and de The 2010), and release histone deacetylase activity from PML-RARa (Grignani F, 1998). The resulting PML/RARa oncoprotein proteolytic degradation through the autophagy-lysosome pathway and the ubiquitin SUMO-proteasome system (UPS) (Isakson P, 2010), as well as caspase 3 (cleavage site Asp522 within a-helics region of PML component of the fusion protein) (Nervi C, 1998)or neutrophil elastase(Lane AA, 2003), or lysosomal protease(Kitareewan S, 2007) enzyme induction. PML protein relocates into the wild-type nuclear body(PML-NB) configuration or/and wild-type RARa upregulated. An effect to relieve the blockade(inhibition) of PML/RARa-mediated RA dependent promyelocytic differentiation, and all-trans retinoic acid in APL therapy (see Figure , George Zhu, 1991). Here, like v-erbA, PML/RARa is a (strong) transcriptional repressor of the RA receptor(RAR) complex (George Zhu, 1991, 2007; Rousselot P, *et al*, 1994; Jing Y, *et al*, 2004; de The H, 2010; Isakson P, *et al*, 2010), and PML/RARa fusion receptor gene act as conditional oncogenic receptor(translocated chimeric retinoic acid receptor a signaling) or oncogenic PML/RARa(George Zhu, 1991, 2007, 2012; Zhu J, Nasr R, 2007;Rabellino A, 2012;Thomas M, *et al*, 2012) may participate in leukemogenesis of APL through blocking RA-

mediated promyelocytic differentiation(Grignani F, *et al*, 1993; Rousselot P, 1994). This molecular genetic regulatory model of RA is the some mimicry of "lac operon" in some ways. This is first described in eukaryotes.

***ADDITIONAL
ABSTRACTS***

PO-83*Track: Others - Food and Fermented beverages***INCREASE IN ANTIOXIDANT AND ANTIHYPERTENSIVE PEPTIDES FROM CABERNET SAUVIGNON WINE BY OENOCOCCUS OENI****Gisselle Raquel Apud, Maria Jose Rodriguez Vaquero and Pedro Adrian Aredes Fernandez***CERELA, Belisario Roldan 396 - San Miguel de Tucuman - Tucuman – Argentina; Email: pedroaredes@fbqf.unt.edu.ar*

The study of peptides with biological beneficial activities to human health, such as antihypertensive and antioxidant activities, present in foods has been a challenge that has been taken up in the past few years. Angiotensin I-converting enzyme (ACE) increases blood pressure by raising vascular resistance, therefore ACE inhibition produces a hypotensive effect. The free radicals, which are physiologically produced, could be result in cellular damage. Antioxidant peptides may function by preventing the formation of radicals or by scavenging radicals or hydrogen peroxide and other peroxides. *O. oeni* m, the major species found in wine during malolactic fermentation, is able to hydrolyze grape juice proteins via extracellular enzyme activity. *O. oeni* m was previously reported as proteolytic strain with relevant technological properties for vinification process. The current study examines the modification of the antihypertensive and antioxidant activities produced by the proteolytic activity of *O. oeni* m on the protein fraction of high molecular weight of a commercial Argentinean wine.

Protein-polypeptide fraction (PPF) was obtained from a commercial red wine, cabernet sauvignon varietal, by dialysis during 48 h through 10000 Da pore size membrane. *O. oeni* m was inoculated at 108 Log ufc/ml in Syntetic wine medium (SWM) in presence of PPF. Microbial growth was determined by optical density measurement and viable cell count. Proteolytic activity, amino acid and peptide concentrations were measured by Doi method. I-ACE activity percentage was measured by Cushman and Cheung method. Antioxidant capacity was evaluated by ferric reducing/antioxidant power (FRAP) and DPPH radical scavenging activity methods.

In SWM added with PPF of cabernet sauvignon wine, *O. oeni* maintains viability after 48 h incubation time and enables the increase of extracellular proteolytic activity and the release of peptide nitrogen fraction of 1.067 mg N/L. After 48 h incubation time, concomitantly with the peptide release, the maximum increase in antioxidant activities (366.1 μ mol FeSO₄/L in the case of ferric reducing antioxidant power and 8.9% in 2,2-diphenyl-1-picrylhydrazyl radical scavenging) were observed. The released peptides from cabernet sauvignon wine also enable the increase of antihypertensive activity of the samples (ACE inhibition of 63.8 %).

Oenococcus oeni m would provide additional benefits to wine, that include the increase of bioactive peptides with multifunctional beneficial activities

PO-85*Track: Medical Biotechnology***EXPRESSION OF CARBOXYL TERMINUS OF HSP70-INTERACTING PROTEIN (CHIP) INDICATES POOR PROGNOSIS IN HUMAN GALLBLADDER CARCINOMA****Zhe Long Liang and Jin-Man Kim***Department of Pathology, Regional cancer center and Infection Signaling Network Research Center, Chungnam National University School of Medicine, 6 Munwha-Dong, Jung-Gu, Daejeon 301-131, Korea; E-mail: jinmank@cnu.ac.kr*

Gallbladder carcinoma (GBC) is a lethal neoplasm, and new prognostic markers are needed. Deregulation of E3 ligases contributes to cancer development, and is associated with poor prognosis. Carboxyl terminus of heat shock protein 70-interacting protein (CHIP) is a U box-type E3 ubiquitin ligase; however, its role in GBC has not been evaluated. Therefore, we investigated CHIP expression in GBC and its prognostic significance. In this study, we examined CHIP expression in 78 tumor specimens with GBC by immunohistochemistry and analyzed the correlation between CHIP expression and clinicopathologic factors. Of the tumor specimens, 26.9% showed strong staining intensity (CHIP high-expression group [CHIP-HEG]). CHIP-HEG was not associated with other common clinicopathologic parameters, such as T stage, nodal and distant metastasis. CHIP-HEG patients had a significantly worse prognosis than patients with low CHIP expression with a median cancer-specific survival time of 8.0 months (range, 1-34) and 13.0 months (range, 1-110), respectively ($P = 0.023$). Multivariate analyses showed that CHIP expression was close to being an independent

risk factor predicting patient survival. CHIP expression may be associated with poor prognosis of GBC. Because CHIP is not associated with other clinicopathologic prognostic factors, it may serve as an ideal molecular marker for predicting patient outcomes.

Keywords: Carboxyl terminus of Hsp70-interacting protein (CHIP), gallbladder carcinoma, prognosis, survival

PO-84

Track: Plant and Environment

USE OF GENETIC MODIFICATION FOR QUALITY IMPROVEMENT OF FLOWERING ORNAMENTALS

Heiko Mibus, Sridevy Sriskandarajah and Margrethe Serek

Department of Natural Sciences, Leibniz University of Hannover, Hanover, Germany

Genetic modification has been proved as an effective way in improvement of quality of a range of ornamental crops. In the last decade several studies have focused on extension of flower longevity in climacteric ornamentals via controlling ethylene synthesis and perception. Use of the mutant ethylene receptor gene, *etr1-1*, from *Arabidopsis thaliana* seems most promising, especially when it is expressed under the control of a flower specific promoter.

We established effective regeneration and transformation systems for popular flowering ornamentals, such as *Campanula* and *Kalanchoë*, in order to introduce the *etr1-1* mutant gene. Fertile transgenic plants of *Campanula carpatia* cv. Blue Uniform and *Kalanchoë blossfeldiana* cv. Debbie were obtained by *Agrobacterium tumefaciens*-mediated transformation, where *etr1-1* gene were expressed under the control of the flower specific *fbp1*-promoter from *Petunia* plants. Flowers of the transgenic plants were tested for their ethylene sensitivity by continuous exposure to 2 ppm ethylene. Non-transgenic flowers of both plant species wilted within 2 and 3 days (*Campanula* and *Kalanchoë*, respectively). The best transgenic line of *Campanula* flowered in ethylene up to four weeks, while the best *Kalanchoë* line had more than 2/3 open flowers for longer than 10 days of continuous ethylene exposure. *Fbp-1* flower specific promoter successfully prevented expression of *etr1-1* in leaves or roots, which allowed cutting propagation of both transgenic species. Additionally, T1 progenies showed stable inheritance and expression of *etr1-1*, which makes the plants useful for future breeding.

Commercial registration of transgenic ornamentals requires, however, solving of several issues, especially in European countries. One of critical issues is the use of antibiotics resistance for selection, which cause public criticisms and registration refusal from authorities side.

Keywords: *Agrobacterium tumefaciens*, *Campanula*, *etr1-1*, flower longevity, flower specific promoter, inheritance, *Kalanchoë*

PO-67

Track: Plant and Environment

NEW TECHNOLOGY OF GEOECOLOGICAL LIFE SUPPORTING OF A MAN: DRINKING WATER AS AN EFFECTIVE HELIO-GEROPROTECTOR

A.V. Trofimov, G.I. Drujinin and K.K. Gibert

International Scientific Research Institute of Cosmic Anthropoecology, LLC "AkvaHelios", Russia, Novosibirsk; Email: isrica2@rambler.ru

In conditions of continued decrease in induction of the Earth magnetic field, reduction of its buffering properties and corresponding increase in access of solar corpuscular energy flows to biosphere, the problem of surviving of humanity and biotechnological protection of all living on our planet is becoming one of the most actual.

The aim of the study was the development and approbation of effective nonmedicinal helioprotective means, widely available to the population of different countries.

As a result of scientific research in 2006-2012 such remedy was found – it is water, which has passed a special technological processing in the installations with weakening of the magnetic field of the Earth many times (patent of RF

№ 2342149). It has been shown that drinking water after its exposure in these conditions acquires the most important qualities of helioprotector, when a new structured water systems of an organism appear to be able to change the character of dependence of biothermodynamic processes, estimated by the ratio of stable isotopes of carbon ($^{13}\text{C} / ^{12}\text{C}$) in the tissues as a markers of biological age, on the intensity of many heliogeophysical factors and thus reduce the rate of aging of an organism.

At taking drinking water, processed in the “open space”, the vector of associations (correlations) of the functional activity of the various systems of plants, animals and human beings with many dangerous to life and health heliogeophysical factors is also positively changing. In particular it has been shown that in patients-volunteers with arterial hypertension (n=76) at testing admission of drinking water “AKVAHELIOS” the correlation dependence of the pulse wave velocity, endothelial function of blood vessels and arterial pressure level, estimated on the device “Tonocard” (Russia), on the value of the solar proton-electron flows, registered by satellites “Goes” (USA, NACA), which sharply increase at periodic energy flares on the Sun, significantly ($p < 0,05$) reduces.

Thus the range of new technologies of preventive medicine is replenished by effective nonmedicinal helio-heroprotective means, which can reduce helio-dependence of an organism, risks of many heliodependent diseases and the rate of age processes.

In March 2013 in Novosibirsk the production of drinking water “AKVAHELIOS” will start. It should become one of the main elements of the global system of geoecological life-supporting in conditions of present and future changes of heliogeophysical and climatic situation, threatening human health and life.

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PO-51

Track: Industrial and Manufacturing

PRODUCTION OF PROTEOLYTIC ENZYMES BY BACILLUS LICHENIFORMIS IN A CLASSICAL CONTINUOUS BIOREACTOR AND MEMBRANE BIOREACTOR

Anna Trusek-Holownia

*Department of Chemical and Biochemical Processes, Wroclaw University of Technology, Poland;
Email: anna.trusek-holownia@pwr.wroc.pl*

The subject of the research was a production of extracellular proteolytic enzymes by the bacterial strain *Bacillus licheniformis* in a classical continuous bioreactor and in a membrane bioreactor. It has been noticed that at the residence time equal to 20 hours the microorganisms produced the biggest mass of proteins and the value of proteolytic activity was the highest. In the study the impact of glucose concentration in dosed stream on the increase of microorganisms, productivity of the proteins and glucose consumption has been also identified.



It has been shown that applying a bioreactor integrated with a microfiltration membrane it is possible to obtain the same amount of proteolytic enzymes in a much shorter time than in the classical flow bioreactor.

The methods which were used for the purification of proteases included ammonium sulfate precipitation, dialysis, ion-exchange chromatography as well as separation on an ultrafiltration membrane. On the basis of the conducted research, it has been stated that membrane separation appeared to be the best method. It has been noticed that the optimal temperature to obtain and purify proteolytic enzymes was 50°C. This preparation occurred to be quite stable because the half-life time lasted for around 140 hours.

Keywords: membrane bioreactor, enzyme production, protease, purification.

SL-77**Track:** Medical Biotechnology**THE RECENT TECHNOLOGICAL ADVANCEMENTS IN DNA SEQUENCING THAT OFFER THE POTENTIAL FOR NEW DISCOVERIES****Laura Ingram**

The AGBL Group of companies is the largest biomedical gateway to the emerging markets of Middle East, Africa and Asia. The group is dedicated to bringing the latest technologies and products to researchers, clinicians, diagnostic users and healthcare providers in the markets it covers. Following a brief introduction to their portfolio, this talk will focus on the recent technological advancements in DNA sequencing that offer the potential for new discoveries. The rapid speed increase and cost reduction of next generation sequencing technology is enabling powerful opportunities to researchers and clinicians. The presentation will focus on the MiSeq instrument; the latest example of Illumina innovation, which combines Illumina's proven sequencing technology and a revolutionary workflow to create a cost-effective, highly accurate system for a wide range of next-generation sequencing applications. The MiSeq offers a robust applications roadmap, covering amplicon sequencing, small genome sequencing, metagenomics, targeted large genome sequencing as well as diagnostic tools. Supported by streamlined sample preparation technologies, these applications enable MiSeq to be a flexible next-generation sequencing system for every laboratory.

PO-87**Track:** Plant & Environment**IMPROVING YIELD PERFORMANCE OF *PLEUROTUS PULMONARIUS* THROUGH HYPHAL ANASTOMOSIS FUSION OF DIKARYONS****E.A. Adebayo, J.K. Oloke, A. Yadav, M. Barooah and T.C. Bora**

Department of Pure and Applied Biology, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Nigeria; E-mail: brogoke2003@yahoo.com



High production and good quality are always the principal goals for agriculturally important crops, without the exception of mushrooms. *P. pulmonarius* is one of the commercially important edible mushrooms throughout the world. The yield performance improvement was carried out by cross bred *P. pulmonarius* with *P. sapidus* and *P. ostreatus*. The highest rate of 0.587 mm/d for spawn ramification and 53.33% for percentage spawn productivity were obtained in hybrids LN LL910. The least day (11th and 12th) of the primordia mushroom sporophore were recorded in LL910 and LN 97 respectively, while longest day of 19th was recorded in wild type (NE 07). The highest biological efficiency (109.30%) and production rate (3.77%) obtained by LL910, while the least of 33.0% and 0.79% were obtained by NE 07 for biological efficiency and production rate respectively. The morphological and molecular characterization of the hybrid strains established their true variation from their wild type. LL 910 (JF68088) is located at seventh subclusters from the root with bootstrap value of 32%, while only one parent (LAU 09: JF736658) out of the two has the close bootstrap value of 43% at the first subcluster to the root, with the other parent LAU 10 (JF736659) shows distance relationship after Blast. LN 97 (JF680992) is located at outgroup, while the parent strains NE 07 (bootstrap value: 11%) and LAU 09 (bootstrap value: 44%) located at tenth and second subclusters respectively. The results obtained from this study have shown the improved performance of the hybrids strain over wild type strains.

PO-88**Track:** Plant and Environment**ASSESSMENT OF DIFFERENT IMMERSION FREQUENCY AND PERIOD ON PISTACHIO MICROPROPAGATION IN TEMPORARY IMMERSION SYSTEM****Hülya Akdemir, Yelda Özden Çiftçi and Veysel Süzerer**

Department of Molecular Biology and Genetics, Gebze Institute of Technology, Turkey;
E-mail: pinarakdemir@gmail.com

RITA[®], temporary immersion bioreactor system has various advantages such as supplementation of temporary and preferable contact between plant and medium, renewal of culture atmosphere on each immersion and reduced cost in comparison to semisolid and liquid culture systems. In this study, effect of different immersion frequencies and periods on pistachio shoot apices and nodal buds were tested in TIS in order to optimize *in vitro* regeneration system. The explants cultured on semisolid standard MS proliferation medium supplemented with 2 mg/L BA and 0.5 mg/L GA3 were used as control of the study. In order to test the effect of different frequency, each RITA[®] unit contained 200 ml liquid proliferation medium and 20 uniform explants was programmed to submerge the explants one period (16 min) per various immersion frequency (8, 16 or 24 h). The best shoot forming capacities (SFC) for shoot tips (2.72) and nodal explants (3.05) were obtained with 16 min of immersion period per 16 h. Furthermore, we tested the effect of reduced (8 h) or elevated (24 h) immersion period per 16 h to improve regeneration capacity. The highest regeneration capacity (92% and 100% for shoot tips and nodal explants, respectively) and SFC (2.60 and 3.55 for shoot tips and nodal explants, respectively) were obtained with the increased immersion period (24 h) in comparison to semisolid medium. The optimized system can be applied to commercially important other pistachio cultivars to scale up micropropagation.

Keywords: Pistacia vera, RITA[®], bioreactors.

PO-89

Track: Industrial and Manufacturing

THERMAL STABILITY OF IMMOBILIZED RAW STARCH DIGESTING AMYLASE (RSDA) FROM ASPERGILLUS CARBONARIUS IN THE PRESENCE OF POLYOLS AND SUGAR

C. Amadi Onyetugo, N. Okolo BarthoSilva, M. Tony, C. Pessela Benevedes, C. Mateo, M. Guisan Jose and N. Moneke Annie

Department of Microbiology, University of Nigeria Nsukka, Nigeria; Email: chioma.amadi@unn.edu.ng

Raw starch digesting amylase (RSDA) from *A. carbonarius* was immobilized on CNBr-activated Sepharose this is a mild covalent attachment as the activity and stability of this derivative are almost identical to ones of soluble enzyme. The aim of the study was to evaluate the thermal stability of the immobilized and soluble amylase in the presence of sugar (trehalose) and different polyols (mannitol, glycerol, polyethylene glycol) in varying conditions of temperature, pH and buffer ionic strength. All additives but Polyethylene glycol showed a protective effect on the immobilized enzyme heat stability, which was strongly dependent on the added compound concentration, temperature, pH and buffer ionic strength. Among all stabilizing compounds investigated, trehalose exhibited the highest protective effect on the soluble enzyme. In the presence of trehalose the soluble RSDA was 10 fold more stable than in the absence the of additives. The best conditions were: 10mM Citrate buffer pH 5.0, containing 30% of trehalose at 53°C over 6 hour incubation period. The stabilizing effect of additives is interesting to improve storage and transportation of immobilized derivatives.

Keywords: Immobilization, RSDA, additives, *Aspergillus carbonarius*

PO-91

Track: Plant & Environment

MACROPROPAGATION OF MEDICINAL PLANTS, TINOSPORA CRISPA FOR DOMESTICATION PURPOSES

H. Aminah, M. S. Ahmad Fauzi, H. Tariq Mubarak and M. Hamzah

*Forest Research Institute Malaysia (FRIM) 52109, Kepong, Selangor;
Email: aminah@frim.gov.my*

Tinospora crispa belongs to the family of Menispermaceae. This tropical liana (woody) with shiny green leaf is also widely distributed in Indonesia, Malaysia including Borneo, Thailand and Vietnam. It has being cultivated as a medicinal plant in Thailand, Sri Lanka and India. In Malaysia, its stem has been traditionally used for various therapeutic purposes such as treatment for diabetes, hypertension, stimulation of appetite and protection from mosquito bites. Recent studies have shown that *T. crispa* has the potential to be a source of natural antioxidants and nutrients, besides having a moderate anti-proliferative effect on selected human cancer cell lines. Besides that, supplementation of *T. crispa* extract was able to



reduce or retard the progression of atherosclerotic plaque development induced by dietary cholesterol. Due to its potential uses, the demand for this species is increasing yearly. To ensure the continuous supply of raw materials, a study was conducted to develop macropagation protocol for *T. crispa* to mass produce planting materials for domestication purposes. An experiment on using three cutting length and two hormones treatments was carried out. The length of cutting used was: 7.5 cm, 15 cm and 22.5 cm. The base of each cutting was treated with 2 commercial powdered hormones and a control (without hormone: 1) Seradix 1 (0.1% indole butyric acid-IBA), 2) Seradix 2 (0.3% IBA) and 3) control (without hormone). Results 12 weeks after planting showed that cuttings of 22.5 cm length produced significantly higher rooting (81%) than the other two length (59% & 38%). Similar results were obtained with the number of roots where 22.5 cm length had significantly most number of roots (1.9) compared to the other two lengths (1.3 & 1.2). This experiment showed that *T. crispa* can be easily mass propagated by cuttings as source of planting materials.

Keywords: Vegetative propagation, stem cuttings, rooting percentage, root number.

PO-92

Track: Plant & Environment

BIODEGRADATION OF POLYCYCLIC AROMATIC HYDROCARBONS PRESENT IN CRUDE OIL BY LOCALLY ISOLATED BACTERIA

A.A. Ayande, O.E. Fagade and H.M. Dionisi

Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomosho, Nigeria;
E-mail: lizdeley@yahoo.com

Ten bacterial strains that were able to utilize crude oil as their sole source of carbon and energy were isolated from soil contaminated soil and used for biodegradation experiment. The bacterial isolates were identified by classical method and molecular techniques of 16SrRNA analysis. The biodegradation experiment showed that the bacterial isolates were able to degrade about 55.2 to 77.1% of hydrocarbons present in the crude oil, with *Providencia* sp 3 having the highest degradation rate of 77.1%, followed by the other two species of *Providencia* species 1 and 2 (69.5%) while *Proteus* sp had the least degradation rate of 55.2%. Gas Chromatography was used to analyze the degraded oil in order to determine the percentage of degraded polycyclic Aromatic Hydrocarbons (PAHs) present in the crude oil and the analysis revealed that about 25.5% to 96.5% of the PAHs present in the crude oil had been degraded by the selected bacterial strains. About 35.1 to 99.6%, 16.2% to 99.6%, 45.6 to 99.1%, 61.0 to 99.7% and 47.4 to 99.7% of Naphthalene, Acenaphthylene, Acenaphthene, Fluorene and Phenanthrene were degraded respectively by the selected bacterial strains.

Keywords: Biodegradation, providencia sp, PAHs, classical method, 16SrRNA, bacterial strains.

PO-93

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

EFFECT OF ORAL ADMINISTRATION OF *SCUTELLARIA BAICALENSIS* GEORGI EXTRACT ON PERFORMANCE AND DISEASE CHALLENGE TEST OF CATFISH (*SILURUS ASOTUS*)

Sung Hwoan Cho, Gyu Ho Jeon and Hee Sung Kim

Division of Marine Environment and BioScience, Korea Maritime University, Korea; E-mail: chosunh@hhu.ac.kr

Effects of various concentrations of *Scutellaria baicalensis* (SB) extract in diets on growth and disease challenge test of far eastern catfish (*Silurus asotus*) were determined and compared to a commercially available immune enhancer. Eight experimental diets were prepared in triplicate: control (Con) diet without supplementation of SB and SB-0.25, SB-0.5, SB-1, SB-2, SB-3 and SB-5 diets containing SB at concentrations of 0.25, 0.5, 1, 2, 3 and 5%, respectively. In addition, 0.1% of a commercial immune enhancer product (CP) was also tested. No significant difference in weight gain of fish was found. Feed consumption, feed efficiency ratio and protein retention of fish was not affected by the experimental diets. At the end of the 8-week feeding trial, ten externally normal fish from each tank were infected by *Vibrio anguillarum* or *Streptococcus iniae*. Cumulative mortality of fish fed the Con diet was higher than that of fish fed the all other diets in 10 and 25 days after *V. anguillarum* or *S. iniae* infection. Results of this study indicated that dietary inclusion of SB extract was effective in improving survival of eastern catfish after *V. anguillarum* and *S. iniae* infection, but the various concentrations of SB did not affect fish performance.

Keywords: *Scutellaria baicalensis*.

PO-94**Track:** Plant & Environment**THE EFFECTS OF CRUDE EXTRACTS AND FRACTIONS OF *ALCHEMILLA ABYSSINICA* ON SMOOTH MUSCLE OF GUINEA PIG ILEUM****Atkilt Esaiyas***Hawassa University, School of Medicine, Hawassa, Ethiopia; E-mail: atkiltesa@yahoo.com*

Background: *Alchemilla abyssinica* is a plant widely used in traditional medicine. Its wide use among the community plus already established scientific evidences for medicinal values of other *Alchemilla* species provided good ground for this investigation. **Methods:** In this research, CHCl₃/EtOAc 1:1 extract of dried aerial parts of *Alchemilla abyssinica*, methanolic extract of the CHCl₃/EtOAc residue and fractions of the methanolic extract were tested on isolated guinea pig ileum (GPI) for possible presence of spasmogenic or spasmolytic effects. Concentrations of each extract and fraction ranging from 20-600 µg/ml final organ bath concentration were tested. The effects of these test samples on the basal rhythmic contractions of the GPI as well as on its contraction elicited using the agonist, histamine, were determined. The antagonist, Papavarine, was also used as a control smooth muscle relaxant. **Results:** While the CHCl₃/EtOAc 1:1 extract showed neither spasmogenic nor spasmolytic result, the methanolic extract showed marked spasmolytic effect. This methanolic extract was fractionated using column chromatography and the fraction eluted using Hexane/EtOAc 1:2 gave greatest spasmolytic result and it was taken as the final test fraction. The final test fraction produced significant ($P < 0.05$) dose-dependent spasmolytic effects on the agonist induced contractions of the GPI to 95.7% at 20 µg/ml, 43.6% at 70 µg/ml and 14.2% at 120 µg/ml in the organ bath. **Conclusions:** the results of the present study showed that *Alchemilla abyssinica* possesses spasmolytic property. Also, the result of the present oral acute toxicity study showed *Alchemilla abyssinica* exhibited no toxicity up to doses of 1,000 mg/kg body weight in Swiss albino mice. It is highly recommended that the fractionation should proceed till final identification of the pure compound and taxonomically closely related plants should also be investigated.

Keywords: *Alchemilla abyssinica*, spasmolytic, guinea pig ileum.**PO-95****Track:** Plant and Environment**POTENTIAL BIOFUEL COMPOUNDS AND NOVEL TRITERPENE FROM A PHOMOPSIS SP. ISOLATE****Jose Guedes de Sena Filho, Jeff Shaw, Dan Spakowicz and Scott Strobel***EMBRAPA-Empresa Brasileira de Pesquisa Agropecuária, Tabuleiros Costeiros, Av. Beira Mar, 3250, Jardins, 49025-040, Aracaju, SE, Brazil; E-mail: Jose-guedes.sena@embrapa*

Biofuel production by microorganisms may provide an alternative source of energy to current fossil-derived fuels. The aim of this work was to identify endophytic fungi that produce Volatile Organic Compounds (VOC) that may serve as fuel surrogates. We screened over one hundred endophytes isolated from plants from the endangered Atlantic Coastal Rainforest of Brazil. One isolate was shown by GCMS to produce a series of monoterpenes, including α -phellandrene, α -terpinene, limonene, cymene, γ -terpinene, terpinolene and sabinene. This class of molecules has been successfully used as a gasoline additive. In addition, we identified a novel triterpene in a dichloromethane extraction and characterized it by NMR. Sequencing of the internal transcribed spacer rDNA region suggests this isolate is a member of the *Phomopsis* genus. We are now pursuing whole genome sequencing to identify the genetic basis of production of these molecules to better engineer their over the production.

Keywords: *Phomopsis*, VOC, triterpene, biofuel.

PO-96*Track: Medical Biotechnology***ANALYSIS OF FILAGGRIN EXPRESSION IN THE SKIN OF NORMAL AND ATOPIC DERMATITIS SUBJECTS****Ji-Sook Lee and In Sik Kim***Biomedical Laboratory Science, School of Medicine, Eulji University, Korea; E-mail: orientree@eulji.ac.kr*

Atopic dermatitis (AD) is characterized by chronically pruritic and inflammatory dermatitis. AD is caused by a variety of pathogenic factors, which include environmental components, such as allergen and genetic factors. Filaggrin is critical for the regulation of epidermal homeostasis. Filaggrin monomers can become incorporated into the lipid envelope, which is responsible for the skin barrier function. In this study, we investigated the expression of filaggrin in the skin of normal and atopic dermatitis subjects by non-invasive method. We examined the usefulness of a new patch to obtain protein sample from the skin of healthy subjects, and the patch is effective on protein acquirement from the skin. We detected the expression of filaggrin in lysates of normal skin by Western blotting. The expression of filaggrin in normal skin is stronger than that of AD skin.

Keywords: Filaggrin, atopic dermatitis, diagnosis.**PO-97***Track: Food; Marine; Bio-safety; Systems Biology; Bioethics***EFFECTS OF THE DIETARY PROTEIN AND LIPID LEVELS ON GROWTH AND BODY COMPOSITION OF RED- AND WHITE-COLORED FANCY CARP, CYPRINUS CARPIO VAR. KOI****Sang-Min Lee, Lee Oh Kim and Choi Jin***Department of Marine Bioscience and Technology, Gangneung-Wonju National University, Korea;
E-mail: smlee@gwnu.ac.kr*

A 8-week feeding trial of four dietary protein levels (20%, 30%, 40% and 50%) and two dietary lipid levels (7% and 14%) factorial design with three replications was conducted to investigate the optimum dietary protein and lipid levels for the growth of juvenile red- and white-colored fancy carp, *Cyprinus carpio* var. koi. (12.1±0.2 g initial weight). Daily feed intake of fish decreased with increasing dietary protein level at both lipid levels. Survival (100%) of fish was not affected by either dietary protein or dietary lipid level. Weight gain, feed efficiency and protein efficiency ratio of fish were affected by dietary protein level ($P<0.001$) but not lipid level ($P>0.05$). Weight gain of fish increased with increasing dietary protein level up to 40%. Feed efficiency of fish increased with increasing dietary protein level up to 50% regardless of dietary lipid level. Protein efficiency ratio of fish decreased with increasing dietary protein level regardless of dietary lipid level. The results of this study indicate that an increase of dietary lipid level cannot improve growth and feed utilization, and the diet containing 40% protein with 7% lipid would be suitable for the optimum growth and effective feed utilization of juvenile red- and white-colored fancy carp, *Cyprinus carpio* var. koi.

Keywords: Dietary protein, lipid, growth, red- and white-colored fancy carp, *cyprinus carpio* var. koi.**PO-98***Track: Food; Marine; Bio-safety; Systems Biology; Bioethics***EFFECTS OF DIETARY INCLUSION OF DISTILLERS DRIED GRAIN ON GROWTH PERFORMANCE, FEED UTILIZATION AND BODY COMPOSITION OF JUVENILE BLACK SEABREAM *ACANTHOPAGRUS SCHLEGELI*****Sang-Min Lee, J. Choi and M.M. Rahman***Department of Marine Bioscience and Technology, Gangneung-Wonju National University, Korea;
E-mail: smlee@gwnu.ac.kr*

This study was conducted to investigate the effects of dietary inclusion of distillers dried grain (DDG) level on growth performance, feed utilization, body composition and antioxidant enzyme activity of juvenile black seabream (*Acanthopagrus schlegeli*). DDG is a solid residue obtained by filtration of an aqueous mixture of fermented rice with

Aspergillus oryzae and yeasts. Five isonitrogenous and isocaloric diets were formulated to contain 0 (control), 60, 120, 180 and 240 g kg⁻¹ DDG designated as DDG0, DDG6, DDG12, DDG18 and DDG24, respectively. Juvenile black seabream averaging 1.2 ± 0.01 g was randomly distributed in fifteen 400-L tanks of a flow-through aquarium system. Three replicate groups of fish fed one of the experimental diets to visual satiation three times a day for 8 weeks. Weight gain was not affected by dietary DDG level ($P > 0.05$). Feed efficiency and protein efficiency ratio of fish fed the DDG24 diet were lower than those of fish fed DDG0 diet ($P < 0.05$). Daily feed intake and daily protein intake of fish fed the DDG24 diet were higher than those of DDG0 ($P < 0.05$). Proximate and amino acid composition of whole body were not affected by dietary DDG level ($P > 0.05$). The activities of superoxide dismutase and glutathione peroxidase in the liver were not affected by dietary DDG level ($P > 0.05$). The results of this experiment suggested that DDG is good ingredient to replace plant origin such as wheat flour and gluten meal and could be used up to 240 g kg⁻¹ for the optimum growth performance of juvenile black seabream.

Keywords: Dietary ingredient, distillers dried grain, growth, black seabream.

PO- 99

Track: Industrial and Manufacturing

COMPARATIVE ETHANOL YIELD IN THE FERMENTATION OF DATE FRUITS AND LEAVES BY *SACCHAROMYCES CEREVISIAE*

Sy Mudi, Md Mukhtar, R. Ajoke and B. Usman

Department of Pure and Industrial Chemistry, Bayero university PMB3011, Kano Nigeria ; E-mail-symudi@yahoo.com

In a quest for available and affordable natural resources that could be used in the production of alternative energy and more industrial raw materials without undue fear of wastage of staple food items, *Saccharomyces cerevisiae* was used in the fermentation of date fruit and leaves to ethanol. This was with a view to selecting the most Biotechnologically viable source among the leaves and the edible fruits. The process was conducted by suspending twenty grams of Date fruit sample in 200ml of water to hydrolyze the starchy content and 200ml of 0.2N NaOH was used to breakdown the lignin content of 20g of the Date leaves. Enzymatic hydrolysis was used to breakdown the cellulosic materials of the Date leaves while acidic hydrolysis was used for the Date fruits. The respective hexose sugars collected were separately fermented for five days (120 hours) at 28°C and pH of 4.8 using yeast (*Saccharomyces cerevisiae*). The liquor was distilled at temperature below 100°C in the fractionation column. It was rectified by re-distillation of the raw ethanol obtained and the remaining water was dried using potassium carbonate and quickly passed through a suction apparatus to minimize the escape of the ethanol into the atmosphere. The purity of the ethanol generated based on density and boiling point was confirmed as 0.787g/L and 78.3°C respectively. Its bactericidal potency was found to be in concordance with that of the standard ethanol. The percentage of ethanol yield from cooked Date fruit gave a maximum yield of 11.25%v/v; the uncooked Date fruit gave 9.47% v/v, while the Date leaves gave 12.67%v/v. It was therefore concluded that Date leaves with the highest ethanol yield would be the cheapest alternative source for ethanol production compared to the date fruit which serves as direct human food. This technology could help in circumventing the sociological fear that staple food items like date fruits are reduced into non consumable items in favour of industrial raw materials or sources of energy.

Keywords: Datefruits,leaves,Bioethanol,Saccharomyces cerevisiae Microbial fermentation

PO-100**Track:** Plant & Environment**EVALUATION OF POTENTIAL OF SOME RHIZOSPHERE BACTERIA AS BIOFERTILIZER STRAINS****MD. Mukhtar and WO. Okeji**

Department of Microbiology, Gombe State University, Gombe, Nigeria;

E-mail: mukhtardaouda@yahoo.com



The present study was prompted by the urgent need of identifying and isolating rhizosphere bacteria that could be reputed as resident soil strains towards biofertilizer development for use in Kano, Nigeria. This was achieved by screening several samples of rhizosphere soil of some garden plants in Mannitol Ashby's and Pikovskaya agar for nitrogen fixing and phosphate solubilizing bacteria respectively. Axenic cultures of the isolates were further tested for increased stimulation on seed germination, plant growth promotion in okro, pepper as well as their inhibitory action on phytopathogenic fungus, *Fusarium* sp by central disk test protocol. Statistical significance in the performance of the isolates was authenticated by ANOVA and Least significant difference test (LSD) at 5% probability level. Three isolates namely *Corynebacterium xerosis* (asymbiotic nitrogen fixer), *Citrobacter freundii* and *Bacillus panthothenicus* (phosphate solubilizer) showed significant properties in the promotion of growth and yield in the test crops. *B. panthothenicus* had highest effect on seed germination rate in pepper. *C. xerosis* exhibited the highest inhibitory activity against *Fusarium* spp while, the highest shoot height was stimulated by treatment with *C. freundii*. The inoculum density of these bacterial strains were standardized by McFarland turbidity procedure and mass multiplied in a constituted medium containing 1:1 (w/w) mixture of poultry dung and saw dust in 100ml distilled water and sterilized by autoclaving. Changes in bacterial population were monitored at 48 hour interval for nine days by plate counting techniques. The experiment was terminated at 10^9 cfu/ml (value exceeding the minimum required standard of 10^8 cfu/ml for fertilizer (British standard institute). Pot trial experiment of the candidate mixture on okra with *Corynebacterium xerosis* gave the highest yield of 61.3% compared to 38.7% in the control. The study shows that these isolates could serve as potential cultures for massive production of biofertilizer.

Keywords: Biofertilizer, biotechnology, *Bacillus panthothenicus*, *Citrobacter freundii*, *Corynebacterium xerosis*.**PO-101****Track:** Plant and Environment**KILLING EFFECT OF COMBINATION OF UV LIGHTS AND SOME CATALYST AGENTS ON SOME MICRO ORGANISMS ASSOCIATED WITH HOSPITAL INFECTION****Muftah Abdel Wahed Nasib**

Department of Microbiology, Omar Elmokhtar University, Libya; E-mail: Malhorer@yahoo.com

Hospital Infection is a one of the most hot area of research as its role in mortality and because of its part in increasing of inpatient health care cost and a new infection control methods must be invented and improved. The study was conducted in Sheffield university hospital in 2005 and the poster will illustrate some trails result that shows of the effect of some catalysis chemical to enhance the effect killing of UV light on the some pathogenic that cause hospital infection. *Staphylococcus aureus* and *C. albican* are both considered as the important source of Nosocomial and a lot of hospital equipment and medical devices can be contaminated by these organisms and play role in distributing them inside hospital wards and then infect patients. Using UV light to eliminate pathogens or reduce their number from devices surfaces is one of the sterilization methodology, in other hand, is more killing effect comparing with other UV lights, UVC light also has a harm effect on the technique users while using UVA is more safety, in this trail, we try to increase the killing effect of UVA light by combine them with Catalyst chemical such Titanium dioxide $TiO_2(B)$ or Psarolen ($C_{11}H_6O_3$). Certain number of Microbial cell was spread in dish and different concentration of the either Titanium dioxide $TiO_2(B)$ or Psarolen ($C_{11}H_6O_3$) added and then well mixed, then hit by UVA, UVB or UVC several hitting viral. Although no significant difference when hit the micro organism by either UVC light alone (control) or combination of UVC and the catalyst agent (treatment), the number of killed microbial cells was significantly increased by combination of catalyst agent and UVA comparing with the control where UVC using alone. The results show that Combination of UV with Psarolen is more significant than $TiO_2(B)$ and *S. aureus* more sensitive for hitting by the UV lights than *C. albican*.

Keywords: Environmental microbiology.

PO-102***Track: Plant and Environment*****EFFECT OF HEAVY METALS ON GLYPHOSATE UTILIZATION BY *PSEUDONOMAS FLUORESCENS* AND *ACETOBACTER* SP.****Gloria Ngozi Okpala and Moneke N. Anene***Department of Microbiology, University of Nigeria, Nsukka, Nigeria; E-mail: glocos2006@yahoo.com*

The effect of heavy metals (zinc, cadmium, chromium and lead) at concentrations 50, 100 and 500 g/ml of the heavy metal salts on glyphosate utilization by some bacterial species isolated from rice fields were studied, the addition of Pb^{2+} , Cd^{2+} and Zn^{2+} to the glyphosate mineral salt medium used in growing the *Acetobacter* sp. significantly ($P < 0.05$) increased the rate of glyphosate utilization as indicated by the increase in the growth of the organism and could be attributed to easy uptake of the metal-glyphosate complex by the organism. The growth of *Pseudomonas fluorescens* was enhanced in the following order: $Zn^{2+} > Pb^{2+} > Cd^{2+} > Cr^{2+}$ when compared with the metal free medium.

Keywords: Heavy metals, glyphosate utilization, *pseudomonas fluorescens*, *acetobacter* sp.**PO-103*****Track: Plant & Environment*****ANTIMICROBIAL ACTIVITY OF POLYPHENOLS FROM ARGENTINEAN FRUIT USING FLOW CYTOMETRIC ANALYSIS****Claudia V. Vallejo, Pedro A. Aredes - Fernandez, Marta E. Farías and María J. Rodríguez-Vaquero***Facultad de Bioquímica, Química y Farmacia. Universidad Nacional de Tucumán (UNT-CONICET), Ayacucho 471, (+54) 381 4247752 - 7067. Tucumán (4000), Argentina; Email: mariajo@fbqf.unt.edu.ar*

The aim of this work is the searching of natural antimicrobial compounds from Argentinean juices effective against eukaryotic and prokaryotic cells, using two different methods. A flow cytometric analysis was compared with the standardized method to determine the minimum inhibitory concentration (MIC) and minimum microbicide concentration (MMC).

Coincident results were found between both methods, which demonstrate that eukaryotic cells were more resistant than prokaryotic cells to juice treatment. Between the 9 juices assayed *strawberry* juices content the highest amount of total phenolic compounds and flavonoid fraction and lowest value was found in *genova* lemon juice. The antimicrobial effect observed with juices was related with phenolic compounds concentration but also with their phenolic profile.

The most important finding is the discovery of natural compounds, with antimicrobial properties, in fruit juices cultivated in northwest region of Argentina, that could replace synthetic antimicrobial agents used in pharmaceutical or food industry. Moreover, the use of a same rapid and reliable method to quantify and discriminate the number of viable, damage or dead cells in eukaryotic and prokaryotic cell suspensions, comparable with standardized assays is a big finding.

PO-90***Track: Plant & Environment*****USAGE OF SYNSEED TECHNOLOGY FOR THE MEDIUM-TERM CONSERVATION OF FEMALE PISTACHIO CULTIVAR****Veysel Süzerer, Hülya Akdemir and Yelda Özden-Çiftçi***Gebze Institute of Technology, Department of Molecular Biology and Genetics, 41400, Gebze, Kocaeli, Turkey; E-mail: beyso1985@gmail.com*

Development of synseed technology is critically important for commercially valuable species such as pistachio not only to maintain germplasm for medium- or long-term conservation but also to reduce cost and labor of micropropagation. In this study, synseed technology is utilized for the optimization of medium-term storage (until 12 months) of pistachio female 'Siirt' cultivar. In the optimized protocol, pistachio shoot tips were incubated in MS medium supplemented with

3% Na-Alg and 0.4 M sucrose for 2-3 minutes and then transferred drop by drop to liquid MS medium enriched with 100 mM calcium chloride where it is held for 25 min. Then the synseeds plated on 1 mg/L benzyl adenine containing MS medium and stored in 4°C without any illumination for different period of times (3, 6, 9 and 12 months). Following to storage period, the synseeds were immediately transferred to the fresh medium under standard culture conditions. After 3 and 6 months of storage, 31.2% and 53.1% of synseeds were proliferated, respectively. Furthermore, more than 80% of plant retrieval was obtained with the storage of synseeds for longer storage times. The relatively higher plant retrieval rates obtained with the longer storage periods could be due to the adaptation of the encapsulated explants to low temperature conditions. Overall results indicated that synseed technology could be used as a complementary strategy for the medium-term conservation of pistachio germplasm.

Keywords: ‘Siirt’, *in vitro* storage, *Pistacia vera*.

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