



**3rd Biotechnology
World Congress**
February 10 - 12, 2014, Dubai, UAE

ABSTRACTS

Dubai Women's College
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ORGANIZERS

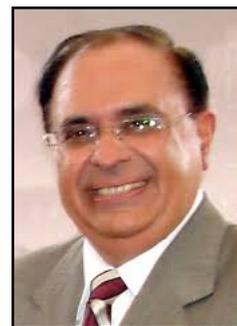


HIGHER
COLLEGES OF
TECHNOLOGY

WELCOME MESSAGE



It is our pleasure to extend a very warm welcome to the honourable scientists and young researchers participating in the two conferences -- the *3rd Biotechnology World Congress* and *6th International Conference on Drug Discovery & Therapy* the 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 Hamdan Bin Mubarak Al-Nahayan**, Honourable Minister of Higher Education and Scientific Research and **His Excellency Mohd. Omran Al Shamsi**, Chancellor, Higher Colleges of Technology for their cooperation. We are also most grateful to all the scientists who have travelled from the four corners of the world to the UAE to participate in these scientific symposia.

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

PROF. FERID MURAD
(Nobel Laureate)
Co-President

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

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PLENARY LECTURES

PL-55**CURRENT AND FUTURE DRUG TREATMENT OF OBESITY****Richard L. Atkinson**

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Obesity is a chronic disease, and like other chronic diseases, will require long term treatment. Single treatments, whether they be lifestyle changes or drugs generally have quite modest effects over the long term. Virtually all other chronic diseases are treated with more than one intervention and clearly obesity will require combination treatment as well. The combination of diet, exercise, and behavior modification (“lifestyle change”) is viewed as “standard treatment”, but has a poor long term success rate. Diet, exercise, and behavior modification combined with obesity drugs is somewhat more positive. The rigid structure of a dietary supplement as a meal replacement helps some people. However, the future of the treatment of obesity likely will reside with drugs and particularly with combinations of obesity drugs. Drugs change the biochemistry of the body and it is clear that obese people have a different biochemistry than do non-obese people. Food intake, energy expenditure, and storage of calories as fat have been so essential to survival of all organisms on Earth that it is not surprising that there are multiple redundant systems to stabilize them. Single drug treatments usually affect only one biochemical pathway or physiologic system. Single drugs currently used for the treatment of obesity all show modest weight loss. The current drugs used singly are adrenergic agonists (eg phentermine); orlistat, a lipase inhibitor; and locaserin, a 5-HT_{2c} serotonin re-uptake inhibitor. Phentermine may produce a 10% or more wt loss, but orlistat and locaserin produce only about a 5% wt loss. Until recently, there have been few combinations of obesity drugs studied or approved. The best known was the combination of phentermine and fenfluramine or dexfenfluramine. Phen-fen produced wt loss of ~16%, the largest wt loss of any obesity treatment except obesity surgery. The removal of fenfluramine and dexfenfluramine from the market relegated obesity treatment to single drugs again since the combinations of orlistat and either sibutramine or phentermine are reported to be ineffective. The combination of phentermine and fluoxetine attempted to reproduce the success of the combination of an adrenergic agent and a serotonergic agent. Phen-flu was not quite as effective as phen-fen, but since both drugs are available on the market, this combination may be used with caution for obese people by physicians willing to run the risk of displeasure of the governmental authorities. Recently the combination of phentermine and topiramate has been approved. Studies show a wt loss of 10%-13% at one year. The combination of bupropion and naltrexone is currently under consideration by the FDA and clinical trials show a weight loss of about 6%-8%. Locaserin is approved only as a single drug, but the combination with phentermine has a similar biochemical profile as phen-fen. It remains to be seen if this combination will give similar results as phen-fen. Potential future agents for obesity may fall into several categories, including CNS active agents, thermogenic agents, and nutrient partitioning agents. A particularly interesting possibility for combination drug therapy are gut hormones or analogues of gut hormones since it appears that the excellent clinical results seen with obesity surgery are due mainly to alterations of gut hormones. Gastric bypass and similar surgical procedures work by altering the biochemistry of the body and not by mechanical mechanisms. It is possible we eventually may be able to reproduce these effects with drugs. In this category, liraglutide, a GLP-1 agonist has been shown to be quite effective for diabetes and is in clinical trials as an obesity drug. Initial reports suggest it alone causes an 8% wt loss. We are in our infancy of understanding obesity, its causes and its treatments. The belated interest of the drug companies and the more favorable attitude of the FDA for obesity drugs predict a bright future.

PL-56**NATURAL-PRODUCT BASED DRUG DISCOVERY - CAN WE AFFORD TO IGNORE CHEMICAL DIVERSITY OF NATURE?****M. Iqbal Choudhary and Atta-ur-Rahman**

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Biodiversity is the outward manifestation of chemical diversity. Nature’s treasure house of diverse classes of chemical has been the main source of blockbuster drugs in most of the 20th century. However, use of various non-validated techniques and emphasis on arsenal of synthetic chemists has led to decline productivity and increased cost. Drugs for most of the tropical diseases and prevailing diseases are simply not available with pharmaceutical pipeline nearly dried out. Question is how

long we can afford to ignore nature's tremendous chemical source as primary source of new pharmacophores. During last two decades we have been focusing on natural products. This has led to the identification of novel lead molecules.

Prolonged hyperglycemia is recognized as the characteristic of diabetes and most important and core cause of diabetes related disorders. It is now recognized that chronic hyperglycemia may trigger long-term damage to different proteins in the body by undergoing non-enzymatic glycation process. There are many factors which increase the worldwide prevalence of diabetes, these includes sedentary lifestyles, obesity, an increase in the aging population, consumption of calorie-rich diets. Diabetes is the third most common killer of mankind, after cancer, cardiovascular and cerebrovascular diseases. According to WHO (World Health Organization) till 2030 about 366 million people will suffer from diabetes.

Glycation is the most common non-enzymatic process, results in the formation of advanced glycation end products (AGEs), which affect all the tissues in the body. Modifications due to glycation accumulate during the life span. Different proteins were reported to undergo glycation when exposed to elevated levels of sugars (e.g. serum albumin, hemoglobin, elastin, crystalline, collagen, tubulin, myelin, fibrinogen, immunoglobulin, insulin, and lipoproteins, etc. Glycation is not only implied to be a marker of the development of diabetic complications, but also found to be the main reason of diabetic associated disorders. No enzyme in the body system is capable of hydrolyzing the AGEs or making their formation a reversible process. Hence, the AGEs accumulate in the body with time. This leads to abnormalities, such as diabetic retinopathy, atherosclerosis, diabetic nephropathy, neuropathy, etc. Therefore inhibition of glycation of proteins and formation of AGEs has attracted considerable scientific attention as a novel approach towards delaying the on-set of the AGEs-mediated late diabetic complications. Keeping in view of the therapeutic significance of antiglycation agents, the prime objective of our on-going research is to evaluate the inhibitory potential of different compounds of natural and synthetic origins. Over 3,000 fully characterized chemical constituents (both of natural and synthetic origin), were systematically evaluated for their antiglycation activity. As a result, a number of new and novel antiglycation agents from *Parmotrema cooperi* J. Steiner & Zahlbr. (lichen), *Ziziphus oxyphylla* Edgw., *Morus mesozygia* Stapf., *Aloe sinkatana* Reynolds. etc. were identified. Along with the natural antiglycation agents, we also identified a number of new and novel classes of synthetic antiglycation agents like anthranilic acid, isatin-3-thiosemicarbazone, bis Schiff bases of isatin, oxindole, chelocardin, flavonoids, thiazolidinone, urea, and benzohydrazide Schiff bases. Similarly a large number of compounds was screened for α -glucosidase inhibition, which may be used for the management of type 2 diabetes, as they have the potential to slow down the absorption kinetics of carbohydrates in the small intestine.

Obesity is an emerging challenge and a serious health problem worldwide. It is associated with many chronic diseases, such as diabetes, cardiovascular disorders and certain cancers. Molecular cascade involves in obesity and associated disorders are still not fully understood. Proliferation of adipocytes plays an important role in the on-set and progression of obesity. Understanding the phenomenon of adipogenesis is of major importance as adipocyte dysfunction makes an important contribution to metabolic diseases. Differentiation of preadipocytes to adipocytes not only results in increasing number of adipocytes but also provide a large pool of fat depots in adipose tissues. Thus one strategy to treat obesity is to reduce the adipocyte numbers and fat content through targeting the mature adipocytes by diverse molecular entities.

Among different therapeutic interventions, the discovery of effective antiadipogenic compounds from various sources is considered to be a promising approach. Our recent research is focused on the study of the inhibitory effects of natural and synthetic compounds, such as steroids, flavonoids, terpenes alkaloids and sulfonamides, on the proliferation of adipocytes, as well as to evaluate their effects on to the mature adipocytes and their capacity to initiate lipolysis process. This study has resulted in the identification of several new inhibitors of adipogenesis process.

During this plenary presentation, underlying philosophy and approach of our research on cost-effective discovery of lead molecules at the interface of chemistry and biology will be discussed.

PL-27

CELL SIGNALING BY PROTEIN PHOSPHORYLATION

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A rapid overview of the past, present and future of signal transduction by protein phosphorylation will be presented. This process represents one of the most prevalent mechanisms by which eukaryotic cellular events are regulated. It is involved in the control of many physiological processes and pathological conditions including various bacterial and viral diseases. After recalling how this field has originated and evolved in the last sixty years, the talk will focus on cellular regulation by protein tyrosine phosphorylation. This process is directly implicated in cell growth, differentiation



and transformation, bringing into play a diversity of tyrosine kinases of viral or cellular origin or linked to growth factor receptors. The receptors transduce their signal by recruiting a multiplicity of adapter proteins interacting with one another in a tinker-toy sort of way through a wide variety of binding modules (SH2, SH3, WW, PH, PDZ, *etc.*) thereby initiating a diversity of signaling cascade pathways.

Regulation also involves protein tyrosine phosphatases, an expanding family of transmembrane and intracellular enzymes that catalyze the reverse reaction. Most receptor forms have highly variable external domains that, surprisingly, display all the structural characteristics of cell adhesion molecules, suggesting that they must be involved in – or regulated by – cell-cell interaction, with the very exciting possibility that they might be directly implicated in contact inhibition that plays such a crucial role in carcinogenesis. Protein tyrosine phosphatases cannot be viewed as simply providing the “off” switch in an “on/off” kinase/phosphatase system: depending on their structure and where they localize within the cells, they can act either positively or negatively in eliciting a particular physiological response.

We begin to understand the links that exist between the disruption of protein kinases and phosphatases and the etiology of certain human diseases. Some of the attempts that are being made to develop therapeutic tools to target these enzymes will be presented.

PL-54

BIOLOGICAL BASIC RESEARCH AND ITS TRANSLATION INTO PRACTICE AND BUSINESS, MY EXPERIENCE

Robert Huber

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As a student in the early nineteen sixties, I had the privilege to attend winter seminars organized by my mentor, W. Hoppe, and by M. Perutz, which took place in a small guesthouse in the Bavarian-Austrian Alps. The entire community of a handful of protein crystallographers assembled in a room which served as living and dining room and as auditorium for the lectures.

Today structural biologists organize large congresses with thousands of attendants and there exist many hundreds of laboratories specialized in this field. It appears to dominate biology and biochemistry very visibly if we count covers in scientific journals displaying macromolecular structures.

Structural biology was successful, because it was recognized that understanding biological phenomena at the molecular and atomic level requires to see those molecules.

Structural biology revealed the structure of genes and their basic mechanism of regulation, the mechanism of enzymes' function, the structural basis of immune diversity, the mechanisms of energy production in cells by photosynthesis and its conversion into energy- rich chemical compounds and organic material, the mechanism that makes muscle work, the architecture of viruses and multi-enzyme complexes, and many more.

New methods had an essential impact on the development of structural biology. Methods seemed to become available in cadence with the growing complexity of the problems and newly discovered methods brought biological problems within reach for researchers, a co-evolutionary process of the development of methods and answerable problems.

An important additional incentive for structural biology came from its potential application for drug design and development by the use of knowledge of drug receptors at the atomic level. The commercial interest in application spurred this direction of research enormously.

My lecture will start out with the history of protein crystallography and describe the major factors contributing to its development, illustrated with examples contributing to our understanding of the physical and chemical basis behind biological phenomena.

I then will let you share my experience with the foundation and development of two biotech companies with different business models, but both based on basic academic research in structural biology:

Proteros (www.Proteros.com) offers enabling technology services for Pharma- and Crop science companies imbedding all steps of the workflow molecular and structural biology can provide and commands and uses its platform for the generation of leads from identified targets to *in vivo* Proof of Concept (PoC).

Supremol (www.Supremol.com) specializes in the development of novel immunoregulatory therapeutics for the treatment of autoimmune diseases on the basis a recombinant, soluble, non-glycosylated version of the human Fcγ receptor IIB.

PL-3

TARGETING PAK1 KINASE IN HUMAN CANCER

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The process of cancer progression to more invasive phenotypes is profoundly influenced by dysregulation of pathways that control cytoskeleton remodeling, cell-cycle progression and mitosis, cell survival, transformation, chromatin remodeling, and genomic stability. Further, devastating influence of cancer invasiveness is also fueled by persistent stimulation of pathways that counteract commonly used cancer therapies targeting receptor-tyrosine kinases, mitogenic and/or survival signaling modules. One of the major signaling nodules of extracellular stimuli that plays an important role commending role in the above cancer phenotypes is p21-activated kinase 1 (PAK1), a widely overexpressed nodular molecule in human cancer. Recent studies have implicated PAK1 in the nucleus with new functions. Interestingly, PAK1 hyperstimulation also leads to mitotic abnormalities such as multipolar spindles and activation of Aurora kinase, an important mitotic regulator. In this context, emerging data suggest that a novel physiologic PAK1 substrate, could effectively regulate Aurora kinase activity and function, and in-turn, and participates in defective mitosis. Since mitotic defects leads to the development of aneuploidy and because such cells are likely to be resistant to anti-cancer drugs, these findings suggest that PAK1 and its targets could be effectively co-targeted to achieve a greater anti-cancer activity and possibly to sensitize tumor cells to anti-cancer modalities. Thus, the levels, subcellular localization, and activation status of PAK1 is likely to be an important determinants of tamoxifen resistance, and that raising the possibility that tamoxifen resistance might be prevented or reversed by PAK1 inhibition. The presentation will summarize the most exciting PAK1 data in the area of cancer biology and provide an up-to-date status of PAK1 as a cancer therapeutic target.

PL-2

SUPRAMOLECULAR AND ADAPTIVE CHEMISTRY BIORGANIC AND DRUG DISCOVERY ASPECTS – RECENT ADVANCES

Jean-Marie LEHN

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Supramolecular chemistry aims at constructing and implementing highly complex chemical systems from molecular components held together by non-covalent intermolecular forces. It has relied on the development of preorganized molecular receptors for effecting *molecular recognition*, catalysis and transport processes. The implementation of molecular recognition and transport processes in systems of biological significance will be described, in particular in the development of a bioactive molecule, *myo*-Inositol TrisPyroPhosphate (ITPP) displaying remarkable properties of interest in cardiovascular diseases and cancer.



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 molecular constituents. The same holds for molecular chemistry when the molecular entity contains covalent bonds that may form and break reversibility, so as to allow a continuous change in constitution by reorganization and exchange of building blocks. These features define a *Constitutional Dynamic Chemistry* (CDC) on both the molecular and supramolecular levels.

CDC takes advantage of dynamic constitutional diversity to allow variation and selection so as to achieve *adaptation*, opening the path towards *adaptive chemistry*. Its combinatorial features led to the development of a *dynamic combinatorial chemistry*, as a novel approach to drug discovery.

Supramolecular chemistry and CDC have also opened novel perspectives in materials science, in particular towards the development of supramolecular and dynamic materials for biological and medical applications.

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PL-29

REWRITING NATURAL PRODUCT DRUG DISCOVERY THROUGH SYNTHETIC BIOLOGY

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Natural product chemicals have historically been discovered based on their structural and biological properties. With the ease and affordability of genome sequencing today, a new era in natural product discovery is unfolding in which genomics and biosynthesis are together fostering new innovations in compound discovery. This orthogonal discovery approach takes advantage of the biosynthetic potential of a genomesequenced organism to design hypothesis-driven experiments to rapidly find new chemical entities that are desperately needed in the drug discovery and development pipeline. Examples from the author's laboratory will highlight the myriad of available and evolving genome mining approaches to connect orphan biosynthetic genes to new natural product molecules.



PL-1

APPLICATION OF NITRIC OXIDE RESEARCH TO DRUG DEVELOPMENT AND DISEASE THERAPY

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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 formulation and metabolism. Such promise and expectations have obviously fueled the interests in nitric oxide research for a growing list of potential therapeutic applications. There have been and will continue to be many opportunities from nitric oxide and cyclic GMP march to develop novel and important therapeutic agents. There are presently more than 80,000 publications in the area of nitric oxide research. The lecture will discuss our discovery of the first biological effects of nitric oxide and how the field has evolved since our original reports in 1977. The possible utility of this signaling pathway to facilitate novel drug development and the creation of numerous projects in the pharmaceutical and biotechnology industries will also be discussed.

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PL-28

TARGETING SOLUBLE GUANYLATE CYCLASE FOR THE TREATMENT OF CARDIOPULMONARY DISEASE

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The soluble guanylate cyclase (sGC) is a key signal-transduction enzyme in the cardiovascular system and activated by NO. It became apparent that many cardiovascular diseases are associated with a dysfunction of the NO/sGC system. Importantly, two different forms of sGC exist *in vivo*, the native and heme-free sGC. sGC activators, such as cinaciguat (BAY 58-2667) are capable of selectively activating the heme-free enzyme via binding to the enzyme's haem pocket. These new compounds selectively target the dysfunctional sGC that is prevalent under disease conditions. Cinaciguat has demonstrated efficacy in patients with acute decompensated heart failure (ADHF), reducing pre- and afterload and increasing cardiac output. sGC stimulators, such as riociguat (BAY 63-2521), show a dual mode of action: they sensitize sGC to the body's own NO while also directly stimulating sGC independently of NO. They may be beneficial in the treatment of a range of cardiovascular and non-cardiovascular disorders. Riociguat had beneficial effects on pulmonary haemodynamics, right heart hypertrophy, and remodeling of the pulmonary vasculature in different experimental models of pulmonary hypertension (PH). In phase III studies riociguat has demonstrated efficacy in patients with pulmonary arterial hypertension (PAH) and, remarkably, also in patients with chronic thromboembolic pulmonary hypertension (CTEPH). Very recently, the Food and Drug Administration (FDA) has approved Adempas[®] (riociguat) for use in these two forms of pulmonary hypertension: The treatment of adults with persistent/recurrent CTEPH after surgical treatment or inoperable CTEPH to improve exercise capacity and WHO functional class; and the treatment of adults with PAH to improve exercise capacity, improve WHO functional class and delay clinical worsening.



KEYNOTE LECTURE

KN-31*Track: Pharmaceutical Biotechnology***REPOSITIONING AND REDEPLOYMENT OF EXISTING PHARMACOPHORES BY MICROBIAL TRANSFORMATION****M. Iqbal Choudhary and Atta-ur-Rahman**

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Drug repositioning is an approach by which new or additional value is created from an existing by targeting diseases other than those for which it was originally proposed. Many companies are now examining the existing pharmacopoeia for repositioning candidates, and there is increased number of success stories in drug redeployment. An important benefit of drug repositioning over traditional drug development is a significant number of toxicity and other steps can be avoided. Its safety is known and the risk of failure for reasons of adverse toxicology are reduced. Preliminary work showed that these approaches can speed up the process of drug discovery at much lower cost.

The transformation/ structural changes in a molecule brought about either by whole cell cultures of fungi or bacteria, isolated enzymes, animals, plant is termed as biotransformation. Biotransformation can be used for drug repositioning as a faster way towards drug discovery of targeting diseases other than those for which its substrate was originally proposed. The process can also be successfully utilized for the expansion of molecular diversity around the pharmacophore of a compound. The process is the best tool in medicinal chemistry for the introduction or modification of specific functionalities at the positions difficult to access by conventional chemical methods. In last two decades, this methodology has become an indispensable tool for asymmetric synthesis, not only in academic research 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 our studies of drug repositioning, we structurally modify a number of existing drugs into their structural analogues by microbial and plant cell suspension cultures. The resulting metabolites have exhibited interesting biological activities, different from their precursor drugs. Oxymetholone which is marketed as anadrol, a synthetic anabolic steroid developed in 1960 by Zoltan' Anadrol Z'F. It has been approved by the US Food and Drug Administration for the treatment of anemias caused by deficient red cell production. Its biotransformation was carried out with various fungi resulted in the production of various new and a known metabolites. Oxymetholone and some of its metabolites showed anti-inflammatory activity. Similarly, exemestane (trade name aromasin) is a steroidal aromatase inhibitor, used for the treatment of breast cancer. Aromatase inhibitors block the synthesis of estrogen. This lowers the estrogen level, and slows the growth of cancers. Exemestane was invented and synthesized by the Italian company using commercially available boldenone (androsta-1, 4-diene-17 β -ol-3-one) FDA approved it in October 2005. After biotransformation cytotoxicity was checked, which showed that new metabolite of exemestane showed activity against Hela and PC3 cell line. Similarly melengestrol acetate is used as a feed additive for feedlot heifers, was found to be a potent anti-inflammatory agent along with its new transformed product. Tibolone is a synthetic steroid hormone drug, used for the treatment of endometriosis and hormone replacement therapy in post menopausal women, we have successfully biotransformed the drug into its new derivatives and find out their potent activity as alpha glucosidase inhibitors. These results showed drug repositioning of already marketed drugs and also showed that resulted new and known compounds can fasten the process of drug development.

During this presentation, underlying philosophy and approach of our research on cost-effective discovery of lead molecules by using drug repositioning strategy will be discussed.

INVITED LECTURES

IL-40

Track: Medical Biotechnology: diagnostics; imaging

CONFOCAL RAMAN AND BRILLOUIN COMBINED SPECTROSCOPY A NEW HORIZON IN BIOTECHNOLOGY**Maher S. Amer**

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The high information content of confocal Raman spectroscopy can characterize biological samples in both *ex-* and *in-vivo* situations. With its ability to probe chemical composition, primary and secondary structures, and the chemical micro-environment of molecular subgroups, Raman spectroscopy has been utilized to provide deep, non-invasive characterization of biological tissues and to study con formation of macromolecules inside living cells with a spatial resolution of 1 μm . In addition, Brillouin spectroscopy with its ability to probe mechanical constants of matter on a sub-micron scale provides crucial information and a non-invasive investigative tool for a deep understanding of the mechanical integrity of biological tissues. Combined confocal Raman/Brillouin spectroscopic investigation is a new leap in biotechnology investigative tools that braves the road to a better understanding of tissue behavior, disease mechanisms, and, most importantly, the correlation between chemical composition, structural characteristics, and mechanical integrity. In this presentation, we will demonstrate the advantages of our recently developed confocal micro-Brillouin spectrometer and its application in measuring mechanical stiffness in bones and other biological tissues such as insect wings. We will also present our data emphasizing the ability and importance of combining, chemical composition, structural characteristic (via Raman) with mechanical properties (via Brillouin) spectroscopy techniques.

IL-19

Track: Regenerative Medicine

3D PRINTING OF ANTIBIOTIC-LADEN BONE SCAFFOLD FOR LIMB SPARING RECONSTRUCTION**Hani Awad**

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Major bone reconstruction procedures often use autografts or allografts to improve bone healing in a critically sized defect or non-union; however, these bone grafts suffer from multiple limitations that make synthetic alternatives an attractive option. Calcium phosphates are a primary focus for synthetic bone graft substitutes because they are biocompatible, osteoconductive, and provide sufficient mechanical strength. Inkjet-based 3D printing has been employed to fabricate calcium phosphate scaffolds (CPS), where the calcium phosphate powder is temporarily bound by an adhesive polymer and then permanently bound by sintering of the printed green body. This technique, however, requires high temperatures that preclude the incorporation of bioactive molecules and drugs during the 3D printing process that could stimulate bone regeneration or combat infection.

Low temperature 3D printing of calcium phosphate scaffolds holds great promise for fabricating synthetic bone graft substitutes with enhanced performance over traditional techniques, including enabling drug incorporation, and the potential to create composites with synthetic or biological polymers such as collagen. Many process parameters have yet to be optimized to ensure maximal biocompatibility and osteoconductivity with sufficient mechanical properties of the scaffolds. In this presentation, we discuss recent advances in optimizing binder solution and powder formulation and physical characteristics to maximize cytocompatibility and mechanical strength. For example, we developed methods to incorporate collagen into the binder solution to fabricate collagen-calcium phosphate composites. Reducing the viscosity and surface tension through a physiologic heat treatment and the addition of a surfactant (Tween 80), respectively, enabled reliable thermal inkjet printing of the collagen solutions. Our studies demonstrate for the first time the feasibility of 3D printing 1-2 wt% collagen into calcium phosphate scaffolds, which significantly improved maximum flexural strength and cell viability. To assess the bone healing performance, we implanted 3D printed scaffolds into a critically sized murine femoral defect for 9 weeks. The implants were confirmed to be osteoconductive, with new bone growth

incorporating the degrading scaffold materials. We also present preliminary data demonstrating the feasibility of 3D printing of antibiotic-laden collagen-calcium phosphate scaffolds, which can be used in reconstruction of infected diaphyseal defects. In conclusion, this study demonstrates strategies for adapting inkjet 3D printing technology and optimization of material parameters to fabricate CPS for applications in preclinical small animal models of bone regeneration.

IL-62

Track: Industrial and Manufacturing

PRODUCTION OF BIOFUEL FROM LIGNOCELLULOSIC BIOMASS USING FUSION TECHNOLOGY TO CREATE NEW THERMOPHILIC CLOSTRIDIA

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Ethanol, the most widely used renewable liquid transportation fuel, has only 70% of the energy content of gasoline and its hygroscopicity makes it incompatible with existing fuel storage and distribution infrastructure. Advanced biofuels with high-energy content and physiochemical properties similar to petroleum-based fuels may be better alternatives, as they would allow use of existing combustion engine designs, distribution systems and storage infrastructure. Recently there has been an increased interest to convert sugars from lignocellulosic biomass into butanol.

Due to its physical properties, the four-carbon butanol is a better replacement for gasoline than ethanol. Many different *Clostridia* have been utilized in butanol fermentation, although these gram-positive anaerobes coproduce butanol with a few byproducts, such as butyric acid, acetone, ethanol, therefore lowering its yield. From a biotechnology perspective, the lack of efficient genetic tools to manipulate *Clostridia* hinders metabolic engineering efforts for the optimization of butanol synthesis and the reduction of by-product formation. In addition, alcohols are toxic to microbes at higher concentrations. Because of these two major hurdles, we created new species of thermophilic *Clostridia* by a novel bacterial fusion technology. Therefore, instead of transfer of enzyme coding genes in cassettes by recombinant technology, we created entirely new microorganisms by fusing two species of *Clostridia*. This new *Clostridia* can carry out fermentation in a single vessel at relatively thermophilic temperatures (i.e. 45⁰ C to 65⁰ C). Use of this microorganism eliminates the need for multistep, multi-vessel, low temperature reaction system and brings about a single vessel system for the direct conversion of lignocellulosic biomass to butanol and other economical important chemicals. Because of its thermophilic quality, the alcohols are evaporated under vacuum at 65⁰ C, eliminating the toxic effects of the alcohols. Since, new *Clostridia* microorganisms not naturally occurring in wild by total genomic fusion process, we have tentatively named these strains *Clostridium therobutanolicum* (which will be subject to verification by Nomenclature Committee of the International Society for Microbiology in the future).

IL-9

Track: 'Other Areas: Clinical Research/clinical trials and bioethics'

THE BIG DATA TSUNAMI: THE KEY ROLE OF DATA ON DEVELOPMENT/REGULATION/MITIGATION OF BIOTECHNOLOGY IN NATIONAL SECURITY AND DEFENSE

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Knowledge's improvement in biosciences and large number of biotechnological application, big part of them over human beings, has a big potential in national security and defense context.

Scientific experiments, often characterized by low invasiveness, are aimed to better understand brain functioning and its relationship with the thought and behavior. These trials have given a significant contribution to the debate in the cognitive field, stimulated a philosophical debate about free will-

responsibility and attracted interest from the public. Scientific knowledge brings every day new amazing biotechnology that enable us to interact and manipulate human brain. Moreover science and biotechnology could become the greatest revolution in military affairs since the atom bomb. Some researcher are sure that we are on the cusp of a massive shift in military technology that will have profound effects on the front lines, as well on politics back home. Science and biotechnology and their use in national security and defense, should be well understood and evaluate to challenge strategic and political questions and to void serial ethical implications.

In this frameworks Big Data offers big benefits:

1. Makes information transparent and usable at much higher frequency.
2. Creation and storage of transactional data in digital form acquires more accurate and detailed performance information - exposing variability and boosting performance.
3. Clearer, narrower customer segmentation - providing customized products or services.
4. Sophisticated analytics to improve high-stakes decision-making.
5. Big data can be used to improve the development of the next generation of products.

As these new possibilities rise, an hard debate emerged, a debate that is too broad to fully explicate here.

I would like, with my proposal, to overcome this positions suggesting the idea that Big Data should play a more direct roles in biotechnology public decision-making or at least engage more deeply with political issues. I believe that this form of critical science, that I will draft in my presentation, can find voice and effective power only through a participatory governance as creation of a national advisory committee on biosecurity. Only if government set out the direction of participatory governance in national advisory committee way we can realize and make effective the key role of Big Data on development/regulation/mitigation of biotechnology in national security and defense.

IL-4

Track: Medical Biotechnology

HUMAN PAPILOMA (HPV) VIRUS BIOLOGY, FDA APPROVED TESTS AND AMERICAN COLLEGE OF GYNECOLOGISTS (ACOG) SCREENING GUIDELINES

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Human papilloma virus biology and oncogenesis have been studied extensively the last ten years because of its association with cervical intraepithelial neoplasia (CIN) [1]. More than 44% women are diagnosed once with HPV infection in the US and HPV related CIN is the 2nd cancer in women . Three coding regions of HPV are necessary for its expression and cancerogenesis. First, it seems that an important role plays the LCR (Long Coding Region) of the virus, which is necessary for virus- epithelial cell “cross-talking” and for the establishment of persistent infection².Second, early regulation proteins are expressed during the early and late infection stages. Third, late proteins (L1 and L2) are expressed at late stage of infection and lead to viral capsid protein assembly and shedding.Regulation of the replication and transcription is mainly played by the E1 and E2 protein [2]. E2 also regulates expression of E6/E7 oncoproteins, which interact with anti-apoptotic, anti-oncogenic proteins pRB (Retinoblastoma protein) and p53. At post transcriptional level several complex events have been elucidated such as the role of exon and intron sequence enhancers and silencers as well as the exon-intron splicing primary and secondary sites [2]. The virus can be finally established in the epithelial cell layer as plasmid, plasmid and integrated or fully integrated in the epithelial DNA when cancer occurs. The oncogenesis process is as long as 10 to 15 years) [1, 2]. Although important advances in biology of HPV have been accomplished, elucidation of HPV mysteries remains fragmentary due to the complexity of key events and the need of all epithelial cell layers for the development of CIN, starting from the basic layer. Several target molecules as tools in diagnosis and topical therapies are under research. Key changes of screening guidelines according to ACOG have been launched in December 2012) [3]. They are based on large scale studies and better knowledge of viral oncogenesis. In brief: No screening test is recommended for girls under 20 years old. From 21 to 29 years Pap test screening every three years, as well as from 30 to 65 years old is recommended every three years by Pap test only, or every 5 years if the test is accompanied by an FDA approved HPV DNA test. An algorithm of patient oriented treatment has been proposed, considering ASCUS, CIN classification, test Pap, HPV DNA testing and colposcopy. FDA approved tests have been

designed according to high technical standards and the detection of more than 13 high risk HPV DNA/RNA. Papanicolaou classical cytology test [4] seems the most important screening test for cervical cancer prevention [3]. ACOG recommends vaccination against HPV since 2010, however, long time is needed to evaluate against cancer prevention) [5].

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IL-8

Track: Pharmaceutical Biotechnology

SITE-SPECIFIC MODIFICATION AND PEGYLATION OF PROTEIN DRUGS MEDIATED BY TRANSGLUTAMINASE

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In recent years many proteins produced in large quantities by recombinant DNA techniques have become important new drugs [1]. Despite these tremendous advances, protein drugs possess several shortcomings, including susceptibility to degradation by proteases, short circulating half-life *in vivo*, low solubility, rapid kidney clearance and propensity to generate neutralizing antibodies. Among the techniques so far explored for the development of safer and more useful protein drugs, undeniably the protein surface modification by covalent attachment of poly(ethylene glycol) (PEG) became an extremely valuable technique for producing protein drugs more water-soluble, non-aggregating, non-immunogenic and more stable to proteolytic digestion [2,3]. Recently, novel procedures of transglutaminase (TGase)-mediated PEGylation of pharmaceutical proteins were proposed [4-6]. The PEG polymer can be covalently linked to protein-bound glutamine (Gln) residues by using a microbial TGase and an amino-derivative of PEG (PEG-NH₂) as an amino donor in the enzymatic reaction, thus resulting in a protein-CONH-PEG conjugate. Thus, TGase can act as a protease, but in the reverse order (coupling instead of hydrolysis).

The TGase-mediated PEGylation reaction allows the modification of proteins under mild conditions and often at specific Gln residues, leading to more homogeneous protein conjugates than those that can be obtained by using acylating derivatives of PEG [5]. Several proteins have been PEGylated by means of TGase, among them human growth hormone, granulocyte colony-stimulating factor, interleukin-2 and erythropoietin. We have analysed our own experimental results and those of other published experiments of TGase-mediated modification and PEGylation of proteins in terms of their known 3D structure and dynamics. We have observed a clear-cut correlation between sites of TGase attack and chain regions of enhanced backbone flexibility, this last detected by the crystallographic profile of the *B*-factor along the protein polypeptide chain. Moreover, we have found that the TGase-mediated reaction often occurs at chain regions characterized by missing electron density, thus indicating that these regions are disordered. We conclude that a mechanism of local unfolding of the polypeptide substrate strongly favours a site-specific modification by TGase, provided that a Gln residue is encompassed in that region [6]. Of interest, chain flexibility was earlier found to dictate the sites of limited or site-specific proteolysis of proteins [7].

We will illustrate the results of TGase-mediated modification and PEGylation of several proteins of pharmaceutical interest. Overall, it is demonstrated that the TGase attack on protein substrates can be very specific and that TGase requires an unfolded polypeptide substrate for its selective enzymatic attack. This is in keeping with a view that the enzymatic reaction involves the binding and adaptation of a 10-12 residue polypeptide substrate at the enzyme's active site. Therefore, it is possible to predict the site(s) of TGase-mediated modification of a protein on the basis of its 3D structure and dynamics and, consequently, the likely effects on its physicochemical and functional properties [5].

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IL-5

Track: Pharmaceutical Biotechnology

NANO BEADS FOR DRUG TARGET PROTEIN COMPLEX DISCOVERY

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To analyze the signal of bio-active small compounds (hormones, amino acids, etc), magnetic nanobeads system was established. Using this bio-active small compound-immobilized nanobeads, its target protein(s) is purified effectively.

1. PGJ2 target proteins purification for osteoporosis In the 2nd Biotechnology World Congress in Dubai PGJ2-immobilized nano beads technology was presented. Mitochondrial PGJ2 target protein was purified and identified from the HEK293 cell extract directly. Using nanotech, 2nd target protein was also purified and identified from osteoblast cells. The mechanism of PGJ2 action will be presented.
2. Arginine target proteins purification for diabetes mellitus Previously reported, one-step-purification system for identification of arginine interacting factor 1 ~3 (AIF1~3) was developed from HeLa cell extract directly.

Using arginine-immobilized magnetic nanobeads, arginine signaling complex purification method was established. Here novel AIF, AIF4, was purified and identified from HUVEC cells. Moreover the cofactor of arginine-AIF4 complex was also purified and identified with nanobeads and recombinant AIF4 protein. The new component of complex was designated AIF4-BP1 (Arginine Interacting Factor-Binding Protein 1). Both of AIF4 and AIF4-BP1 locate in endoplasmic reticulum (ER), because of their ER retention signals. Using nanobeads and recombinant proteins, Arginine signal complex of AIF4 and AIF4-BP1 was confirmed *in vitro* as well as in cells. This technology is more than 30-fold efficient purification efficient than general purification technology. Taken together arginine/AIF4/AIF4-BP1 complex is observed in ER.

IL-32

Track: Industrial and Manufacturing

A BIOPROCESSING STRATEGY FOR THE BIOCONVERSION OF GRAPE MARC INTO PROTEIN RICH ANIMAL FEED

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This investigation was to establish bioprocess engineering strategy for the bioconversion of grape marc into protein rich animal feed. We experimentally identified influential operation parameters in solid state fermentation process, including medium substitutions composition: nutrient, inoculum size, water content, and operation conditions: fermentation time and temperature, and steam treatment time. Three pre-selected microbial fungal strains *Aspergillusoryzae*DAR 3699, *Aspergillusoryzae*RIB 40 and *Trichodermaeesei* RUT C30 were employed in this study. Overall results revealed that

these fungal strains performed an excellent bioaccessibility to use grape marc as carbon sources. The inoculum size, steam treatment, temperature and water content were found to be the key parameters, which can significantly affect fungal cell growth and protein enrichment. Under optimized conditions, protein content can be increased from 7% to 32% after 5 days of fermentation, resulting in 360% protein improvement. It was expected that the fungi-enriched grape marc can be suitable as highly valuable animal feedstock.

IL-58

Track: Medical Biotechnology

NORMAL AND CANCEROUS STOMACH STEM CELLS

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In the stomach of mice and humans, the glandular epithelium is perpetually replenished by multipotent stem cells. They actively proliferate to maintain themselves and to produce four main cell lineages secreting mucus, acid, pepsinogen and various hormones. Dysregulation of this cellular proliferation/differentiation program in genetically engineered mice induces events similar to those preceding gastric carcinogenesis such as loss of acid-secreting parietal cells. In addition, the gastric epithelial stem/progenitor cells undergo hyperplasia and express glycoconjugates specific for adhesins of *Helicobacter pylori*. Eventually, cellular changes evolve and gastric adenocarcinoma develops suggesting a role for stem cells in carcinogenesis.

In humans, gastric biopsies with normal mucosa, mild superficial gastritis, severe atrophic gastritis, and intestinal metaplasia show progressive increase in mucosal thickness and amplification of dividing stem/progenitor cell population. The expression pattern of the stem cell transcription factor Oct4 in these tissues along with gastric cancer tissues (safe resected margin, tumor edge and tumor center) confirms the amplification of epithelial stem cells during carcinogenesis. Interestingly, up-regulation of Oct4 expression is associated with a block in nuclear translocation. In conclusion, studies in mice and humans provide evidences for the role of stem cells in gastric carcinogenesis.

IL-6

Track: Other Areas: Clinical Research/Clinical trials; Nano-biotechnology

SYSTEMATIC LIGHT EXPOSURE IN THE TREATMENT OF CANCER-RELATED FATIGUE

William H. Redd, Heiddis Valdimarsdottir, Lisa Wu, Gary Winkel, Emily E. Byrne, Melba Beltre, Elizabeth S. Liebman, Tanya Erazo, Judy A. Hayes, Luis Isola, Eileen Scigliano, Susan Lutgendorf and Sonia Ancoli-Israel

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Introduction: Cancer-related fatigue (CRF) is the most commonly reported side effect of cancer treatment. It is a persistent sense of physical, emotional, and cognitive tiredness and exhaustion. Patients report diminished concentration and feel tired even after resting. Unfortunately, there are no effective treatments for this serious public health problem. This preliminary randomized clinical trial investigated the impact of systematic bright white light exposure (BWL) on CRF. **Methods:** After informed consent was obtained, 31 cancer survivors were randomly assigned to receive daily 30 minute exposure to either BWL or to a dim red light (DRL --- comparison condition traditionally used in studies of systematic light exposure). Both BWL and DRL treatment were administered via a commercially available Litebook 1.2 (Litebook®, Ltd. Medicine Hat, Canada). For the BWL condition, the Litebook® used 60 premium white light emitting diode (LED) lights which mimic the visible spectrum of sunlight (full spectrum white light) for minimum glare and maximum eye comfort. An identical-appearing device utilizing red LEDs emitting <50 lux was used for the DRL comparison group. Participants were instructed to self-administer the light treatment by placing the light box at a 45 degree angle, 18 inches from their face, for 30 minutes every morning throughout the four week intervention period. DRL has been used as a comparison for BWL as photoreceptors are relatively insensitive to red light frequency. The

impact of BWL and DRL was assessed using the FACIT-Fatigue scale, a validated measure which is commonly used in cancer research studies. Assessments were conducted at four points: baseline, after two weeks of exposure, after four weeks of exposure and at three weeks post light treatment follow up. Results: Linear Model analysis of baseline fatigue indicated no significant ($p=0.5454$) difference between the DRL and BWL groups at baseline. There was also no significant ($F(1,18)=1.77$; $p=0.1999$) difference between the two light conditions at baseline in their belief that the treatment to which they had been assigned would successfully reduce their fatigue. Effect of light on CRF: There was a significant time effect ($F(3,56)=6.08$; $p=0.0012$) suggesting that fatigue levels changed over time. There was a significant group effect ($F(1,23)=8.20$; $p=0.0088$) suggesting that across all time points, the BWL group had less fatigue than the DRL group. In addition, there was a significant time x treatment effect ($F(3,56)=6.82$; $p=0.0005$) suggesting that the groups differed with respect to change in fatigue over time. There was a significant reduction in fatigue over time for those in the BWL condition. For those in the DRL condition, fatigue improved at two weeks but became worse at 4 weeks and at 3 weeks following completion of the intervention. There was a large effect size ($d= 1.15$). At the end of the 4-week intervention, no patients in the BWL condition were still clinically fatigued (FACIT-Fatigue score >30) while 62% of patients in the DRL condition were still clinically fatigued. Conclusions: Although other studies have examined non-pharmacologic treatments of fatigue, the effect size in this study is substantially larger than the small to moderate effects seen in other interventions. What is needed is a larger study to determine possible biological and psychological mediators of the observed effects of BWL on CRF and effective methods of dissemination. It is important to note that the BWL intervention is a low cost and low burden intervention which is more effective than available alternatives.

IL-46

Track: Medical Biotechnology

MAGNESIUM DEFICIENCIES AND CATECHOLAMINE CARDIOMYOPATHY: A DEADLY COMBINATION

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Catecholamine cardiomyopathy (CC) is an acute, usually self-limiting, heart failure syndrome characterized by hypocontractile apical and midventricular regions of the left ventricle, with a compensatory hypercontractile base. Recently I published that space flight (SF) can trigger CC, based on data of the first moon walker Neil Armstrong; this showed during Armstrong's last 20 lunar minutes, before potential confounding by inhaling toxic iron-laden dust brought into the habitat on space suits, both dyspnea consistent with left ventricular dysfunction and tachycardia (130-160/min.) This shows that just 5 SF days are sufficient to trigger CC. Since with SF, C levels are twice supine Earth levels and SF- serum magnesium (Mg) is reduced ($p < 0.0001$), there is a predisposition for CC. Four vicious cycles may occur: 1. oxidative stress, endothelial dysfunction with insulin resistance, 2. Decreased Mg. ions and increased C 3. Ischemia and increased C 4. Decreased Mg. ions and increased angiotensin effect. I have published that by using the International Space Station (ISS) with life expectancy possibly shortened by a factor of 10, conducting rat studies might show life may be prolonged by correcting these invariable Mg. deficiencies. Prolonging a rat's life span with Mg with telomeres and telomerase studies might also suggest that their potential for CC has been offset. Since its been shown that at least 60 % of those in the U.S. have a Mg. deficiency, its conceivable that correcting Earth Mg deficiencies may reduce the potential for CC.



IL-39*Track: Plant and Environment***REGULATION OF CARBON METABOLISM IN MICROALGAE UNDER STRESS****Virginie Pasquet, Parisa Heydarizadeh, Virginie Mimouni, Lionel Ulmann, Freddy Guiheneuf, Brigitte Moreau, Justine Marchand, Véronique Martin-Jezequel, Gérard Tremblin, Annick Manceau and Benoît Schoefs***MicroMar, Mer Molécules Santé/Sea, Molecules & Health, EA2160, IUML – FR 3473 CNRS, University of Le Mans, IUT, Department Génie Biologique, F-53020 Laval cedex 9, France; E-mail: benoit.schoefs@univ-lemans.fr*

The diversity of high valued molecules by microalgae is huge [1]. Astaxanthin, β -carotene, lutein or omega 3 are probably the most popular examples of marketed molecules from microalgae. If some of these high valued molecules are synthesized under control conditions, stress conditions usually enhance their production [2]. For instance, a temperature stress triggers an increase of the eicosapentaenoic acid (EPA) by the marine diatom *Ondotella aurita* [3]. The enhancement of the production of the high valued molecules cannot be envisaged without considering a metabolic shift i.e. a reorientation of the carbon atom to one or more selected biosynthetic pathways. A good example is the green freshwater microalga *Haematococcus pluvialis* that starts to synthesize the red carotenoid astaxanthin under nutrient limitation or high light conditions [4]. From the scientific point of view, the regulatory mechanisms involved in the metabolic shifts are crucial for the understanding of the algal physiology. Those data are also very important to understand the alga ecology. From the applied science point of view, the knowledge of these regulatory mechanisms would allow to applications in which the carbon atoms are better channeled into selected biochemical pathways leading to high valued compounds.

The first part of the lecture will be dedicated to the diversity of high valued molecules synthesized by microalgae and why it is still interesting to screen microalgae for interesting metabolites. Then, the effects of growth conditions on the algal metabolism will be illustrated with the impact of stress factors (i.e. high light, alkaline pH, nutrient limitation) on lipid and carotenoid syntheses, respectively. The third part of the communication will be dedicated to signaling and the molecular regulation of the metabolism in microalgae. The conclusion and perspective section of the communication will explain in which applications a deep knowledge about the mechanism regulating the metabolism can be useful.

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IL-11*Track: Pharmaceutical Biotechnology***PEPTIDE-POLYMER CONJUGATES AS SELF-ADJUVANTING VACCINES****Mariusz Skwarczynski***The University of Queensland, School of Chemistry and Molecular Biosciences, Brisbane QLD 4072, Australia; E-mail: m.skwarczynski@uq.edu.au*

Classical vaccines incorporating live or attenuated microorganisms possess several disadvantages (e.g. production and stability complications, autoimmune and allergic side effects) and cannot be applied against cancer and some pathogens. Modern vaccines utilizing immunogenic subunits derived from a particular pathogen are able to overcome these obstacles. However, such vaccines need delivery system and immunostimulant (adjuvant) to trigger desired immune responses.

We designed new vaccine delivery system-based on the polyacrylate polymer conjugated to peptide epitope. We applied this system to Group A Streptococcus (GAS) vaccine and have demonstrated that peptide subunit vaccine formulated into nanoparticle was able to induce desired humoral immune response in mice after single immunization when administered subcutaneously or intranasally without help of any external adjuvant. We have shown that immune responses induced against desired peptide epitope were dependent on particle size. Interestingly, our delivery system induced strong and protective immune response without help of classically use immunostimulating agents, highlighting the potential of nanoparticle-based construct as a peptide vaccine delivery platform.

Recently, we applied this delivery strategy to create therapeutic peptide-based subunit anticancer vaccine. The synthesis of peptide epitopes was greatly improved by the change of standard SPPS procedure and application of the isopeptide method under microwave irradiation condition. The most promising antigens were identified, conjugated to polymer, and self-assembled into the microparticles. Modification of immunogenic epitope allowed the elimination of undesirable disulfide bond-based aggregation/polymerization of the peptide-polymers conjugates. Polyacrylate conjugated to the selected epitope produced excellent therapeutic effect against established tumour without help of any external adjuvant. Thus, we developed the first self-adjuvanting delivery system for the therapeutic vaccine against cervical cancer, demonstrating that our vaccine delivery system is also able to induce potent cellular immune responses.

IL-59

Track: Industrial and Manufacturing

BIOTECHNOLOGICAL TOOLS FOR THE SUSTAINABILITY OF INTEGRATED BIOREFINERIES

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With the increasing global energy demand, the need of sustainable alternative fuels made from renewables has been more urgent recently. In this sense the emergence of biorefineries has become an attractive and environmentally friendly option compared to the petroleum refineries where biomass is converted to fuels, energy and chemicals. In the past few years we have been working on the bioconversion of crude glycerol (the main byproduct of biodiesel production) to succinic acid by *Actinobacillus succinogenes* [2,4] and we have studied its economic feasibility through plant-wide simulations of integrated biorefineries [1]. To improve the sustainability of this bioprocess, experimental studies and detailed simulations have been carried out. In particular, an unstructured kinetic model that takes into account all the important physical conditions that affect the extracellular availability of nutrients has been proposed [4]. This can also be used as a predictive and design tool for effective scale up. Moreover, a detailed model of the cellular metabolism has been developed in order to understand the biochemical mechanism and to investigate the conditions that maximize the formation of the desired product [3]. Furthermore, we have constructed a new model of the biochemical reactions under intracellular conditions, which allows the evaluation of the volume exclusion effects on the diffusion and reaction rates [5] providing additional information for the potential of genetic manipulations and giving feedback for the optimal environmental conditions of the process.



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IL-66

Track: Business Development

PROCESS LOSS MONITORING AND DIAGNOSIS USING A WEIGHTED-LOSS CONTROL CHART

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Devising a single chart, instead of \bar{X} and R charts or \bar{X} and S charts, to simultaneously monitor the process mean and variability would reduce the required time and effort. A number of studies have shown that adaptive control charts can detect process shifts faster than the traditional Shewhart chart. This paper proposes a weighted-loss (WL) control chart to effectively monitor and diagnose whether the out-of-control process loss is caused by the changes in the difference of the process mean and target, the increase in process variability, or both. Furthermore, a WL chart with the optimal variable sampling intervals (VSI s) is also proposed. An example is used to illustrate the application and evaluate the performance of the proposed VSI WL control chart in monitoring and diagnosing the out-of-control process loss. Numerical analyses demonstrated that the optimal VSI WL chart outperforms the fixed sampling interval WL chart and the Shewhart joint \bar{X} and S charts in detecting the out-of-control process loss. Therefore, the (optimal) VSI WL chart is recommended.

Keywords: Control chart, loss function, Markov chain, variable sampling intervals.

IL-43

Track: Medical Biotechnology

CELL FRACTIONATION ON PH-RESPONSIVE CHITOSAN MEMBRANE

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The purpose of this study is to demonstrate pH-responsive chitosan is able to control cell behavior in response to small changes in environmental pH, which is at useful pH suitable for recovering cultured cells without additional enzymatic treatment and extensive washing steps. HeLa cells attached and spread well on chitosan at pH 6.99. When the pH was increased to 7.65, over 90% of cells would rapidly detached from chitosan surface within 1 h. Similarly, fibronectin adsorbed on chitosan at pH 6.99 also rapidly desorbed after increasing the medium pH. Most importantly and interestingly, the rate of fibronectin adsorption/desorption and cell attachment/detachment could be facilitated by altering chitosan/PVA and chitosan/PCL blended rate. It was found over 75% of HeLa cells could be recovered from 5-20% PVA surface within 0.5 h and just 62% of HeLa cells could be recovered from 2% PCL surface within 2 h. Additionally, chitosan/PVA and chitosan/PCL blended surfaces could effectively control attachment/detachment of various types of cells including cell lines HaCaT, 3T3, and primary adipose-derived stem cell, indicating the technology described here is easily reproducible and should be promising for controlling rapid fibronectin adsorption/desorption and cell attachment/detachment for tissue engineering applications.

Keywords: Chitosan, Fibronectin, Cell adhesion, pH-responsive materials, Cell culture system.

SESSION LECTURES

SL-51*Track: Plant and Environment***THE ARABIDOPSIS EXPANSIN-LIKE A2 GENE LINKS PLANT DEVELOPMENT AND DEFENSE****Synan F. AbuQamar, Suad Ajeb and Arjun Sham***Department of Biology, UAE University, P.O. Box 15551, Al-Ain, UAE;
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Expansins are cell wall loosening agents, known for their endogenous function in cell wall extensibility. The *Arabidopsis expansin-like A2 (EXLA2)* gene was identified by its down-regulation in response to infection by the necrotrophic pathogen *Botrytis cinerea*, and by the reduced susceptibility of an *exla2* mutant to the same pathogen. The *exla2* mutants also enhanced tolerance to the phytoprostane-A1. This suggests that the absence or downregulation of *EXLA2* leads to increased resistance to *B. cinerea* in a CORONATINE INSENSITIVE 1 (COI1)-dependent manner, and this down-regulation can be achieved by phytoprostane-A1 treatment. *EXLA2* is induced significantly by salinity and cold, and by the exogenous application of abscisic acid. The *exla2* mutant also showed hypersensitivity towards increased salt and cold, and this hypersensitivity required a functional abscisic acid pathway. The differential temporal expression of *EXLA2* and the phenotypes in transgenic plants with altered expression of *EXLA2* indicate that plant cell wall structure is an important player during *Arabidopsis* developmental stages. Our results indicate that *EXLA2* appears to be important in response to various biotic and abiotic stresses, particularly in the pathogenesis of necrotrophic pathogens and in the tolerance to abiotic stress.



SL-13*Track: Medical Biotechnology***NEW BIOMARKER VEA 195 AS EARLY SEROLOGIC INDICATION OF ORGAN TRANSPLANT REJECTION****F. Agbalika, M. Carmagnat, D. Bengoufa, M. Stern, F. Desgrandchamps J. Vérine, D. Charron and D. Glotz***Department of infectious Diseases, Saint-Louise Hospital, Paris, France;
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The identification of a new biomarker to predict and monitor renal status in transplant recipients is a challenge to overcome. We present immune protein detection, VEA 195, as a serologic biomarker in a blind test study for early diagnosis of rejection in comparison with histomorphology data of selected patients. We measure the level of VEA 195 in patients' residual sera by immunochromatographic assay on a strip using two monoclonal antibodies (MAbs) 7G9 and 10E5. For a total of 77 patients' serum specimens collected from 33 recipients documented for various stages of rejection and 17 controls, the following results were obtained: Sensitivity 96.3% (n=77) Specificity 95.2% (n= 43) The VEA level measured, with previously calibrated densitometer, varied from 45 to 300 ng/ml of serum (recipients), and with 0 to 2 ng/ml of serum (controls). The test had excellent accuracy for borderline stage, which can be difficult to diagnose. VEA 195 is reactive even before rejection confirmation by histological diagnosis on renal biopsies. The relevance of VEA 195 to graft loss makes this diagnostic test valuable for the management of transplanted patients by physicians. It can also be used for initial diagnosis.



SL-25

Track: Regenerative Medicine

AUTOLOGOUS MESENCHYMAL STEM CELL SHEETS AS A TECHNOLOGY PLATFORM FOR REGENERATIVE MEDICINE: A FOCUS ON PERIOSTEAL TISSUE ENGINEERING**Hani Awad**

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Massive bone loss in extremities as a result of trauma, war injuries, or cancer is a challenging clinical problem. In many cases, these injuries involve diaphyseal gap defects. The standard of care is the transplantation of a processed, devitalized allograft. With about a million allografts transplanted annually in the US alone, these scaffolding allografts are fraught with complications, including fractures and non-unions that render their average survival rate at 7 years to less than 40%. Often, the outcome of a failed extremity reconstruction is amputation. Over the past 10 years, we have been interested in understanding the limitations of processed, devitalized allografts. Studies we pioneered in a murine model of intercalary femoral allograft model have uncovered important differences in the impaired healing of the allograft compared to the robust healing of the viable autograft. The most relevant to this presentation is the finding that the robust autograft healing, which the allograft lacks, is potentiated by the periosteum. The lack of a periosteum in processed allografts results in deficits in osteogenesis, angiogenesis, and osteoclastic remodeling. We also demonstrated that restoring the periosteum on processed allografts (via stem cell and biomaterial hydrogel approaches) can rescue these deficits and stimulate an autograft-like healing of allografts. A major obstacle to translation in this approach is identifying an optimized mode of cell delivery. While in theory the use of biomaterials can enhance localized delivery and immobilization of cells to intended sites of implantation, they do illicit and inflammatory response as they degrade, and this typically leads to incomplete or delayed healing.

To address limitations of cell delivery, we have adapted a cell sheet technology that was invented in Japan to create pseudo-periosteal membranes. The premise of this technology is that cell culture surfaces can be covalently coated with nano-polymers (isopropylacrylamide and derivatives) that exhibit thermo-reversible hydrophobicity. At 37 °C, the coated surface allows cell attachment, and a drop of temperature to <32 °C causes the cells to detach. If enough time is allowed for the cells to reach confluence and synthesize ECM, the cell sheet and the deposited ECM can be lifted intact as a membrane. This technology has been tried and has shown promising results in numerous regenerative medicine applications, which will be reviewed in this presentation. Further, we recently demonstrated that growing marrow-derived MSCs in monolayer cell sheets does not alter their surface markers or their expression of gene markers of stemness. We have also demonstrated that MSC-sheets can be engrafted on decellularized allografts as an engineered periosteum and transplanted to reconstruct femoral diaphyseal defects in the mouse successfully. In just 6 weeks after transplantation, the MSC sheets induced robust callus formation, improved graft incorporation, and enhanced the torsional strength of the reconstructed femurs significantly over naked allografts or allografts directly seeded with MSCs, reaching about 70% of the strength of normal bones. This rate of healing is comparable to what is typically observed in fracture or autograft healing. These exciting findings provide merit and support autologous mesenchymal stem cell sheets as a technology platform for regenerative medicine in general, including the repair of segmental defects in bone.

SL-44

Track: Industrial and Manufacturing

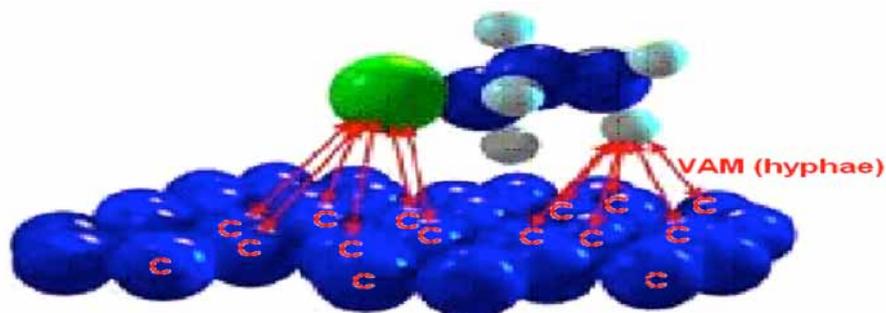
A PLAUSIBLE MECHANISM OF BIOSORPTION IN DUAL SYMBIOSES BY VESICULAR-ARBUSCULAR MYCORRHIZAL IN MOMORDICA CHARANTIA**Rafia Azmat**

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Dual symbioses of vesicular-arbuscular mycorrhizal (VAM) fungi with growth of *Momordica charantia* were elucidated in terms of plausible mechanism of biosorption in this article. The experiment was conducted in green house and mixed inoculum of the VAM fungi was used in the three replicates. Results demonstrated that the starch contents were the main source of C for the VAM

to builds their hyphae. The increased plant height and leaves surface area were explained in relation with an increase in the photosynthetic rates to produce rapid sugar contents for the survival of plants. A decreased in proline, protein, and amino acid contents and protease activity in VAM plants suggested that these contents are the main bio-indicators of the plants under stress. A greater rate of nutrient absorption by the plant is well explained by the adsorption of C and in place of C the other nutrients may take part which results in the increase leaf area and the development of lateral roots. The decline in protein may be due to the degradation of these contents which later on converted into dextrose where it can easily be absorbed by the period of symbioses. A mechanism of C chemisorption in relation with physiology and morphology was discussed.



Keywords: Dual symbioses, VAM fungi, biosorption, C, degradation.

SL-67

Track: Medical Biotechnology

ROLE OF PARATHYROID HORMONE-LIKE HORMONE IN STEM CELL PROLIFERATION AND GASTRIC CARCINOGENESIS

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The gastric stem cell plays a fundamental role in stomach homeostasis by maintaining normal rates of cell proliferation and differentiation. This perpetual role of stem cells is supported by many key molecular signaling pathways. Although the structure and secretory function of the gastric gland have been well studied, the pathways controlling stem cell proliferation and differentiation are not fully understood. It is generally accepted that cross-talks between epithelial and mesenchymal tissues regulate cell proliferation/differentiation through a number of secreted factors. The growth factor parathyroid hormone-like hormone (Pthlh) is expressed in different organs during development and adulthood, orchestrating key cellular events, including cell proliferation and differentiation. Unsurprisingly, imbalanced Pthlh gene regulation leads to the development of cancer in some organs. The aim of this study is to test whether the expression pattern of Pthlh receptor is altered during gastric carcinogenesis. Tissues obtained from consented gastric cancer patients undergoing gastrectomy were processed for immunohistochemistry using a monoclonal anti-Pthlr antibody. Our results demonstrated over-expression of Pthlr in precancerous and cancerous gastric mucosal tissues. These data suggest that Pthlh and its receptor are essential members of the signaling pathways involved in control of stem cell proliferation and gastric carcinogenesis and, therefore, Pthlr could represent a new biomarker for early detection of gastric cancer. (This study is supported by grants from UAEU and Terry Fox Foundation).



SL-16

Track: Medical Biotechnology

GBV-C-MEDIATED INHIBITION OF HIV-1

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Recent studies have shown that co-infection with GBV-C in individuals infected with HIV-1 results in significant longer survival compared to mono-infected individuals. Here we show that GBV-C infection upregulates several HIV-1 homologous miRNAs, that inhibit HIV-1 replication in several CD4+ T cell lines. Comparative global miRNA PCR-based microarray analysis of GBV-C infected Jurkat cell line infected with GBV-C showed 11 miRNAs were differentially overexpressed which shared >75% mutual homologies to HIV-1 and GBV-C. The stable expressions of each of the 11 miRNAs into three CD4+ cell lines—Sup-T1, Jurkat and HeLa-CD4+ cell lines showed significant long-term inhibition of HIV-1 replication ($p < 0.001$). Intracellular localization of HIV-1 in pre-GBV-C infected cells showed trapping of the HIV-1-provirus in cytoplasmic-perinuclear areas, whereas in post-infected cells HIV-1 integration was dominant. This is the first study that identifies the mechanism that enables the beneficial effects of GBV-C at intracellular levels by miRNAs. We propose that utilization of a genetically modified version of GBV-C could be a powerful tool in HIV-1 prevention in high risk groups.

SL-47

Track: Industrial and Manufacturing

WASTEWATER TREATMENT AND BIOENERGY RECOVERY USING BIOELECTROCHEMICAL SYSTEM

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A Microbial fuel cell (MFC) is a promising biotechnology to recover electricity or bioenergy from wastes. However, its practical application is greatly restrained by the low power outputs, which mainly arise from the low electron transfer rates in the anode electrodes and the low cathode performance. The cathode accounts for the main part of these problems due to the high cost of its components and slow kinetics of oxygen reduction at neutral medium (Logan, 2005, Zhang, 2009). With a continuous improvement in cathode performance, it is expected that the power production will eventually be limited by the maximum rate of electron transfer that can be sustained by anodic bacteria. Anodic bacterial attachment, biofilm formation and electron transfer efficiency are directly affected by the physicochemical properties of electrode surface. Therefore, effective anode material is critical for a high anode performance. Hence pre-treatment of the anode materials is desired. On the other hand, carbon material has low conductivity comparing with metal materials. The leading-out terminal of anode could be big factor effecting on the power output of scale up MFC when the carbon based materials were used as anode.

We focus here on examining the effects of anode leading-out terminal, pre-treat anode and hydraulic pressure on power output of scale-up MFC. Hydrogen peroxide or sodium hypochlorite pre-treatment offers an environment friendly and low-cost approach to improve the anode performance.

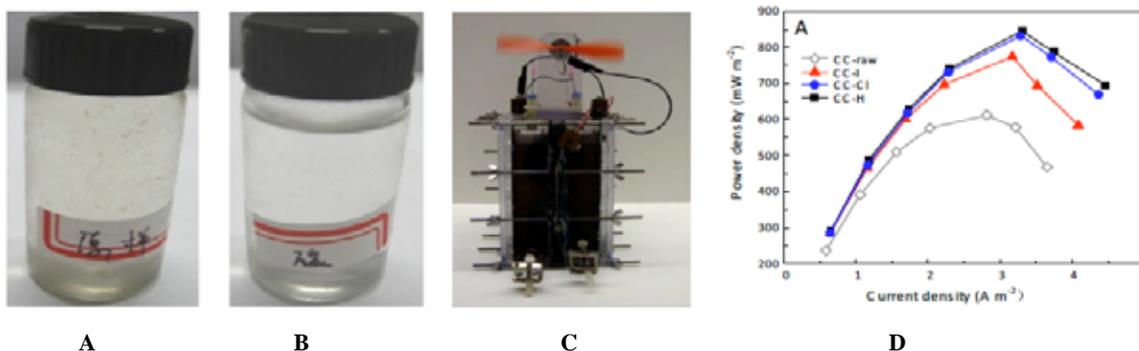


Fig. (1). Bioelectrochemical system for Wastewater treatment: (A) before treatment;(B) after treatment; (C) power from wastewater to drive a fan; (D) Power density for MFCs with the raw and treated anodes.

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SL-48*Track: Plant and Environment***USING LICHENS AS BIOINDICATORS OF AIR BORNE POLLUTION - SPECIATION OF CHROMIUM (VI) / (III) IN LICHENS AND SOIL SAMPLES****Ewa M. Cukrowska, Hlanganani Tutu and Luke Chimuka***Molecular Science Institute, University of the Witwatersrand, 2050 Johannesburg, South Africa;
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The use of lichens as bio-accumulators of pollutants has proved their efficiency in environmental sciences. They are an extraordinary symbiotic association of fungi and algae with peculiar physiology and morphology, which forces them to absorb and accumulate chemical elements in gaseous, liquid or particulate form from the atmosphere. Owing to the absence of excretion mechanisms in lichens, the xenobiotic substances cannot be expelled and accumulate over the years providing integrated measurements over a long period.

While many data have been published on heavy metals content and their redox compositions of plants and airborne particles only a few papers deal with heavy metal ratios in lichens. No study involving lichens and chromium redox compositions was previously reported.

We conducted the research on use lichens as bio indicators for study of the origin of the chromium contamination in the atmosphere in order to understand the distribution and speciation of chromium in soils and lichens in the vicinity of Tubatse Ferrochrome smelter in SA.

Conditions such as wind directions, soil pH, redox conditions, and solar radiation can alter the ratio of Cr(III/VI). For this reasons the soils cannot be used as indicator of Cr(VI) air borne pollution.

Samples of lichens (parmoterma) and soils were analysed for Cr_{tot} and Cr(VI) after digestion and leaching using sequential extraction procedure.

The results for lichens clearly show the differences in chromium speciation depending on the site and conditions, which was not shown in soils.

SL-15*Track: Others - Clinical Research/Clinical trials; Nano-biotechnology***FUNCTIONALISED SURFACES COUPLED TO ENHANCED SPECTROSCOPY FOR FOOD SAFETY ANALYSIS****Philippe Daniel, Fabienne Lagarde, Romain Chauvet, Gerald Thouand and Michele Lees***Institute for Molecules and Materials of Le Mans, IMMM UMR CNRS 6283, Université du Maine, France; E-mail: philippe.daniel@univ-lemans.fr*

The contamination of food by pathogenic bacteria or viruses appears as a huge subject of research, especially because it could have economic consequences but also can induce very important public health problem. Of course, analyses can be achieved by using standard microbiological methods; however the time of detection is generally minimum 2 days. A new approach can be based on the coupling between dedicated functionalized surfaces to enhanced Raman spectroscopy.

Then this presentation will show how the functionalization of gold surfaces but also polymeric surfaces allow to specifically fixe microorganisms which can be detected by Raman spectroscopy, which is a non-destructive and non-invasive technique to characterize any material using vibrational information. Additionally it will be demonstrated how the coupling with functionalized nanoparticles including magnetic nanoparticles covered by gold or silver could bring a valuable information by strongly enhancing the Raman signal (SERS effect). However if this SERS effect could be easily applied to living samples, like bacteria, a focuss will be especially done on the design of experiment and the data treatment using chemometric methods, in order to develop a robust protocol which could be developed in the next future as an alternative method to detect pathogens.

Keywords: Food safety, Nanoparticles, Spectroscopy, SERS effect.



SL-71*Track: Other Areas***THE EVALUATION OF MARGINAL FIT OF FULL CERAMIC CROWNS BY MICRO-CT TECHNIQUE****Necla Demir, Meral Arslan Malkoc and A.Nilgün Ozturk***Department of Prosthodontics, Faculty of Dentistry, Selçuk University, Konya, Turkey; E-mail: necladt@gmail.com*

Objectives. To measure the marginal gap (MG) and absolute marginal discrepancy (MD) values of full ceramic crowns using the Micro-CT technique before and after cementation, testing the null hypothesis that there is no difference in the adaptation between the ceramic systems studied.

Methods. Sixty extracted human maxillary premolar teeth with no caries or anatomical defects were divided into two groups according to the finish line design (Group I: 90° shoulder, Group II: 135° chamfer) to receive 3 types of full ceramic crowns; Group A; Vitablocs Mark II; Group II; In Ceram 2000 AL and Group C; IPS e-Max Press produced by using CEREC inLab according to the manufacturer's instructions. All crowns were veneered using the recommended porcelain. 5 crowns of each group 30 crowns totally were scanned using micro-CT before and after cementation with self-cure resin cement (Multilink Automix) and thermal cycle procedure. Micro-CT scanning was performed from each side (Buccal, Lingual, Mezial, Distal) in two sections; sagittally and coronally in order to determine the MG (Marginal Gap) and MD (Marginal Discrepancy) values. The measurements were done from 10 points; 80 points in totally for each side of the crown to evaluate the MG and MD values (mm) (Sagittal Buccal; Sagittal Lingual; Coronal Mezial and Coronal Distal regions). Files were processed using NRecon and CTAn software. Results were statistically analyzed using one-way, two-way ANOVA and Tukey HSD tests ($p=0.05$).

Results. All ceramic systems showed clinically acceptable marginal adaptation values. Vitablocs Mark II ceramics generally presented the lowest variance when compared to the other ceramics except the MG values in the mezial surface of the coronal section.

SL-38*Track: Industrial and Manufacturing***SUSTAINABLE PRODUCTION OF BIOBASED ORGANIC ACID MONOMERS FOR BIOPLASTICS****André de Haan***Corbion Purac, Arkelsedijk 46, 4206AC, Gorinchem, The Netherlands; E-mail: andre.de.haan@corbion.com*

Corbion is the global market leader in lactic acid, lactic acid derivatives and lactides, and a leading company in functional blends containing enzymes, emulsifiers, minerals and vitamins. The company delivers high performance bio-based products made from renewable resources. Recently Corbion announced the expansion of its activities towards other biobased organic acid monomers such as succinic acid and FDCA.

Aim of this lecture will be to provide an overview of recent developments within Corbion's biobased organic acid monomer portfolio and the corresponding sustainable production technology platforms. Next to developments in our fermentation processes also Corbion's ambitions to move towards non-food future generation feed stocks will be presented. Specific topics that will be discussed include Corbion's:

- Technology developments in stereo chemically pure lactides for high-heat PLA.
- Universal gypsum/salt free technology platform for fermentation pH control and acid recovery.
- 10 kT succinic acid production technology.
- >99% yield bio-oxidation process for HMF conversion into FDCA.

- Technology developments and strategic partnerships for the conversion of second generation fermentable sugars into organic acids.
- Sustainability and value chain challenges in applying second and future generation feed stocks.

SL-45*Track: Plant and Environment***IN VITRO TECHNIQUE FOR SELECTING ONION (*ALLIUM CEPA* L.) FOR WHITE ROT DISEASE-RESISTANCE****Kasem Z. Ahmed, Sayed A.M. Osman, Abdel-Rahem T. Abdel-Rahem Elrefai and Abdel-Rahem. A. Mohamed***Department of Genetics, Faculty of Agriculture, Minia University, El-Minia, Eg-61517, Egypt; E-mail: drelrefai@hotmail.com*

In vitro selection is one of the most effective and efficient methods for plant improvement due to its ability of changing the plant to the desired character(s), either by applying a selection agent on the culture media to change the somaclones toward the required character(s) or by giving particular condition to change the somaclones toward the required character(s). In the present work, we established a suitable protocol for *In vitro* selection for *Allium* white rot disease (*Sclerotium cepivorum*) tolerant for different commercial Egyptian onion (Giza 20, Giza 6 and Beheri Red). Oxalic acid (phytotoxic of this fungus) was used as selective agent. Seed of these 3 different Egyptian varieties were germinated on four different concentrations (0.0, 0.02, 0.2, 2 and 20 mM) of Oxalic acid (OA). Among the tested genotypes, Beheri Red showed the highest germination frequency (60%) at all tested concentration, followed by Giza 20 (58.2%), while, Giza 6 was the lowest cultivar (53.3%). Response of onion explants to OA were studied, Cotyledon explants from 3 different Egyptian varieties were cultured on different toxic MSBDK medium supplemented with 0, 3, 6 and 12 mM OA. The mean of calli percentage on MSBDK free toxic medium was 70.7 % calli in over all tested onion genotypes, however, MSBDK stressed medium with 3 mM OA produced calli in percentage of 42.1% only, the highest OA concentration (12 mM) was completely inhibited calli induction from cotyledons explants. Toxicity of OA on onion calli first were determinate the LD80 of OA, the result showed that medium supplement with 3 mM OA nearly retarded 80% of growth calli which was more suitable for *in vitro* selection protocol. Among 156 tested calli of Beheri Red only 23 calli (14.7%) were survived after growing on toxic medium for 45 days and 15.6% were survivors for Giza 20, while 40.1% calli were survived of Giza 6. Many plantlets were regenerated from survivors calli which transplanted to ex vitro and formed bulb after acclimatization.

Keywords: Onion, *In vitro* selection, oxalic acid (OA), *Sclerotium cepivorum*, Egypt, plant regeneration.**Abbreviations:** AWR, *Allium* white rot; OA, oxalic acid; MSBDK, Murashige and Skoog (1962) stressed medium with OA; 2,4-D, 2,4-dichlorophenoxyacetic acid; KIN, kinetin. BAP, benzylaminopurine.**SL-64***Track: Medical Biotechnology***THREE DIMENSIONAL SCAFFOLD-FREE MODELS FOR BONE REGENERATION****Ahmed T. El-Serafi***M27-138, College of Medicine, University of Sharjah, Sharjah University City, P.O. Box 27272, Sharjah, UAE; E-mail: aelserafy@sharjah.ac.ae*

Three dimensional tissue culture is gaining more interest as being more physiological, increases cell to cell contact and consequently enhances stem cell differentiation and matrix formation. Such model can be of particular interest for bone regeneration. We reported different 3D models for osteogenesis, based on pellet cultures system of either human bone-marrow-derived stromal cells (HBMSC) or human fetal-femur-derived cells (FC). HBMSC pre-treated with 5-Aza-dC (a DNA demethylation agent) showed enhanced osteoid formation compared with controls. 5-Aza-dC was not required for FC, which produced a configuration comparable to the cross section of a fetal bone. In a different 3D culture system,

plastic rods were inserted into the pellets. FC formed aggregates that migrated ~5 mm against gravity to cover the plastic rod and form a band of osteogenic tissue. When FC were cultured on flat bottom glass tubes, the cells formed a monolayer, which ultimately formed a 3D sphere after 10 days that had at least 2.5 times the diameter of pellets formed in similar conditions on a plastic surface. Extensive osteoid was observed in these self-assembled spheres, which had folded into sheets with chondrogenic matrix in between. These unusual constructs offer novel approaches for stem cell differentiation.

SL-24

Track: Other Areas

IMPLEMENTATION OF HACCP REQUIREMENTS IN NUTRITION DEPARTMENTS OF SELECTED HOSPITALS OF ISFAHAN UNIVERSITY OF MEDICAL SCIENCES

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Nutrition department is one of the most important parts in a hospital since the performance of it has great impact on patients' satisfaction. HACCP standard is one of tools for management and monitoring of the nutrition department. The aim of this study is reviewing the condition of HACCP standard requirements in nutrition departments of selected hospitals of Isfahan University of Medical Sciences. In this cross-sectional study, nutrition department of 12 hospitals of Isfahan University of Medical Sciences were studied. Data was gathered using questionnaires including 156 questions covering 5 domains through observation and interview. Statistical analysis was done using SPSS software and Kruskal-Wallis test. In total, rate of adherence to HACCP standard requirements in the studied hospitals was found as following: Modares (53.7%) and Seyedoshohada (53.1%) Hospitals, unacceptable conditions; Azahra (56.5%), Feiz (60.7%) and Amin (57.9%) Hospitals, moderate; Noor (68.2%), Ali-Asghar (68.2%), Eisabnmaryam (68.7%), Chamran (70.1), Shahid Beheshti (70%), Imam Musa Kazem (69.3%), Farabi (68.2%) and Kashani (68.5%) Hospitals, acceptable conditions. According to Kruskal-Wallis statistical tests, scores from the five studied domains were not significantly different while the scores of the 12 hospitals were significantly different ($P < 0.001$). Using HACCP system as a food safety management system in nutrition departments of the hospitals, not only leads to safe and healthy food production, but also results in improved patients' satisfaction, decreased complaint rates, preventing food poisoning episodes and avoiding unnecessary costs. Hospitals must work to establish HACCP standards. Educational courses for staff and enforcement of the prerequisites are recommended.

SL-10

Track: Medical Biotechnology

EFFECT OF IONIC LIQUIDS ON TYPE I COLLAGEN AT DIFFERENT HIERARCHICAL ORDERING

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The interaction of ionic liquids (ILs) with proteins has been gaining huge interest recently due to its easy tunability of cation and anion thus providing an opportunity for generating desired effect in proteins. Collagen has been widely explored in tissue engineering for its excellent biocompatibility and biodegradability. In this study, we have investigated the effect of alkyl imidazolium chloride ILs on collagen at the molecular, inter-fibrillar and skin matrix level. Circular Dichroic spectral studies reveal that at the molecular level, the secondary structure of collagen was not affected by ionic liquids. Also, there was no change observed in the thermal stability of collagen treated with ionic liquids at the molecular level. However, collagen at the inter-fibrillar level behaved differently on treatment with imidazolium ILs. An increase in concentration of ILs, led to decrease in thermal stability of rat tail tendon (RTT) collagen fibers. Scanning electron microscopy studies of skin matrix treated with imidazolium IL revealed an increase in the pore size of skin.

This kind of exquisite behavior of ionic liquids at different hierarchal order of collagen indicates that ionic liquids are endowed with potential lyotropic action, which can aid in use of collagen for various biomedical applications.

SL-21

Track: 'Other Areas'

SIGNIFICANT EVALUATION OF CHINESE HERBAL MEDICINE IN HEPATIC CELL CARCINOMA (HCC)

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3 cases of male underwent surgery for primary liver cancer, following post surgery interventional chemotherapy was administered. Months later, multiple hepatic *in situ* metastatic lesions had been found by CT and the level of AFP elevated, none of those patients were received the treatment of kinase inhibitor sorafenib target treatment, due to their economic carrying capacity. Alternatively, we administered Chinese herbal medicine (Gan Decoction, mixed a variety of effective herbal components) to help them to recover from the poor condition. After taking the Chinese herbs for 2 months, the tumour marker (CEA, AFP, CA19-9) dramatically decreased, with the result to the normal range. Most residual liver metastatic sites reduced according to CT imaging, and the patient felt free from the complaint of abdominal discomfort. The quality of life has been greatly improved, we managed to have prolonged the PFS (Progression-Free-Survival) and TTP (Time-to-Progression) from the onset to date. In the course of this individual treatment, we evaluated significance of Chinese herbal medicine the therapy of liver cancer. Chinese herbs might be an additional choice with its better benefits and tolerability in the treatment of recurrent hepatic cell carcinoma.

Keywords: Chinese medicine, hepatic cell carcinoma, interventional chemotherapy.

SL-52

Track: Other Areas

PSYCHOTROPIC DRUGS – SIDE EFFECT ON MOUTH

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Depression is the commonest form of affective disorders, and may range from discrete to severe. Various studies have worked with the hypothesis that depression arises from the deficiency of monoamines (noradrenaline, serotonin and dopamine), the most adequate treatment being to raise the supply of these neurotransmitters in the central nervous system (CNS). The treatment of psychiatric disorders mainly involves antidepressant, antipsychotic and anxiolytic drugs. Among the undesirable effects, the psychotropic drugs have affinity for the adrenergic receptors present in the salivary glands leading them to produce a more viscous secretion with low flow rates. A reduction in the salivary flow can lead to many complications, including dry and burning mouth, as well as difficulty in speaking, swallowing, eating and sleeping. In addition, it has been associated with an increase in the prevalence and severity of periodontal disease and an increase in the susceptibility to root caries, erosion and dental hyperaesthesia, chronic mucositis, trauma of the soft tissues, and oral candidosis.

Despite the well-state knowledge that psychotropic drugs induce mild-to-severe reduction in salivary secretion rates, until the present date, no studies prospected the histological alterations in salivary glands. To our knowledge, this is the first report that evaluated the architectural alterations provoked by a psychotropics in such glands.

In this study, the histomorphometric results revealed that, after the long-term treatment with anxiolytics drugs the parotid glands exhibited a disorganised parenchyma with loss of inter-lobular limits and a decrease and/or the disappearance of the central lumen. This anomalous architecture plus the results of acinar cell counts that did not vary suggest an increase in the size of the serous cells. These findings led to the idea of hypertrophy and refute the

supposition of hyperplasia. It can be postulated that such hypertrophy derives from the anticholinergic effect of the psychotropic drugs administered, via interaction with M3 muscarinic receptors, which reduces the release of saliva and leads to its retention within the gland. When we administrated an alkaloid present in the leaves and calluses of Brazilian Rutaceae plus antidepressants this effects diminished. Its therapeutic properties are due to its direct and unselective action on cholinergic muscarinic receptors, where alkaloid acts qualitatively as similar acetylcholine in the parasympathetic pathway.

SL-70

Track: Medical Biotechnology

GROWTH AND DIFFERENTIATION OF GASTRIC STEM CELLS ON BIODEGRADABLE SCAFFOLDS

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Recent therapeutic trends for some congenital and acquired diseases include stem cell therapy and tissue engineering. This is an interdisciplinary field that combines the technology of cell culture with material sciences to generate artificial tissues that can be used to partially or completely replace an organ. This study aimed at the cultivation of our recently established and immortalized mouse gastric stem (mGS) cells on an artificial scaffold to test whether it will be possible to support their growth and induce their differentiation on electrospunmicrofibrouspolycaprolactone (PCL) scaffolds. Non-porous, porous and a three-dimensional electrospunmicrofibrouspolycaprolactone (PCL) scaffolds were prepared and tested their suitability to grow the cells by cell viability/ toxicity assays and toluidine blue staining. The mGS cells were seeded on sterile PCL scaffolds and allowed to grow for up to 12 days *in vitro*, then processed for various methods. Cell proliferation and differentiation were monitored using PicoGreen assay of DNA quantification, scanning electron microscopy and lectin-/immuno-histochemistry. Results revealed the attachment, growth and differentiation of mGS cells preferentially on the microfibrous scaffolds in the standard culture conditions. Fluorescence and confocal microscopic analysis of cryosections probed with N-acetyl-D-glucosamine-specific lectin and anti-trefoil factor 2 antibodies indicated that, within 9-day culture, more than 50% of the mGS cells differentiated into mucous neck cells. Therefore, PCL scaffolds support not only adhesion and growth of mGS cells, but also their differentiation into mucous-secreting cells.



SL-69

Track: Marine Biotechnology

COMPARATIVE STUDY OF THE BIODEGRADATION OF ortho AND meta CRESOL BY WASTEWATERS MICROFLORA.

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Biodegradation of *o*-cresol and *m*-cresol by microflora of wastewaters taken from one sewage treatment plant of Constantine/Algeria is studied. A genomic identification test is applied to the main bacterial populations involved in the process. The cultures are performed in miniaturized fermentors, using a minimum medium, where cresols are the sole source of carbon and energy. The kinetic of the biodegradation is followed for 30 days of incubation at 30°C, in a batch and static system. Cresols concentration in the medium is measured by spectrophotometry.

The obtained results show the capacity of microorganisms to develop by degrading cresols. More than 90% of initial concentration of *m*-cresol are decomposed at the end of 14 days of incubation. After a lag phase of 24 hours, 75% of *o*-cresol are decomposed at 30 days of incubation. An abiotic degradation was also recorded, proceeding to a percentage of 25% of *o*-cresol and 41% of *m*-cresol, during the same incubation period. However, the difference between the two processes of degradation is statistically very significant ($p < 0.05$). Genomic analysis of bacterial isolates can assign two genera: *Alcaligenes* and *Pseudomonas*.



SL-12*Track: 'Other Areas: Environmental/Medical Microbiology'***NOVEL HOST FOR PATHOGENIC AND NON-PATHOGENIC ENVIRONMENTAL LEGIONELLA PNEUMOPHILA: IMMUNOLOGICAL IMPLICATIONS****Mahmoud Ali Halablab and Alia M.A. Aldahlawi***Rafik Hariri University, P.O. Box: 10, Damour, Chouf 2010, Lebanon; E-mail: halablabma@rhu.edu.lb*

Legionella pneumophila is a widely spread environmental bacterium. It is mainly transmitted through aerosolisation of water contaminated by Legionella cells. Aerosols can be generated by different means including, cooling towers, air-conditions, humidifiers, cutting glass procedures, water fountains etc. The organism is transmitted by inhalation of small aerosols containing the organism and may result in a severe form of pneumonia (Legionnaires'disease). The bacterium is a facultative intracellular pathogen that can infect and/or multiply in different host cells including alveolar macrophages, human monocytes, and murine dendritic cells (DCs). DCs are potent antigen presenting cells (APC) that are able to activate resting T cells to initiate primary immune response. DCs are also a major source of IL-12 that is required for the sensitization of Th1 cells, which play a critical role in immune response against intracellular pathogens. Moreover, it has been documented that DCs are more potent antigen presenting cells than macrophages.

So far, interaction of Legionnaires'disease bacterium with human DCs required further investigation. In the current study, the interaction was studied using a strain of Legionella that was pathogenic (virulent) and another non-pathogenic (avirulent) counterpart. After infection of human monocyte-derived DCs, the uptake, intracellular growth of the bacteria and the induction of apoptosis (programmed death) were investigated. The results obtained provide evidence that human monocyte-derived DCs can be infected by both virulent and avirulent strains of *L. pneumophila*. Although, human DCs were able to take-up both strains, only the virulent strain was able to multiply during 48h of infection as indicated by the viable count. Such infection resulted in the reduction of the viability of DCs. In contrast, avirulent strain did not affect the viability of the latter cells. Moreover, induction of apoptosis in human DCs, infected by virulent *L. pneumophila*, was evident after 2h of initial infection but the avirulent strain did not induce DC-apoptosis. Interestingly, intracellular multiplication of *L. pneumophila* inside DCs was not correlated with apoptosis which indicates early down regulation of cellular and humoral immune responses of DCs. In conclusion, the results suggest that the virulent *L. pneumophila* may subvert human DCs function as antigen presenting cells by the early induction of DC apoptosis that may contribute to the pathogenicity of the bacterium.

SL-18*Track: Others -Clinical Research***ADHERENCE TO ANTIRETROVIRAL TREATMENT AND ITS CORRELATES IN AN ERA OF SECOND-LINE ANTIRETROVIRAL DRUGS BEEN COMMON AMONG HIV/AIDS PATIENTS IN HUNAN, CHINA****Guoping He, Zhuying Li, Xianhong Li, Dan Liu, Honghong Wang and Siyuan Tang***School of Nursing of Central South University, 172 Tong Zi Po Road, Changsha 410013, Hunan, China; E-mail: hgpcsu@aliyun.com*

Background: The second-line antiretroviral drugs were developed because of its better efficacy of depressing HIV and fewer side effects. It has also been considered to have the benefits of improving antiretroviral treatment (ART) adherence, which is very important for high quality of life of the AIDS patients.

Methods: A cross-sectional survey was conducted in two major CARE sites of Hunan from July 2010 to Oct 2011 by face-to-face interviews, where the second-line antiretroviral drugs were widely used.

Results: The average ART time was 18 months (range: 1,73). According to the 30-day-VAS, 72% (301/418) of subject maintained more than 90% adherence, but during past 7 days, 90% (375/418) reported more than 90% adherence and 78% (325/418) reported 100% adherence. The three measures of self-reported adherence were highly correlated. On



multivariate analysis, heroin abuse (odds ratio [OR] =1.7; 95% confidence interval[CI]=1.1, 2.7, $p<0.05$), less educated (OR=1.3; 95%CI=1.0. 1.7; $p<0.05$) and lack of remind from family members (OR=1.5; 95%CI=1.0. 2.4; $p<0.05$) were associated with 90% or less adherence.

Conclusion: The adherence to ART among AIDS patients in Hunan was improved compared to that of 5 years ago by the same measurement. The widely used second-line antiretroviral drugs might be a contributor.

SL-49

Track: Medical Biotechnology

EFFECT OF 50 HZ MAGNETIC FIELD ON THE TESTIS AND SPERM MORPHOLOGY OF ADULT ALBINO RATS

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This study was conducted to evaluate the effect of 50 Hz magnetic field on the testicular gross morphology, histology, sperm morphology and meiotic chromosomes of adult Albino rats. The rats were divided into three groups; Control, Low intensity exposed group in which rats were exposed to a magnetic field of 0.15 mT and high intensity exposed group with rats exposed to a magnetic field of 20 mT. 37 rats were included in each group. The exposure time lasted for 2 weeks, 3 days /week and for 1/2 hour per day. After the exposure period the rats were sacrificed by cervical dislocation and handled for evaluation. One of the testes was examined for gross morphological and histopathological changes. The other one was handled for evaluation of the meiotic chromosomal abnormalities in the spermatocytes and the rat sperms were extracted from the epididymis and vas deferens to evaluate their morphological changes.

There were no observed gross morphological changes. The histopathological evaluation revealed a picture of maturation arrest at the level of the round spermatids with slight degenerative changes. These changes were more marked at the high intensity exposed groups with progress to totally lost seminiferous tubules. The morphometric study showed reduction in the mean cellular surface area of the seminiferous tubules and an increase in each of the thickness of the basal lamina of seminiferous tubules, the number of the Leydig cells in the interstitial space and the surface area of the blood vessels. There was no effect on neither the seminiferous tubular diameter, nucleo-cytoplasmic ratio of Leydig cells nor the Sertoli cell number. The sperm morphological evaluation showed increased number of abnormal forms in the low intensity exposed group versus the control one with higher increase in the high intensity exposed group. The same conclusion was conducted from the chromosomal study as it showed increase in the chromosomal abnormalities in the exposed groups with higher increase in the high intensity exposed one.

Keywords: Magnetic field, spermatocytes, testicular gross, chromosomes.

SL-68

Track: Other Areas - hydraulic conductance mechanism

DETERMINATION OF THE HYDRAULIC CONDUCTANCE OF THE DENTIN DISCS OBTAINED FROM PRIMARY AND PERMANENT TEETH AND THE BOND STRENGTH OF RESTORATIVE SYSTEMS APPLIED TO THESE DISCS

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Aim: It was to establish the permeability of dentine discs obtained from primary teeth, to determine the bond strength of three different restorative systems applied to these discs.

Methods: 60 carious free primary second molar teeth were used. Dentine discs were obtained from the occlusal surface of these teeth. Dentine permeability of discs was evaluated by hydraulic conductance mechanism. Then, the discs were randomly divided into three groups. Under artificial



pulpal pressure, restorative systems [Clearfil SE Bond+Clearfil AP-X (CSEB + CAPX), AdheSE+Compoglass F (ASE + Comp F), Prime Bond NT+Dyract AP (PBNT + DAP)] were applied. The dentine discs which the restorative systems applied on were cross-sectioned to 1 mm² using the low speed diamond saw. The bond strengths of 60 samples were measured by means of micro shear test method.

Results: The mean of the dentine permeability of the dentine discs was found 0.014 $\mu\text{cm}^2\text{cmH}_2\text{O}^{-1}\text{dak}^{-1}$. The bond strength of CSEB + CAPX was 13.68 \pm 5.25 MPa, the bond strength of ASE + Comp F was 16.02 \pm 8.19 MPa and the bond strength of PBNT + DAP was 17.03 \pm 3.55 MPa. It was found that there was statistically no difference between the bond strengths of three different restorative systems ($p>0.05$). Also it was determined that there was no relation between the values of the dentine permeability of dentine discs and the bond strengths of applied restorative systems.

Conclusion: The dentin permeability that a biological factor has no effect on the bond strength.

SL-23

Track: Pharmaceutical Biotechnology

THREE-ARMED PROTOCOL FOR CYTOGENOTOXICITY STUDIES OF 5-AZACYTIDINE IN MOUSE BONE MARROW CELLS

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5-aza-2'-cytidine (5-aza-C), an anticancer cytidine analog, was tested for its cytogenetic toxicity in mouse bone marrow cells using a colcemid-fixative-air drying-fluorescent technique. The cytogenetic end points selected were cell survival, cell replication index (CRI) and sister chromatid exchanges (SCE) induction. The study was conducted under three different experimental conditions. In the first (*in vivo/in vitro* protocol), mice were intraperitoneally (ip) injected with increasing doses of 5-aza-C; 0.00625, 0.0625, 6.25, 12.5, 25, 50 and 100 μM for 8 h, followed by a 28 h-culture period. In the second and the third (*in vitro/+S9*, *in vitro/-S9* protocols, respectively), cells were treated with the same 5-Aza-C concentrations for 1 h in the presence or absence of a metabolic activation system (rat S9 fraction), followed by a culture period of 35 h. Exactly matching positive controls were exposed to mitomycin C (MMC; 0.005 μM) and cyclophosphamide (CP; 0.025 μM); the first does not require metabolic activation while the second does. Negative controls received only distilled water. Relative to the negative control, percent cell survival significantly decreased only at concentrations equal or greater than 50 μM . At 100 μM 5-Aza-C, cell viability was 86.71%, 43.64% and 45.15% for the *in vivo/in vitro*, *in vitro/+S9* and *in vitro/-S9*, respectively. At levels equal or higher than 6.25 μM , 5-aza-C few or none of the cells reached their third cycle under both *in vitro* conditions. Cytotoxicity was too excessive to score SCE. In the *in vivo/in vitro* experiments, cytotoxic effect was less pronounced; some cells completed their third division cycle and this effect was not significant. Mean SCE rate/ cell at 0.625 μM 5-aza-C was 2-fold with and 3-fold without S9 metabolic activation; 4.86 and 7.40, respectively as compared to that in the *in vivo/in vitro* system 2.43) suggesting that the drug is converted into a less mutagenic metabolite. Collectively, 5-aza-C can be considered a cytostatic/cytotoxic weak mutagen.

SL-65

Track: Industrial and Manufacturing

MOLECULAR CHARACTERIZATION OF POLYGALACTURONASE PRODUCING BACTERIAL STRAINS COLLECTED FROM DIFFERENT SOURCES

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Seventy-two bacterial strains were isolated from twenty various sources including soil, water, rotten fruits, vegetables etc. Preliminary screening for pectinase producing bacterial strains was done by well plate method and twenty-six bacterial strains gave zones on MS medium supplemented with 0.2% pectin. Most of the isolates belonged to plant origin, either rotten fruits or vegetables due to high pectin presence as compared to other samples. Amongst the bacterial

isolates, ZP-F5 and ZP-F6 gave the largest zone sizes i.e., 45 mm and 40 mm respectively. All selected strains were subjected to cellular, morphological and biochemical characterization. Polygalacturonase production by the selected strains was measured quantitatively by DNS method. Strain ZP-F5 and ZP-F6 were producing maximum amount of enzymes i.e., 1.85 U/ml and 1.86 U/ml respectively. Identification of pectinase producing bacteria was confirmed by amplification and sequencing of their 16S rRNA gene. The sequences obtained were BLAST which revealed that ZP-F5, ZP-F6, ZP-F14, ZP-F24 and ZP-F25 were found homologous to *Bacillus subtilis*. Whereas, ZP-F10, ZP-F16 and ZP-F18 were homologous to *Klebsiella variicola*, *Brevibacillus laterosporus*, *Ewingella americana* respectively. Some of the novel strains obtained like *Klebsiella variicola*, *Brevibacillus laterosporus*, *Ewingella americana*, *Providencia vermicola* and *Klebsiella oxytoca* demonstrated significant levels of pectinolytic activity. The results show that the natural frequency of the genus *Bacillus* species but other bacterial strains are also capable of hydrolyzing pectin.

Keywords: Pectinase, polygalacturonase, Pectin.

SL-50

Track: Industrial and Manufacturing

HYDROCARBON BIOFUELS PRODUCTION FROM WASTE ANIMAL FATS VIA PYROLYSIS REACTOR: AN ENVIRONMENTAL APPROACH

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Recently, biofuels has become more attractive because of its environmental favor and for overcoming the energy needs. In this context, this study aims the conversion of waste animal fats from Tunisian Meat Industry into biofuels (bio-oil, syngas and biochar) via pyrolysis process. Experiments were conducted in a fixed-bed reactor at 500°C as final temperature and 5°C/min as heating rate, in order to study the products distribution and their chemical compositions.

The study showed that: (i) Fatty wastes are precious raw materials for biofuels production by pyrolysis method, (ii) The physical and chemical characteristics of the obtained bio-oils are very similar to those of Tunisian Diesel (according to the JORT standard) and European biodiesel (according to the EN14214 standard), which makes them a good alternative for fossils fuels, (iii) The pyrolysis process is a simple and environmental technology to produce renewable energy.

Keywords: Biofuels; pyrolysis; waste animal fats; characterisation.

SL-30

Track: Plant and Environment

CONTROLLING FUSARIUM PATHOGENS AND MYCOTOXINS VIA MOLECULAR BIOTECHNOLOGY

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Fusarium pathogens are ascomycetes that cause Fusarium head blight (FHB) in wheat, barley, maize and other cereal crops world-wide. They produce various types of mycotoxins in the grain, posing a serious threat to human and animal health. FHB epidemics occur frequently in central China, especially along the middle and lower reaches of the Yangtze River. Since the mid-1990s, FHB has re-emerged as a serious problem to agriculture in North America and Europe, causing huge economic losses. However, innate resistance in nature against FHB pathogens is inadequate. Current protective measures rely on chemical control, producing undesirable environmental consequences. We have developed an alternative strategy to control them. Fusarium-specific recombinant antibodies were isolated by phage display and the generated antibodies were assayed for their binding and antifungal activity when fused to antifungal proteins. Immunofluorescence labeling and immunoblot analyses revealed a specific binding of the antibodies to the surface targets of the pathogens. The antibody fusions had both the binding capability and antifungal activity. Plants expressing

the antibodies or fusions displayed significantly enhanced resistance against *Fusarium* pathogens and contained less mycotoxins. These results indicated that antibodies and fusions can be used to effectively control *Fusarium* pathogens and mycotoxins in agriculture.

SL-73

Track: Medical Biotechnology

QUANTITATION AND COMPARISON OF INSULIN ANALOGUES GLARGINE, ITS METABOLITES M1 AND M2, ASPART AND PORCINE INSULIN BY USING TRIPLE QUAD 6500 AND TRIPLE TOF 5600 LC-MS/MS SYSTEMS AND ELISA IN A DOG TOXICOKINETICS STUDIES

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Glargine, Aspart and porcine insulin are used as long lasting, fast acting or other especially medical treatment of insulin-dependent diabetes as bioengineered insulin analogue. Quantitative evidences of presences and concentrations after doping multiple concentrations are important information. Such information can also help to understand the pharmacokinetics, pharmacodynamics, and toxicology during the drugs developments. Due to challenges of selected quantitation without interferences, not many validated methods have been published so far. In this presentation bioanalytical methods coupled with solid-phase extraction and LC-MS/MS for the quantitation of above intact insulin analogues in plasma are discussed from their developments, validations and applications. The multiple-reaction-monitoring (MRM) experiments performed on a triple-quadruple mass spectrometry instrument were used for quantitation with range from 0.2 to 20 ng/mL corresponding to the normal non-fasting insulin levels in plasma. This validated MRM method was applied for toxicity studies of insulin analogues in dogs. Moreover, we compared the MRM results to the MS quantitation acquired on a hybrid quadruple-quadruple time-of-flight and showed the high resolution instrument can be an option for peptide and biotherapeutic protein quantitation. Toxicokinetics in dogs are also compared between LC-MS/MS and ELISA methods from our work which will provide important information for such protein therapy developments.

SL-37

Track: Pharmaceutical Biotechnology

DEVELOPMENT OF POLYMER-PEPTIDE CONJUGATES AS THERAPEUTIC VACCINE AGAINST CERVICAL CANCER

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Cervical cancer is the second most common cancers in women worldwide and this cancer is caused by high-risk types of human papillomavirus (HPV), most commonly HPV-16. Prophylactic HPV vaccines were commercialized and are clinically effective in preventing HPV infection but do not have a therapeutic effect against established HPV infection. The development of therapeutic vaccines that eliminate HPV infected cells and eradicate established HPV-associated tumors would therefore be beneficial and desirable.

The aim of this study is to develop a novel therapeutic vaccine strategy based on polymer conjugated to synthetic peptide epitopes derived from HPV-16 E7 proteins. The polyacrylate polymer has been selected as the most promising candidate because it has little or no toxicity and the self-assembling and self-adjuvanting ability of polyacrylate amphiphilic dendrimers has been reported. The most promising antigens were identified, conjugated to polymer, and self-assembled into the microparticles. Several constructs have been synthesized; each contained multiple copies of the same epitope conjugated to the polymer. These conjugates were examined and the lead conjugate produced a robust therapeutic effect against a tumor without the help of any external adjuvant after single immunization. This delivery system overcomes the lack of immunogenicity of peptide-based vaccines. The presented vaccine candidates were able to



completely eradicate tumor in mice model. Thus, we developed the first self-adjuvanting delivery system for therapeutic vaccine against cervical cancer.

SL-61

Track: Medical Biotechnology

HEDGEHOG SIGNALING REGULATES MYOD EXPRESSION AND ACTIVITY DURING SKELETAL MYOGENESIS

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The inhibition of MyoD expression is important for obtaining muscle progenitors that can replenish the satellite cell niche during muscle repair. Progenitors could be derived from either embryonic stem cells or satellite cells. Hedgehog (Hh) signaling is important for MyoD expression during embryogenesis and adult muscle regeneration. To date, the mechanistic understanding of MyoD regulation by Hh signaling is unclear. In this study, we demonstrate that the Hh effector, Gli2, regulates MyoD expression and associates with MyoD gene elements. Gain- and loss-of-function experiments in pluripotent P19 cells show that Gli2 activity is sufficient and required for efficient MyoD expression during skeletal myogenesis. Inhibition of Hh signaling reduces MyoD expression during satellite cell activation *in vitro*. In addition to regulating MyoD expression, Hh signaling regulates MyoD transcriptional activity, and MyoD activates Hh signaling in myogenic conversion assays. Finally, Gli2, MyoD, and MEF2C form a protein complex, which enhances MyoD activity on skeletal muscle-related promoters. We therefore link Hh signaling to the function and expression of MyoD protein during myogenesis in stem cells.

SL-26

Track: Other Areas

BIOTECHNOLOGICAL BASIS OF RESOURCE-SAVING IN MORI-AND SERICULTURE

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Two biological species, mulberry tree *Morus alba* L and mulberry silkworm *Bombix mori* L are the basis for present-day silk production.

Some applications of biotechnological approaches are presented in this work as a solution of topical problems in traditional moriculture, sericulture and silk technology [1, 2].

Assimilability of natural and artificial diets, elaborated silkworm bioregulators as well as enhancers of cocoon productivity were improved particularly on the basis of physico-chemical, biochemical and biophysical researches of mulberry leaves composition, potential components of artificial diets (AD), digestion and respiration processes in different stages of mulberry silkworm development. Silkworm rearing and silk producing in the space condition by AD showed possibility to breed it everywhere.

Biocontrol methods of mulberry pyralid including by wild and genetically-modified viral and some other bioinsecticides in IPM system were studied.

Methods of bioprotective post harvest cocoon processing, utilization of sericulture and silk industry wastes for obtaining of new biotechnological and nanotechnological products were developed.

Structural organization of developed fibroin biosorbents, drug-, protein-, enzyme- and cell entrapped composites was studied by electron microscopy.

Keywords: Mulberry, silkworm, composition, feeding, physiology, biochemistry, bioregulators, artificial diet, space experiments, mulberry pyralid, biocontrol, silk, wastes, resource-saving, biotechnology, nanotechnology.

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SL-57

Track: Plant and Environment

APPLICATION OF RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) TO ANALYZE GENETIC DIVERSITY IN *SPHENOSTYLIS STENOCARPA* (HOCHST EX A. RICH) HARMS (AFRICAN YAM BEAN) CULTIVARS

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The African yam bean (*Sphenostylis stenocarpa*) is a leguminous plant grown for its edible seeds and tubers in Central and West Africa, especially Nigeria. The plant has more potentials than other grain group legumes, but it is highly understudied and underutilized. Differentiating among the various varieties of *S. stenocarpa*, which appear similar, is one of the major challenges facing its improvement. The current study was aimed towards developing a random amplified polymorphic DNA (RAPD) assay to analyze the genetic diversity which exists in the crop. For the RAPD analysis, nucleic acids were isolated from three cultivars and various amounts were used as templates in optimizing the polymerase chain reaction (PCR). Two random primers were designed and used in various combinations: forward primer only, reverse primer only and combination of both forward and reverse primers for the PCR. From the results obtained, polymorphisms became evident as the presence and/or absence of DNA fragments in the different cultivars were compared among one another. Finally, this study indicated that RAPD analysis can be used to evaluate the genetic variability that exists in the various cultivars of the different species of *S. stenocarpa* in order to harness its full potential.

SL-60

Track: Plant and Environment

EFFECT OF INCREASING BIOMASS OF SELECTED PROTOZOAN ISOLATES ON BIODEGRADATION OF CRUDE OIL (PETROLEUM) SPILL BY-PRODUCTS POLLUTED WASTE WATER

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Water pollution through oil and oil based by-products as a result of human and industrial activities has become a daily occurrence in the past decades. There is therefore a greater need for wastewater management and protection of existing water resources in the environment from harmful hydrocarbon contaminants. This study investigated the role of three protozoan isolates in the biodegradation of crude oil (petroleum) from polluted wastewater. The study was conducted using three protozoan isolates (*Aspidisca sp.*, *Trachelophyllum sp.* and *Peranema sp.*) where their growth and biodegradation abilities were screened using industrial rich oily wastewater in shaking flasks at pH 8 and incubated at 30 °C with a speed of 100 rpm for 5 days. Chemical Oxygen Demand, Dissolved Oxygen and growth of protozoan isolates were determined using standard methods. Crude oil concentrations in the samples were determined using partition gravimetric method. The study revealed that up to 80% COD released and almost 100% DO were removed from wastewater. There was a direct relationship between the increase in population growth of the protozoa and their oil biodegradation capacity. The protozoan cell density of 1.00×10^3 cells/ml was able to degrade crude oil (initial concentration: <50 mg/L) from wastewater at average rates ranging from 6.41 to 35.5%, from 11.0 to 38.5%, from 11.7 to 30.1% and from 21.2 to 55.1% for *Aspidisca sp.*, *Trachelophyllum sp.*, *Peranema sp.*, and a consortium of the three isolates, respectively. An increase in oil concentrations in wastewater mixed liquor (>50 mg/L) decreased their



biodegradable capability (ranges: 3.40 to 23.2%, 3.50 to 25.9%, 3.04 to 20.7% and 9.50 to 40.5%, respectively). An increase in protozoan cell densities of 1.00×10^4 cells/ml and 1.00×10^5 cells/ml resulted in oil biodegradation rates ranging from 11.4 to 66.0 %, 15.0 to 58.9%, 13.7 to 49.0% and 28.0 to 73.2%, respectively. Statistically, the microbes were found to have a significant difference in their biodegradation ability in different oily wastewater concentrations ($p < 0.001$).

SL-42

Track: Plant and Environment

BIOTECHNOLOGY OF CEREALS: IMPLEMENTATION OF RNAI TECHNOLOGY FOR FUNCTIONAL GENOMICS AND IMPROVEMENT OF SELECTED TRAITS

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Biotechnology offers a range of tools for functional analysis and for improvement of selected traits such as yield, product quality, resistance to biotic and tolerance to environmental stresses. One of the most promising, especially useful in allopolyploid cereals, is RNAi-based gene silencing.

We proved its applicability by silencing two groups of genes: *Pina / Pinb* in wheat and their orthologs in triticale and, in another line of experiments, *HvCKX1 / HvCKX2* in barley. The *Pin* genes determine grain hardness in wheat, which is important technological trait. Silencing of the genes in wheat resulted in reduction of the transcript exceeded 90% what caused significant reduction or lack of both puroindoline proteins and increased grain hardness up to the level of *T. turgidum* var. *durum* cultivar, which lacks of the genes (Gasparis *et al.* 2011). Unexpectedly silencing of *Sina* and *Sinb* genes in triticale however resulted in significant reduction of transcripts and secaloidindoline proteins, did not prove their role in grain hardness.



The second group of silenced genes belongs to the *HvCKX* family of barley. They encode cytokinin dehydrogenase enzymes (CKX), which regulate cytokinin level in different tissues of developing plants. The detailed functions of the genes are not known. We have already documented that silencing of the *HvCKX1*, which expression was the highest in the roots and developing spikes of wild plants, decreased cytokinin dehydrogenase level in these tissues. This led to higher plant productivity expressed as the yield, the number of seeds per plant and the 1000 grain weight and greater mass of the roots (Zalewski *et al.* 2010). Silencing of *HvCKX2* (Zalewski *et al.* 2012), which expression was the highest in the developing spikes as well as in the young and fully developed leaves of wild plants, decreased transcript and CKX level in these tissues. There was a positive correlation between the low level of transcript in spikes 7 DAP and the higher plant productivity. The data of productivity of modified lines up to T₃ generation will be presented and discussed.

Acknowledgements

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SL-22

Track: Regenerative Medicine

TRACKING AND MRI VISUALIZATION OF MESENCHYMAL STEM CELLS LABELED WITH IRON OXIDE NANOPARTICLES IN THE HEPATOCELLULAR CARCINOMA MODEL

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Introduction: Recently, a significant increase in the incidence of hepatocellular carcinoma (HCC) has been reported worldwide. The recent reports related to the use of mesenchymal stem cells (MSCs) to reduce tumor size in experimental models of HCC stimulated us to document these effects. The development of a tracking system that maintains cell viability and is easy to follow and document is essential.

Methods: We injected MSCs labeled with iron oxide nanoparticles, intravenously, in the rat model of HCC. A time course visualization and tracking of the MSCs was done using magnetic resonance imaging (MRI). Histopathological examination of the liver and organ samples was performed.

Results: We detected a significant tumor-mass reduction in the group which received MSCs compared to the control groups. Labeled MSCs were traced to the affected liver and they participated actively in reducing the tumor size.

Conclusion: The results of this work support the use of iron oxide nanoparticles in tracking and visualization of MSCs to the site of the lesion, which may provide a documentation of their therapeutic effect.

SL-72

Track: Pharmaceutical Biotechnology

ANTI-VIRAL VACCINES BASED ON INDUCED INHIBITION OF VIRAL RECEPTORS

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Recent studies have shown that co-infection with GBV-C in individuals infected with HIV-1 results in significant longer survival compared to mono-infected individuals. Here we show that GBV-C infection upregulates several HIV-1 homologous miRNAs, that inhibit HIV-1 replication in several CD4⁺ T cell lines. Comparative global miRNA PCR-based microarray analysis of GBV-C infected Jurkat cell line infected with GBV-C showed 11 miRNAs were differentially overexpressed which shared >75% mutual homologies to HIV-1 and GBV-C. The stable expressions of each of the 11 miRNAs into three CD4⁺ cell lines—Sup-T1, Jurkat and HeLa-CD4⁺ cell lines showed significant long-term inhibition of HIV-1 replication ($p < 0.001$). Intracellular localization of HIV-1 in pre-GBV-C infected cells showed trapping of the HIV-1 provirus in cytoplasmic-perinuclear areas, whereas in post-infected cells HIV-1 integration was dominant. This is the first study that identifies the mechanism that enables the beneficial effects of GBV-C at intracellular levels by miRNAs. We propose that utilization of a genetically modified version of GBV-C could be a powerful tool in HIV-1 prevention in high risk groups.



SL-14

Track: Pharmaceutical Biotechnology

WIDE ANGLE NEUTRON AND X-RAY DIFFRACTION ARE SENSITIVE TO CHANGES OF BIOLOGICAL ACTIVITY OF X-MAB SOLUTIONS

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A series of X-MAB protein (aqueous) solutions, with varying biological activities, have been investigated by synchrotron X-ray diffraction and reactor-based neutron diffraction. Corrected and normalised total scattering structure factors show systematic variations over the scattering vector range between 10 and 35 inverse nanometers as the biological activity of the samples varies between "perfect" and "unacceptable". The scattering vector range mentioned above is much higher than that would be for the more conventional small angle scattering region (which is below about 5 inverse nanometers). This is the first experimental evidence for the given series of protein solutions that the variations in terms of the biological activity is most probably caused by changes in terms of the solvation structure and NOT by alterations of the shape and/or aggregation of the protein molecules.



SL-7*Track: Medical Biotechnology***LACTAPTIN – A NOVEL PEPTIDE FOR ANTICANCER THERAPY****Vladimir Richter, O.A. Koval, A.V. Tkachenko, A.S.Fomin, D.V. Semenov, A.A. Nushtaeva, E.V. Kuligina and E.L. Zavjalov***Institute of Chemical Biology and Fundamental Medicine SB RUS, 8 Lavrentiev Avenue, Novosibirsk, 630090, Russia. E-mail: richter@niboch.nsc.ru*

Lactaptin - peptide, induced apoptosis in cultured tumor cells, was isolated from human milk. Recombinant analog of lactaptin RL2 was designed. The mechanism of RL2 inducing apoptosis has been studied. It was shown RL2 effectively penetrated into the cells, interacted with cytoskeleton proteins, induced dissipation of mitochondrial membrane potential and caused apoptosis via the activation of initiator caspases 8, 9 and effector caspases 3 and 7. Dynamic changes in the mRNA and protein levels of apoptosis-related genes were estimated. We observed RL2 constitutively suppressed *bcl-2* mRNA expression and down regulated Bcl-2 protein expression in MDA-MB-231 cells.

To test whether the RL2 delays tumor growth *in vivo*, a mouse xenograft model of breast cancer was used. A suspension of MDA-MB-231 cells was subcutaneously injected into female SCID mice to form solid tumors. Tumor-bearing mice were injected RL2 intravenously three times. RL2 injections significantly (up to 43%) delayed tumor growth compared to the control group. Furthermore, RL2 treatment extended the lifespan of animals with tumors. The antitumor RL2 based drug was developed and the preclinical trials have been successfully finished. The study was supported by RFBR grants No's. 13-04-01313 and 13-04-01457 and by Russian Federal Target Program "Farma-2020" grant No. 16.N08.12.1009 <<http://www.sciencedirect.com/science/article/pii/S030090841>>.

SL-63*Track: Plant and Environment***VIOLAXANTHIN AND DIADINOXANTHIN DE-EPOXIDASES: MECHANISM OF ACTION AND SIGNIFICANCE FOR PHOTOPROTECTION IN PLANTS****D. Latowski, M. Bojko, M. Olchawa-Pajor, W. Strzalka, P. Kuczynska and K. Strzalka***Department of Plant Physiology and Biochemistry and Department of Plant Biotechnology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland; E-mail: kazimierzstrzalka@gmail.com*

All photosynthetic organisms developed photoprotective mechanisms with carotenoids playing a fundamental role in the dissipation of excess light energy. Photoprotection is mainly connected with the de-epoxidized forms of the xanthophyll pigments which are formed by enzymatic removal of epoxy groups from epoxy carotenoids under high light conditions. These reactions occur in the processes known as the xanthophyll cycles, the most common among them being the violaxanthin and diadinoxanthin cycles. In violaxanthin cycle present in all vascular plants and in many groups of algae, violaxanthin is converted to zeaxanthin *via* antheraxanthin by violaxanthin de-epoxidase, whereas in diadinoxanthin cycle epoxy group is removed from diadinoxanthin and diatoxanthin is created. This conversion takes place *e.g.* in diatoms with involvement of the enzyme diadinoxanthin de-epoxidase. Previously, only one gene of this de-epoxidase, designed DDE, was postulated. Today in one of the diatoms, *Phaeodactylum tricorutum* (CCAP 1055/1 strain with all genome sequenced) three forms of de-epoxidases were identified but only one of them corresponded to VDE. This form of DDE is also called VDE, and its gene is marked as *PtVDE*. The two other VDE-like de-epoxidases (designated as *PtVDL1* and *PtVDL2*) may be more specialized in the chromist-specific diadinoxanthin cycle.

The de-epoxidases operating in the xanthophyll cycles are the lipid-dependent enzymes. Our data show that they are active only in the presence of the lipids which are able to form inverted hexagonal phases. Lipids forming bilayers do not support the activity of these enzymes. Violaxanthin and diadinoxanthin which in photosynthetic membranes are bound to proteins of chlorophyll-protein complexes, are not available for de-epoxidation by the respective enzymes. To be converted to de-epoxidised forms of xanthophylls in high light conditions, they must be released from the binding proteins to the lipid phases. A molecular mechanism of xanthophylls cycle based on the data obtained from model systems as well as from native photosynthetic membranes will be discussed.

SL-41

Track: Industrial and Manufacturing

PILOT PLANT FACILITY FOR THE SCALE-UP OF CONTINUOUS MODE LACTIC ACID FERMENTATION**Joachim Venus**

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A multifunctional pilot plant that converts renewables such as starchy materials, green biomass, several residues/wastes/by-products and lignocellulosics.

The entire processing chain has been implemented: from the feedstock, the pre-treatment/hydrolysis for releasing C5 and C6 sugars, the (continuous mode) fermentation to lactic acid and the downstream processing of fermentation broth to generate marketable lactic acid of high enantiopurity (L and D) and quality.

With the pilot plant a new biotechnological procedure is realized, allowing an efficient refinement of ingredients from plant biomass and residues into industrial products according to the process steps of a sugar-based fermentation technology. One of most interesting issues is the long-term stability of the continuous fermentation process leading to much higher volumetric productivity.

SL-33

Track: Plant and Environment

PHYLOGENETIC POSITION OF AMPHORA SENSU LATO (BACILLARIOPHYCEAE) SPECIES AND COMPARATIVE ANALYSIS OF MORPHOLOGICAL CHARACTERISTICS**Pengbin Wang, Bum Soo Park, Jin Ho Kim, Joo-Hwan Kim and Myung-Soo Han**

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Amphora Ehrenberg ex Kützing *sensu lato* is a common and widespread benthic diatom genus with a taxonomy that has been under perpetual revision, particularly by means of molecular analyses. Though *Amphora* species have been studied using modern microscopy in recent years, there has not been much progress on molecular characterization of the species, especially in Asia. In this study of *Amphora*, sampling was carried out from September 2009 to August 2010 in Korean coastal waters using sampling tools and the brush method. The morphological and molecular characteristics of eight *Amphora sensu lato*, *Amphora marina*, *A. proteus*, *Halamphora eunotia*, *H. terroris*, *H. holsatica*, *H. costata*, *H. coffeaeformis*, and *Halamphora* sp. were examined. Their morphological characteristics, according to previous description from other publications, suggest that *A. marina* and *A. proteus* belong to the subgenus *Amphora* Cleve, which have smooth cingula and rather coarse and very distinct areolae on the valve. The other species, *H. coffeaeformis*, *H. costata*, *H. eunotia*, *H. holsatica*, *H. terroris* and *Halamphora* sp. belonging to the subgenus *Halamphora* Cleve, recent elevation to generic status by Levkov 2009, have plicate cingula, puncta which do not form straight longitudinal lines, valves which have a narrow ventral portion and apices that are generally rostrate-capitate and recurved. In agreement with analysis based on morphological characteristics, phylogenetic analysis based on small subunit (SSU) rDNA suggested that the eight *Amphora sensu lato* species were not a monophyletic group as the morphological classification. In this study, morphological features of all eight species were studied and comparatively analyzed. The results of molecular work and statistical analysis on all these *Amphora sensu lato* combined with phylogenetic analysis on our geographically representative samples and the results of previous studies give more strong evidence on that the subgenera *Euamphora* Cleve and *Halamphora* Cleve should be treated as two independent genera.

SL-20*Track: Pharmaceutical Biotechnology***NOVEL PHOSPHORUS-CONTAINING AMINO ACID AND DIPEPTIDE MIMETICS AS HIGHLY POTENT INHIBITORS OF NEISSERIA MENINGITIDIS ALANYL AMINOPEPTIDASE****Ewelina Węglarz-Tomczak, Stamatia Vassiliou, Łukasz Berlicki, Małgorzata Pawelczak, Bogusław Nocek, Rory Mulligan, Andrzej Joachimiak, Paweł Kafarski and Artur Mucha***Department of Bioorganic Chemistry, Faculty of Chemistry, Wrocław University of Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland; E-mail: ewelina.weglarz-tomczak@pwr.wroc.pl*

Alanyl aminopeptidase (APN, E.C.3.4.11.2) is a zinc-containing protease that selectively hydrolyzes an amino acid residue from the N-terminus of peptides and proteins. Deregulation of the mammalian ortholog activity is associated with angiogenesis, cancerogenesis, malignancy and metastasis [1]. The counterpart enzyme expressed by microorganisms, including human pathogens, is mostly responsible for proteolysis and nutrition delivery. Infection of *Neisseria meningitidis*, a gram-negative bacterium that causes meningitis, can be an example of such a disease. Blocking the activity of *N. meningitidis* alanyl aminopeptidase might appear a new strategy of highly specific control of meningococcal meningitis and can allow to recognize new tools to identify the precise role of the enzyme in the bacterial life cycle. Recently, recombinant NmAPN was isolated, its sequence, the crystal structure and the substrate specificity were investigated [2].

Phosphorus-containing, amino acid and short peptide mimetics represent an interesting class of potent inhibitors of aminopeptidases. Being transition state analogues they act in a competitive and noncovalent manner. In this work we present two strategies of rational modifications of canonical organophosphorus inhibitors that led us to identify highly effective and selective inhibitors of NmAPN, with inhibition constants at the nanomolar range. The modifications involved either introduction of the α,β -diaminoethyl portion as the specific P1 residue dedicated to interact with glutamate-rich region of the active site or P1 and P1' optimization by heteroatom addition to exploit favorable contacts with the distal part of the S1 and S1' binding pockets.

Keywords: Alanyl aminopeptidase, *Neisseria meningitidis*, transition state inhibitors, organophosphorus inhibitors, amino acid and pseudodipeptide analogues.

Acknowledgements

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SL-17*Track: Pharmaceutical Biotechnology***VIRUS-LIKE PARTICLES AND CAPSOMERES FOR ENGINEERED DELIVERY OF ANTIGEN****Nani Wibowo, M.R. Anggraeni, N.K. Connors, Y. Wu, F.K. Hughes, Y.P. Chuan, L.H.L. Lua, A.P.J. Middelberg***Australian Institute for Bioengineering and Nanotechnology, Centre for Biomolecular Engineering, The University of Queensland, St Lucia, QLD 4072, Australia; E-mail: n.wibowo@uq.edu.au*

Vaccine development is increasingly based on modern concepts of rational design which use minimal components, such as antigenic peptide, in vaccine formulation. Peptide however exhibits lower immunogenicity and requires adjuvant or delivery system to boost its immunogenicity. Virus-like particles (VLPs) and their sub-unit, capsomeres, are promising delivery systems formed by self-assembly of viral capsid proteins. VLPs and capsomeres harness the molecular signature and particulate nature of

a virus, which induce potent immune response, without infectivity. This work demonstrates the potential of murine polyomavirus VLPs and capsomeres as vaccine delivery systems for influenza antigens. Helix 190 from influenza hemagglutinin receptor binding region and M2e from extracellular domain of influenza matrix protein 2 were modularised for presentation on VLP and capsomere, respectively. A number of strategies were used to modularise single or multiple copies of antigen. *In vivo* testing of modular VLPs, supported by prediction from molecular dynamic simulations, shows that different modularisation strategies elicited different quality of immune responses. *In vivo* testing of modular capsomeres presenting 15 copies of M2e antigen induced high titre of antigen-specific antibody response, leading to reduced viral titre in the lungs of infected mice. MuPyV capsid protein can be produced at gram-per-litre levels in microbial expression system, enabling rapid and cost effective vaccine manufacturing. This technology is potentially suitable for addressing not only influenza but numerous other diseases that require large-scale and low-cost vaccine manufacture that is rapidly adaptable to match pathogen variation.

SL-35

Track: Industrial and Manufacturing

FERMENTING KELP CARBOHYDRATES INTO BIOETHANOL: AN AVENUE FEASIBLE IN CHINA

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Fermenting starch and sugar into ethanol once paved an avenue of biofuel production; however the raising concerns on food-fuel conflict, land usage and among others have narrowed this avenue. Fermenting lignocellulose into ethanol is a dream; unfortunately, people are enjoying its beauty due to the difficulty of pretreating lignocellulose and concerns on ecological function maintenance. Culture of such brown algae as laminaria (kelp or konbu) does not use freshwater, fertilizer and territorial land, which also facilitates animal culture. Previously, less attention was paid to brown algae as the feedstock of bioethanol production; their major carbohydrate, alginate, cannot be fermented by the tactical industrial microorganisms. At present, well engineered *E. coli* can simultaneously ferment all brown algal carbohydrates, alginate, mannitol and glucan (laminarin) into ethanol, which is pleasing the whole world. China is a country culturing laminaria on a large scale and uses it either for the extraction of mannitol and alginate or as food. Breeding of laminaria hybrids and high-temperature resistant varieties and year-round cultivation trials may increase the unit yield to the theoretical, thus producing abundant algal feedstock for bioethanol production. Fermenting algal biomass into ethanol should be an avenue feasible in China.

SL-53

Track: Others

THE NEW PARADIGM IN THE MANAGEMENT OF WOUND USING COFFEE POWDER

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Background: Many patients complaint inconvenience and discomfort resulting from the replacement frequency of wound dressings, wound odor and sticky wound dressing. Coffee has high bactericidal capacity against MRSA, antioxidants, deodorize. Coffee powder has the capacity to resolve the complaint to be a paradigm in the management of wounds.

Methods: 105 patients not willing to do skin grafting, with a variety of acute & chronic wounds, productive wound fluid or not, purulent or not, type 2 diabetes mellitus, or autoimmune, were treated topically, using coffee powder as wound dressing replaced every 3-5 weeks.

Results: After trying with many different wound dressings, after debridement, the use of coffee powder in large quantities to cover the surface of the wound, resulting in healing without dressing replaced every day or every week, be

kept dry, not visited by flies, smelled coffee, did not cause pain, speed recovery, simple, without any adverse complication found.

Robusta coffee and arábica coffee powder mixed with wound fluid causing the properties:

- Much longer dressing change
- Maintaining moisted wound
- Antioxidants
- Antimicrobial
- Absorbing wound fluid
- Eliminate wound-odors (deodorize)
- Non-traumatic
- Cost-effective
- Autolytic debridement
- Minimized pain from frequent dressing change
- Reduce scarring



Figure 1. Inhibition zone in MRSA culture: Coffee, Bumazin, Betadine 3%

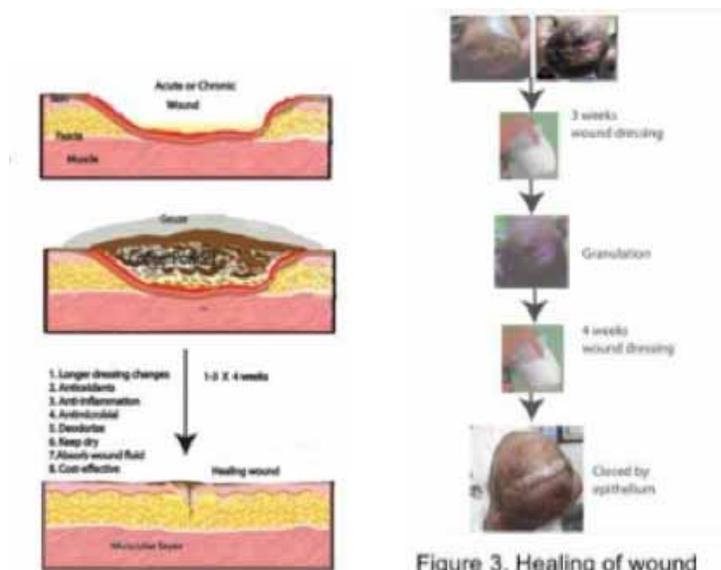


Figure 2. New paradigm in wound management using coffee powder.

Figure 3. Healing of wound dehiscence in Juvenile rheumatoid arthritis of 17 year-old female .



Figure 5. Experimental burn injury to Wistar rats.

Conclusion: Coffee powder as a wound dressing has a strong influence on the emergence of a new paradigm of thinking in the management of wounds: longer dressing change, maintain moistened wound, many antioxidants, antimicrobial, absorbing, deodorize, autolytic debridement, reduce scarring, minimize pain by less frequent dressing change, cost-effective, no adverse reaction.

SL-36

Track: Plant and Environment

PREPARATION OF β -1,3-OLIGOSACCHARIDES FROM CURDLAN BY ALKALI-NEUTRALIZATION TREATMENT AND ACID HYDROLYSIS AND THE ELICITATION EFFECTS OF OLIGOSACCHARIDES ON THE DEFENSE RESPONSES IN POTATO (*SOLANUM TUBEROSUM* CV. MCCAIN)

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It is recently confirmed that β -1,3-glucan oligosaccharides possess a variety of biological activities. The bacterial polysaccharide, curdlan, is a linear β -1,3-glucan which has the potential to produce oligosaccharides in large-scale, whereas the insolubility impedes the application of curdlan.

To enhance the hydrolysis efficiency, an alkali-neutralization treatment was applied to increase the stability of curdlan suspension. According to the sedimentation and scanning electron microscopical results, a speculated double-layer structure model comprising a compact core and a hydrated outer layer was proposed to describe the treated curdlan particles. This model was then verified by the identification of curdlan-acid hydrolyzate. Furthermore, an effective HPLC procedure was established to purify individual β -1,3-oligosaccharides with degree of polymerization (DP) from 2 to 10 from the hydrolyzate.

Subsequently, the elicitation effect of curdlan oligosaccharides (CurdOs) on the defense responses in potato (*Solanum tuberosum* cv. McCain) was evaluated. The results indicated that the CurdOs elicited the defense responses at a higher level than the Laminarin (from *Laminaria digitata*) did. The DP 5 oligosaccharide was the smallest unit that had the full elicitation activity. Furthermore, the induced responses in potato leaves by CurdOs were observed only in short term, as no remarkable changes were detected after 3-week cultivation.

Keywords: Curdlan, β -1,3-oligosaccharide, double-layer structure, *Solanum tuberosum*, elicitation, defense response.

SL-34

Track: Pharmaceutical Biotechnology

THE BEAUTY OF WINE: THERMODYNAMICS OF GRAPE AND WINE TANNIN INTERACTIONS AND WHAT IS HIDDEN BEHIND THEM

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Wine has been an integral part of human civilization for thousands of years and it is the oldest biotechnological process, with humans having been involved in wine production for at least 7000 years [1]. Wine can also benefit our health only if it is consumed with cautions. According to "Dietary Guidelines for Americans 2010" published by the US Department of Agriculture, and "The National Health Service" in UK: "If alcohol is consumed, it should be consumed in moderation - up to one drink per day for women and two drinks per day for men". The medical profession has recognized the healthful and nutritive properties of wine for thousands of years. Recent archeological evidence shows wine was in use as a pharmaceutical as early as 3,150 B.C [2].

The aim of our research was to determine thermodynamics of grape and wine tannin interactions, especially with the poly-(L-proline) peptides (PLP), major part of salivary proteins [3], to understand the mechanisms behind red wine astringency, known as an important indicator of wine quality and to change over time. The astringency of red wine softens with wine aging and yet the mechanisms for this change remain unclear [4]. Therefore, we applied isothermal titration calorimetry to demonstrate differences in binding between PLP and variety of tannins by analyzing thermodynamics of the interactions of PLP with grape seed tannins (preveraison PV) or Cabernet Sauvignon wine tannins (T08 from 2008 and T04 from 2004). Our investigation performed in the solutions containing various concentrations of ethanol indicated that all tannin-PLP complex formation processes being spontaneous, as indicated by negative Gibbs free energy, and exothermic with enthalpy as a driving energy. The aged wine tannin T04 bound to fewer PLP units than younger wine and grape seed tannins, and demonstrated also the smallest enthalpy and the binding association constant values of complex formation. This suggested that changes in the tannin structure with wine aging, such as the formation of intramolecular bonds, create more folded structures with fewer binding sites available for interaction with PLP. Increase of ethanol concentration resulted in weaker tannins binding to PLP and decrease in Gibbs free energy by increasing the negative entropy.

Lower PLP association with aged wine tannins might have an implication in the wine astringency softening over the time. Winemaking is a performance of art and we believe that our findings provide some insight into the understanding of wine astringency phenomenon and that will drive into manufacture of great wines tuned in to harmony.

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POSTERS

PO-44*Track: Industrial and Manufacturing***COLD-ADAPTED SERINE PROTEASE FROM ANTARCTIC YEAST *LEUCOSPORIDIUM ANTARCTICUM* STRAIN PI12****Raja Noor Zaliha Raja Abd. Rahman and Norsyuhada Alias***Enzyme and Microbial Technology Research Centre, Universiti Putra Malaysia, Malaysia; E-mail: rmzaliha@upm.edu.my*

Leucosporidium antarcticum strain PI12 was isolated from Casey Station, Antarctica. It was shown to be protease-producer. Full-length cDNA of PI12 protease gene was amplified by rapid amplification of cDNA ends (RACE) strategy with an open reading frame (ORF) of 2892 bp that coded for 963 amino acids. Homology search by BLAST web server has showed the PI12 protease sequence shared a significant homology to the subtilisin protease family from fungus but no similarity with other psychrophilic protease. The protease sequence was deposited into the GenBank Database with the accession no. CAQ76821. The gene encoding mature PI12 protease was cloned into *Pichia pastoris* expression vector, pPIC9, and positioned under the induction of methanol-alcohol oxidase (AOX) promoter. The recombinant PI12 protease was efficiently secreted into the culture medium driven by the *Saccharomyces cerevisiae* α -factor signal sequence. The highest protease production was obtained from *P. pastoris* GS115 host (GpPro2) with 20.3 U/mL activity at 15°C after 3 days of induction time. The expressed protein was detected by SDS-PAGE and activity staining with a molecular weight of 99.3 kDa.

Keywords: Antarctica, psychrophilic yeast, serine protease, RACE, protein expression.

PO-45*Track: Pharmaceutical Biotechnology***PANCREATIC LIPASE INHIBITORY POTENTIAL OF SELECTED MALAYSIAN PLANTS****Raja Noor Zaliha Raja Abd. Rahman, Norsyuhada Alias, Mohd Shukuri Mohamad Ali, Adam Leow Thean Chor and Asilah Ahmad Tajudin***Enzyme and Microbial Technology Research Centre, Universiti Putra Malaysia, Malaysia; E-mail: rmzaliha@upm.edu.my*

Pancreatic lipase inhibitors from natural sources are potential tool for the treatment of obesity. The inhibitory activity of 24 crude extracts of selected Malaysian plants against porcine pancreatic lipase (PPL) was successfully screened, *in vitro*. *Orthosiphon stamineus*, *Phyllanthus niruri*, *Murraya paniculata* and *Averrhoa bilimbi* leaves extracts showed more than 70% inhibitions when incubated with PPL at a final concentration of 500 $\mu\text{g/mL}$ for 15 minutes at 37°C. *P. niruri* crude extract exhibited strongest lipase inhibitory activity, with an IC₅₀ value of 27.65 $\mu\text{g/mL}$ followed by *O. stamineus*, *M. paniculata* and *A. bilimbi* with IC₅₀ values of 34.74, 41.45 and 55.18 $\mu\text{g/mL}$, respectively. Inhibition mode study disclosed that *O. stamineus* and *A. bilimbi* could act as uncompetitive inhibitor while *P. niruri* and *M. paniculata* could act as noncompetitive inhibitor. These results suggested that these four potent plant extracts may be useful for the treatment of obesity.

Keywords: Obesity, Pancreatic lipase, Orlistat, Inhibition mode.

PO-82

Track: Others - Environmental Biotechnology

NEW POLYHYDROXYALKANOATES SYNTHETIC GENE FROM WILD TYPE YEAST IN *E. COLI*

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This study aims to isolate, confirm and characterize the genes responsible for biosynthesis of the polyhydroxyalkanoates (PHA) biopolymers in wild type yeast. Therefore, yeast strain DGM1 was isolated and its ability to form PHA was confirmed by using Nile-red viable colony staining assay, fluorescence microscopy, TEM analysis and its extracted and purified PHA was characterized by FTIR, as well. The results confirmed the ability of strain DGM1 to produce PHA. Molecular identification of strain DGM1 put it under the non-saccharomyces yeast *Hanseniaspora valbyensis*. Subsequently, a sub-genomic library of the *Hanseniaspora valbyensis* strain DGM1 partial BamHI digested g-DNA was constructed in pBK phagemid vector. The genomic library in *E. coli* was screened for PHA genes using Nile-red viable colony staining assay. Physical characterization of PHA by FTIR, ¹H NMR and ¹³C NMR of the purified PHA showed peaks typical to the isotactic PHA copolymer. TEM analysis confirmed the presence of PHA granules in uniform spherical shape. The 0.5 kb insert in PUC18 plasmid was subjected to automatic sequencing and the complete sequence was found to be 452 bp. However, no significant similarity was found between the sequence of the new gene and previously characterized bacterial PHA biosynthetic genes. Thus, it would be categorized as novel PHA biosynthetic gene. Therefore, further studies to identify and characterize the role of this clearly unique PHA synthase-like gene in yeast are currently underway.

Keywords: Polyhydroxyalkanoates, *Hanseniaspora valbyensis*, phagemid vector.

PO-65

Track: Pharmaceutical Biotechnology

WATER-SOLUBLE CHIRAL RUTHENIUM (II) PHENYLOXAZOLINE COMPLEX: REUSABLE AND HIGHLY ENANTIOSELECTIVE CATALYST FOR INTRAMOLECULAR CYCLOPROPANATION REACTIONS

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Cyclopropanes are useful building blocks for the synthesis of natural and synthetic products due to their unique structures and reactivities. They are also easily converted into useful compounds that have medical, pharmaceutical, and insecticidal properties. In addition, cyclopropanes fused to five- or six-membered lactone rings are widely applied for the synthesis of natural products. Recently, we reported the efficient use of polymer-supported chiral Ru(II)-pheox complex in asymmetric inter- and intramolecular cyclopropanation. Cyclopropanation reactions are usually performed in organic solvents, which are less favored than in aqueous media in the context of green chemistry. Herein, we report an intramolecular cyclopropanation catalyzed by water-soluble R(II)-pheox in ether/water biphasic medium. A variety of Ru(II)-pheox complexes with different functional groups were studied. By using the water-soluble Ru(II)-hydroxymethyl phenyloxazoline catalyst, excellent enantioselectivities were achieved (up to 98% ee) in quantitative yields with a broad class of trans-allylic diazoacetates and alkenyl diazoketones. The catalyst could be reused at least 5 times.

Keywords: Asymmetric synthesis, biphasic medium, cyclopropanation, ruthenium, water-soluble catalyst.

PO-87

Track: Medical Biotechnology

STUDY VIRULENCE FACTORS RESPONSE OF *S. AUREUS* AND *E. COLI* AFTER EXPOSURE TO NONTHERMAL DBD PLASMA SYSTEM

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This study has been designed for inactivation of pathogenic bacteria in atmospheric air by direct exposure to air plasma produced by Atmospheric pressure parallel- plate Dielectric Barrier Discharge (DBD) at various time periods. Both of *Escherichia coli* and *Staphylococcus aureus* isolates were used as tested bacterial models to evaluate antibacterial properties of DBD. The germicidal efficiency was 100% for *E.coli* at 6 seconds of exposure to plasma and 8 seconds for *S. aureus* obtained by PCT (plate count technique) method, and losing some of virulence factors of *S. aureus* and *E. coli* isolates that survived after plasma exposure as compared to parental cells due to efficiency of using DBD plasma.

Keywords: Dielectric Barrier Discharge, virulence factors, *E. coli*, *S. aureus*.

PO-66

Track: Regenerative Medicine

CHICK EBF1 GENE EXPRESSION IN FEATHER DEVELOPMENT

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The chick Ebf1 (Early B-cell Factor-1) gene is a member of a novel family of helix loop helix transcription factors. The expression profile, regulation and significance of this gene have been extensively studied in lymphatic, nervous, adipose and muscular tissues. However, cEbf1 expression, regulation and function in the feather of chick embryo have not investigated yet. cEbf1 expression was first detected throughout the mesenchymal core of some few feather placodes (D7-D7.5). After feathers became mature and grew distally (D9-D10), the mesenchymal expression of cEbf1 became confined to the caudal margin of the proximal half of all formed feather buds. Because this dynamic pattern of expression resembles that of Shh and Bmp4 plus the crucial role of these two major signals in feather development, we hypothesized that cEbf1 expression in feather may be regulated by Shh and Bmp4. In feather explant culture system, Shh signals are necessary to initiate and maintain cEbf1 expression in the posterior half of the feather bud, while Bmp4 is crucial for the initial cEbf1 expression in the anterior half of the feather bud. Inhibition of Shh, does not only down-regulate cEbf1 but also change the morphology of feather buds, which becomes irregular and fused. This is the first study to demonstrate that cEbf1 expression in the feather bud is under the control of Shh and Bmp4 signals and that expression may play a role in normal development of the feather.

PO-60

Track: Pharmaceutical Biotechnology

ANTIBACTERIAL AND ACUTE TOXICITY EFFECTS OF SOME PLANT EXTRACTS

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Plant extracts are much known about their many therapeutic and pharmaceutical effects. In this work, were tested the antibacterial activity of the ethanolic and methanolic extracts of some plants by the agar diffusion method. Then, we measured their acute toxicity effect on mice and realized a phytochemical screening for all the extracts.

The results showed that the methanolic extract of *Punica granatum* was more active against the Gram-positive and Gram-negative bacteria. The ethanolic extract of *C. sinensis* and the methanolic extract of *P. granatum* had the best acute toxicity values. Also, we had observed that the two cited extracts contained mainly flavonoids, phenolic acids and

tannins. As conclusion the methanolic extract of *P. granatum* was the most interesting extract for the both tested activities.

Keywords: Plants, extracts, antibacterials, acute toxicity, phytochemical screening.

PO-12

Track: Plant and Environment

ALLELOPATHIC EFFECT OF CALLIGONUM COMOSUM EXTRACT ON SEED GERMINATION OF RHANTERIUM EPAPPOSUM

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Some plants may beneficially or destructively affect other plants through allelochemical compounds which may be released directly or indirectly from live or dead parts and cause significant effects.

This study was conducted to estimate the effects of different concentrations of *Calligonum comosum* (leaves, stem and roots) extracts on the seed of *Rhanterium epapposum*. Germination indicators (Percentage of germination, germination start, Coefficient of velocity (CV)) were evaluated. Results showed that the stem and leaves extracts, have negative and significant effects on *Rhanterium epapposum* seeds" growth, one the other hand, highly positive effect was observed for the application of the root extract on the seeds. The significant allelopathic effect remained up to 21 days, CV decreased with the allelochemicals from the stem and leaves, furthermore increased CV compared to control when the seed treated with the rood extract was documented.

Based on the study results, stem and leaves residues of *Calligonum comosum* should be eliminated from the field to avoid negative allelopathic effects of *Calligonum comosum* on *Rhanterium epapposum* growth.

Keywords: Allelopathic, *Calligonum comosum*, Seed Germination, *Rhanterium epapposum*.

PO-89

Track: Medical Biotechnology

STUDY ON CULTURED MUSCLE CONTRACTION INHIBITORY EFFECT OF GRAYANOTOXIN

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Muscle disease treatment are expected in the field of regenerative medicine and applied research of cultured muscle to bio actuator is performed in Biomedical Engineering as applied research of cultured muscle. This study is intended to cultured myoblast C2C12 from mouse striated muscle, and a mechanism of cultured muscle contraction by electric stimulation is investigated. Changes in membrane potential is measured using a micro glass tube electrode of myotubes cells induced by applying an external electrical stimulation. The contraction and membrane potential change by injection of current using a micro glass electrode are also measured. A spike response to a injected negative short pulse of current is observed in membrane potential before the contraction. We are investigating in detail muscle contraction and this spike of membrane potential change a machine of the spike potential preceding the contraction is also discussed.

Keywords: Cultured muscle contraction.

PO-83*Track: Pharmaceutical Biotechnology***IN VITRO ANTIDERMATOPHYTIC ACTIVITY OF AQUEOUS EXTRACT FROM THYMELAEA HIRSUTA LEAVES****N. O. Amari, M. Bouzouina, A. Berkani and B. Lotmani***Department of Biotechnology, University of Abdelhamid Ibn Badis, Mostaganem, Algeria;
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Infectious diseases treatment by plants became traditionally a current practice. Thymelaea Hirsuta, a rich plant flavonoids, is considered among the plants which present therapeutic properties. The aim of this study is to evaluate, in vitro, by direct method, the anti fungal activity of leaves aqueous extract on the behavior of fungi responsible for the human surface mycosis: *Microsporum audouinii*. The results showed that the solutions used in this experiment act according to their concentrations on mycelial growth and spores germination of the studied dermatophyte, with interesting IC50 and IC90. . Because of its crude chemical composition, the natural product seems to be effective, and records $315,41 \pm 3,86\mu\text{g/ml}$ (IC50) and $672,80 \pm 0,98 \mu\text{g/ml}$ (IC90), respectively. The flavonoid compounds resulting from the secondary metabolism of Thymelaea hirsuta can be used advantageously in the treatment of fungal skin infections.

Keywords: Thymelaea hirsuta - *Microsporum audouinii* - Aqueous extract - Antifungal activity.

PO-61*Track: Medical Biotechnology***CRYPTOSPORIDIUM INFECTION AND CORRELATION WITH CD4+ T-CELL COUNT AMONG HUMAN IMMUNODEFICIENCY VIRUS PATIENTS WITHIN KADUNA METROPOLIS, NIGERIA****M. Aminu, C.M. Ndaks and E.E. Ella***Department of Microbiology, Ahmadu Bello University, Zaria-Nigeria; E-mail: maryamaminu@yahoo.com*

Cryptosporidiosis being an opportunistic infection is becoming more prevalent in Human Immunodeficiency Virus (HIV) seropositive patients. This study was carried out to determine the prevalence of Cryptosporidium infection and the correlation with CD4+T-cell count among HIV seropositive patients within Kaduna metropolis, Nigeria. Stool specimens were collected from 300 HIV seropositive patients (study population) and 30 HIV seronegative patients (control population) between October 2011 and January 2012, and examined for oocyst and antigen of Cryptosporidium by microscopy and ELISA method. Microscopy was used as the Gold standard and its sensitivity was compared with that of ELISA. Participants' blood samples were also analyzed for CD4+ T-cell count by flow cytometry. Prevalence of 15% (45/300) was obtained by microscopy. Sensitivity of microscopy when compared to ELISA was found to be 24.9%. For the control population, a prevalence of 3.3% (1/30) was obtained. Cryptosporidium infection was associated with those who defecate in open layouts (50%: 3/6) and those who drank river water without boiling (50%: 1/2). There was a significant association between Cryptosporidium infection and diarrhoea ($\chi^2 = 104.669$, $df=1$, $p=0.000$) and also the duration of diarrhoea ($\chi^2 = 117.073$, $df=4$, $p=0.000$). The oocysts were detected more frequently in males (19.8%: 18/91) than female patients (12.9%: 27/209) while patients within age group 16-25 years were most affected (25.7%: 9/35). Cryptosporidium infection was not associated with occupation, marital status, sex, age, education, animal contact, overseas travel and swimming ($p>0.05$) in this study. There was a decrease in prevalence with longer duration of being on HAART. The mean CD4+ count of patients was 409.86 ± 14.1 while the median was 382. There was a strong association between cryptosporidiosis and CD4+T-cell count ($\chi^2 = 58.478$, $df=10$, $p=0.000$) with the highest prevalence recorded among patients with CD4+T-cell count $<200 \text{ cell}/\mu\text{l}$. This finding indicates that there is low opportunity for this parasite to get established as the patients CD4 +T-cell count increases and confirms the organism opportunistic nature.

Keywords: Prevalence, Cryptosporidium, HIV patients, Microscopy, ELISA, CD4+ count, Kaduna, Nigeria.

PO-20*Track: Plant and Environment***EFFECTS OF HEAT STRESS ON PHYSIOLOGICAL RESPONSES OF GOATS****D. Amiri and M. Et Ben M'rad***National Institute of Agricultural Science Tunisia, 43, avenue Charles Nicole, 1082 -Tunis-Mahrajène Tunisia; E-mail: dorra.amiri86@yahoo.com*

The aim of the study is to compare the physiological, parameters of goats raised in the north of Tunisia according to THI (Temperature Humidity Index). The study was conducted from July until Decem ber 2010. Twenty five goats raised indoors away from direct exposure to sun. Rectal temperature, the temperature of the udder, respiratory rate, and heart rate, temperature of the forehead, the food intake and the quantity of water intake were measured during the experiment.

The results showed that rectal temperature increased ($P < 0.05$), according to the THI index, from 38.6 °C to 39.3 °C respectively when the THI increased from 22 °C to 30 °C.

The results also showed that heatstress increased ($p < 0.05$) significantly the respiratory rate (42 breath / min vs. 64 breaths / min for an index THI which varied from 22° C to 30 °C (and heart rate increased from 99 beats / min to 107 beats / min when the THI increased from 22 ° C to 30°C). Statistical analyses showed significant differences ($P < 0.05$) between the temperature of the udder, (alveolar or cisternal) left and right, according to the THI (respectively 35, 34.8, 34.9, 34.6 vs 30, 37.9, 37.4, 37, 36.8 for a THI of 22 °C and 30 °C). Statistical analysis ($P < 0.05$) showed a correlation between temperature of the forehead and rectal temperature, which allows us to conclude that it is possible to estimate the rectal temperature from the estimation of the temperature of the forehead with a coefficient correlation equals 0.21. The results showed that heat stress reduced food ingestion from 18.2 Kg to 13.2kg respectively for concentrate and hay to 15kg and 10kg respectively, for the concentrate and hay. The overall results showed a significant effect of heat stress on physiological and behavioral parameters of goats.

Keywords: Goats, physiological parameters, ingestion, thermal stress.

**PO-86***Track: Pharmaceutical Biotechnology***MOLECULAR CHARACTERIZATION OF HONEY BEE (*APIS MELLIFERA*) GUT BACTERIAL FLORA****Syed Ishtiaq Anjum¹, M. Kamran Azim², Abdul Haleem Shah, Ayaz Ahmad and Junaid Ahmad Kori**¹*International Center for Chemical and Biological Sciences, University of Karachi Karachi-75270, Pakistan;*²*Department of Zoology Kohat, University of Science and Technology, Kohat, KPK; E-mails: ishtiaq11@gmail.com; kamran.azim@iccs.edu*

The gut microbiota of honey bees (*Apis mellifera*) can be symbiotic or pathogenic and, therefore, important for bee survival and honey production. The information regarding the role of bee gut microbiota in the bee physiology and/or pathology is very limited. Losses of honey bee colonies all over the world have brought attention for the need of understanding the symbiotic and pathogenic aspects of honey bee associated microbes. Our recent surveys of honey producing areas in Pakistan showed a decline in the bee colonies due to various reasons.

In order to study the cultivable honey bee gut bacteria, 45 honey bee samples were collected from northern Pakistan. The complete digestive system of bees were dissected and processed for bacterial isolation. A total of 250 bacteria were isolated and identified by a variety of morphological and biochemical parameters. In addition, bacterial isolates were identified using PCR amplification of 16S ribosomal DNA. The resulting sequences were analyzed using bioinformatics tools to enumerate isolates identification.

Blast sequence searching revealed the presence of following bacterial genera in honey bee alimentary canal; *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Kocuria*, *Ochrobactrum*, *Brucella*, *Sphingomonas*, *Shigella*, *Citrobacter*, *Salmonella*, *Paenibacillus*, *Ralstonia*, *Cupriavidus*, *Brucella*, *Corynebacterium*, *Mycobacterium* and *Micrococcus*. Presence of an array of bacteria in bee gut indicated diverse roles of these microbes in bee physiology.

PO-32*Track: Medical Biotechnology***QUANTIFYING THE EFFECT OF CANCER ON ELASTICITY OF BREAST TISSUE BY ATOMIC FORCE MICROSCOPY****Arian Ansar-Damavandi, Ramin Omidvar , Mohammad Tafazzoli-Shadpoor and Issa Jahanzad***Department of Biomedical Engineering, Amirkabir University of Technology, Iran; E-mail: ansari.arian@aut.ac.ir*

Different behaviors of cells such as growth, differentiation, metastasis and apoptosis (predefined death) widely differ due to pathological diseases. The mechanical properties of cells and tissues can be used as a clue for diagnosis of diseases. Here, we implemented Atomic Force Microscopy to evaluate the extent of alteration in mechanical stiffness of tissue layers from patient affected by breast cancer. Fuzzy-logic algorithm was used to categorize the derived Young's modulus coefficients (E) to a number of clusters.



Three distinct categories were defined ($E < 3000$ Pa, $3000 < E < 7000$ Pa). The first cluster was assumed as the cellular region while the last cluster was referred to the fibrous parts of the tissue (non-cellular region). Results indicated the significant difference of average Young's modulus of cellular region between cancerous and healthy tissues. In the other words, cancerous breast cells (0.71 ± 0.34 kPa) exhibit a lower elastic behavior rather than normal cells (1.43 ± 0.25 kPa). While the average Young's modulus of non-cellular area of tissues was not noticeably changed due to cancer (16.06 ± 1.37 kPa and 14.45 ± 1.2 kPa).

Keywords: Cancer disease, cells and tissues, atomic force microscopy, elastic modulus, fuzzy-logic algorithm.

PO-62*Track: Plant and Environment***CHARACTERIZATION, ANTIBACTERIAL AND BIOLOGICAL ACTIVITIES OF PHENOLIC FRACTION OF ARGENTINEAN RED WINES****M.G. Stivala, M.B. Villecco, M. Fanzone, V. Jofre, M.J. Rodriguez-Vaquero and P. Aredes-Fernández***Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 491, Tucumán, Argentina; E-mail: pedroaredes@fbqf.unt.edu.ar*

For a first time, the qualitative and quantitative characterization of phenolic compounds of lower molecular weight fraction (LMF) was evaluated in commercial Cabernet Sauvignon (CS) and Tannat (T) wines varieties produced in Cafayate, Argentine. The “*in vitro*” antioxidant and antihypertensive activities as well as the antimicrobial activity against *Pediococcus pentosaceus* 12p, spoilage wine bacteria, were also evaluated.



The composition of LMF obtained by liquid-liquid extraction with ethyl acetate was determined by HPLC-DAD, showing a total content of phenolic compounds of 229.4 and 298.1 mg/L for CS and T wines respectively. LMF of CS and T wines have antioxidant and antihypertensive activities. Synthetic simil-wine media (SWM), pH 4.5 was supplemented with LMF at equal concentration that wine (1X) and eight times concentrated (8X), and inoculated with *P. pentosaceus* 12p at 10^6 ufc/mL. At different times, samples were collected to determine cell viability in MRS-agar medium and damage of the cell membrane integrity was determined by electron microscopy observation. In presence of LMF at 1X concentration, both fractions diminish growth rate without cellular damage. At 8X concentration, LMF produce microbial cellular death and plasmatic membrane disruption with cellular lysis.

The phenolic compositions of LMF of studied wines are capable of produce death of spoilage wine bacteria and, additionally have beneficial properties to human health, like antioxidant and antihypertensive activities.

PO-26*Track: Plant and Environment***A STUDY OF IONIC LIQUID TOXICITY ON THE BIOTRANSFORMATION AGENT, RHODOCOCCUS****Hazeeq Azman, Anthony Cass and Jason Hallett**

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There has been a lot of interest recently on using ionic liquid for processing lignocellulose biomass. While cellulose has the obvious application in generating biofuel, it will be valuable to use the abundant lignin as well. *Rhodococcus* has been reported previously to degrade lignin. Therefore, it is attractive to think about a scheme where ionic liquid is also used to enhance the microbial breakdown of lignin. As the initial step in this study, the action of *Rhodococcus* on lignin derived compound (vanillic acid) in ionic liquid has been investigated. Results show that *Rhodococcus* UKMP-5M is able to degrade vanillic acid as a sole carbon source of 10mM concentration to give the highest growth rate of bacteria. Different ionic liquids show varying toxicity to the bacteria. This study has demonstrated that an increased number of carbon atoms in the cation of ionic liquids has a direct correlation with the toxicity, but is not dependent on the structure or the distribution of those atoms. In addition, whilst the anion of ionic liquids does correlate with toxicity, no clear link with its physicochemical properties has yet been identified and the mechanism of toxicity is still being studied.

PO-38*Track: Pharmaceutical Biotechnology***COMPUTATIONAL IDENTIFICATION OF MICRORNAS FOR TARGETING KEY SMALLPOX GENES****Omar Bagasra**

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Background: Smallpox or Variola virus (VACV) belongs to the family Poxviridae, complex virion that contains a linear double-stranded DNA and mRNA synthesis enzymes. Poxviruses are exceptional among DNA viruses, as they replicate in the cytoplasm within distinct cytoplasmic factories (CFs), where RNA transcripts, protein synthesis and DNA replication are coupled. Transcription, followed by protein synthesis, is characterized by three phases: early, intermediate and late. VACV competes with the host translation machinery and guarantees viral proteins synthesis through enhanced degradation of host mRNAs and sequestering crucial translation initiation factors within the cytoplasm to the VACV-CFs. The enhanced mRNAs turnover is assisted by two decapping enzymes encoded by the virus, D9 and D10. Both enzymes contain a Nudix hydrolase motif and show 25% sequence similarity. D9 is an early protein, while D10 is an intermediate-late protein. As a consequence of read through transcription, at the intermediate and late phases of infection, double-stranded RNAs (dsRNA) are formed that are confined to the CF. These double-stranded transcripts can activate interferon (IFN)-dependent resistance of the host to infection. The counter-activity against IFN is mediated through the expression of viral proteins such as B8R, an IFN- α soluble receptor analogue; K3L, an eIF-2 α homolog; or E3L, a dsRNA-binding protein.

MiRNAs are key regulators of gene expression by posttranscriptional suppression, a process that is highly conserved throughout evolution. They are involved in almost every biological pathway, including developmental timing, cell differentiation, cell proliferation, cell death, metabolic control, transposon silencing and antiviral defense. Transcription of miRNAs is typically performed by RNA polymerase II. The primary capped and polyadenylated transcripts (pri-miRNA) are processed in the nucleus by Drosha to one or more precursors of miRNAs (pre-miRNA), which are then further processed in the cytoplasm by Dicer1 to form the mature miRNAs. miRNAs and siRNAs are incorporated into a ribonucleoprotein complex known as the RNA induced silencing complex (RISC), and they direct the RISC to downregulate gene expression by either of two posttranscriptional mechanisms: mRNA cleavage or translational

repression. Since, miRNA silencing takes place in the cytoplasm we hypothesize that miRNA based post exposure therapy for smallpox or related chimera viruses can be used in case of bioterrorism or accidental, zoonotic event.

Methodology: The target genes for the *hsa-miRNAs* were identified by retrieving the whole genome for Variola from NCBI Site (<http://www.ncbi.nlm.nih.gov/>). Each human miRNA found within the Sanger database (version 20.0) was determined to be identical to the Coding DNA Sequence (CDS) of the target gene by matching with 19-23 bp. The lengths of the 19-23 bp *hsa-miRs* were identical to those of the target genes of both viruses, as identified by alignments, according to the full length CDS of the three viral genes using the specific alignment tools.

Results: These sequence alignments with the viral genome and with miRNAs showed that 26 miRNAs have complete identities to the virus. The functional profiles of each of the miRNAs revealed that these miRNAs target multiple genes in the virus including 5' ITR (inverted tandem repeat, A, B, C, D, E, F, G, H, I, J, K, M, N, O, P and Q, in variola virus. Most importantly, five miRNAs: *hsa-miR-32-5p*, *hsa-miR-337-3p*, *hsa-miR-1205*, *hsa-miR-6824-3p* and *hsa-miR-4719*, targeted the C gene of variola. These miRNA targeted serine protease inhibitor protein, extracellular envelope virus maturation protein, Serine/threonine kinase protein, DNA binding virion core protein, ds-RNA binding protein, early and late transcription elongation factors, zinc finger protein and DNA polymerase enzyme. Most importantly, it blocked the interferon resistant factor transcribed by the virus, allowing the most powerful host anti-viral innate defense cytokine to quell the virus at the outset.

Conclusion: We believe that utility of these 26 miRNAs may prove to be an excellent post exposure treatment against smallpox or other related chimera bioweapon or a natural zoonotic outbreak.

PO-30

Track: Pharmaceutical Biotechnology

ANTIOXIDANT ACTIVITY AND ANTICANCER POTENTIAL IN B16F10-NEX2 AND 4T1 CANCER CELLS OF ANNONACEAE SPECIES

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Traditional modalities of metastatic cancer treatments have been presenting unsatisfying results, not resulting on a cure in the majority of the cases. Therefore, a promising area in therapeutic research against cancer is the phytopharmacology. According to the World Health Organization, medicinal plants are the primary source for medicines for 70% of the world population, and Brazil is seen as a major study focus in herbal-based treatment, in which a variety of plants' compounds might present effects on animal organisms, like active principals, that depending on the concentration, may be useful in therapy. Regarding that, the present study aimed, firstly, to assess antioxidant and anticancer activities of ethanolic extracts from leaves of *Annona crassiflora*, *Annona muricata* and *Annona squamosa*, by analysis of DPPH radicals' kidnapping and antitumoral activity by the tumor induction's inhibition by *Agrobacterium tumefaciens* in discs of *Solanum tuberosum*. Furthermore, had the objective of evaluating the treatment's death induction in melanoma cells of murin B16F10-Nex2 tumor and mamma 4T1 tumor *in vitro* and also the evaluation of cellular toxicity by hemolysis test. Finally, it aimed to quantify total phenols, flavonoids and tannins in the different extracts concentrations. The results showed that the *Annona muricata* had the highest values of DPPH, phenols, flavonoids and tannins, 44.72%, 5.34mg of galic acid/ gram of extract, 297.2mg of rutin/ gram of extract and 8.17mg of catequin/ gram of extract respectively, at a dilution of 1000µg of extract/mL of ethanol. The highest potential of tumor induction's inhibition by *Agrobacterium tumefaciens* in *Solanum tuberosum* discs was found also in *Annona muricata*, with 94.95% at 1mg of extract/mL of ethanol. The antitumor test for B16 cells presented to be time dependent and concentration dependent for all three extracts, in which the lowest IC50 was found in *Annona squamosa*, with 29.7 to 24 hours, and 18.41 for 48 hours. Related to 4T1 cells, all three extracts showed to be concentration dependent, however, just *Annona muricata* was time dependent (IC50=31.54 for 24 hours and 15.95 for 48 hours). *Annona crassiflora* (IC50=19.52 and 42.18 for 24 and 48 hours) and *Annona squamosa* (IC50=24.79 and 25.72 for 24 and 48 hours) extracts didn't present time dependence behavior. Lastly, all different concentration extracts for all three plants didn't lyse more erythrocytes than negative control, except for *Annona muricata* at 100µg of extract/mL. The study showed that the three plants have ambitious antitumor activity. The future directions for this work are the isolation of active principles and *in vivo* assay, and lastly, the development of a topic antimurine lotion.

PO-31

Track: Pharmaceutical Biotechnology

ANTIOXIDANT AND PHOTOPROTECTIVE ACTIVITY IN LEAF AND PULP EXTRACTS OF *ANNONA MURICATA*

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Introduction: Oxidative stress is correlated to the development of diseases may cause cell damage and lead to accelerated aging of cells. Studies have showed that plant extracts may contain the same compounds of pharmacological interest to treat and / or prevent cellular damage caused by oxidative stress. The antioxidant activity of plants is mainly due to the presence of phenolic compounds, especially flavonoids. Studies seeking new natural compounds, found in the leaf extract of soursop (*Annona muricata*) substances of pharmacological interest that can be applied in different therapies, including the treatment of cancer

Objective: The present study aimed to determine the antioxidant and photoprotective activity in leaf and pulp extracts of *A. muricata* through DPPH test and FRAP (ferric reducing antioxidant power) and also contents of total phenolics and flavonoids.

Material and methods: The antioxidant capacity was assessed using the Test of DPPH and FRAP (ferric Reducing antioxidant power), the determination of total phenolic compounds was performed by the Folin-Ciocalteu method, total flavonoid content was measured both with a spectrophotometer UV / VIS. For photoprotective activity was measured maximum wavelength arrived at between 200 and 400 nm.

Results: The ethanol extracts of the pulp and leaf showed better values related to antioxidant activity with EC50 of 4503 µg/mL and 3270 µg/mL, respectively, confirmed by FRAP test, which showed in concentration of 2000 µg/mL values of 101,84 µM/g dry extract, for the ethanol extract of the pulp, and 1698,05 µM/g dry extract for the ethanolic leaf. About the content of total flavonoids, the ethanol extract of the pulp showed 69,27 mgEAG/g extract, and ethanolic leaf 176,22 mgEAG/g extract. On phenolic composition, the ethanol extract of the pulp showed 42,25 mgRE /g extract and ethanolic leaf with 62,52 mgRE / g extract, which are the highest contents. The peaks of absorbance were 2,4 in the ethanol extract of the pulp at a wavelength of 226nm, and the ethanol extract of the leaf reached 4,5 at 231nm.

Conclusion: In conclusion, the extracts of *Annona muricata* possess antioxidant properties associated with the high content of phenolic compounds and flavonoids, and absorbance in the area of incidence ideal for sunscreens.

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Keywords: Photoprotective, Antioxidant, Annonaceae.

PO-81

Track: Others - Clinical Research

EFFECT OF NEW RUSSIAN FIBRINOLYTIC TROMBOVAZIM® ON INTRAOCULAR INFLAMMATION INDEXES AFTER CATARACT SURGERY

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Background: Intraocular inflammation is a one of the early cataract surgery complications, the standard therapy of which includes antibiotics, anti-inflammatory drugs, mydriatics and fibrinolytics. In 2007 Siberian Center of Pharmacology and Biotechnology (Novosibirsk, RF) made the first “per oral” fibrinolytic Trombovazim® by means of a classic Nano-Bio Technology (AXIS®).

Aim: is to estimate the influence of Trombovazim® on the ocular inflammatory process after cataractous lens removal.



Methods: 71 patients with postoperative intraocular inflammation were examined and treated. The main group was formed by the patients who were treated with standard therapy plus Trombovazim[®] at dose 400 IU (2 tablets) 2 times per day for 5-7 days, while only routine treatment was used in the comparative group of patients.

Results: At the end of the therapy the main group demonstrated more significant increase in visual acuity than the comparative one. The first group of patients was characterized by more evident decrease in the pro-inflammatory markers levels (C-reactive protein, fibrinogen in blood and TNF- α in lacrimal fluid) and more significant elevation of anti-inflammatory factor (IL-4) concentration in the lacrimal fluid in contrast to the second group.

Conclusion: Anti-inflammatory effect of fibrinolytic Trombovazim[®] was revealed as additional, that's quite advisable to use this drug in combined therapy of postoperative intraocular inflammation.

Key words: cataract surgery, Trombovazim[®], intraocular inflammation.

PO-11

Track: Plant and Environment

RHIZOSPHERE SYMBIOTS VALORISATION: EXPLOITATION OF COMMON BEAN-RHIZOBIA SYMBIOSIS ADAPTED TO P DEFICIENCY CONSTRAINT

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Leguminous have a high environmental, dietary and socio-economic importance especially for the African countries. Despite of their interest, during these last years, their culture is decreasing caused by biotic and abiotic stresses: Temperature variations, impairment of Mediterranean soils in minerals especially phosphorus. The ability of a symbiotic association with rhizobia allows the biological nitrogen fixation resulting can be exploited to improve plant growth and fertility soil. The inoculation by rhizobia plays an important role in improving and increasing the potential of fixing the atmospheric nitrogen through increasing number and weight of nodules. In this context that is our study whose objective is to select an efficient rhizobia and bean genotypes to improve growth and production of this very important beans and adapt it to address constraints in particular soil phosphorus deficiency with reasoned inoculation with natives' rhizobia from selected agroecosystem to preserve of biodiversity and agroecosystem functions.

Six recombinant inbred lines, namely RILs: (CIAT) and one common bean variety widely cultivated in Algeria. Lines 115, 104 and 75 have been characterized as P-efficient whereas 147, 83 and 29 have been categorized as P-inefficient based on plant growth and seed yield in relation to the availability of P. This RILs was sowing both in vitro trapping and in natura multilocus test for 20 plots from Ain Temouchent agroecosystem chose in northwest of Algeria. After 45 days after transplanting the nodules are collected, according to macroscopically aspect 40 strains was selected to PCR-RFLP analysis amplified 16S rDNA genes. Total DNA was extracted as Laguerre (1992) described. Aliquots of PCR products were digested with restriction endonucleases. The following enzymes were used: Msp I and Nde II.

Small collection of isolates revealed an interesting diversity. For 40 strains studied five were identified as *Rhizobium etli*, 3 *R. leguminosarum*, 11 *R.gallicum*, 1 *R.loti*, 1 *R. ciceri* and 6 *Agrobacterium* and 10 strains remaining will be sequenced to be identified. For this strain studied, 29 can establish a nodule and 17 and 2 strains were more efficiency that was identified as *R. etli*.

All strains were tested in glass house at hydroponic cultural condition. Plants were subjected to two P deficiency levels: moderate and severe. After 5 weeks of growth under greenhouse conditions, oxygen consumption measures related to nitrogen fixation were performed on the whole plant as Vadez (1996). O₂ consumption of root nodules of inoculated beans was measured at flowering stage 45 days after sowing (DAS). Results show's That P deficiency decrease nodule-root respiration and affects growth parameters especially nodulation by lowering the number and size of nodules and nodule growth is more sensitive to P deficiency than the plant growth.

Keywords: Common beans, nodulation, phosphorus, rhizobia.

PO-85*Track: Industrial and Manufacturing***MILK-CLOTTING PROTEASE PRODUCED BY *ASPERGILLUS TAMARII*: EFFECT ON COW MILK AND COMPARISON WITH RENNET****Aïcha Benlounissi, Aïcha Mechakra-Maza, Loïc J. Blum and Christophe A. Marquette**

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A strain *Aspergillus tamaris* was isolated from Algerian soil and identified using both microscopic observation and ICT sequence identification. A potential milk-clotting activity on casein was demonstrated and attributed to an acidic protease activity. After production by fermentation at acid pH on medium containing whey and, purification by molecular exclusion chromatography, affinity chromatography and SDS-PAGE, an enzyme having, approximately, molecular weight of 35KDa was found, with optimum pH of activity 5.5 and optimum temperature 50°C. This enzyme hydrolyses total casein and shows on SDS-PAGE three bands corresponding to α , β and κ caseins; and can coagulate fresh cow milk in very short time, 2min, comparing with rennet, with 0.83 UP/ml clotting activity, and 184.44 coagulating power.

Keywords: Acid protease, *Aspergillus tamaris*, milk clotting, rennet, comparison.

PO-67*Track: Marine Biotechnology***PREVALENCE OF THE PROTOZOAN *PERKINSUS SP.* IN CULTURED PACIFIC OYSTER *CRASSOSTREA GIGAS* IN SINALOA, MEXICO****Cesar Marcial Escobedo-Bonilla, Lizeth Carolina Villanueva-Fonseca and Andres Martin Gongora-Gómez**

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Crassostrea gigas is a bivalve mollusk of great commercial importance. In northwest Mexico its production is affected by mortalities whose infectious origin has not been clearly determined. This study determined the prevalence and intensity of infection with *Perkinsus sp.* in a culture of *C. gigas* in 2011-2012.

The culture was performed in a long-line system with densities of 28 and 42 oysters/tray. A sample size of 30 oysters per month was determined. Diagnosis of *Perkinsus sp.* was done according to the protocols of the World Organization for Animal Health (OIE) for Fluid Thioglycolate Medium and PCR. Both methods were able to determine the prevalence of *Perkinsus sp.*, which ranged between 3.3 and 40%. The infection intensity was low (levels 1-2) according to the Mackin scale. Cumulative mortality in densities between 28 and 24 oysters per tray was 4 and 6% respectively. The highest mortality of oysters and the highest prevalence of *Perkinsus sp.* occurred in September (2.7 and 16.6%) and October (1.5 and 23.3%), respectively, when the temperature was high.

In conclusion, *Perkinsus sp.* was detected in a *C. gigas* culture in the Estero La Pitahaya, with moderate prevalence, low infection and increased presence in the warmest months of the culture cycle.

Keywords: *Crassostrea gigas*, *Perkinsus*, prevalence, Thioglycolate fluid medium, aquaculture, Mexico.

**PO-50***Track: Industrial and Manufacturing***WASTE COOKING OIL AS SUBSTRAT TO PRODUCTION OF BIODIESEL****Bronislaw Tomasz Buczek**

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The economic effectiveness of the production of fatty acid methyl esters (FAME) applied in the production of biofuel has been diminishing. One way of reducing the production costs for biodiesel fuels is the use of non-edible oils, which tend to be considerably cheaper than edible vegetable oils. By the end of the 1990s, the technology of simultaneous transesterification of used cooking oils together with fresh ones had been implemented in industry.

New conversion technologies of used edible oils and waste animal fats into a biofuel appropriate for use in standard diesel engines have been developed, taking into consideration environmental requirements and improvement in the economics of current transesterification technologies. The variation in the properties of substrate made from used rape oil after treatment with mixed adsorbents (active carbon, magnesium silicate) was studied in this work. The obtained results are compared with the quality requirements for the substrates used in Vogel & Noot GmbH technology for transesterification of oils and fats.

Keywords: Used cooking oil, adsorptive treatment, biodiesel.

Acknowledgement

The author is grateful to the AGH (Project 11.11.210.128) for financial support of this work.

PO-90

Track: Pharmaceutical Biotechnology

SYNTHESIS OF NOVEL HETEROCYCLIC COMPOUNDS BEARING ISOXAZOLE, ISOINDOLE AND PYROZOLE AND ASSESSMENT OF RELATIONSHIP BETWEEN STRUCTURE AND BIOLOGICAL ACTIVITY

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Chalcones are convenient intermediate compounds for the synthesis of five-, six-, and seven-membered heterocycles exhibiting different biological activity. Pyrazole, isoxazole and isoindole derivatives constitute an interesting class of heterocycles due to their synthetic versatility and effective biological activities. Pyrazoles display various biological activities such as antimicrobial, antifungal, antidepressant, immunosuppressive, anticonvulsant, antitumor, antiamebic, antibacterial, and anti-inflammatory. Isoxazole derivatives represent a unique class of nitrogen- and oxygen-containing five-membered heterocyclic, and they are associated with a wide spectrum of biological effects such as antiviral, anthelmintic, anti-inflammatory, and anticonvulsant, anticancer.

Therefore, last decades chalcones, pyrazole, isoxazole and isoindole derivatives have been very attractive in medicine and organic chemistry. Researchers have been making many researches on synthesis and biologic activities of these compounds for a long time. However, in the research, investigation of biological activity of these units have done one by one or two units in one molecule.

In this study, novel chalcone, isoxazole, isoindole and pyrazole derivatives will be synthesized and antiproliferative activity (against HeLa and C6 cancer cell lines) tested. Later, novel heterocyclic compounds containing chalcone, isoxazole, isoindole and pyrazole units will be synthesized and activities investigated.

Our aim in this project is to know that individual pharmacological performance of these four functional groups how can be change when this four functional groups in one molecule. Addition, structure-activity relationships will be investigated.

Keywords: Kalkon, Isoksazol, İsoindol, Pirazol, Antiproliferatif.

PO-41

Track: Industrial and Manufacturing

TIME-COURSE ADDITION OF L-PHENYLALANINE IN YARROWIA LIPOLYTICA CULTURES FOR IMPROVEMENT OF 2-PHENYLETHANOL PRODUCTION

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2-phenylethanol (2-PE) is a valuable aroma compound, widely used in food and cosmetics production. Recently we have shown that *Yarrowia lipolytica*, which is an industrially accepted microbe, widely used in multiple industrial applications, is able to produce considerable amounts of 2-PE. In the yeast cell, 2-PE is primarily produced via Ehrlich pathway of bioconversion from L-phenylalanine (L-Phe) via two consecutive steps. In this study we applied time-course addition of L-Phe, in order to investigate if supplementation with the precursor at any specific growth phase of the culture promotes greater 2-PE production. To this end, we set shake flask cultures of *Yarrowia lipolytica* with varying time-points of L-Phe supplementation. During the whole culturing time (336 h) samples were collected to monitor cultures in a kinetic manner. L-Phe consumption, 2-PE and intermediate production were monitored using HPLC technique. Biomass formation was monitored through turbidity measurement at 600 nm wavelength. The cultures were carried out in duplicate. Our results indicated that addition of L-Phe at the beginning of culturing time brought the highest titer of 2-PE, but such cultures were characterized by the worst volumetric productivity parameter, which, on the opposite was significantly improved, when L-Phe was provided at the stationary phase of growth. (Project No OPIE01.01.02-00-074/09).

Keywords: *Yarrowia lipolytica*, 2-phenylethanol.

PO-51

Track: Medical Biotechnology

HEPATITIS B AND HEPATITIS C MOLECULAR DETECTION IN GREECE, A DEMOGRAPHIC CHANGING MEDITERRANEAN COUNTRY

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AIM: The aim of this study was to evaluate the prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection and epidemiological parameters in a three year pilot study. This observational study was carried out in a reference laboratory of Athens, in order to estimate prevalence of them in a demographic changing Mediterranean country, Greece.

SETTING, PATIENTS AND METHOD: *In vitro* Labs is a reference Lab in Athens, Greece, accomplishing routine and molecular medical diagnosis. Its stakeholders are clinics, doctors and small routine labs within Greece and Cyprus. The lab is an ISO 15189 and 9001 accredited. Patients in the current study are coming from different regions, different social levels, pregnant women and 1st and 2nd generation migrants. Molecular diagnosis with serology was suggested by the family doctor. Real time PCR was used to detect viral load according to international and WHO guidelines.

RESULTS AND DISCUSSION: During 2011 -2013 a total of 314 and 146 blood samples have been tested by RT PCR respectively for hepatitis B and hepatitis C to confirm serology. Men and women were almost represented in equal numbers, immigrants represented about 15% of the screened population. Fifty seven % have been positive and 45% have been positive for HBV and HCV respectively. Viral load was ranging from $1,2 \times 10^2$ to $3,5 \times 10^8$ and $2,8 \times 10^2$ to $2,2 \times 10^8$ copies/ml respectively to HBV and HCV. It seems that HBV and HCV are both emerging in general population. Clinical diagnosis of liver disease and viral hepatitis by the family doctor a key actor seems accurate and highly sensitive. In several Mediterranean countries, the disease burden due to viral hepatitis remains largely unrecognized



because of a lack of epidemiological data. Targeted surveillance and vaccination strategies for hepatitis B virus (HBV) among risk groups and education at large scale are needed.

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PO-52

Track: Medical Biotechnology

HPV DNA TESTING AND TYPING IN A COHORT OF 566 WOMEN IN GREECE

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Introduction: Prevention programs for cervical cancer (CIN), which are mostly based on cytological examination have achieved a more than 30% reduction in incidence and mortality of invasive cervical cancer but still with limitations due to low sensitivity and the false negative rate. HPV detection and typing seems to be an important test enhancing diagnosis and follow-up of patients and it is recommended by ACOG (American College of OBGYN). The aim of this study was to evaluate the prevalence of HPV high- and low- risk human papilloma virus in a cohort of women by using molecular detection and typing.

Materials and Methods: Five hundred and six women, aged from 19 to 62 years, have been tested for the presence of HPV in cervical samples, by using the Linear Array method (ROCHE molecular diagnostics). The method includes the detection of 13 high-risk and 24 low-risk HPV types. The study was carried out during two years.

Results: Out of 566 cervical samples, 298 have been found positive for HPV. High-risk HPV types (HR-HPV) were detected in 62 women, Low-risk HPV types (LR-HPV) in 89 and High+Low risk in 147 women samples. Among the high-risk-only HPV group, 44 shared DNA sequences of the 16 and 8 shared the 18 subtypes. Typing of the mixed group of subtypes detected in 63 samples the G16, in 15 samples the G18, in 33 the G31, in 14 the G33 and in 19 samples the G45 respectively. Co-infection with two strains, either high- and/or low risk, was detected in 57 samples, with three genotypes in 45 samples and with four or more genotypes in 18 samples. Three samples with colposcopy HSIL findings but negative for high-risk strains were retested by PCR. One was positive and two had different low-risk HPV types.

Conclusions: This was a pilot study accomplished in our laboratory. By using the linear array method we detected high- and low-risk HPV types in women. The most prevalent HPV types were the low-risk ones independently of colposcopy findings. Interestingly, in most of the cases, high- and low-risk types have been detected in the same sample. This typing profile seems to be the most prevalent. We aim in the upcoming future to co-evaluate clinical, cytological and colposcopy findings with the results of Linear Array Method in the diagnosis of HSIL and also to correlate any immunohistochemical patterns (e.g. p16 and Ki-67 expression) with HPV status in cervical biopsies or cervical colozation material performed after a diagnosis of CIN and/or HPV. In our setting , IASO we aim to promote and apply the 2012 ACOG cervical screening guidelines.

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PO-69

Track: Marine Biotechnology

ISOLATION, CHARACTERIZATION AND ANTIMICROBIAL SCREENING OF FUCOXANTHIN FROM *ULVA LATUCA* FROM THE WEST AFRICAN COAST

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Marine algae are gradually receiving increased attention in a bid to find new antimicrobials. The West African Coast is blessed with a diverse range of flora of marine algae which have not been well explored. As part of a study to explore the antimicrobial potential of marine algae of the West African coast we have investigated the antimicrobial activity of *Ulva latuca* against selected microorganisms. The dried algal material was extracted with dichloromethane-methanol (2:1) and the organic extract was further fractionated into eight different fractions by step gradient silica gel column chromatography. Fraction E which eluted with 40:60 Hexane/Ethyl acetate was further purified by silica gel column fractionation to obtain a pure compound. The antimicrobial activity of the purified compound was assessed by the agar well diffusion method while structural characterization was done mainly by NMR spectroscopy.

PO-58

Track: Pharmaceutical Biotechnology

SYNTHESIS AND SELECTIVE INTERACTIONS OF SOME NEW BENZOQUINONE DERIVATIVES WITH G-QUADRUPLEX DNA AS PROMISING ANTICANCER AGENTS

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The end caps of chromosomes "telomeres" are consisting of tandem repeats enriched in guanine bases that fold up under physiological conditions to form four-stranded G-quadruplex' structures. Folding single stranded telomeric DNA into the G- quadruplex structure has shown to inhibit telomerase enzyme overexpressed in 85-90% of cancer cells. It has also been hypothesized that formation of G-quadruplex DNA structures in the promoter region of some oncogenes plays an important role in regulating the transcription of the corresponding gene. Consequently, small molecules selectively bind and stabilize G-quadruplex DNA are potential for developing new selective and efficient anticancer therapeutic agents.

In this work, fourteen new BQ₍₁₋₁₄₎ derivatives were synthesized. The obtained BQs were examined against human pancreatic cancer cells L3.6pL and MiaPaCa-2, human lung cancer cells H1299, human prostate cancer cells C4-2B and breast cancer cells MCF7. Most of benzoquinone derivatives exhibit potent anti-proliferative activities against the examined cancer cell lines which open up the possibilities of optimizing application of these analogs.

Interaction between BQ derivatives and telomeric quadruplex was investigated. Selectivity of BQ derivatives towards quadruplex over DNA duplex, binding affinity, binding constant and binding stoichiometry was studied. Most of BQ derivatives have shown higher binding affinity than parental compound and better selectivity towards G-quadruplex over ct-DNA.

PO-74

Track: Pharmaceutical Biotechnology

DETERMINATION OF CHOLESTEROL LEVEL IN LIBYA'S FARM'S HEN EGGS COMPARED WITH OTHER TYPES

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Hen eggs are considered to be among human first food as they highly nutritious, cheap and easy to obtain. They are a chock full of proteins, minerals and vitamins..etc. However, eggs cholesterol content in eggs yolk is very high and this is often a cause for concern. With the aim of evaluating the cholesterol content of the Libya farm's hen eggs from Benghazi Region in Libya, (as the hen farm's eggs are Libyans favorite type). The eggs were collected from 20-50 weeks old. Hens were fed by the farmers own food. Eggs were boiled and egg yolk was separated. Three yolk samples were taken from each egg yolk. The cholesterol was extracted from boiled egg yolk used isopropyl alcohol. The isolated cholesterol was recovered by evaporated alcohol using rotator evaporator equipment, and then the concentration of cholesterol was estimated in terms of mg/g yolk. The overall average of the cholesterol content of the Libya hen egg was 16.0-18.0 mg/g yolk and that of the other eggs is 11-13mg/g yolk.

Cholesterol contents were also determined by the traditional colorimetric methods and found to be 16-20mg/ g yolk. No free fatty acids or triglycerides were detected in isopropyl alcohol extracts. The concentration of calcium and phosphorus in egg shells were also estimated.

Keywords: Cholesterol, cholesterol extract, egg yolk, eggshells, spectrophotometer.

PO-75

Track: Plant and Environment

ANALYSIS OF VITAMIN C (ASCORBIC ACID) CONTENTS IN VARIOUS FRUITS, VEGETABLES AND PACKED FRUIT JUICES IN BENGHAZI-LIBYA MARKETS AND STUDYING THE EFFECT OF COOKING AND FROZEN OF SOME VEGETABLES BY UV-SPECTROPHOTOMETRY AND REDOX TITRATION METHODS

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Total vitamin C (ascorbic acid + dehydroascorbic acid) has been determined by UV-spectrophotometric method in various fruits and vegetables. In this method, a blended sample is homogenized with 3% metaphosphoric acid-10% acetic acid solution. Then addition of bromine water oxidizes ascorbic acid to dehydroascorbic acid in presence of acetic acid and the excess of bromine is removed by a few drops of 10% thiourea. After coupling with 2,4-dinitrophenyl hydrazine at 37OC temperature for three hours, the solution is cooled in an ice bath and is treated with chilled 85% H₂SO₄ to produce a red color complex and the absorbance is measured spectrophotometrically at 521 nm. This method is obeyed Beer" Law as was tested by using a standard ascorbic acid. The effect of cooking and frozen of vegetables on vitamin C was determined.

Also ascorbic acid can be determined by redox titration. We performed an iodimetric back-titration in which we generated a measured excess of iodine in the sample solution and then we titrated the unreacted iodine with a standard sodium thiosulfate solution. This back titration was used for fruit juices and many fruits and vegetables.

Keywords: Vitamin C determination, Vegetables, Fruits, UV-spectrophotometer, iodimetric titration

PO-76

Track: Pharmaceutical Biotechnology

NATURAL PRODUCTS FROM THUJA ORIENTALIS

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Natural products have been important molecules in drug discovery. Thuja orientalis (Cupressaceae) has been used as a traditional medicine for the treatment of haemorrhages, dermatitis, gout and chronic tracheitis. In this work, plant materials were collected from Tokat region. The air-dried aerial part of the plant materials were boiled in water, filtered and the aqueous part was extracted with ethyl acetate, dried (MgSO₄) and the solvent was removed by rotary evaporator. A phytochemical investigation on the ethyl acetate extract resulted in the isolation and identification of four flavonoids, quercitrin (1), quercetin (2), apigenin-7-O-glycoside (3), quercetin-3-O-glycoside (4), taxifolin (5).

Keywords: Thuja orientalis, flavonoid

PO-77

Track: Pharmaceutical Biotechnology

ISOLATION AND IDENTIFICATION OF CHEMICAL CONSTITUENTS FROM ORIGANUM CYRIACUM L.

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Origanum genus belonging to the Lamiaceae family is represented by 23 species and six hybrids in the flora of Turkey, 14 of which are endemic. Origanum species have been used for treatment of a very large variety of diseases for years. They are also employed as powerful disinfectants, flavouring agents, in perfumes and in scenting soaps. These species are well known for their essential oils which have been applied in the flavouring of various foods, particularly soups, sauces, meat, fish, canned foods, liqueurs, vermouths and bitters. In this work, the plant materials were collected from Aromatic and Medicinal Plant Field of Gaziosmanpasa University.

Dried and powdered aerial parts of *O. cyricum* were extracted sequentially with hexane, ethyl acetate and methanol. The methanol extract was subjected to column chromatography, eluted with hexane, followed by increasing polarity with ethyl acetate and methanol (hexane, hexane-ethyl acetate, ethyl acetate-methanol, and methanol) to isolate β -sitosterol- β -D-glycoside (1), 2,4,6-trihydroxyacetophenone-2-O-D-glycoside (2), vitexin (3) and rosmarinic acid (4). The structures of isolated compounds were identified by spectroscopic methods including 1D-NMR, 2D-NMR, IR, UV and LCQTOF.

Keywords: Origanum cyriacum, isolation.

Acknowledgements:

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PO-08

Track: Medical Biotechnology

SPECIES AND VIRULENCE DETERMINATION OF *LISTERIA MONOCYTOGENES* ISOLATED FROM GOAT MEAT IN PORT HARCOURT, NIGERIA

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The speciation and virulence determination of four *Listeria monocytogenes* strains isolated from goat meat alongside *L. monocytogenes* PCM 2191 serovar 01/2, which served as positive control, was done using internalin genes *inlA*, *inlB*, *inlC* and *inlJ* primers individually. The *inlA* and *inlB* gene primers formed the expected bands of 800bp and 884bp respectively with the genomic DNA of *L. monocytogenes* PCM 2191 serovar 01/2 but not with genomic DNA of the 4 *L. monocytogenes* isolated from goat meat. The *inlJ* gene primer generated a 238bp fragment with the DNA of *L. monocytogenes* PCM 2191 serovar 01/2 and the DNA of 3 of the 4 isolated *L. monocytogenes* while *inlC* gene primer generated a 517bp fragment with the DNA of *L. monocytogenes* PCM 2191 serovar 01/2 and 2 of the 4 isolated *L. monocytogenes*.

Only the DNA of 1 of the 4 isolated *L. monocytogenes* alongside *L. monocytogenes* PCM 2191 serovar 01/2 formed the expected fragment with the *inlJ* and *inlC* gene primers. The inability of the isolated strains to produce the expected fragments with the *inlA* and/or *inlB* gene primers remained to be unravelled.

Keywords: Goat meat, internalin genes, *Listeria monocytogenes*, primer, virulence.



PO-88*Track: Medical Biotechnology***A MATERNALLY INHERITED DIABETES AND DEAFNESS PATIENT WITH THE 12S RRNA M.1555A>G AND THE ND1 M.3308T>C MUTATIONS ASSOCIATED WITH MULTIPLE MITOCHONDRIAL DELETIONS****Faiza Fakhfakh, Najla Mezghani, Mouna Mnif, Emna Mkaouar-Rebai, Nozha Kallel, Nadia Charfi and Mohamed Abid***Laboratoire de Génétique Moléculaire Humaine, Faculté de Médecine de Sfax, Université de Sfax, Tunisia; E-mail: faiza.fakhfakh@gmail.com*

Maternally inherited diabetes and deafness (MIDD) is a mitochondrial syndrome characterized by the onset of sensorineural hearing loss and diabetes in adults. Some patients may have other additional clinical features common in mitochondrial disorders such as pigmentary retinopathy, ptosis, cardiomyopathy, myopathy and renal affections. We report a 40-year-old Tunisian patient presenting maternally inherited type 2 diabetes and deafness (MIDD). A molecular genetic analysis was conducted in the patient and his twin sister, but no reported mutations in the tRNA^{Leu}(UUR) and tRNA^{Glu} genes were found, especially the two mitochondrial m.3243A>G and the m.14709T>C mutations in muscle and blood leukocytes.

The results showed the presence of the mitochondrial NADH dehydrogenase 1 (ND1) homoplasmic m.3308T>C mutation in the 2 tested tissues (blood leukocytes and skeletal muscle) of the proband and in the patient's sister blood leukocytes. In addition, we identified the mitochondrial 12S rRNA m.1555A>G mutation in muscle and blood leukocytes. The Long-range PCR amplification revealed the presence of multiple deletions of the mitochondrial DNA extracted from the patient's skeletal muscle removing several tRNA and protein-coding genes. Our study reported a Tunisian patient with clinical features of MIDD in whom we detected the 12S rRNA m.1555A>G and the ND1 m.3308T>C mutations with mitochondrial multiple deletions.

PO-15*Track: Plant & Environment***IMPROVABILITY OF FIBER QUALITY BY OVER-EXPRESSION OF *GhUGP1* IN COTTON****Ling Fan, Bo Li, Yang Yang, Wen-Ran Hu, Xiao-Dong Li and Jia-Qiang Cao***Institute of Nuclear and Biological Technologies, Xinjiang Academy of Agricultural Sciences, 403 Nanchang Road, Urumqi 830091, China; E-mail: fanLing@xaas.ac.cn*

Cellulose is an important component of cotton fiber cells, and UGPase is one of the important enzymes for cellulose synthesis. In this context, the plant expression *GhUGP1* vector was constructed and introduced into cotton via agrobacterium-mediated transformation, and the transgenic cotton lines were obtained. Southern blotting had been confirmed that *GhUGP1* was single copy insert in the genome of transgenic cotton lines. The test results showed that: (1) The expression of *GhUGP1* was higher in transgenic lines than those in wild type at 15 DPA, and lower than those in wild type at 20 DPA; (2) Agronomic trait observation indicated that the height of transgenic cotton lines increased by 18%-62%; (3) The quality analysis showed that the fiber length increased by 8.22%-18.50%, and the strength increased by 8.22%-18.50%, respectively. These improved quality traits lasted stable for 3 generations already; (4) Chemical analysis indicated that the content of soluble sugar and reducing sugar decreased by 35%-60% and 34%-48%, respectively, and the content of cellulose increased by 0.8%-1.1% in mature transgenic fibers. Therefore, we consider that over-expression of the key *GhUGP1* gene could improve the change ratio from oligosaccharides to polysaccharides in developing cotton fiber. As results, the sugar content decreased and the cellulose content increased in cotton fiber, the fiber quality was significantly improved.



PO-94*Track: Medical Biotechnology***ASSESSMENT OF QUALITY OF LIFE IN CANCER PATIENTS FROM MAZANDARAN PROVINCE IN 2013****Fereshteh Farzianpour, Jalil Shojai, Ghasem, Abedi and Farideh Rostami***School of Public Health, Tehran University of Medical Sciences, Iran; E-mail: farzianp2@yahoo.com***Background:** These days one of the most important issues in clinical researches is the quality of life. That is as effective aspects of the patient's cares.

The aim of this study was to analyze the regression model of the quality of life in cancer patients from Mazandaran province in 2013.

Methods: This descriptive cross-sectional study was carried out on 185 cases after a chemotherapy treatment session during in the first three months that was referred to Rajaee Chemotherapy Center in 2013. The method of sampling was Purposive.

General quality of life was assessed using WHO questionnaire (WHOQOL-BREF) and particular life quality was assessed using researcher-developed questionnaire. Data analysis was consisted of a multiple regression method and for comparison one-sample test of Kolmogorov-Smirnov was used.

Results: Statistical analysis showed that the average of general life quality, particular life quality and total average was evaluated, $1 < 0.96 < 5$, $1 < 1.13 < 5$ and $1 < 1.04 < 5$, respectively.**Conclusion:** Due to the low quality of general & particular life, fully integration of the care program of patient care in primary health care system, easy access and facilitation in intervention to improve the quality of life is offered.**Keywords:** Assessment, regression, quality of life, patient, cancer.**PO-92***Track: Others: Sports Medicine***EXERCISE IS MEDICINE: ARM-CRANKING REDUCED SYSTEMIC INFLAMMATION IN ADULTS WITH CHRONIC SPINAL CORD INJURY****G. Fornieles, A.J Diaz, I. Rosety, M.A. Rosety, S. Elozegui, M. Bernardi, M. Rosety-Rodriguez and F.J. Ordonez***School of Sports Medicine, University of Cadiz, Cadiz, Spain; E-mail: gabriel.fornieles@uca.es*

This study was designed to ascertain the effect of arm-cranking exercise on improving plasma levels of inflammatory cytokines and adipokines in untrained adults with chronic spinal cord injury (SCI).

Seventeen male adults with complete SCI at or below the 5th thoracic level (T5) volunteered for this community-based supervised intervention. Participants were randomly allocated to the intervention (n=9) or control group (n=8) using a concealed method. The intervention consisted of a 12-week arm cranking exercise program 3 sessions/week, consisting of warming-up (10-15 min) followed by a main part in arm-crank (20-30 min [increasing 2 minutes and 30 seconds each three weeks]) at a moderate work intensity of 50-65% of heart rate reserve (HRR) (starting at 50% and increasing 5% each three weeks) and by a cooling-down period (5-10 min). Plasma levels of leptin, adiponectin, PAI-1, TNF- α , and IL-6 were determined. Furthermore, physical fitness (VO₂max) and body composition (Anthropometric Index [AI], waist circumference [WC] and BMI) were also assessed. Plasma levels of leptin, TNF- α , and IL-6 were significantly decreased after the completion of the training program. Similarly, AI and WC were diminished too. A moderate correlation was found between leptin and AI. Finally, VO₂max was significantly increased suggesting an improvement of their physical fitness in the intervention group. No changes were found in the control group.

In conclusion, arm cranking exercise improved low-grade systemic inflammation by decreasing plasma levels of inflammatory cytokines. Furthermore, it also reduced plasma leptin levels. Long-term, well-conducted SCI studies are still required to determine whether these changes may improve clinical outcomes of adults with chronic SCI.

Keywords: Spinal cord injury, Low-grade systemic inflammation, Exercise.

PO-84*Track: Medical Biotechnology***NEW BADNAVIRUS SPECIES INFECTING BANANA IN BRAZIL****Antonia dos Reis Figueira, Patrick Callegari Magnani, Priscilla Geraldino de Sousa Duarte, Daniele Costa Pompeu and Suellen B.F. Galvino-Costa***Dept. Fitopatologia, Universidade Federal de Lavras, Lavras, Brazil, Cep. 37200-000. E-mail: antonia@dfp.ufla.br*

The *Banana streak virus* (BSV) is one of the most important viruses which affect banana in Brazil, due to its high genetic variability and integration into the nuclear genome of plants. Several new related species have been found in other parts of the world, based on molecular analysis of RT/RNase H gene. However, in Brazil, these genetic variants have not been investigated. In this study, 10 virus isolates, chosen among 40 banana samples collected in distinct Brazilian regions, were sequenced and analyzed. Four virus isolates were classified as *Banana streak Uganda virus C* (BSUgCV), two were *Banana streak obino/Ewai virus* (BSOLV) and one was *Banana streak Mysore virus* (BSMyV). According to ICTV rules, two new species were also found: the isolates PR-CAT1 and SE-PAN1, showing 92% of identity with each other, for which the name *Banana streak Brazilian A virus* (BSBrAV) is being proposed. The CE-PRCA1 isolate, which presented less than 50% identity with the Brazilian and GenBank virus isolates, was named *Banana streak Brazilian B virus* (BSBrBV). It is the first time that badnaviruses causing streak in banana are characterized in Brazil, based on genomic analyzes, revealing new species not yet described elsewhere.

PO-13*Track: Plant and Environment***AOX2a AS SOURCE OF POTENTIAL FUNCTIONAL MARKERS FOR DAUCUS EFFICIENT SOMATIC EMBRYOGENESIS****António Miguel Frederico***ICAAM - Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Universidade de Évora, Portugal; E-mail: amcmf@uevora.pt*

Daucus carota is a model species for somatic embryogenesis (SE). Evidences support SE as a stress response and key molecules were identified during SE as playing crucial roles on improving stress tolerance responses. However, SE cannot always be efficiently induced. *Daucus* genotypes can show recalcitrant behaviour under identical inducing conditions. Metabolic reorganisation is supposed to be crucial. Thus, mitochondria and components of the alternative respiration should be highly involved and the alternative oxidase (AOX) as key enzyme is suggested to play an important role. AOX differential expression through polymorphic sites or gene variants could be crucial for SE efficiency.

D. carota cultivars and subspecies and *Daucus* species were screened for the differential capacity to develop calli and embryos. Embryogenic and non-embryogenic cell lines were selected through SE efficiency phenotyping.

AOXs are grouped in two families, AOX1 and AOX2s are predominantly related to developmental patterning. Therefore, the complete genomic DNA structure of AOX2a genes was isolated from the selected cell lines and sequenced. The existence of regulatory motifs was explored in AOX2a polymorphic sites. Those can be applied as potential functional markers for experimental validation. Progress of this work will be reported.

Keywords: *Daucus*, somatic embryogenesis, AOX, functional markers.



PO-33*Track: Medical Biotechnology***NEPHROPROTECTIVE EFFECTS OF ALKALOID BERBERINE AGAINST CISPLATIN-INDUCED KIDNEY DAMAGE IN MICE****Robert Domitrović, Ivana Gobin, Olga Cvijanović, Ester Pernjak-Puge, Marko Skoda and Lorena Mikelić***Department of Microbiology, University of Rijeka, Medical Faculty, Hungary; E-mail: ivana.gobin@uniri.hr*

The aim of this study was to investigate the therapeutic activity of isoquinoline alkaloid berberine against cisplatin (CP)-induced kidney injury in mice. Berberine was administered at daily doses of 1, 2 and 3 mg/kg by oral gavage for two successive days, 48 h after intraperitoneal CP injection (13 mg/kg). Mice were sacrificed 24 h after the last dose of berberine. Histopathological changes and the increase in serum creatinine and blood urea nitrogen (BUN) induced by CP were significantly ameliorated by berberine in a dose-dependent manner. Additionally, oxidative/nitrosative stress, evidenced by the increase in renal 4-hydroxynonenal (4-HNE), 3-nitrotyrosine (3-NT), cytochrome P450 E1 (CYP2E1) and heme oxygenase (HO-1) expression, was significantly reduced. The expression of nuclear factor-kappaB (NF-κB), tumor necrosis factor-α (TNF-α), cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) was markedly suppressed by berberine, indicating the inhibition of inflammatory response. Treatment of CP-intoxicated animals with berberine also significantly reduced the expression of p53, active caspase-3 as well as autophagy marker light chain 3B (LC3B) in the kidneys. The results of the current study show the nephroprotective activity of berberine against CP-induced kidney injury, which could be attributed to the inhibition of oxidative/nitrosative stress, inflammation, autophagy and apoptosis.

**Keywords:** Berberine, cisplatin-induced kidney injury, mice.**PO-47***Track: Plant and Environment***SLOW RELEASE FERTILIZERS PRODUCED FROM WASTE ENCAPSULATED OR COATED WITH BIODEGRADABLE POLYMERS - NOVEL ENVIRONMENTAL USE OF POLYHYDROXYALKANOATES****Katarzyna Gorazda, Zbigniew Wzorek, Anna K. Nowak, Joanna Kulczycka, Anna Henclik and Ipsita Roy***Department of Inorganic Technology and Environmental Biotechnology, Cracow University of Technology, Poland; Email: gorazda@chemia.pk.edu.pl*

Nowadays alternative phosphorus source are in wide demand because of its natural reserve depletion. Phosphate rich waste is one of such alternative for fertilisers' production.

In EU 10-16% of the whole sewage sludge amount is incinerated, bringing nearly 0.6 mln tons of sewage sludge ash (SSA) per year. In October 2012 the EU started the realization of the P-REX programme, whose main goal is an improvement and industrial-scale implementation of phosphorus recovery methods concerning water treatment by-products like sewage stream, sewage sludge and sewage sludge ash as alternative sources of this element.

Proposed by Cracow University of Technology technology transfers unwanted waste into phosphorus products-fertilisers that are identified as a priority resource for long-term food production by European Environment Agency. It was shown that there is a possibility of phosphorus recovery from sewage sludge with its autothermic combustion and further utilization of ash in the extraction processes with the use of inorganic acids both nitric and phosphoric. Phosphorus recovery efficiency is greater than 92%. Obtained products in the form of NP and NPK fertilisers are characterized by high purity and phosphorus availability compatible with the phosphate standard.

Fertilizers with controlled release of nutrients have many advantages over the conventional type of fertilizer such as reducing the rate of the nutrient released from the soil by rain or irrigation, uniform supply of nutrients over a longer



period of time, increasing the fertilizer efficiency, lower frequency of use, minimizing the negative effects associated with overdose and lower toxicity. Polymer-coated or encapsulated controlled-release fertilizers can cause an environmental problem since undesirable residues of the coating material may accumulate in the fields.

Caring for the environment and reducing the adverse effects of the use of common fertilizers such as soil acidification, the introduction of excessive amounts of heavy metals, salinity and over-fertilization of plants causes considerable interest in efficient fertilizer of controlled release coated with biodegradable polymers. PHAs as a water-insoluble polyesters characterized by a non-toxicity and biodegradability under both aerobic and anaerobic conditions are relevant materials for coating and encapsulation of fertilizers. Moreover PHAs can be produced from renewable raw materials what enables the creation of waste management systems in a closed cycle and is consistent with sustainable development.

In the on-going research at Cracow University of Technology using the ashes after thermal processing of sewage sludge as alternative raw material, different phosphate fertilizer products are produced: liquids and solids in the form of ammonium phosphate or calcium and ammonium nitrate originating from renewable sources.

In the presented research P(3HB) and P(3HO) derived respectively from *Bacillus Cereus* SPV and *Pseudomonas Mendocina* were used as a coating (in case of solid fertilizers) or encapsulating agent (in case of liquid fertilizers). Microspheres and coated granules of fertilisers were investigated for the nutrients release according to standard EN 13266:2001:Slow-release fertilizers. Structure and other properties of granules and microspheres were also investigated using: XRD, SEM, TA methods.

It was revealed that PHAs as coatings for solid or liquid fertilizers have a very good potential in future use. Moreover the combination of biopolymers derived from waste with the fertilizers produced from waste materials will contribute to the creation of entirely new fertilizer products on the market with potentially lower costs of production and compatible with the expectations of the European market for fertilizers.

Keywords: Sewage sludge ash, fertilisers, phosphorus recovery, PHAs, Polyhydroxyalkanoates, slow release fertilizers, microsphere.

PO-43

Track: Industrial and Manufacturing

MODIFICATION OF GLYCEROL CATABOLISM IN YARROWIA LIPOLYTICA THROUGH GENETIC ENGINEERING APPROACH

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High supply of raw, residual glycerol from biodiesel production plants promote the search for novel methods of its utilization. Numerous attempts towards biotechnological valorization of this bioresource are currently being developed. *Yarrowia lipolytica* is a dimorphic, nonconventional yeast species with unique metabolic properties, known for its efficient growth on raw glycerol from biodiesel production plants. In this study we attempted modification of *Yarrowia lipolytica* glycerol catabolism towards its more efficient utilization. To this end we developed a genetic construct bearing three heterologous genes natively involved in glycerol metabolism, cloned under a glycerol-induced promoter. The genetic construct was completed using standard genetic engineering protocols. The final expression cassette (of more than 13 kbp) was subsequently transformed into host *Yarrowia lipolytica* A18 competent cells. Further studies comprised estimation of copy number of the cassette integrated with the host genome, stability of the cassette and assessment of the three genes' expression level. Finally the modified strain's performance in glucose- and glycerol-based media during shake flask cultivations was investigated. (Project No. OPIE01.01.02-00-074/09).

Keywords: *Yarrowia lipolytica*, genetic engineering, glycerol.

PO-42

Track: Industrial and Manufacturing

THE USE OF CLOSTRIDIUM BUTYRICUM DSP1 FOR THE PRODUCTION OF 1,3-PROPANEDIOL FROM CRUDE GLYCEROL

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The current tendency to use alternative energy sources has resulted in a significant increase in the production of biofuels that are a wide range of fuels derived from biomass. The most common biofuel in Europe is biodiesel, made from vegetable oils, animal fats or recycled greases. However, a rise in biodiesel production generates a huge amount of crude glycerol. One way of utilization of glycerol generated in biodiesel production is its microbial conversion to 1,3-propanediol (1,3-PD). In this work, a newly isolated *C. butyricum* strain was used to convert crude glycerol to 1,3-PD. The main aim of the research was to investigate the efficiency and other vital parameters of 1,3-PD production in bioreactors of various capacity (6.6 L, 42 L, 150 L) in order to determine the possibility of achieving desired production parameters on a given scale. The values of effectivity parameters for 1,3-PD synthesis in batch fermentations carried out in 6.6 L, 42 L and 150 L bioreactors were similar. The parameters obtained during fed-batch fermentations in the 150 L bioreactor differed in the rate and percentage of substrate utilization. The analysis of cell proteins demonstrated that a number of multifunctional stresses occurred during fed-batch fermentations in the 150 L bioreactor, which suggests the possibility of identifying the key stages in the biochemical process where inhibition of 1,3-PD synthesis pathways can be observed.

Keywords: Clostridium, glycerol, 1,3-propanediol.

PO-29

Track: Medical Biotechnology

PREPARATION AND OPTIMIZING THE CHARACTERISTICS OF IRINOTECAN-LOADED PLGA-PEG-FOLATE NANOPARTICLES USING BOX-BEHNKEN STATISTICAL DESIGN

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One of the most important goals in novel drug delivery systems is to target anticancer drugs specifically to the site of tumors in order to improve antineoplastic effects and reduce systemic toxicity of chemotherapy. The purpose of this study is to prepare and optimize the characteristics of irinotecan nanoparticles (NPs) using Poly lactide-co-glycolide-folate (PLGA-PEG-folate) to be used in active targeted drug delivery.



The drug-polymer NPs were fabricated by an emulsification/solvent evaporation method. Organic phase consisted of drug and polymer was driply added to the aqueous phase and after sonication the resultant nanoemulsion was placed on an stirrer at room temperature to ensure that the organic phase is totally evaporated. NPs were then centrifuged and lyophilized. Finally, some properties such as morphology, zeta potential, particle size, drug loading and encapsulation efficacy was determined and optimized using a Box-Behnken statistical design.

The range of particle size was between 121-274 nm. The zeta potential of NPs was negative. The prepared NPs were spherical with smooth surfaces and Box-Behnken design showed a reasonable correlation between the predicted and experimented values.

Evaluating different characteristics of NPs demonstrates that they can be considered as suitable drug delivery systems for active targeted drug delivery in different types of cancers.

Keywords: PLGA-PEG-Folate, irinotecan, emulsification/solvent evaporation, box-behnken.

PO-25*Track: Plant and Environment***IN VITRO MANIPULATION OF IMPATIENS GLANDULIFERA POLLEN FOR TRANSPORTING EXTRACELLULAR SUBSTANCES TO THE EMBRYO SAC****Noreldaim Hussein***Faculty of Science, Al Jouf University, KSA; E-mail: nourguneid@yahoo.com*

Pollen from *Impatiens glandulifera* was manipulated *in vitro* to investigate the possibility of using them as a vector for transporting extracellular substances to the site of gamete fusion in the embryo sac. Manipulation of plant male and female gametophytes included studies on pollen culture *in vitro*, pollen viability and developmental state and loading of fluorescent probes by plasmolysis/endocytosis via germinating pollen.

Keywords: Pollen, plasmolysis, endocytosis, fluorescent probes.



PO-17*Track: Pharmaceutical Biotechnology***RESEARCH AND DEVELOPMENT OF A THERAPEUTIC ANTIBODY CHA21****Liu Jing***School of Life Sciences, University of Science and Technology of China, China; E-mail: jliu@ustc.edu.cn*

Structure basis for recognition and down-regulation of ErbB2 by a potential therapeutic antibody chA21 and its development of antibody therapy drug.

A novel anti p185^{her2/neu} antibody chA21 was constructed by assembling a single-chain Fv (scFv) of mAb A21 with human IgG1 Fc fragment. The chA21 recognized and down-regulated ErbB2 receptor on the surface of tumor cell, inhibited the growth of p185^{her2/neu} overexpress cancer cells *in vitro* and *in vivo* specifically and induced the conversion of the malignant to normal phenotype. So it can be developed a potential therapeutic antibody for breast, ovarian and gastric cancer with overexpressed p185 on their cell surface. The crystal structure of the chA21 in complex with an N-terminal fragment of p185 reveals that chA21 binds a region opposite to the p185 receptor dimerization interface indicating that chA21 does not directly disrupt the dimerization as the antibody drugs Trastuzumab and Pertuzumab. In contrast, the bivalent chA21 leads to internalization and down-regulation of p185 strongly. The supernatants collected from Bioreactor were purified with a three-step chromatography. The preclinical studies such as immunogenicity and pharmacokinetics/ pharmacodynamics have been assessed. It will be going to the clinical studies.

PO-28*Track: Industrial and Manufacturing***BIO-ESTERS FORMATION IN TRANSESTERIFICATION AND ESTERIFICATION REACTIONS ON POLYANILINE DOPED WITH VARIOUS SULFONIC ACIDS****A. Drelinkiewicz, Ż. Kalembe-Jaje, E. Lalik, A. Zięba and J. Stejskal***Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Niezapominajek 8, 30-239 Kraków, Poland; E-mail: zaneta.kalembe@op.pl*

Introduction: Biodiesel fuel consisting of methyl esters of fatty acids is formed in transesterification of triglycerides with methanol [1,2]. Nowadays, substitution of industrial homogeneous catalysts by heterogeneous ones is a desirable goal. Solid acid catalysts are preferred as they are able to catalyze both transesterification of triglycerides and esterification of free fatty acids. Among variety of solid acid catalysts the sulfonic acid-based ones seem to be the most promising candidates. The examples include sulfate treated inorganic oxides, organosulfonic groups-containing polymers and mesoporous silica. Here, another polymer,



polyaniline doped with organosulfonic acids is studied for transesterification of triglycerides (triacetin (TACT) and castor oil) and esterification of fatty acid (ricinoleic acid) with methanol. Polyaniline doped with methanesulfonic (**pani-MSA**), camphorsulfonic (**pani-CSA**) and sulfuric acids (**pani-S**) in the form of powder and deposited on silica, carbon and multiwall carbon nanotubes (CNT) are examined [3-5].

Experimental/methodology: Polymer coating (15 – 20 wt %) was obtained by *in situ* polymerization [6] of aniline [(NH₄)₂S₂O₈ + appropriate sulfonic acid] in the presence of carriers (carbon 734 m²/g; d~1-2 nm; SiO₂ 325 m²/g, d = 9.8 nm; CNT 173 m²/g, d = 3.8 nm). The textural, morphological and acid (capacity and strength) properties of catalysts were examined by FT-IR, XRD, SEM, DSC/TG and microcalorimetric ammonia sorption techniques. The transesterification and esterification with methanol were carried out at 55 °C, methanol to triglyceride molar ratio of 29, catalyst concentration (0.33 - 1 wt. %) The composition of reaction mixture was analysed by GC and HPLC methods [3].

Results and discussion: Polyaniline salts (**pani-X**) (Fig. 1) exhibit good environmental and thermal stability (to 300 °C), are insoluble in most of organic solvents and could be easily obtained by doping of **pani** with protonic acids. Strong adhering ability of **pani-X** towards variety of materials (glass, ceramics, inorganic oxides) allowed preparation of samples with the solvent resistant **pani-X** coating (µm thickness) [6]. An interesting property of polyaniline sulfate is high content of acid sites (4.75 mmol/g), close to that of Amberlyst-15. However, the powder **pani-X** samples exhibit low surface area (8 - 26 m²/g) [3-6] and does not swell in reaction mixture; methanol, methyl esters or triglycerides thus giving low utilization of active sites. Therefore, in the present work the catalysts with **pani-X** coatings deposited on supports; carbon, silica and CNT are studied. The using of organosulfonic MSA and CSA acids of various alkyl groups and H₂SO₄ allows tuning of hydrophobic/hydrophilic balance of the catalysts surface, which according to the literature affects the interaction of active sites with the reagents. This could essentially determine the activity especially for bulky reactants, like triglycerides, fatty acids. Easily accessible active sites and mesoporous structure are considered to be preferred. Because of different properties of the C, CNT and silica carriers (textural and hydrophobic) the **pani-X** coatings of various morphologies were obtained (Fig. 2). The morphology of polymer coating was also acid-type dependent. In most of studied catalysts the polymer nanorods agglomerated into interconnected networks forming branched fibrous structures appear (Fig. 2, left). In agreement with [6], the carbon-based C and CNT supports produced less aggregated and thus more extended polymer overlayers. This is evidenced by dramatic reduction of surface area and porosity of carbon-based supports due to deposition of polymer (from 734 m²/g to 347 and 59 m²/g for **C-pani-S** and **C-pani-CSA**). In silica-supported catalysts apart from the branched structures also rice-type spherical grains of polymer appear. Definitely lower reduction in surface area and porosity observed for silica (from 325 to 288 and 237 m²/g for **SiO₂-pani-S** and **SiO₂-pani-MSA**) suggest preferential deposition of **pani-X** coatings onto outer most surfaces of silica particles. In both studied reactions, methanolysis of triglycerides (triacetin and castor oil) and esterification of ricinoleic acid **CNT-pani-S** and **SiO₂-pani-MSA** catalysts with “rice-type” polymer morphology and mesoporous structure are much more active (Fig. 1) compared to all other studied catalysts with branched fibrous structures of polymer coating. The course of TACT methanolysis on the former is similar to that on soluble sulfuric, MSA and CSA acids giving the maximum yield of methyl esters ca. 91-95 %.

On catalysts with **pani-X** fibrillar structures the accumulation of partial glycerides/glycerol appears resulting in a blockage of active sites. This accumulation effect is especially strong for sulfuric acid doped polyaniline and becomes evidently weaker for organosulfonic MSA, CSA-acid doped polymer thus proving advantageous effect of hydrophorous alkyl groups in acid groups.

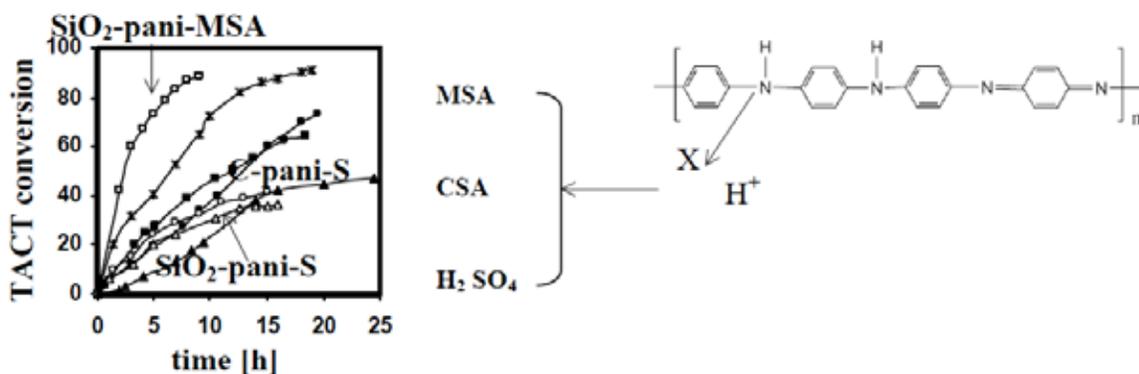


Fig. (1). The methanolysis of triacetin in the presence of **pani-X** catalysts; the schematic structure of **pani-X**.

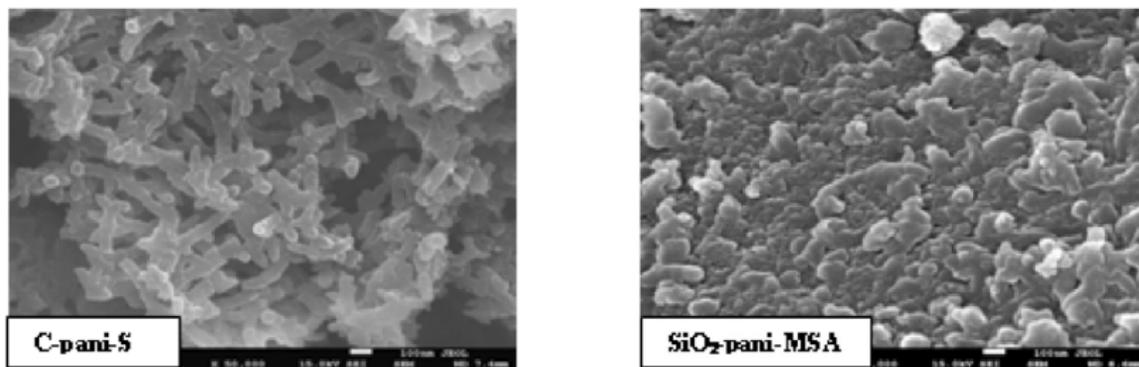


Fig. (2). SEM micrographs of SiO₂-pani-CSA and SiO₂-pani-MSA.

From the observed effects of catalysts concentration, temperature of reaction and the role of added diacetin and glycerol a strong interaction of active sites with glycerol via a system of hydrogen bonds giving a diffusion barrier for the reaction could be suggested. This is facilitated either by fibrillar morphology of polymer and microporous structure as in case of carbon-supported catalyst. Fibrillar, branched structures of polymer create a specific spatial organization of the polymer chains resulting in locally increased density of active sites. This facilitates interactions between active sites and polar reagents and in particular with glycerol. On the other hand, a combination of well extended *pani-X* coating together with the mesoporous structure provides conditions for almost uniform surface distribution of active sites. This gives easily accessible active sites and almost effectively prevents the accumulation of polar reagents. These features make mesoporous silica supported *pani-MSA* an interesting catalyst for methanolysis of triglycerides and esterification of ricinoleic acid

Conclusions: Polyaniline salts prove to be active and stable (~90 %) solid acid catalysts for transesterification of triglycerides and esterification of fatty acid with methanol. Morphology of polymer coating and porous structure of catalysts are crucial for activity and interactions of active sites with reagents. In particular they affect interactions with glycerol resulting in a blockage of catalysts. These interactions are facilitated by the polymer morphology in form of branched networks of *pani-X* nanorods owing to locally increased density of active sites. To prevent blockage of catalyst due to accumulation of glycerol almost uniform surface distribution of active sites is needed.

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PO-24

Track: Medical Biotechnology

THE EFFECT OF HIV COINFECTION, HAART AND TUBERCULOSIS (TB) TREATMENT ON CYTOKINE/CHEMOKINE RESPONSES TO TB ANTIGENS IN ACTIVE TB PATIENTS AND LATENT TB INFECTED (LTBI) INDIVIDUALS: THEIR USEFULNESS AS BIOMARKERS FOR TB

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Background: Identification of *Mtb* specific cytokine/chemokine biomarkers for latent and active



TB could assist to develop novel diagnostic, prognostic and therapeutic tools for TB.

Methods: Levels of IFN- γ , IL-2, IL-17, IL-10, IP-10 and MIP-1 α (pg/ml) in response to ESAT-6/CFP-10 were measured using Luminex assay in 7 day culture supernatants of whole-blood collected from HIV positive TB patients (HIV+TB+; n=26, 14 were on anti-TB treatment (ATT), 12 on ATT plus HAART); HIV-TB+ patients (n=14, all on ATT); HIV+TST+ patients (n=19, all on HAART); HIV-TST+ individuals (n=10); and HIV-TST- controls (n=10).

Results: Compared to Controls, HIV-TST+ individuals showed elevated IP-10, IFN- γ and IL-17, but lower MIP-1 α ; while HIV-TB+ patients showed raised IL-2 and IP-10, but lower MIP-1 α and IL-10 ($p<0.05$). The levels of IFN- γ , IL-17, MIP-1 α , and IL-10 were elevated in HIV-TST+ individuals compared to HIV-TB+ patients ($p<0.05$). HIV coinfection suppressed IFN- γ , IL-17, IP-10 and IL-2 production in HIV+ individuals with or without TB ($p<0.05$). By six month (M6) of ATT alone, the level of IFN- γ , IL-10, and MIP-1 α increased, and IL-2 and IP-10 decreased in HIV-TB+ patients ($p<0.05$). However, there was no significant change in any of the cytokines in HIV+TB+ patients, except for MIP-1 α , which was increased ($p=0.04$).

By M6 and M18 of ATT plus HAART, HIV+TB+ patients showed a progressive increase in MIP-1 α and IL-10 ($p<0.05$), but the levels of IFN- γ , IL-17, IL-2 and IP-10 remained impaired. Moreover, in HIV+TST+ patients on HAART, while IFN- γ , IL-10 and MIP-1 α levels increased progressively, IL-2 and IL-7 decreased ($p<0.05$).

Conclusions: We showed distinctive cytokine/chemokine expressions during LTBI and active TB. Simultaneous measurement of ESAT-6/CFP-10 induced IFN- γ , IL-17 and IP-10 may assist to diagnose LTBI; IL-2 and IP-10 to diagnose active TB; and IFN- γ , IL-17, MIP-1 α , and IL-10 to distinguish LTBI and active TB. HAART adjusts cytokine/chemokine production in HIV+TST+ patients except IP-10. However, ATT plus HAART did not restore IFN- γ , IL-17, IP-10 and IL-2 response in HIV+TB+ patients. Combined measurement of ESAT-6/CFP-10 induced IL-2, IFN- γ and IP-10 in HIV-TB+ patients; and IL-10 and MIP-1 α in HIV-TB+, HIV+TB and HIV+TST+ patients can be useful surrogate markers to monitor therapeutic responses.

PO-39

Track: Medical Biotechnology

ANALYSIS OF APOPTOSIS, ADHESION AND IMMUNE RELATED PROTEINS IN UTERUS ENDOMETRIUM WITH NORMAL OVARIAN FOLLICLES AND OVARIAN CYST IN HANWOO

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Cows may suffer impaired ovarian function, often accompanied by reduced conception rates and increased embryonic loss. Cystic ovarian disease (COD) is one of the most frequently diagnosed gynecological findings in dairy cattle. It causes temporary infertility and is likely to affect reproduction as well as production parameters in cattle. Therefore, the purpose of this study was to determine the expression patterns of apoptosis (Bcl-xL, Bax), implantation (E-cadherin) and immune related proteins (TNF- α , IL-10) in uterus endometrium of Hanwoo (Korean native cattle) with ovarian cyst and normal ovarian follicles. In the Western blot analysis, the expression of anti-apoptotic Bcl-xL protein was significantly increased in endometrium with normal ovarian follicles, whereas expression of pro-apoptotic Bax protein was significantly decreased. On the other hand, adhesion related protein (E-cadherin) and immune related proteins (TNF- α , IL-10) was significantly increased in uterus endometrium with normal ovarian follicles. Taken together, these results suggest that the expressions of apoptosis, adhesion and immune related proteins in uterus endometrium with ovarian cyst were showed the aberrant patterns.

Keywords: Ovarian cyst, Endometrium, Apoptosis, adhesion, immune.

PO-70*Track: Plant and Environment***CHARACTERIZATION AND ANTI-INSECT ACTIVITY OF A TUBER LECTIN FROM *DIOSCOREA MANGENOTIANA*****Adenike Kuku and Kehinde Akinyoola**

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Insect pests are a major constraint to increased crop production in tropical and subtropical regions. Plant resistance to insect pests is mediated by a range of primary metabolites among which are lectins. Thus, there is intensified research in the use of lectins in protecting crop plants from the attack of insects and fungal pathogens. This study purified a tuber lectin from *Dioscorea mangenotiana*. The lectin was composed of two isoforms, DM I and DM II. The apparent native molecular mass of DMI was estimated as 56 kDa while it showed as a single protein band with subunit molecular mass of 27 kDa on SDS - polyacrylamide gel electrophoresis under reducing conditions. The carbohydrate content was 2.54%, thus is a dimeric glycoprotein. The lectin agglutinated erythrocytes from human and rabbit, but the activity was inhibited by N-acetyl-D-glucosamine with minimum inhibitory concentration (MIC) of 50 mM. The lectin was heat stable up to 90°C and within the physiological pH range. When incorporated in artificial diet, DMI significantly affected the development of the larvae of sugarcane stalkborer/maize stemborer, *Eldana saccharina* (Lepidoptera; Pyralidae) indicating the possibility of being useful in a biotechnological strategy for the pest management. Larvae fed on artificial diet containing sub-lethal dose of DMI showed a significant decrease in acid phosphatase and alkaline phosphatase activities while esterase activity markedly increased as compared to larvae fed on diet without the lectin.



Keywords: Lectin, tuber, anti-insect, *Dioscorea mangenotiana*, *Eldana saccharina*.

PO-37*Track: Medical Biotechnology***A PATCH-LESS FULLY EMBEDDABLE BIODEGRADABLE MICRONEEDLE INSERTION DEVICE FOR (TRANS) DERMAL DRUG DELIVERY****Shayan Fakhraei Lahiji and Hyungil Jung**

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Microneedles (MN) have been introduced as a means to overcome limitations of traditional transdermal drug delivery. Dissolving microneedle patch provides self-administration of therapeutic macromolecules in a painless and patient friendly manner. Almost all microneedles employed for commercial purposes are placed on a patch. However, due to the heterogeneity and anisotropy of skin, the percentage of therapeutic drug delivered into body is less than the fabricated amount. On the other hand, the wide base geometry of MNs decreases the possibility of complete skin insertion. The incomplete insertion of dissolving MNs lowers the efficacy of drug delivery. Chemicals used in patches as well as topical agents used for treatment of superficial wounds may cause irritation and allergy in skin. In addition, patches cannot be applied to hairy parts of body. Accordingly, patients must wait from minutes to hours for MNs to be fully dissolved before removing the patch. In this study, we have developed a self-administrable patient friendly device (Micropiercer) to insert MNs into skin without the necessity of patch in less than one second. Unlike single layered microneedle patches that lack efficiency in delivering drugs, a single layered dissolving microneedle inserted into skin through Micropiercer showed higher accuracy and efficiency. The depth of MN insertion can be adjusted to be superficial or deep up to 2 millimeters. Any type of MN, regardless of fabrication method can be inserted into skin through Micropiercer. Through this device, MNs can be inserted into hairy skins without shaving in a painless manner. To compare the insertion depth of traditional MN patches and Micropiercer, we have performed hematoxylin and eosin (H&E) staining and brightfield imaging studies. Results showed deeper penetration and higher preciseness of MNs inserted into skin through Micropiercer compared to the traditional approach. The drug delivery efficiency and drug release profile of microneedles inserted into skin through Micropiercer compared



to the traditional method was carried out via *in vivo* studies. We have studied the effects of insulin MN on diabetic mice. Increased down regulation of blood glucose level was confirmed in diabetic mice followed by Micropiercer MN insertion. To our knowledge, this is the first time that such a device is designed and manufactured. Micropiercer offers a simple and attractive approach to improve therapeutic drug efficiency while reducing the risk associated with traditional injections.

Keywords: Micropiercer, Dissolving microneedle, Drug delivery, Insulin.

PO-64

Track: Plant and Environment

APPLICATION OF MOLECULAR BREEDING TO IMPROVE SUBMERGENCE TOLERANCE IN VIETNAMESE RICE CULTIVAR TO COPE CLIMATE CHANGE

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Introduction: Submergence stress caused by climate change is the major hindrance to enhancing rice production of Vietnam. Hence, the objective of this study was to improve submergence tolerance of Khang dan 18, an elite Vietnamese rice cultivar.

Methodology: Transferring the QTL/Sub1 submergence tolerance into a popular Vietnamese cultivar by using marker-assisted backcross (MABC) from the donor parent PSB-Rc68 Sub1.

Results: Four backcrosses and selfing were conducted to transfer positive alleles of QTL/Sub1 into Khang dan 18. Few individual plants of BC4F2 generation carrying QTL/sub1 and attaining the maximum recurrent genetic background (approximately 100%) were identified. The improved lines exhibited high submergence tolerance in the artificial assay and ongoing to screen in the field trial.

Discussion and Conclusion: All improved lines efficiently converted to submergence tolerance similar to the donor parent PSB-Rc68 Sub1, whereas their agronomic performances were similar as the original Khang dan 18.

PO-05

Track: Regenerative Medicine

HLA-E EXPRESSION ON PORCINE CELLS PREPARED FOR XENOTRANSPLANTATION

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Human NK cells play an important role in the cell-mediated rejection of pig-to human xenografts. The inhibitory receptor CD94/NKG2A expressed on a majority of activated human NK cells binds specifically HLA-E. *In vitro* studies demonstrated that the expression of HLA-E on porcine endothelial cells provide partial protection against xenogenic human NK cell cytotoxicity. The aim of study was to prepare the pHLAE-GFPBsd genetic construct containing a gene encoding HLA-E under a control of elongation factor-1 alpha (EF-1 α) promoter, which was subsequently transferred to male pronuclei of the fertilized ova by a microinjection. The bioplates of the potentially transgenic animals were examined to confirm the integration of the genetic construct with genomic DNA. Transgenic animal was subjected to the molecular and cytogenetic characteristics which comprise of fluorescence *in situ* hybridization (FISH), reverse transcriptase PCR analysis (RT-PCR) and flow cytometry. Seventy-seven piglets of F0 generation were obtained and investigated. Analysis indicated that only one transgenic sow (TG1151) had pHLAE- GFPBsd genetic construct integrated with genomic DNA. The next stage of research included comparison of transgene function with previously obtained three lines transgenic animals with expression of α 1,2-fucosyltransferase, α -galactosidase and CD59. This work was supported by Project no. N R12 0036 06.

Keywords: Enotransplantation, pigs, transgenesis, gene expression.

PO-92*Track: Plant and Environment***AGROBACTERIUM RHIZOGENES-MEDIATED TRANSFORMATION - A PLATFORM FOR DEVELOPING COMPACT ORNAMENTALS AND BOOSTING BIOACTIVE COMPOUNDS IN MEDICINAL PLANTS****Henrik Lütken, Uffe Bjerre Lauridsen, Martin Himmelboe, Josefine Nymark Hegelund and Renate Müller***Crop Sciences Section, Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Højbakkegård Allé 9-13, DK-2630 Taastrup, Denmark; Email: hlm@plen.ku.dk*

Compact growth is a desirable trait in ornamental plant production because it is preferred both by producers, distributors and consumers. The growth of many potted plants is currently regulated by application of chemical growth retardants; however, several of these compounds are potentially harmful to both the environment and human health. In addition to chemical growth regulators, compactness can also be targeted in conventional or molecular breeding programmes. An upcoming alternative biotechnology with very promising results is *Agrobacterium rhizogenes*-mediated transformation. In this method, the soil borne bacterium *A. rhizogenes* inserts T-DNA, containing four root loci (rol)-genes rolA, rolB, rolC and rolD among 18 ORFs, into the plant genome. Generally, rol-genes cause stunted plant height, short internodes and reduced apical dominance. Infection of plants by *A. rhizogenes* induces hairy root (HR) growth at the infection sites. The HRs and regenerated plants derived from HRs often contain a higher content of secondary metabolites compared to wild type roots. Hence, this method also presents a tool for boosting high value compounds in plants. An important aspect of the HRs is their distinct morphology, which can be used directly in the selection process as a primary indicator of a successful transformation as an alternative to antibiotic resistance marker genes. We have developed an optimised *Agrobacterium rhizogenes*-mediated transformation platform useful for a wide range of ornamentals. *Kalanchoë* was the starting point and the effect of the rol-genes has now been followed in three progeny generations. Other ornamental plant species e.g. *Campanula* containing rol-genes are currently being generated from the same transformation platform. For the bioactive compounds focus has been on roseroot species (*Rhodiola* sp.). These plants have for centuries been utilised in biomedicine against depression and for improving mental abilities. Specifically, the root of *R. rosea*, containing the two bioactive compounds salidroside and rosavin, has been used. Due to excessive collecting, the natural populations have been declining and there is an urgent need to find alternative sources of plant material. Hence, the purpose of this study is to obtain HR cultures from roseroot and regenerate whole plants containing rol-genes for future sustainable production of bioactive compounds.

Keywords: *Agrobacterium rhizogenes*, bioactive compounds, compactness, *Kalanchoë*, non-GM, rol-genes, roseroot.

PO-27*Track: Medical Biotechnology***ADVANCED VACCINE PLATFORMS TO CONTROL ARENAVIRAL HEMORRHAGIC FEVERS****Igor S. Lukashevch, Peter Bredenbeek and Peter Pushko***Pharmacology and Toxicology, University of Louisville, KY, USA;
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Arenaviruses are rodent-borne emerging human pathogens. The diseases caused by these viruses, Lassa (LASV), Junin (JUNV), Machupo (MACV), Guanarito (GTOV), as well as lymphocytic choriomeningitis virus (LCMV), are a major public health problem in areas of South America and West Africa. Currently there are no FDA approved vaccines to control these infections. In attempts to develop safe and efficacious arenaviral vaccines, we employed replication-competent and replication-deficient platforms to design vaccine candidates potentially targeting different groups "at risk". Reassortant technology was used to make ML29 virus encoding the major antigens of LASV and the RNA polymerase of Mopeia virus (MOPV), a naturally attenuated genetic relative of LASV. In non-human primates the ML29 vaccine was safe and induced robust sterilizing cross-protective cell-mediated immune responses against LASV strains. This vaccine was safe and immunogenic in SIV-infected monkeys and also effective for post-exposure treatment.



Recently developed advanced reverse genetics system applicable for human vaccine development provides a great opportunity to re-design ML29 and JUNV Candid #1 vaccines in compliance with FDA regulatins.

The 2nd vaccine technology is based on Yellow Fever (YF) 17D vaccine. We designed YF17D-based recombinant viruses expressing major antigens of LASV, JUNV, and MACV. These vaccines candidates can be used as bivalent vaccines to control YF and Arenaviral HFIs in co-endemic areas. The 3rd platform is based on the new generation of alphavirus-based Virus-Like-Particles Vectors (VLPV). This technology was developed using backbone of human VEEV TC-83 vaccine: 26S promoter for helper genes was substituted with alphavirus-non-related CMV promoter; and the replicon was redesigned to express two or more antigens from LASV, JUNV, and MACV. Development of multivalent VLPV-based vaccines can potentially control several infections in overlapping endemic areas.

Keywords: Arenaviruses, Hemorrhagic Fevers, Vaccines.

PO-78

Track: Medical Biotechnology

EX VIVO EFFECTS OF FLAVONOIDS EXTRACTED FROM ARTEMISIA HERBA ALBA ON CYTOKINES AND NITRIC OXIDE PRODUCTION IN ALGERIAN PATIENTS WITH BEHÇET'S DISEASE

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Background: Behçet's disease (BD) is a chronic multisystemic inflammation of unknown pathophysiology. This disorder is associated with a dysregulation of the cytokine network that hyperactivates neutrophils and macrophages. In this study, we investigate the modulatory effects of flavonoids compound extracted from Algerian medicinal plant *Artemisia herba alba* on Th1 and Th2 cytokines and nitric oxide production.

Methods: The modulatory effects of flavonoids extracted from *Artemisia herba alba* on cytokines and nitric oxide production by peripheral blood mononuclear cells isolated from Algerian BD patients and healthy controls was respectively measured by means of ELISA assays and Griess modify method.

Results: Our results show that flavonoids reduce significantly the production of interleukin (IL)-12, the key effector for T helper (Th)1 cells in Behçet's disease. In contrast, the production of IL-4, the key marker of Th2 cells is increased. In addition, flavonoids also decrease the production of nitric oxide in a dose-dependent manner.

Conclusion: This study suggests that *in vitro* supplementation with flavonoids extracted from *Artemisia herba alba* has an immuno-modulatory potential via Th1 cytokine down-regulation and Th2 cytokine up-regulation and probably prevent nitric oxide induced damages.

Keywords: Behçet's disease; *Artemisia herba alba*; Flavonoids; Immunomodulation; IL-4; IL-12; nitric oxide

PO-10

Track: Plant & Environment

VEHICULAR EMISSION ON CLIMATIC CHANGE: CASE STUDY OF LAGOS STATE, LAGOS

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Climate is dynamic in nature has various factors are responsible for the climatic situation of a region so also technology is advancing and new innovation come up from time to time. For most Lagosian's, the automobile is an essential part of daily life. It has shaped our culture, our landscape and air quality. The Lagos metropolis is highly built up with millions of residence. The automobile is not without its faults, but they often are concealed by the styling, performance and other features that make today's vehicles so desirable. Still, when a product is so widely used, its faults can add up to massive unwanted side effects.

Some of the factors that bring about exhaust/fume emission is incomplete combustion of the fuel (which usually means not enough air in the fuel mixture), too much air in the fuel mixture, dirty fuel (due to bad distillation or oil in the fuel), misfiring of the spark plug (in gas engines) before complete compression of the fuel, and incomplete compression of the fuel mixture. These are just a few of the major causes. The origin of vehicular emissions is the result of the interaction of (1) the fuel used by (2) vehicles with a certain technology under (3) certain conditions of use (traffic).

The parameters monitored are Carbon Monoxide (CO), Nitrogen Dioxide (NO₂), Sulphur Dioxide (SO₂) and Volatile Organic compounds (VOC). Using gas measuring equipment specified for each e.g GasAlertMicro5 PID, ToxiRAE II PGM-1130 series, Chy670 which measures 0 to 1000ppm of CO and so on. Data was collected at varying time of the day; early morning rush hour, mid-day, evening rush hour and at night when traffic was very minimal. The sample locations were GRA Ikeja (control site), Mile 2, Mile 12, Ketu / Ojota, Oshodi and Victoria Island (these sites were chosen randomly putting into consideration that air cannot be trapped at a location). The readings show that CO was present in the air at all sample locations; at Victoria Island NO₂ is present at 0.084ppm, SO₂ at 0.174ppm while VOC was present at Mile 12 at 2.34ppm, Ketu at 3.0ppm and Victoria Island at 15.21ppm

The finding of this study reveals that CO carbon Monoxide, NO₂ Nitrogen Dioxide, SO₂ Sulphur Dioxide and VOC's are present in the ambient air in Lagos State at a concentration that is above EPA standard and will affect the weather of the state as well as of neighbouring environment, it is also a means of worry since these gases are associated with many health issues.

Some of the recommendation is that we should drive less, use public transport sometimes to reduce the number of vehicle on the road, if we must drive we should drive moderately, keep our vehicles well-tuned, not carry unnecessary weight around in the cars, refuel when the temperature is low and park in shades to minimize evaporation of fuel and ultimately we could switch to renewable fuels and change our cars to fuel efficient cars like the hybrids, vehicles using high technology such as the turbocharging compressor cars.

PO-57

Track: Medical Biotechnology

CHIP-BASED IONIZATION ELECTRON TRANSFER/COLLISION INDUCED DISSOCIATION MASS SPECTROMETRY ANALYSIS OF THE NONCOVALENT INTERACTION BETWEEN CHOLERA TOXIN AND G1 GANGLIOSIDE CLASS

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The noncovalent interaction between the subunit B of Cholera toxin (Ctb) from *Vibrio cholerae* and a native complex mixture of gangliosides extracted and purified from adult human cerebellum was studied using an analytical platform encompassing fully automated chip-nanoelectrospray ionization (nanoESI) on a NanoMate robot coupled to a high capacity ion trap mass spectrometer (MS). The interaction assay involved the incubation of Ctb and gangliosides dissolved in 10 mM ammonium acetate buffer pH 5.8. Aliquots of the reaction products were collected after 10, 30 and 60 min of incubation directly in the 96-well plate of the NanoMate robot and immediately submitted to MS analysis. Except for the Ctb envelope, positive ion mode chip-nanoESI mass spectra presented 25 signals corresponding to multiply protonated ions of the Ctb complexes with different gangliosides, all belonging to G1 class. Certain complexes revealed Ctb interaction with G1 species of high sialylation degree or modified by fucoylation which were not discovered in human cerebellum before. Multistage fragmentation carried out by electron transfer dissociation (ETD) followed by collision induced dissociation (CID) on the Ctb complex with Fuc-GT1 (d18:1/18:0) provided data supporting the binding of the c isomer of Fuc-GT1 (d18:1/18:0) to histidine 14 (H14), most probably via Neu5Ac.

Keywords: Cholera toxin, gangliosides, noncovalent interaction/complex, NanoMate robot, chip-nanoelectrospray, ion trap mass spectrometry, collision-induced dissociation, electron-transfer dissociation.

PO-68*Track: Plant and Environment***DETOXIFICATION OF HALOGENATED COMPOUNDS BY *RHODOCOCCUS*****Jayasudha Nagarajan, Anthony E.G. Cass and David J. Leak***Imperial College London, London, UK; Email: j.nagarajan10@imperial.ac.uk*

Members of the genus *Rhodococcus* are well known for their high metabolic capabilities to degrade wide range of organic compounds including aromatic hydrocarbons and polychlorinated biphenyls. Their ability to exhibit novel enzymatic capabilities for the transformation of hazardous substances makes them an ideal candidate for bioremediation. *Rhodococcus* UKMP-5M, an actinomycete isolated in Malaysia shows great potential towards degradation of cyanide, hydrocarbons and phenolic compounds. In this study, the capacity of the strain to degrade halogenated compounds was explored. Our preliminary studies have proven that *R. UKMP-5M* is capable of dehalogenating compounds like haloalkanes, haloalcohols and haloacids. It was interesting to discover that 1-Chlorobutane acts as an inducer for the degradation of halomethanes and haloethanes in *R. UKMP-5M*. Metabolism of 1-Chlorobutane in *Rhodococcus* in many cases has been reported to take place via hydrolysis, yielding an alcohol (1-butanol) which can then be assimilated for growth. In contrast to these reports, results from this research show that *R. UKMP-5M* metabolises 1-Chlorobutane but is unable to grow on it as sole carbon and energy source- giving rise to a possibly unique degradation pathway. Hence, this project uses proteomic and molecular biology tools to unravel the mechanism of haloalkane degradation in *R. UKMP-5M*.

PO-09*Track: Marine Biotechnology***OPTIMIZATION OF THE EXTRACTION, PARTIAL CHARACTERIZATION AND ANTICOAGULANT ACTIVITY OF FUCOIDAN *SARGASSUM* SPP.****J.A. Nieva, J.T. Narsico, N.C.F. Gomez, J.H. Bacurio and M.N.E. Montano***The Marine Science Institute, College of Science, UP Diliman, Quezon City, Philippines;
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Fucoidan is a sulfated polysaccharide found among brown seaweeds. In this study, we optimized the extraction of fucoidan from *Sargassum spp.* by subjecting the macroalgal samples into different hydrolysis time and pH conditions. The structural characterization of the extracted samples was conducted through sulfate and carbohydrate content, specific rotation and infrared spectroscopy. The anticoagulant activity of fucoidan extracted from *Sargassums spp.* was also investigated *in vitro* with heparin as positive control. Results showed that fucoidan yield was highest at highly acidic conditions (pH=2) using a hydrolysis time of 6 hours. Partial characterization of fucoidan from the species showed it is a left-circularly polarized light type of compound composed of ~75% and ~16% of carbohydrates and sulfates, respectively. Spectroscopic analysis of extracts indicates the presence of carbonyl groups and carboxylic acid, suggesting the presence of gluconic acid. Sulphate ester groups were also observed based on the IR spectrum. Fucoidan from *Sargassum spp* also showed a potent anticoagulant activity by increasing the coagulation time. To further improve the study, the association fucoidan activity and structure must be thoroughly investigated. In the evaluation of the anticoagulant activity of fucoidan, extracts must be purified and assessed for their ability to prolong the intrinsic coagulation pathway.

PO-72*Track: Medical Biotechnology***PALPITATION DUE TO THE CAVITATION PHENOMENON, CASE OF A FLEXIBLE PUMP****Khodjet-Kesba Omar***Laboratory of Mobilization and Value of Water Resource, High Hydraulique School, ENSH, B.P. 3109000, Blida Algeria; E- mail: okhodjet@yahoo.fr*

Our study consists essentially on the principle of a flexible pump working which looks like greatly to the function of a heart with a closed circuit.

After many efforts, we remarked the effects of cavitation either by strangulation of the flexible structure 'vessel'; or by pressure just at the pump opening (entrance).

The cavitation manifestation occurred through secondary efforts (i.e: vibration, sound, palpitation, heating of the liquid vein, depression just at the entrance of the flexible pump) We didn't follow our experiment until the end, that means at the stopping of the flexible pump. (heart disease) by suffocation, strangulation, non baiting.

By making comparison of the functioning of a flexible pump and a heart, it's the same principle or effect the simulating is similar, as far as the installation and functioning are concerned.

Keywords: palpitation, cavitation, flexible pump, heart.

PO-01

Track: Plant & Environment

FERRIPYOVERDINE RECEPTORS AND GENERAL METABOLISM IN *PSEUDOMONAS AERUGINOSA*, PRELIMINARY RESULTS

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Pseudomonas aeruginosa is a human opportunistic pathogen ubiquitously distributed in nature. Under iron limiting conditions, this organism secretes pyoverdine which helps to chelate iron to form ferripyoverdine complexes recognized at the outer membrane by ferripyoverdine receptors, these receptors help to transport iron bound to pyoverdine into the internal milieu. This work used *P. aeruginosa* wildtype and ferripyoverdine receptor mutants (for the first time) cultures as inoculums for the VITEK 2 (bioMérieux) biochemical identification system to study the possible role ferripyoverdine receptors might play in the ability of *P. aeruginosa* to utilize substrates impregnated in the VITEK 2 GNI cards, in the presence of iron and gentamicin, differential utilization of substrates were observed for *P. aeruginosa* wildtype and ferripyoverdine receptor mutants.



Keywords: General metabolism, Ferripyoverdine receptors, *Pseudomonas aeruginosa*, iron, gentamicin.

PO-22

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

RADICAL SCAVENGING ACTIVITIES OF EXTRACTS FROM FERMENTED *UNDARIA PINNATIFIDA* WITH *CORDYCEPS MILITARIS* MYCELIA

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In Oriental medicine, *Undaria Pinnatifida* (*U. Pinnatifida*) has been used for blood purification, intestinal strength, skin, hair, reproductive organs and menstrual regularity. Also, it is popularly consumed by women after giving birth as it contains high content of calcium and iodine, nutrients that are important for new nursing mothers in Korea. The major bioactive compound of *Cordyceps militaris* (*C. militaris*) is cordycepin, which has been reported to display many biological and pharmacological activities, such as immunological stimulating, anti-virus, and anti-cancer activities. In this study, *U. Pinnatifida* was fermented with *C. militaris* mycelia using a solid culture. The resulting fermentation was determined compared to unfermented *U. Pinnatifida* and mycelia for anti-oxidant activities. The various radical scavenging activities of the extracts from fermented *U. Pinnatifida* with *C. militaris* mycelia (FUCM) were evaluated by electron spin resonance (ESR). The antioxidant activities of the extracts of FUCM were also determined based on the ferric reducing antioxidant power (FRAP), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity. The free radical scavenging activity of FUCM extracts were higher than *C. militaris* mycelia or *U.*

Pinnatifida alone. These results indicate that FUCM extracts have different chemical ingredients from the *U. Pinnatifida* and might provide beneficial anti-oxidant activity.

Keywords: Radical scavenging, *Undaria Pinnatifida*, *Cordyceps militaris*.

PO-21

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

ANTICANCER ACTIVITY OF SALIDROSIDE PURIFIED FROM THE BARK OF ACER TEGMENTOSUM MAXIM

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This study aimed to isolate a single compound with an anticancer activity, from the bark of *Acer tegmentosum* Maxim (ATM). Phytochemicals from the ATM were first extracted with 70% ethanol as an initial solvent. Anticancer activity of the ethanol extracts were assessed by flow cytometric analysis of the apoptosis-inducing ability against the human hepatoblastoma, HepG2 cells. In order to isolate the active compound, the ethanol extracts were partitioned into different solvents including hexane, ethyl acetate, butanol, and water. The butanol fraction showed the most potent anticancer activity in various fractions. We isolated a compound, which strongest anticancer activity by reverse phase high performance liquid chromatography (RP-HPLC) from the butanol soluble fraction. The isolated compound was identified by ¹H, ¹³C, COSY, HSQC, HMBC, Nuclear Magnetic Resonance (NMR), and Electrospray ionization mass spectroscopy (ESI/MS) to be salidroside. We evaluated the effects of salidroside on cell proliferation, cell cycle analysis, nuclear staining, and apoptosis in HepG2 cells. These results indicate that salidroside, an anticancer compound from ATM, exhibits high apoptosis activity in HepG2 cells. Therefore, ATM could be a promising candidate for liver cancer treatment. Taken all together, the present results not only constitute the first evaluation of salidroside as a potential anticancer agent, but also establish useful procedure to extract salidroside from the bark of ATM.

Keywords: *Acer tegmentosum* Maxim., Anticancer activity, Salidroside.

PO-54

Track: Medical Biotechnology

PLASMA BIOMARKERS FOR PREDICTING PROGRESSION FROM COLORECTAL ADENOMA TO CARCINOMA IN HUMAN PATIENTS

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In the present study, we screened proteomic and cytokine biomarkers between patients with adenomatous polyps and colorectal cancer (CRC) in order to improve our understanding of the molecular mechanisms behind tumorigenesis and tumor progression in CRC. To this end, we performed comparative proteomic analysis of plasma proteins using a combination of 2-DE and MS as well as profiled differentially regulated cytokines and chemokines by multiplex bead analysis. Proteomic analysis identified 11 upregulated and 13 downregulated plasma proteins showing significantly different regulation patterns with diagnostic potential for predicting progression from adenoma to carcinoma. Some of these proteins have not previously been implicated in CRC, including upregulated leucine-rich α -2-glycoprotein, hemoglobin subunit β , Ig α -2 chain C region, and complement factor B as well as downregulated afamin, zinc- α -2-glycoprotein, vitronectin, and α -1-antichymotrypsin. In addition, plasma levels of three cytokines/chemokines, including interleukin-8, interferon gamma-induced protein 10, and tumor necrosis factor α , were remarkably elevated in patients with CRC compared to those with adenomatous polyps. Although further clinical validation is required, these proteins and cytokines can be established as novel biomarkers for CRC and/or its progression from colon adenoma.



PO-40*Track: Plant and Environment***COMPARATIVE ANALYSIS OF TWO ALGICIDAL BACTERIA ACTIVE AGAINST THE *MICROCYSTIS AERUGINOSA* FOR THE BIOLOGICAL CONTROL OF FRESHWATER HARMFUL ALGAL BLOOMS****Chong-Sung Park and Myung-Soo Han***Department of Life Science, College of Natural Sciences, Hanyang University, Seoul 133-791, Republic of Korea; E-mail: maeruginosa@gmail.com*

Harmful Algal Blooms (HABs) of freshwater cyanobacteria including *Microcystis aeruginosa* cause ecological disturbance and deterioration of water quality. In an effort to detect a bio-agent capable of controlling the *M. aeruginosa*, we used direct-(lawn, modified MPNs, and alginate bead) and indirect-method (isolation of algicidal bacteria from many candidates based on bioinformatics).

First, bacterium HYD0802-MK36 isolated directly from sediment in Daewang reservoir was identified as *Aeromonas bestiarum* using 16S rDNA sequencing data. Next, isolates *Pseudomonas syringae* KCCM 10921 showed strong algicidal activity among 56 *Pseudomonas* species which known as the major members of algicidal bacteria in freshwater.

Growth of *M. aeruginosa* ($\approx 10^5$ cells ml⁻¹) was strongly suppressed by HYD0802-MK36 ($\geq 10^7$ cells ml⁻¹) and KCCM 10921 ($\geq 10^6$ cells ml⁻¹), respectively. Host range assays revealed that isolate MK36 appeared to attack *M. aeruginosa* (NIES 298) in a strain-specific manner, while KCCM 10921 has relatively broad algicidal range against *M. aeruginosa* (NIES 298) as well as *Scenedesmus actus*, *Navicula* sp., *Alexandrium* sp. and *Akashiwo sanguinum*.

The 10, 50% diluted culture filtrates, in which *M. aeruginosa* (NIES 298) had been killed by the bacterium HYD0802-MK36, had no inhibitory effect on *M. aeruginosa* (NIES 298). Moreover, *M. aeruginosa* (NIES 298) even grew in the presence of 90% culture filtrate without any inhibitory effect being detected. In contrast, the more filtrate (10 < 50 < 90%) from KCCM 10921 was added, the host algal cells was died (algicidal activity: 27 < 77 < 95%). Biochemical assay revealed that the algicidal substance seemed to be localized in the cytoplasmic membrane of this newly identified algicidal bacterium HYD0802-MK36, while the main algicidal substance from KCCM 10921 was likely to be extracellular substance (mix-culture filtrate).

In conclusion, the most distinct characteristics between two algicidal bacteria were their algicidal mechanism including direct attack (HYD0802-MK36) and indirect attack (KCCM 10921), and available prey range as specialist (HYD0802-MK36) and generalist bacteria (KCCM 10921). In spite of their different characteristics, however, our results also suggest that they can tract *M. aeruginosa*, and terminate cyanobacterial blooms as a potential bio-agent in future use.

PO-94*Track: Others: Sports Medicine***EXERCISE IS MEDICINE: ARM-CRANKING REDUCED OXIDATIVE STRESS IN ADULTS WITH CHRONIC SPINAL CORD INJURY****M. Rosety-Rodriguez, A.J. Diaz, I. Rosety, G. Fornieles, M.A. Rosety, S. Elosegui, M. Bernardi and F.J. Ordonez,***Department of Medicine, School of Sports Medicine, University of Cadiz, Cadiz, Spain; E-mail: manuel.rosetyrodriguez@uca.es*

This study was undertaken to assess the influence of a 12-week arm-cranking exercise program on reducing oxidative damage in untrained adults with chronic spinal cord injury (SCI).

A total of 17 male adults with complete SCI at or below the 5th thoracic level (T5) volunteered for this community-based supervised intervention. Participants were randomly allocated to the intervention (n=9) or control group (n=8) using a concealed method. The intervention consisted of a 12-week arm cranking exercise program, 3 sessions/week, consisting of warming-up (10-15 min) followed by a main part in arm-crank (20-30 min [increasing 2 minutes and 30 seconds each three weeks]) at a moderate work intensity of 50-65% of heart rate reserve (HRR) (starting at 50% and increasing 5% each three weeks) and by a cooling-down period (5-10 min).

Plasmatic levels of total antioxidant status (TAS) as well as erythrocyte glutathione peroxidase (GPX) activity were measured. Lipid and protein oxidation were determined as malondialdehyde (MDA) and carbonyl group levels respectively. Furthermore, physical fitness and body composition were also assessed.

When compared to pre-test results, $VO_2\max$ was significantly increased ($p=0.031$) suggesting an improvement of their physical fitness in the intervention group. Regarding antioxidant defense system it was found both TAS ($p=0.014$) and erythrocyte GPX activity ($p=0.027$) were significantly increased at the end of the training programme. As a consequence, plasmatic levels of malondialdehyde ($p=0.008$) and carbonyl groups ($p=0.022$) were significantly reduced.

It was concluded that a 12-week arm cranking exercise program improved the antioxidant defence system in adults with chronic SCI, which may finally attenuate both lipid and protein oxidation in this population.

Keywords: Spinal cord injury; Oxidative stress; Exercise.

PO-63

Track: Plant & Environment

BIOLOGICAL ACTIVITY OF PHENOLIC COMPOUNDS FROM ARGENTINEAN HERBS INFUSIONS

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Argentinean herbs infusions have been studied for their phenolic compounds concentration, antihypertensive, antioxidant and antibacterial activities in order to find out new natural products with beneficial activities. The modifications of antihypertensive and antioxidant activities by the addition of additives (lemon, sugar and sweetener) used commonly were studied also. Among the 13 infusions assayed *Ilex paraguariensis* present the highest amount of total phenolic compounds and flavonoid fraction, lemon addition increased the phenolic compound concentration. All infusion showed high DPPH radical scavenging assays and additives addition did not modified significantly this activity. *I. paraguariensis* infusion is the most effective to reduce the viability of *Escherichia coli* and *Staphylococcus aureus* and the herbs combinations *Lippia integrifolia-Ilex paraguariensis* was the most effective to reduce *S. aureus* and *E.coli* viability. The antibacterial effect is related with phenolic compounds concentration but also with its profile. The high correlation between antioxidant and antihypertensive capacities with total phenolic contents indicate that phenolic compounds are the major contributors of these capacities. These results permit propose these herbs as new sources of safe natural antioxidants and antihypertensive compound that could be used in pharmaceutical and food industries.



PO-56

Track: Others - Polymerase chain reaction

AMPLIFICATION OF *BRUGIA MALAYI* DNA BY USING HHA1 PRIMER AS A TOOL

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Lymphatic filariasis is a mosquito borne parasitic disease caused by tissue dwelling human filarial nematodes such as *Wuchereria bancrofti*, *Brugia malayi* and *B. trimori* and are considered to be a major complication for the socio-economic development in developing countries. A number of inflammatory responses are associated with the disease that is recurrent attacks of adeno-lymphangitis, lymphoedema, hydrocele and elephantiasis. The present study is directed at the parasite DNA templates in their respective reaction mixtures with the different composition and temperature conditions. The specific primer Hha1 specific to *Brugia malayi* was used for detecting the parasites and was found to give optimum yield in the positive control samples. This result was confirmed from the amplified fragment having size of 322 bp of *B. malayi*. Using this primer as a diagnostic tool for the detection of filariasis could be the most promising aspect of this study and offers scope for detection of both the parasites even at low levels of infection.

Keywords: Polymerase chain reaction, Diagnostic, *Brugia malayi*.

PO-18*Track: Plant and Environment***A PRELIMINARY STUDY ON THE EFFECTS OF DROUGHT STRESS ON SOME PHYSIOLOGICAL CHARACTERISTICS OF THREE MOSS SPECIES****Chunhui Guo, Wei Sha and Xiaokai Li***Key Laboratory of Genetic Engineering , College of Life Science and Forestry , Qiqihar University, Qiqihar Heilongjiang 161006 , China; E-mail: shw1129@263.net*

Three kinds of mosses including *Racomitrium japonicum* Dozy & Molk , *Grimmia pilifera* P.Beauv. and *Tortula ruralis* Gaertn were subjected to dry treatment by silica gel for three months, chose different time gradients and measured six physiological indexes of them including relative water content (RWC), malondialdehyde (MDA) content , free proline (Pro) content , soluble protein (SP) content, soluble sugar (SS) content and PODactivity. The results showed that the malondialdehyde content in the three mosses treated with silica drought stress was gradually decreased and then increased and reveal the same trend once again along with the relative water content reduced. PODactivity of *Tortula ruralis* is lower than those of the other two mosses, and changes inconspicuously, while the PODactivity of *Racomitrium japonicum* and *Grimmia pilifera* in different periods showed great changes. Among osmoregulation substances of the three moss species, the change of free proline content and soluble sugar (SS) content were inversely related, however, change of soluble protein (SP) content and PODactivity is positively related .To some extent, the six physiological indexes indicate that the three mosses may have some special physiological mechanisms those are different from other plants .

Keyword: Silica gel dry stress; *Racomitrium japonicum*; *Grimmia pilifera*; *Tortula ruralis*; physiological characteristics.

PO-19*Track: Medical Biotechnology***CYTOTOXIC EFFECTS OF NUCLEOSIDE ANALOGUES ON THE MAIN ELEMENTS OF DRUG RESISTANCE IN HEPATOCELLULAR CARCINOMA****Nasrin Shojaie and Ghaffari S. Mahmoud***Department of Biochemistry, Institute of Biochemistry and Biophysics (IBB), Tehran, Iran; E-mail: nshojaie@ibb.ut.ac.ir*

Introduction: Nucleoside analogues such as Gemcitabine and 5-Fu are common anti-cancer drugs which can inhibit tumor growth by intervening in DNA replication but Multi-drug resistance (MDR) feature of immortal CSCs is the main obstacle against their function in HCC as a kind of malignant cancer.

Material and methods: Cultured PLC/PRF5 tumor cells in RPMI with 5% FBS treated with Gemcitabine and 5-Fu and their survival determined using MTT assay. We performed simultaneous staining of AO/EtBr and Hoechst for evaluation of apoptosis in the treated cells. Moreover, the alterations of some important factors involved in MDR genesis such as β -catenin, Lgr5, ABCG2, mdr-1, Oct-4, Nanog, p65, p50, c-Rel, HIF-1 α , Bcl-2, BIRC7 and α -AFP have been determined using RT-PCR.

Results and discussion: Our data analysis introduced PLC/PRF5 cells as sensitive tumor cells to 5-Fu and resistant to Gemcitabine which their canonical Wnt pathway significantly was downregulated in presence of the nucleoside analogues. On the other side, anti-apoptotic proteins and RelA/c-Rel complex promoted the establishment of Gemcitabine resistance and α -AFP presence contributed to settle well-differentiated and epithelial-like state of PLC/PRF5 cells. It seems Nanog behaved like a bridge between EMT and anti-apoptotic state in MDR genesis by its direct impact on bcl-2.

Keywords: Nucleoside analogues, MDR genesis, PLC/PRF5

PO-71*Track: Plant and Environment***PARTIAL PURIFICATION AND KINETIC STUDY OF CELLULASES EXTRACTED FROM *TRICHODERMA VIRIDE* AND ITS HYPERACTIVE MUTANTS****Shazia Shafique and Sobiya Shafique***Institute of Agricultural Sciences, University of the Punjab, Quaid-e-Azam Campus Lahore 54590, Pakistan;**E-mail: shazia.iags@pu.edu.pk*

Cellulases are the enzymes that cleave β -1, 4 linkages of cellulose, and carbohydrate that is main part of plants' cell walls. Presently, cellulase isolation and partial purification was executed through ammonium sulfate precipitation. The isolated protein of parental and derived mutants conferred molecular weights of 30, 45 and 55 kDa. The optimum temperature for maximal cellulase activity was 50 °C with E_a for substrate hydrolysis of 77.73, 83.97 and 83.14 kJ mol⁻¹ and temperature quotient of 1.0020, 1.0022 and 1.0022 by *T. viride* FCBP-142, Tv-UV-5.6 and Tv-Ch-4.3, respectively. The enzyme was stable at 50 °C for about 60 min but rapid denaturation occurred above 55 °C. The enzyme showed optimum activity at pH 4.0 and involved two types of acidic and basic limbs with pKa₁ and pKa₂. The pKa₁ of active site presented a significant shift from 2.55 to 2.9 and 3.1 by Tv-UV-5.6 and Tv-Ch-4.3, respectively in comparison to parental strain. Likewise, pKa₂ moved from 6.05 to 6.5 and 6.4. Enzyme kinetics displayed Michaelis-Menten constant K_m 0.6, 0.5 and 0.28 mg mL⁻¹ and V_{max} value of 8.33, 10 and 9.09 Units mL⁻¹ for parental, Tv-UV-5.6 and Tv-Ch-4.3, respectively.

Keywords: Cellulase enzyme, Partial purification, *Trichoderma viride* FCBP-142, Enzyme kinetics.**PO-73***Track: Plant and Environment***STRAIN IMPROVEMENT OF TOXIGENIC *ALTERNARIA TENUISSIMA* AND ITS GENETIC CHARACTERIZATION FOR HYPER-ACTIVE α -AMYLASE****Sobiya Shafique and Shazia Shafique***Institute of Agricultural Sciences, University of the Punjab, Quaid-e-Azam Campus Lahore, Pakistan;**E-mail: sobiya.iags@pu.edu.pk*

Production of extra cellular α -amylase enzyme by a filamentous fungus, *Alternaria tenuissima* was studied in solid-state fermentation (SSF) as well as submerged fermentation (SmF). The potential strain was successfully mutated by UV and ethyl methanesulfonate (EMS). High-level of α -amylase activity was obtained by the mutant At-Ch-5.6 (76.75 Units ml⁻¹) after chemical treatment followed by UV mutant At-UV-2.8 (63.12 Units ml⁻¹) which was significantly higher than parental *A. tenuissima* FCBP-252 (32 Units ml⁻¹). These mutants with high levels of **activity** were genetically characterized using RAPD-PCR. The expression pattern of mutants exhibited that the mutants were isogenic variants of parent strain and out-performance of the mutants could be attributed to change in genetic makeup. This work represented the first report of strain improvement in *Alternaria* for hyper activity of α -amylase enzyme and suggested that this fungus could be used to extract purified enzyme.

Keywords: *Alternaria tenuissima*, UV mutagenesis, EMS mutagenesis, RAPD-PCR.**PO-59***Track: Plant and Environment***SOIL PROPERTIES CAN CONTRIBUTE TO DISEASE-REDUCING CAPABILITY OF TOMATO (*SOLANUM LYCOPERSICUM* L.) AGAINST *VERTICILLIUM* COLONIZATION****H.O. Shittu, G.C. Mgbeze and G.C. Onwuzama***Department of Plant Biology and Biotechnology, University of Benin, P.M.B. 1154, Benin City, Nigeria; E-mail: shittuh@yahoo.com*

The strategy underlying disease-reducing capability of soils was investigated. Soils, having disease reducing and enhancing capabilities were collected from Ibadan, Oyo State and Warri, Delta State, Nigeria, respectively. These soils were used for both biological experiment and laboratory analysis. Tomato (*Solanum lycopersicum* L.) seedlings of same physiological age were infected with *Verticillium dahliae* race 1 at 4-leaf stage and grown in different Oyo and Warri soils; untreated, washed and autoclaved. Plant height and symptom expression score were recorded, as well as the amount of fungal spores in infected plant stems were determined at 5 and 10 days post inoculation. The results showed that both nutrient composition and soil microorganisms can contribute to disease reducing / enhancing property of a soil. Infected plants grown in the Oyo soils were generally taller and less symptomatic. When Oyo soil was repeatedly washed to get rid of the mineral composition, it was observed that infected plants grown in it became more symptomatic with high amounts of pathogen, suggesting a role of soil nutrients in restricting pathogen colonization. Similar trend was observed in the infected plants grown in the autoclaved Oyo soil, which had both high level of symptom expression score and amount of pathogen. The laboratory analysis indicated that Oyo soil possessed more soil microorganisms and mineral nutrients, especially carbon, nitrogen, calcium and potassium; properties which may contribute to its disease reducing ability. Since breeding for resistant cultivars remains the only effective control method for *Verticillium* wilt disease, the results from this study suggests cultivation of tomato in a fertile soil can ameliorate the devastating effect of the pathogen.

PO-06

Track: Industrial and Manufacturing

THE USE OF *ESCHERICHIA COLI* FOR THE PRODUCTION OF 1,3-PROPANEDIOL FROM GLYCEROL

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1,3-propanediol (1,3-PDO) is a chemical compound commonly used as a monomer in the synthesis of polyesters, polyethers and polyurethanes, that find many applications in chemical, textile, cosmetic industries and food production. Currently, 1,3-propanediol is obtained by chemical synthesis, which leads to the formation of toxic wastes and inquires high production costs. Solution to this problem is production of 1,3-PDO from glycerol - a by-product generated during the biodiesel manufacturing process, using genetically modified microorganisms. The use of natural 1,3-PDO producers, such as *Citrobacter freundii* and *Klebsiella pneumoniae* is limited due to their pathogenicity. The aim of the study was to obtain a genetically modified strain of *Escherichia coli* capable of converting glycerol to 1,3-PDO. Gene constructs carrying genes of 1,3-PDO operon from *Citrobacter freundii* were transferred into *E. coli*, which consumed 35 g/L glycerol and produced from 9.1 to 11.65 g/L 1,3-propanediol. This work was prepared as a part of project No. POIG 01.01.02-00-074/09 co-financed by the European Union of 1052 the European Regional Development Fund under the 1053 Innovative Economy Operational Programme 2007-2013 and was co-financed by European Union under the European Social Fund (Sub-measure 8.2.2 Human Capital Operational Programme).

Keywords: 1,3-propanediol, glycerol, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Escherichia coli*.

PO-48

Track: Medical Biotechnology

EVALUATION OF PERFORMANCE AND SAFETY OF THREE BLOOD WARMERS USING OUTDATED HUMAN RED BLOOD CELL : ENFLOWTM, BUDDY LITETM, THERMOSENSTM

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Background: Hypothermia results in adverse clinical outcome those who patients in surgery or illness. Therefore, several fluid/blood warmer was developed to maintain body temperature. However, a few studies was available to evaluate safety of fluid warmers with red blood cell(RBC). Purpose of this study is to investigate safety of three blood

warmers(enFLOW™, buddy lite™, Thermoses™) using outdated pack RBC(pRBC) by analysis of hematologic and hemorheologic data.

Methods: Outdated pRBC(320ml) was diluted with 100ml isotonic saline. Diluted RBC at room temperature(21°C) was pass through blood warmer by gravity and pressurized with 300mmHg. Blood was sampled before and after warming by devices. All values was collected such as hemoglobin, hematocrit, lactate dehydrogenase, plasma hemoglobin, hemorheologic data(deformability, critical stress, aggregation index).

Results: Maximal temperature of devices outlet (enFLOW, buddy lite, Thermoses) are 42.5 ± 0.9 , 37.1 ± 0.2 , 38.6 ± 0.7 , with gravity, and 40.1 ± 0.6 , 37.4 ± 0.3 , 38.1 ± 0.4 with pressurized 300mmHg, respectively. There was no significant difference of all hematologic and hemorheologic values between before and after warming.

Conclusion: Three devices showed good performance (maintain more than 37°C) and safety by test using diluted pRBC at room temperature.

PO-34

Track: Industrial and Manufacturing

STRUCTURE AND FUNCTION OF A NOVEL THERMOSTABLE PULLULANASE

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Research on novel pullulanase has major significance on the domestic industrialization of pullulanase and the breakdown of foreign monopoly. A thermophilic bacteria LM 18-11 producing thermostable pullulanase was isolated from Lunma hot springs of Yunnan province. It was identified as *Anoxybacillus* sp. by 16S rDNA phylogenetic analysis. Full-length pullulanase gene was cloned from *Anoxybacillus* sp. LM18-11. The optimum temperature of the pullulanase was between 55 and 60 °C with a half-life as long as 48 h at 60 °C; and its optimum pH was between 5.6 and 6.4. V_{max} and K_m of the pullulanase was measured as 750 U/mg and 1.47 mg/mL, which is the highest specific activity reported so far. The pullulanase crystals structure showed a typical α -amylase family structure. The N-terminal has a special substrate binding domain. Activity and substrate binding were decreased when the domain was deleted, the V_{max} and K_m were 324 U/mg and 1.95 mg/mL, respectively. The pullulanase was highly heterologous expressed in *Bacillus subtilis* by P43 promoter. The extracellular enzyme activity was 42 U/mL, which increased more than 40 times compared to the initial strain. This pullulanase has good application prospects.



PO-35

Track: Industrial and Manufacturing

THE WHOLE-CELL BIOTRANSFORMATION OF ALLITOL USING MULTI-ENZYME COUPLING REACTION

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Allitol is a kind of rare polyols which is rarely found in nature. It is useful not only as a sweetener, but also as the raw material for chemical compounds. In the present study, the construction of recombinant plasmids and engineered strains to co-express the genes for converting D-fructose to allitol using the whole-cell biotransformation inexpensively and conveniently instead of extracellular conversion. The biotransformation conditions for allitol production were optimised using D-fructose as the substrate. D-Psicose-3-epimerase and ribitol dehydrogenase were jointly overexpressed in a recombinant strain of *E. coli*, in which D-fructose was converted into allitol from an intermediate D-psicose. To ensure sufficient cofactor nicotinamide adenine dinucleotide in ketohexose reduction, the formate dehydrogenase gene from *Candida methylica* was co-expressed. The glucose/fructose facilitator gene from *Zymomonas mobilis* was also expressed to increase the intracellular D-fructose concentration. With the application of a recombinant *E. coli* strain that overproduces the participating enzymes for allitol production, the disadvantages of enzymatic synthesis for allitol can be successfully overcome using a whole-cell biotransformation system. This multi-enzyme coupling system produced

allitol with a yield of 48.62 g/l in 500 mM initial substrate concentrations. Therefore, the genetic engineering strain could be more effective in producing inexpensive sugar to rare polyol using the whole-cell biotransformation.

PO-03

Track: Industrial and Manufacturing

NOVEL RECOMBINANT *ESCHERICHIA COLI* STRAIN CAPABLE OF PRODUCING 1,3-PROPANEDIOL FROM GLYCEROL

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1,3-propanediol is an important intermediate chemical, which could be used for synthesis reactions, in particular as a monomer to produce polyesters, polyethers, and polyurethanes. 1,3-propanediol has been mainly manufactured by high-cost chemical synthesis. Compared with chemical synthesis, fermentation has the advantage of mass production at low cost, and has been used in production of various industrial chemicals. The use of natural 1,3-PDO producers, such as *Citrobacter freundii* and *Klebsiella pneumoniae* is limited due to their pathogenicity. The aim of the study was to obtain a genetically modified strain of *Escherichia coli* capable of converting glycerol to 1,3-PDO. Gene constructs carrying genes of 1,3-PDO operon from *Citrobacter freundii* and *Klebsiella pneumoniae* were transferred into *E. coli*, which consumed 35 g/L glycerol and produced 10.6 g/L 1,3-propanediol. Recipient bacteria were fully characterized for their production of various metabolites. This work was prepared as a part of project No. POIG 01.01.02-00-074/09 co-financed by the European Union of the European Regional Development Fund under the Innovative Economy Operational Programme 2007-2013 and was co-financed by European Union under the European Social Fund (Sub-measure 8.2.2 Human Capital Operational Programme).

Keywords: Glycerol conversion, 1,3-propanediol, *Escherichia coli*

PO-46

Track: Medical Biotechnology

BIOCOMPATIBILITY STUDY OF ZIRCONIUM OXIDE USED IN TEETH IMPLANTATION

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Dental implants have enabled people who are missing or have lost teeth to have permanently fixed replacement teeth rather than wearing dentures. In the last decade, spotlights have fallen upon zirconium, as implanted materials, which is a grey-white transition metal or metalloid in its pure form and may cause cytotoxicity and immune response. In this study, bacterial adhesion and antibacterial activity of eight prepared models of Zirconium in the presence or absence of Yttrium have been evaluated against *Streptococcus mutans* and *Lactobacillus*. On the other hand, cytotoxicity assay was carried out against adult human fibroblast cell line. Apoptosis and necrosis effects were determined using Annexin-V-FITC Apoptosis Detection kit and analyzed in Flowcytometer. All examined samples of Zirconium revealed not less than 96.9% of non-toxic effects. The lower apoptosis value (0.13%) was observed with the sample that includes Zirconium, Yttrium and 15% MgAl₂O₄. Mutagenic effects of different Zirconium models are still under investigation.

Keywords: Zirconium, teeth implantation, antibacterial activity, apoptosis.



PO-36

Track: Plant and Environment

BIO-TECHNOLOGY FOR RECOVERY OF PULP AND PAPER INDUSTRY WASTES

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The aim of our work is the development of bio-technology for recovery of wastes of pulp and paper industry forgetting the fertile substrate for seedlings and bio- fertilizer for the high productive tree plantations.

The main idea of recovering is the successive development of appropriate fungi and earthworm communities in pulp and paper wastes. It will allow us to get environmentally friendly and fertile substrate for high-productive tree plantations. Our experience on the degradation of chemicals by white rot fungi demonstrated that these fungi degrade DDT, PCB, Lindane, dioxin, and benzopyrene. Its ability to oxidize extremely chlorinated chemicals has been shown. At the stage of worm introduction, fungi can be considering a fodder for worms. The results showed a decrease in the levels of dioxins and increasing the fertility of the substrate (N, bio-available P, K, Ca and etc.) after the two-stage recovery with using fungi and worms. The structure of getting biocompost is grainy. So, we can get a fertile substrate with improved structure, aeration, and therefore, reduced compaction, the wastes were converted into finely structured humic-like material.

Keywords: Pulp and paper industry wastes, fungi, earthworm, dioxin, fertility.

PO-23

Track: Other areas/System Biology

COMPARATIVE TRANSCRIPTOME ANALYSIS OF *GASTRODIA ELATA* (ORCHIDACEAE) IN RESPONSE TO FUNGUS SYMBIOSIS

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Gastrodin, a pharmacologically active constituent, are the major phenolic components of gastrodia (*Gastrodia elata*). Under symbiotic with unique fungus *Armillaria mellea*, gastrodia will switch on the biosynthesis of gastrodin and develop from small protocorms to tubers. To understand the gene regulation in gastrodin biosynthesis in gastrodia, we conducted comparative transcriptome analysis for *Armillaria mellea*, small protocorms, and tubers of gastrodia. Transcriptome comparison between tubers and small protocorms of gastrodia revealed 1070 differentially expressed unigenes, of which 491 were up-regulated in tubers whereas 579 were down-regulated. KEGG pathway analyses were conducted for the up- and down regulated unigenes. Forty-nine up-regulated unigenes were assigned to 116 different pathways, and 55 down-regulated unigenes were assigned to 200 different pathways. Inspection of aforementioned up- and down-regulated pathways, two unigenes named locus 25051 and locus 22288 which may participate in the hydroxylation and glucosylation of gastrodin biosynthesis pathway were focused. Real-time PCR analysis for the two unigenes was conducted to confirm the differential expression between tubers and small protocorms. As the result, gene expression of unigene locus 25051 in tubers are higher than that in small protocorms about 3.6 fold, and gene expression of unigene locus 22288 are about 6.5 fold.

Keywords: *Gastrodia elata*, *Armillaria mellea*, gastrodin biosynthesis, comparative transcriptome, symbiosis.



PO-80*Track: Others – Bioremediation***MOLECULAR FUNCTION AND BIOLOGICAL PROCESS PREDICTIONS FOR NOVEL ALKB GENE METAGENOMIC FROM THE SOIL CONTAMINATED AUTOMOTIVE LUBRICANTS****Silvana Pompeia Val-Moraes and Eliana Gertrudes de Macedo Lemos***Department of Technology, Univ Estadual Paulista FCAV / UNESP, Brazil; Email: valmoraes.silvana@gmail.com*

Many functional metagenomic approaches rely on the use of *Escherichia coli* as a host for the expression of metagenome encoded proteins. The *alkB* gene has been reported as one of those responsible for the bioconversion of hydrocarbons and microorganisms are the key players of biodegradation of contaminants including oil derivatives, they are able to use this combination as a complex carbon source thereby minimizing the impact environment. The soil used for obtaining the consortium was collected from the soil contaminated by the factory that manufactured automotive lubricants site in Ribeirão Preto County, São Paulo State (Brazil). The consortium was harvested after seven days of incubation and used for construction of a metagenomic library in the fosmid vector pCC2FOSTM. We have been customised nylon macroarrays containing fluorescent probes and the DNA, from the genes hybridized followed the experiments. The gene from the metagenomic library was sequenced and submitted to NCBI / BLAST and was chosen to be an identified undescribed species according to the similarity which showed 91 % similarity to *alkB* gene from *Pseudomonas denitrificans* to be considered the same species the percentage is above 98% similarity. PCR primers targeting the metagenomic library were designed and the *alkB* gene was amplified and sub cloned into the expression vector pET 28a using *E. coli* BL21 (DE3) as the host strain. The Bioinformatics is a step potential for innovative in silico experiments, for obtain function predictions for sequence was used CombFunc (<http://www.sbg.bio.ic.ac.uk/~mwass/combfunc/>) is an automated method for the prediction of protein function.

The novel protein alkane hydroxylase was characterized computationally to predict the Molecular Function and Biological Process: For Molecular Function predictions is GO:0018685 - alkane 1-monooxygenase activity and SVM Probability was 0.808 the reaction catalyzed is Octane + reduced rubredoxin + O(2) <=> 1-octanol + oxidized rubredoxin + H(2)O and Biological Process predictions is GO:0043448 - alkane catabolic process that chemical reactions and pathways resulting in the breakdown of an alkane, any acyclic branched or unbranched hydrocarbon having the general formula C_nH_{2n+2} and SVM Probability was 0.380. This suggests that our work in situ will be enzymatic characterization and experiments for verify potential biodegradation and biosurfactant.

Keywords: Alkane hydroxylase, Biosurfactant, Biodegradation**PO-55***Track: Medical Biotechnology***THE γ -TERPINEOL INHIBITS CELL GROWTH AND INDUCES APOPTOSIS IN HUMAN LIVER CANCER BEL-7402 CELLS *IN VITRO*****Qin Wei, Kui-Chuan Ye, Yonghua-Du, Li-Jun Zhou, Zhong-Qiong Yin, Ren-Yong Jia, Jiao Xu, Xu Song and Chao-Feng Shi***Key Lab of Southwest Special Economic Plant Protection & Utilization Yibin College, Yibin, China; E-mail: 249300824@qq.com***AIM:** To study the effects of γ -terpineol on cell proliferation and apoptosis of human hepatoma BEL-7402 cells and to elucidate its molecular mechanism.**METHODS:** BEL-7402 cells were incubated with various concentrations (40, 80, 160, 320 and 640 μ g/ml) of γ -terpineol. After 48h, cell proliferation was determined by 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT) assay. Cell colony inhibition was determined by soft agar assay. Apoptosis and possible molecular mechanisms were evaluated by morphological observation, flow cytometry analysis, and DNA fragmentation assay.**RESULTS:** The γ -terpineol significantly suppressed BEL-7402 cell proliferation in a dose-dependent manner. Characteristic morphological and biochemical changes associated with apoptosis such as cells shrinkage, deformation and vacuolization of mitochondria, nuclear chromatin condensation and fragmentation, formation of apoptotic bodies

were observed after BEL-7402 cells treated by γ -terpineol for 24h and 48 h. Cell cycle were displayed by flow cytometry analysis, the γ -terpineol resulted in accumulation of cells at G₁ or S phase and a blockade of cell proliferation compared with control group. After treatment of BEL-7402 cells with 320 μ g/ml of the γ -terpineol for 36h and 48h, a typical apoptotic "DNA ladder" was observed using DNA fragmentation assay.

CONCLUSION: The present study demonstrated that anti-cancer mechanism of γ -terpineol on human hepatoma cells through suppress tumor cell growth by inducing cell apoptosis.

Keywords: γ -terpineol; Human hepatoma; BEL-7402; Cell proliferation; Apoptosis.

PO-07

Track: Plant and Environment

EFFECTIVE REGENERATION OF SORGHUM FOR THE PURPOSE OF BIO-ETHANOL PRODUCTION

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Growing importance of bioethanol production from sorghum resulted in increased interest in methods for improvement of this species. Application of routine genetic transformation technology is expected to facilitate the enhancement of sorghum. This study describes an efficient and reproducible plant regeneration system developed from tissues of *Sorghum saccharatum* and *Sorghum bicolor* genotypes. Plant regeneration was achieved from epicotyls, coleoptiles and apical buds from aseptically germinated 7-day-old seedlings. The explants were cultured on MSS1 medium: MS medium supplemented with 0.5 mg/l 2,4-D, 2 mg/l BA, 3% sucrose. Although production of secondary metabolites by all type of explants inhibited their growth and development, modification of MSS1 medium with 0.05 mg/l ascorbic acid, 1 g/l proline, 1 g/l polyvinylpyrrolidone (PVP) and 0.3 g/l casein hydrolysate resulted in improvement of in vitro culture conditions. The highest regeneration efficiency was observed for coleoptiles (92%). Plant regeneration from epicotyls and apical buds remained at lower level: 64% and 54% respectively. Root induction with 90% efficiency was achieved on MS medium containing 3% sucrose. Rooted plants were successfully acclimatised, with the survival rate reaching 85% (*Sorghum saccharatum*) and 68% (*Sorghum bicolor*).

Acknowledgement

The studies were funded by Polish National Centre for Research and Development within the project PBS1/A8/0/2012.

Keywords: Sorghum, bioethanol, plant regeneration.

PO-49

Track: Pharmaceutical Biotechnology

CHARACTERIZATION OF DENGUE VIRUS TYPE 1 SPECIFIC MONOCLONAL ANTIBODIES AND THEIR IMMUNOCAPTURE COUPLES WITH MALDI-TOF MASS SPECTROMETRY FOR RAPID DETECTION OF TYPE 1 DENGUE VIRUS

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Dengue infection is the most important mosquito-borne viral infection worldwide, especially in the tropics and subtropics. It is estimated that more than 2.5 billion people living in over 100 countries are at high risk for endemic dengue transmission. There are four serotypes of dengue virus (DV-1, 2, 3, 4), which are antigenically related but distinct from each other. Repeatedly infection with different DV serotypes may lead to severe diseases such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Annually, there are more than 200,000–500,000 cases of DHF, causing 22,000 deaths mainly among children under the age of 15. The case fatality rates of DHF and DSS are as high as 10–15% if no proper treatment is given in early stage. The E protein of DV, which plays an important role in viral assembly, receptor attachment, entry and viral fusion, is responsible for eliciting a neutralizing antibody response.

The development of serotype-specific assay for DV detection is of importance both to the treatment of patients and to epidemiological surveillance. In this study, we immunized mice with DV1 E protein and identified two neutralizing mAbs specifically against E protein of DV1. In addition, we developed a simple method for DV1 detection using magnetic bead conjugated type 1 specific monoclonal antibody coupled with MALDI-TOF MS. The neutralizing epitopes of both mAbs were further identified with synthetic peptides. These findings may be useful for understanding the mechanism of viral entry and the development of vaccines.

PO-14

Track: Others - Analytical chemistry

A NOVEL FLUOROMETRIC DETERMINATION OF HYDROGEN SULFIDE BY N-(9-ACRIDINYL)-MALEIMIDE (NAM) WITH HIGH SPECIFICITY

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Hydrogen sulfide(H₂S) is one of the final products of reductive decomposition of organic matter in the biological world. It is famous as toxic gas and is found in volcanic gas and in hot spring waters. H₂S assay is of considerable interest in the field of environmental protection. In biochemistry, H₂S is known as a potential intracellular messenger as a neuromodulator, smooth muscle cell relaxant and regulator of insulin release. Generally two difficulties exist in H₂S assay. It is an extremely low concentration in cells and instability oxidized with dissolved oxygen. Many methods are available for H₂S assay such as colorimetry, fluorometry and gas chromatography. However they have some drawbacks such as lack of sensitivity, selectivity and simplicity.

Our research group developed N-(9-acridinyl)maleimide(NAM), a detective reagent for sulfhydryl group(SH). We have been studying a micro assay of SH compounds such as metallothionein, glutathione, carnitine and Coenzyme A at pico-mole level. In this study we describe a highly sensitive fluorometric determination method of H₂S with NAM under mild conditions. The result is as follows. H₂S reacted with NAM, a Michael addition reaction to give strongly fluorescent derivatives in buffer solution of pH 5.2-7.0 at room temperature. The sensitivity was 10 pico-mole/ml. There was no interference by the inorganic salts, cations and anions nor by the reducing, oxidizing and organic substances present in natural samples such as waste water, hot spring water and marine sediment.

Keywords: Fluorometry, N-(9-acridinyl)-maleimide(NAM), Hydrogen sulfide.

PO-02

Track: Plant and Environment

A STUDY OF FIVE ANTIOXIDANT ACTIVITIES AND MAJOR CHEMICAL COMPONENT ANALYSES OF TWENTY-SEVEN COMMONLY USED ESSENTIAL OILS

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Twenty-seven commonly used essential oils and major components were subjected to screening for their possible antioxidant activities by measuring from their reducing power (RP), β -carotene bleaching test (B CB), total phenolic contents (TPC), trolox equivalent antioxidant capacities (TEAC), and DPPH free-radical scavenging ability (DFRS). The RP, BCB and TPC assays reveal the essential oil clove bud and thyme borneol are the best two among these essential oils. The TEAC and DFRS respectively reveal the essential oil clove bud, ylang ylang complete and clove bud, jasmine absolute are the best two essential oils among the twenty-seven commonly used essential oils.

At a concentration of 2.5 mg/mL, the clove bud and thyme borneol essential oils showed $103.56 \pm 0.06\%$, $24.64 \pm 0.03\%$ and $96.83 \pm 0.01\%$, $89.33 \pm 0.09\%$ of RP and BCB, respectively. At a concentration of 1 mg/mL, the clove bud and thyme borneol essential oils showed 220.10 ± 0.01 mg/g and 69.05 ± 0.01 mg/g of TPC relative to GAE,

respectively, while the clove bud and ylang ylang complete showed TEAC values of 809.00 ± 0.01 and 432.33 ± 0.01 μM of trolox, respectively. The clove bud and jasmine absolute showed $95.13 \pm 0.01\%$ and $78.62 \pm 0.01\%$ of DFRS, respectively.

Phenolic components of the essential oils clove bud, thyme borneol and jasmine absolute were eugenol (76.08%), thymol (14.36%) and carvacrol (12.33%), and eugenol (0.87%). It is clear that phenolic compounds in essential oils yield a positive correlation with the RP, BCB, TPC, TEAC and DFRS assays.

Keywords: Clove bud, jasmine absolute, thyme borneol, RP, TPC, BCB, TEAC, DFRS, essential oils.

PO-79

Track: Industrial and Manufacturing

MEDITERRANEAN LIGNOCELLULOSIC FEEDSTOCKS FOR BIOFUELS PRODUCTION: BIOETHANOL 2ND GENERATION

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Bio-based products made from renewable resources especially those obtained from biorefineries, such as: biomaterials, green chemistry reagents and bioenergy as well as biofuels are considered as revolutionary products ensuring sustainable development for future generations. Actually, with the increasing of greenhouse gas emission and the high price of fossil fuels, the production of biofuels from biomass sources became a necessity. In fact biofuels first, second and third generation could be respectively obtained from food and non-food-crops as well as from algal biomass. To join these strategic and scientific contexts, the main target of this work is to produce bioethanol second generation from available Mediterranean feedstocks using wastes of terrestrial and marine dry grass plants having an average of lignocellulosic content about 90% related to their dry matter. Moreover, the conversion process of the two substrates to ethanol was performed through many steps. Firstly, the mechanical pretreatment of lignocellulosic fibers was achieved using hammer crusher in order to obtain diameter fibers around 5 mm, after that an acid thermochemical pretreatment was carried out in diluted acid conditions which was about 1% (v/w) of reagent/dry matter. Secondly, the fibers pretreated were converted to ethanol by Simultaneous Saccharification and Fermentation SSF using commercial enzymes with high cellulases activities and adequate yeast strains. At the end of this investigation, the maximum yield of ethanol obtained is about 75% compared to the theoretical yield of glucose conversion to ethanol.

Keywords: Biorefineries, Mediterranean Lignocellulosic Feedstocks, Cellulosic Bioethanol, pretreatments, Enzymes, Yeast Strains and SSF.

PO-04

Track: Regenerative Medicine

DOUBLE TRANSGENIC PIGS WITH COMBINED EXPRESSION OF HUMAN α 1,2-FUCOSYLTRANSFERASE AND α -GALACTOSIDASE DESIGNED TO AVOID HYPERACUTE XENOGRAFT REJECTION

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The shortage of organs for transplantation means that despite the progress in the development of stem cell research and artificial organs, the xenotransplantation research using pigs is still valid. Methods eliminating hyperacute xenograft rejection of pig cells include GGTA1 inactivation, regulation of the complement system and modification of the oligosaccharide structure of surface proteins. The aim of the study was to create a molecular and cytogenetic profile of a double transgenic animal with α 1,2-fucosyltransferase and α -galactosidase expression. As a result of interbreeding of an individual with α 1,2-fucosyltransferase expression with an individual with α -galactosidase expression 12 piglets were

obtained. PCR revealed the pCMVFUT gene construct was present in 4 individuals and pGAL-GFPBsd in 3, including 1 with a confirmed integration of both the gene constructs. Karyotype analysis did not show any changes in the structure or the number of chromosomes ($2n=38,XX$). Expression analysis demonstrated reduction of Gal epitope level on the surface of cells, whereas human serum cytotoxicity tests revealed only the small decrease in longevity of cells in the obtained double transgenic individual (4.35%). Co-expression of both the transgenes leads to a considerable reduction of the Gal antigen level on the surface of cells and a decrease of xenotransplant immunogenicity.

Keywords: α 1,2-fucosyltransferase, α -galactosidase, hyperacute xenograft rejection, xenotransplantation.

PO-53

Track: Pharmaceutical Biotechnology

ENHANCED PRODUCTION OF VITAMIN K₂ FROM *FLAVOBACTERIUM MENINGOSEPTICUM* BY ION BEAM IMPLANTATION

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Low-energy ion implantation as a novel mutagen has been increasingly applied in the microbial mutagenesis for its higher mutation frequency and wider mutation spectra. In this work, N⁺ ion beam implantation was used to enhance *Flavobacterium meningosepticum* in the yield of vitamin K₂, which plays important roles in blood coagulation, bone metabolism, and also in mitochondrial dysfunction repair. The optimization of process parameters was carried out to further improve the vitamin K₂ yield. The results indicated that an excellent mutant FM5-632 with a yield of 42.3 ± 1.2 mg/L, that is 14 times that of the original strain, was achieved by eight successive implantations under the conditions of 15 keV and $60 \times 2.6 \times 10^{13}$ ions/cm². Moreover, production of the mutant FM5-632 was increased by 55% and reached 125.1 ± 1.2 mg/L by using the Plackett-Burman and Box-Behnken designs to optimize the fermentation medium. The optimal fermentation culture medium was composed of (per liter): 10 g glycerol, 10 g peptone, 2 g yeast extract, 5 g K₂HPO₄, 1 g NaCl, 0.5 g MgSO₄·7H₂O, 2.58 g polyoxyethylene oleyl ether (POE) and 0.045 g Cedar wood oil, and fermented at 33 °C and at 250 rpm for 120 h. The results showed that the low-energy ion beam implantation mutations and optimizing fermentation medium using response surface methodology were all effective methods to enhance vitamin K₂ production.

Acknowledgements: The study was supported by the Knowledge Innovation Project of the Chinese Academy of Sciences (No. Y09FCQ5121) and Presidential Foundation of Hefei Institutes of Physical Science, Chinese Academy of Sciences (No. Y29YJ23132).

Keywords: vitamin K₂, *Flavobacterium meningosepticum*, low-energy ion implantation, optimization.



PO-16

Track: Pharmaceutical Biotechnology

GENE SILENCING OF CLASS III CHITIN SYNTHASE AFFECTS HYPHAL DEVELOPMENT AND PENICILLIN PRODUCTION IN *PENICILLIUM CHRYSOGENUM*

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Penicillium chrysogenum, as an important β -lactam antibiotics producing strain, is one of the most economically important filamentous fungi. Previous study shows that penicillin secretion is closely correlated with fungal morphology, such as hyphal extension rates and sub-tip growth. The mycelial morphology plays a significant role on medium rheology, and thereby affecting the mixing and mass transfer within the bioreactor, but also influences metabolite productivity, resulting in either lower specific growth rate, or enhanced production by strains with altered morphology. Therefore, it



would be of great interest if one could devise a way of optimizing the hyphal morphology for mass production. Several studies have shown that chitin synthases are structurally and functionally divergent and play crucial roles in the growth and morphogenesis of the genus *Aspergillus* although little research has been done in *P. chrysogenum*. Understanding of this process is essential if we are to consider optimization of penicillin production via morphological engineering.

We used BLAST to find the genes encoding chitin synthases in *P. chrysogenum* related to chitin synthase genes in *Aspergillus nidulans*. Three homologous sequences coding for a class III chitin synthase CHS4 and two hypothetical proteins in *P. chrysogenum* were found. The gene whose product showed the highest identity and encoded the class III chitin synthase CHS4 was studied in detail. To investigate the role of CHS4 in *P. chrysogenum* morphogenesis, we developed an RNA interference system to silence the class III chitin synthase gene *chs4*. After transformation, mutants exhibited a slow growth rate and shorter and more branched hyphae, which were distinct from those of the original strain. The results also showed that the conidiation efficiency of all transformants was reduced sharply and indicated that *chs4* is essential in conidia development. All mutants exhibited increased sensitivity to chitin-binding dyes Congo red and Calcofluor white. These results demonstrated that *chs4* plays a crucial role in the hyphal tip growth and cell wall integrity. Though manipulating chitin synthases can generate direct morphological changes, so far there have been no reports of chitin synthase manipulated strains grown in submerged bioprocesses. This makes it interesting to examine its connection with product secretion. Therefore, the morphologies of all transformants and the original strain in penicillin production were investigated by light microscopy, which showed that changes in *chs4* expression led to a completely different morphology during fermentation, and eventually caused distinct penicillin yields, especially in the transformants PcRNAi1-17 and PcRNAi2-1 where penicillin production rose by 27% and 41%, respectively, suggesting that morphological changes have some effect on penicillin production which may be attributed to altered mixing and mass transfer in the culture fluid.

In conclusion, class III chitin synthase is essential in hyphal development and conidia formation. Silence of *chs4* gene can cause severe morphological changes (such as highly branched hyphae, lower growth rate and reduced conidiation efficiency) and subsequently affect penicillin production which might be the result of increased number of tips and lower viscosity of the fermentation culture. If all above results prove to be applicable to other fungi and processes, it will be possible to apply a much more directed approach to the development of better industrial strains.

Keywords: Chitin synthase, hyphal development, penicillin production, RNA interference.

***ADDITIONAL
ABSTRACTS***

SL- 74

Track: Regenerative Medicine

OSTEOBLASTIC DIFFERENTIATION OF HUMAN DENTAL PULP STEM CELLS ON DEXAMETHASONE LOADED PLGA MICROSPHERES AS INJECTABLE CELL AND DRUG CARRIERS

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The multipotent nature and self renewal capacity of human dental pulp stem cells in addition to more ease of extraction makes them an attractive cell source for tissue engineering. Dexamethasone can induce osteoblastic differentiation of stem cells. PLGA microspheres as injectable cell and drug carriers are one of the best scaffolds for tissue engineering and cell therapy provide a suitable surface for cell attachment and a controlled drug and growth factor release system. PLGA microspheres which contain dexamethasone were prepared using emulsion/solvent evaporation technique. The *in vitro* release profile was obtained by measurement of UV absorbance at 241 nm. For *in vitro* examinations, dental pulp stem cells were seeded on them. SEM and RT-PCR analyses for osteogenic specific genes were carried out. SEM micrographs of the scaffolds showed a diameter in range of 13–100 μm . Based on the release profiles obtained, the concentration of Dexamethasone released from microspheres reached 10^{-8} to 10^{-9} $\mu\text{g/ml}$. RT-PCR analyses for Osteopontin, Osteocalcin, Osteonectin and Alkaline phosphatase indicated differentiation of dental pulp stem cells into osteoblast on the microspheres. Controlled degradation and following prolonged and continuous drug delivery in addition to its injectability makes PLGA microspheres effective tools for tissue engineering and cell therapy.

SL-26

Track: Other Areas

DETERMINATION OF METABOLIC RESPONSE OF CATFISH (*Clarias gariepinus*) TO *Elizabethkingia meningoseptica* INFECTION VIA LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY QUADRUPOLE TIME-OF-FLIGHT (LC–MS, QTOF)

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Background: Metabolic changes in the host in response to *Elizabethkingia meningoseptica* infection play an important role in the pathogenesis of bacteria. Metabolomic profiles of fish infected with *E.meningoseptica* are recorded.

Methodology: The metabolic changes induced by bacterial infection of *Elizabethkingia meningoseptica* in fish were studied. A physiological dose 4×10^5 cfu mL^{-1} was intraperitoneally injection, and blood-serum, kidney, skin and bacteria cell extractions were collected.

Fish tissue samples were rapidly harvested and frozen in liquid nitrogen and the cells were lysed by homogenizing before extraction. The step process of metabolite sampling of bacterial cells was consisting of quenching to stop metabolism and allow medium removal, followed by extraction to recover the metabolites from the cells. Serum sample proteins were precipitated from the serum samples by cold ACN. Serum, kidney, and skin metabolic profiling was applied in healthy and infected fish to elevated levels of the biomarker. Bacterial metabolic profiling was compared between virulent and nonvirulent bacteria. Liquid chromatography mass spectrometric methods were applied to detect high and low abundant serum, kidney, and skin metabolites both in healthy and infected group during an experimental bacterial challenge test. This is based on the concept that the resilience of the immune system can be assessed after bacterial infection.

Results: Analyses of overall changes in serum, kidney and skin profiles after infection that infected group show a significant in metabolism as compared to healthy control. Analyses of individual metabolites show significant differences in the levels of two types of metabolites: diacylglycerols (DAG), 1-hexadecanoyl-sn-glycerol and 1-(11E-octadecenoyl)-rac-glycerol, were lower in infected groups as compared to the healthy controls. Significant increase in the levels of alpha-amyrin in serum of catfish infected by *Elizabethkingia meningoseptica* isolated from skin. 11-Dehydrocorticosterone was up-regulated in skin group compared to kidney group. Corticosteroids were up regulated in



both kidney samples and skin samples as compared to healthy control. Homosalate metabolite was down regulated in skin group as compared to kidney group. Higher levels of tacrine in bacteria isolated from kidney. *Excoceria agallochia* extracts has antimicrobial activity.

Conclusions: This study has focused on biochemical changes in different situations with significant metabolic disturbances. These changes are seen as metabolic markers of the underlying pathogenetic processes, offering new insights into disease mechanisms and, with further research, even therapeutic potential.

Keywords: Metabolic profiling, Liquid chromatography/Mass spectrometry, *Elizabethkingia meningoseptica*, Catfish (*Clarias carepinus*), pathogenicity.

SL- 50

Track: Other Areas

B.F. NANO V: *BEACKEA FRUTESCENCE* NANOVESICLES: EMERGING OF THE SECRET OF RAPID SKIN REJUVENATION

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BF Nano-V is a hybrid cosmeceutical skincare invention consist of antiseptic effects plus rapid skin rejuvenation. Consumer would portray themselves looks twice younger, fresh, clean and even smooth looking skin within 24 hours! [via surface evaluation of living skin (SELS) test]. This remarkable product is formulated with distinctive technology of nano size vesicles with an extract of *Beackea frutescence* or commonly known as *Cucur atap* [which is a common edible herb species of family Mrytaceae comparable to *Melaleuca alternifolia* (tea tree) that can be found everywhere within Kuala Terengganu district]. This rapid absorbance effect is due to the latest technology breakthrough called "Nanovesicles" which contains three unique components; Nanovesicles, Antimicrobial Agent and Nerolidol (active ingredients of *Beackea frutescence*). Nanovesicles are crucial in transporting the Beackea Frutescence (BF) active ingredients more effectively deeper into the root of the epidermis. Vesicular drug delivery system using colloidal particulate carriers have distinct advantages over conventional dosage forms because these colloidal particles can act as booster to increase the absorbance effect of skin rejuvenation. The nano size vesicles formed are very unique which can penetrate the pores and break easily upon releasing their fluid onto the skin. Indeed, this product was design to create a new hybrid formulation of antimicrobial and moisturizing cream with distinctive technology. The Antimicrobial effect is proven by the antimicrobial test of the emulsions with at least 2% of the essential oil of *Beackea frutescence* proved to be susceptible in inhibiting the growth of *E. coli*, *B. subtilis* and *S. aureus*. These bacteria species were chosen as they were the most common bacteria that cause skin infection. Nerolidol compound helps to reduce the appearance of uneven skin tones and brown spots and also opens up the pores for better absorption of the nerolidol, giving the skin an instant five times smoothness from within.

Undeniable, BF Nano-V can be an alternative product for beauty treatment in order to look younger, fresh and clean not only for appearance purposes but also, germ-free and healthier looks within 24 hours.

Keywords: *Beackea frutescence*, nanovesicles, antiseptic, rapid absorptions.

SL- 25(a)

Track: Pharmaceutical Biotechnology

COST EFFECTIVE AND UNINTERRUPTED PRODUCTION OF ANTIBODIES FOR CLINICAL TRIALS

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Biologic drugs (including antibodies, fusion proteins, growth factors, and cytokines) have proven to be successful in treating many severe diseases including cancers and autoimmune diseases. This clinical success is reflected in the tremendous market size of these products. Compared to small molecule drugs, the production of protein-based biologic drugs is complex and expensive, and this can present many challenges for clinical stage companies. Biologics are

typically produced using mammalian expression systems in large scale cell cultures, then purified and formulated in complex multi-step processes. The facilities required to produce even modest amounts of these drugs to supply clinical trials can cost tens of millions of dollars – clearly, this cost is prohibitive to small emerging biotechnology companies. Faced with this financial barrier, many small companies have outsourced the manufacturing of their biologic drug candidates to contract manufacturing organizations (CMOs). However the use of CMOs entails a different set of issues and costs – most notably, longer development timelines and inflexible manufacturing schedules.

With the use of single-use technologies, it has been possible for small companies to reduce the costs of in-house manufacturing overcoming barriers to the efficient and timely production of clinical drug supplies. However, the successful use of these production technologies depends on successful collaborations with vendors to develop scalable technologies, as well as a productive dialog with regulators to set appropriate regulatory guidelines that support the flexible and cost-effective manufacturing facilities of the future.

The complexity of biologics manufacturing and the use of mammalian cell culture introduce other issues which can interrupt the supply of clinical materials for trials. One type of rare but high impact, problem that has impacted the industry is the contamination of bioreactors with adventitious viral contaminants. This contamination poses potential risks to patients; both as a direct risk from the contaminating virus and as an indirect risk from interruptions in drug supply. Despite industry efforts, viral contamination events continue to occur, and anecdotal data suggest that these occurrences are more common than have been reported. The successful resolution of these contaminations requires enormous resources and time before the facility can be brought on line for further manufacturing.

Merrimack Pharmaceuticals' experiences in developing a highly efficient biologics production facility and its experience with a viral contamination and subsequent solutions will be presented.

PO- 71

Track: Pharmaceutical Biotechnology:

DISCOVERY OF THE MOLECULAR BASIS OF RETINOIC ACID ACTION (RETINOID SIGNALING) - A GENETIC REGULATION OF EUKARYOTES IN TRANSCRIPTION

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Acute promyelocytic leukemia (APL), a specific characteristic of t(15; 17) chromosome translocation, an alternative is to consider retinoic acid (RA) plus chemotherapy or RA plus As2O3 regimens as now novel therapy. Molecular gene analyses are conclusive *in vivo* evidence that oncogenic PML/RARa plays a crucial role in APL leukemogenesis. As a novel approach to APL treatment, one possible the action of RA, A consense sequence (5'-TCAGGTCATGACCTGA-3') has been postulated for thyroid hormone (TRE) and retinoic acid responsive element (RARE) containing half palindromes, which located in the promoter region of target genes. High dose of RA-RARE -PML/RARa complex in intracellular localization appears to relieve repressor from DNA binding, including corepressors N-CoR, SMART, and HDACs, release PML/RARa-mediated transcriptional repression, and release histone deacetylase activity from PML-RARa. The resulting PML/RARa oncoprotein proteolytic degradation through the autophagy-lysosome pathway (Atg1 ULK1- ptdIns-3-kinase PI3K3-Atg5) and the ubiquitin SUMO-proteasome system (UPS) as well as caspase 3 (cleavage site Asp522 within a-helices region of PML component of the fusion protein) or neutrophil elastase, or lysosomal protease (cathepsin D, cathepsin L) enzyme; or ubiquitin-binding adaptor protein 62 (p62) or E1-like ubiquitin-activating enzyme (UBE1L) induction. An effect to relieve the blockade of PML/RARA-mediated RA dependent promyelocytic differentiation, and all-trans retinoic acid in APL therapy (see figure, George Zhu, 1991). Here, PML/RARA is a (constitutive) transcriptional repressor of the RA receptor (RAR) complex, and PML/RARA fusion receptor gene act as conditional oncogenic receptor (translocated chimeric retinoic acid receptor a signaling) or oncogenic PML/RARA may participate in leukemogenesis of APL through blocking promyelocytic differentiation; or oncogenic receptor are locked in their "off" regular mode thereby constitutively repressing transcription of genes or key enzymes that are critical for differentiation of hematopoietic cells. This is first described in eukaryotes.

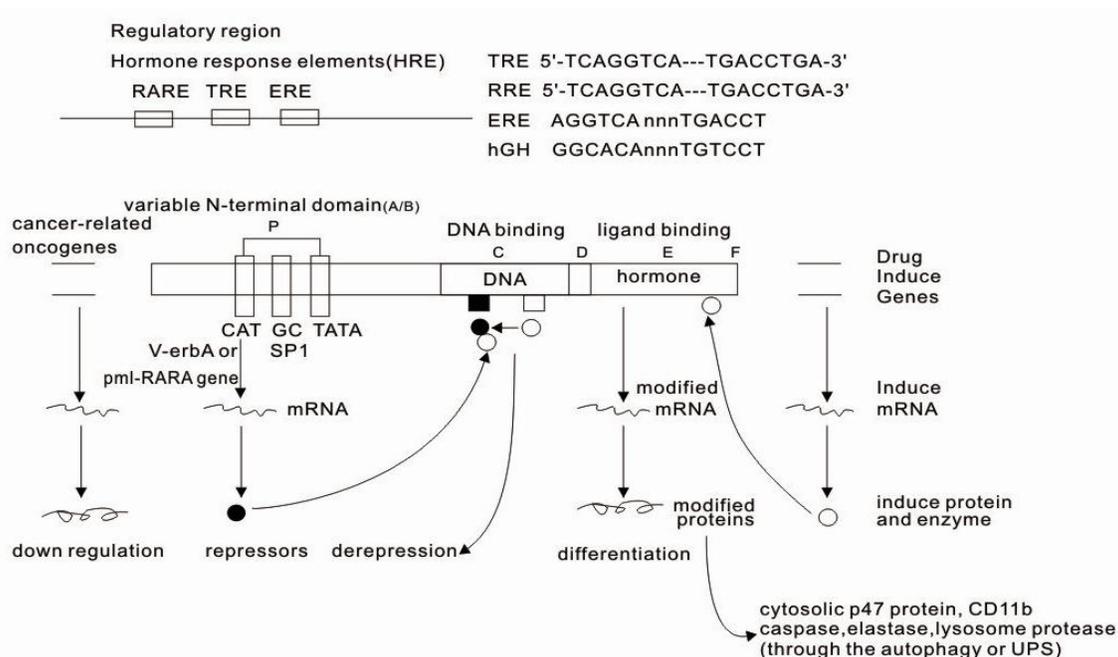


Figure: Hypothetical model of the regulation of retinoic acid action (George Zhu, January 1991, revised in 2012).

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PQ- 73

Track: Medical Biotechnology

THE EFFECT OF MAGNESIUM IONS ON PROLIFERATION AND DIFFERENTIATION IN HUMAN BONE MARROW STROMAL CELLS

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This study is to identify whether magnesium ions, released abundantly from metals or alloys, affects proliferation and differentiation in human bone marrow stromal cells (BMSCs). Our findings demonstrated that high levels of magnesium ions (1-10 mM) did not induce cytotoxicity of hBMSCs. Proliferation of hBMSCs treated by magnesium was shown to have significantly increased at the 2.5 mM – 10 mM of ion content. The expression of integrin alpha-2 and alpha-3 were up regulated compared to the control and were shifted slightly from alpha-3 to alpha-2 in hBMSCs treated by magnesium ions. Knock-down for integrin alpha-2 and/or alpha-3 significantly reduced magnesium-induced proliferation in hBMSCs. We found ALP gene expression and activities were profoundly enhanced in response to magnesium. In particular, ALP activities increased even when exposed to a relatively lower level (2.5 mM) of

magnesium ions. In conclusion, exposure to magnesium facilitated proliferation rates via integrin alpha-2 and alpha-3 expression, and partially promoted differentiation into osteoblast via the alteration of ALP expression and activity. Accordingly, magnesium can be utilized as a useful biomaterial for orthopedics and dentistry.

Acknowledgment

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PO-??

Track: Pharmaceutical Biotechnology

THE PROPHYLACTIC EFFECT OF OIL EXTRACTED FROM *SYZYGIUM AROMATICUM* ON BLOOD LIPID LEVEL IN MICE FED HIGH FAT DIET

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Adverse effect of therapeutic drug renewed an interest in the use of medicinal plant product for treatment of a variety of chronic disorders. One such bioactive plant is *Syzygium aromaticum* (clove). The oils extracted from *S. aromaticum* possess a variety of pharmaceutical activities. In this study we investigated the hypolipidemic effect of the volatile (essential) and fixed oils extracted from the dry buds of *S. aromaticum* by studying the prophylactic effect of these oils in male albino mice for two weeks.

Mice were fed a high-fat/cholesterol diet (1% cholic acid and 3% cholesterol). Blood was collected through inferior cava veins.

The results of the present study indicate that serum TC, TG, LDL, VLDL and lipid peroxide (MDA) were significantly increased ($p < 0.05$) than control, while the serum HDL level and antioxidant enzymes (SOD, GR, GPX and CAT) values were significantly decreased ($p < 0.05$). These results suggest that fixed and volatile oil extracted from clove reduced blood lipid parameters and MDA level and that this effect was associated with improvement in the level of antioxidant enzymes and HDL level. The histopathological studies also supported the prophylactic effects of *S. aromaticum*.

Keywords: *Syzygium aromaticum* (clove), hyperlipidemia, antioxidant.

PO- 78

Track: Industrial and Manufacturing

PRODUCTION OF SECOND GENERATION BIOETHANOL FROM BAGASSE AND RICE HUSK USING *PICHA STIPITIS* AND *SACCHAROMYCES CEREVISIAE* THROUGH OPTIMIZED PRETREATMENT STEP

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The success of bioethanol process depends on the amount of fermentable sugars and inhibitor level obtained from lignocellulosic material, during hydrolysis process. Response surface methodology (RSM) was employed for the optimization of pretreatment factors when phosphoric acid, hydrochloric acid, maleic acid and NaOH were used as catalyst. The hemicellulose hydrolyzates have 37.73 ± 0.45 – 38.12 ± 0.68 g/L and 32.37 ± 0.83 – 33.18 ± 0.53 g/L fermentable sugars along with 1.02 – 2.44 g/L of furfural and 5-hydroxymethyl furfural, during pretreatment of bagasse & rice husk while NaOH degraded them. A quadratic model was proposed for each catalyst to predict the hydrolysis yield, which had high F and R² (0.953–0.9857 for bagasse & 0.9287–0.9847 for rice husk) values along with a low p values, indicating the reliable predictability of the model. The factors seemed to be significant as indicated by their p-value, i.e. $P < 0.05$ excluding NaOH conc. for bagasse and incubation time for rice husk. The pretreated bagasse and rice husk credited 38.54 ± 0.37 – 42.32 ± 0.32 g/L and 35.96 ± 1.53 – 37.92 ± 0.65 g/L of reducing sugars with 71.92–84.64% enzymatic hydrolysis yield. Type of sugar monomers obtained depends on the substrate and the catalyst used for pretreatment. *Pichia stipitis* yielded 14.51 – 15.31 g/L of ethanol from detoxified hydrolyzate and *saccharomyces cerevisiae* produced 18.88 g/L of ethanol with 95.0% conversion yield.

Keywords: Lignocellulosic, Optimization, Maleic acid, Enzymatic Hydrolysis, *Saccharomyces cerevisiae*.

PO- 75

Track: Marine Biotechnology

TRANSGLUTAMINASE ACTIVITY AND COAGULATION REDUCTION ASSOCIATED TO REPRODUCTION IN THE PRAWN MACROBRACHIUM AMERICANUM

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The prawn *Macrobrachium americanum* is a native crustacean in the rivers of northwest México. In the Instituto Politécnico Nacional, the Aquaculture department is working in establishing conditions for breeding *Macrobrachium americanum*. Actually, several variables have been controlled for management, for mate, and egg and larval development. During these studies, mortality of berried females was common and deserved our attention. The goal of this study was to analyze the pro-Phenol-Oxidase system, the total hemocytes count and coagulation in reproductive females during reproduction. Four groups of 10 organisms were arranged: 1. females with immature gonad, 2. females after five days of ovulation, 3. females before five days of harvest and 4. males. Organisms were obtained from Sinaloa River with a mean weight of 74g, they were reared during 2 months feeding tilapia meat and pellets for shrimps, at 29±2 °C. Sample of 50 µL of haemolymph was withdraw to analyze the coagulation time between the groups and immediately a sample of 0.5 µL of hemolymph was withdrawn from the ventral sinus in an anticoagulant solution. Haemolymph was processed to determine the haemocytes account, pro-Phenol-Oxidase content, Phenol-Oxidase activity, and protein concentration. Results showed uniform conditions variables in groups 1, 2 and 4. Five days before harvest females group 3, showed a general alteration, pro-Phenol-Oxidase decreased significantly ($p < 0.05$) in content, meanwhile Phenol-Oxidase activity, the number of hemocytes and the time in coagulation increased ($p < 0.05$). It was interesting why the increase in Phenol-Oxidase activity without apparent difference in contact with non-self material between groups, since they were reared in the same container. But this is not the cause of mortality. If females do not coagulate, they have to face bleeding and the introduction of non-self material. Looking for the cause of the coagulation time alteration, hemolymph samples of females before five days of harvest were used as control and treated with Transglutaminase from Guinea Pig Liver. Results indicate a Transglutaminase malfunction during the process of incubation.

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Keywords: *Macrobrachium americanum*, Transglutaminase, Coagulation

PO- 74

Track: Marine Biotechnology

FEEDING RATE AND AMYLASE ACTIVITY IN THE WHITE SHRIMP LITOPENAEUS VANNAMEI

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Introduction: Shrimp culture as part of aquaculture has been growing in the last years from 4 to 11 percent, thus being the most important animal food production activity regarding its possible scope. Shrimp farming, despite production variations of the last years, constitutes one of the most important culture activity worldwide. In this field the feed factor represents over 60% of production costs in shrimp farming. Feed digestion to obtain nutrients needed for growth, upkeep, movement and reproduction is one of the most important functions in an organism's physiology. The Pacific White Shrimp *Litopenaeus vannamei* has a digestive system that takes less than one hour to digest the consumed feed. The digestive enzymes that shrimps have are capable of hydrolyzing a variety of substrates and several factors are implied in their regulation, among those factors is diet. α -amylase is the main enzyme that hydrolyses sugars in *L. vannamei*. So far the α -amylase enzyme behavior is unknown according to different feeding regimes.

Objective: To determine the existing relationship between feeding rate and α -amylase activity.

Methodology: The organisms were cultivated in commercial 100 m³ plastic containers, two plastic containers for each feeding rate every 3, 6, 12 and 24 hours, samples were taken from each treatment and α -amylase activity was measured by spectrophotometry.

Results: In general, α -amylase activity showed fluctuations during the night and few or none fluctuations were registered during the day in organisms treated with the 4 feeding rates. The night fluctuations are more frequent as the feeding rate's frequency increases. Minimum activity units recorded in this experiment showed that with a higher feeding frequency the enzymatic activity was lower.

Discussion: The fluctuation in amylase activity in this paper demonstrates the capacity of the species to acclimate to different feeding frequencies and to endure their daily activities as acclimatization occurs with proteases too, however, it is interesting that while proteases continue hydrolyzing protein during the day, amylase doesn't. This suggests a diurnal adaptation in carbohydrate utilization and acclimatization to feeding frequency.

Conclusions: α -amylase's enzymatic activity in nocturnal period showed higher fluctuations than during diurnal period. It was observed that in the higher frequency feeding rates a higher wastage of the amylase enzymatic activity. The amylase activity is conditioned by the feeding frequency and by the diurnal cycle.

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Keywords: α -amylase, *Litopenaeus vannamei*, feeding rates.

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