## Book of Abstracts

## 4<sup>th</sup> International Conference on Bioscience and Biotechnology (BioTech 2019)

21<sup>st</sup> and 22<sup>nd</sup> February 2019

Kuala Lumpur, Malaysia

Committee of the BioTech - 2019

The International Institute of Knowledge Management (TIIKM)

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Official website of the conference

www.bioscienceconference.com

Book of Abstracts of 4<sup>th</sup> International Conference on Bioscience and Biotechnology (BioTech 2019)

Edited by Prof. Mark Smales, Dr. James Budge & Dr. Edward Ooi Chien Wei

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#### **MESSAGE FROM CO-HOSTING PARTNER BioTech 2019**



Dear Participants,

The University of Kent is delighted to welcome you to the 4<sup>th</sup> International Conference on Bioscience and Biotechnology 2019, Kuala Lumpur, Malaysia, as a hosting partner of the event. This meeting offers an exciting opportunity for the University of Kent to help foster and develop interactions between research scientists and to share knowledge and instigate new collaborations. The development of biotechnology, such that precision genome editing can be undertaken and large scale sequencing is becoming a 'routine' tool of the trade, has given scientists an unparalleled ability to manipulate and harness the power of biosciences and apply this to health, agriculture and environmental issues (to name but a few) whilst ensuring such tools are used in a responsible manner.

The meeting here brings together delegates from a wide range of disciplines to discuss their latest findings and interpretations across the bioscience and biotechnology remit, providing a forum for the exchange of knowledge and forging of new ideas. Please take the opportunity to engage in the discussions, ask questions and make new friends. We look forward to an exciting 2 days and welcome you again to the conference and wish you a successful and productive meeting in Malaysia.

#### **MESSAGE FROM CO-HOSTING PARTNER BioTech 2019**



The Department of Chemistry, NED University of Engineering and Technology, Karachi, Pakistan is pleased to welcome you to the 4<sup>th</sup> International Conference on Bioscience and Biotechnology 2019, Kuala Lumpur, Malaysia, as a Co-hosting partner of the event. The meeting here brings together delegates from a wide range of disciplines to discuss their latest findings and interpretations across the bioscience and biotechnology remit, providing a forum for the exchange of knowledge and forging of new ideas. The breath of topics covered reflects the fact that bioscience and biotechnology plays an important role in many facets of everyday life and offers the potential to solve local and global challenges that face us all.

We hope Biotech-2019 will provide a perfect blend for novel discovery in both basic and applied research in the Bioscience and Biotechnology. Your presence and deliberation will make this conference remarkably successful in all aspects of Biosciences and Biotechnology.

Dr. Kashif Ahmed Associate Professor Department of Chemistry NED University of Engineering and Technology Karachi Pakistan

#### **MESSAGE FROM CO-HOSTING PARTNER BioTech 2019**



On behalf of Al-Farabi Kazakh National University I would like to warmly welcome all the participants of the 4<sup>th</sup> International Conference on Bio Science and Biotechnology 2019 (BioTech 2019) to the wonderful and dynamic city of Kuala Lumpur, Malaysia (February 21-22 2019).

I wish to extend my deep appreciation to the International Institute of Knowledge Management (TIIKM) for the initiative in organizing this meaningful Conference to learn and discuss methodologies, approaches and problems of biotechnology, human health and sustainable development that will make our research endeavors more effective and relevant.

I congratulate the organizing committee for its dedication and indeed very privileged to participate in this conference as a co-host.

University scientists conduct research in various areas of biotechnology, a lot of work is being done in the field of ecology and resource conservation based on the "Green Bridge through Generations" Eurasian Platform. It is my pleasure to inform you that the Al-Farabi Kazakh National University is a Hub in the field of sustainable development in the Central Asian region.

The problems that will be discussed at this conference require cooperation between scientists to promote scientific information exchange and new approaches to achieve best results in the development of new biotechnologies.

Therefore, to all participants, I wish you a productive Conference. May the deliberations at this event lead to further success in your most valuable work.

Jour /

**Prof. Galym Mutanov** Rector, Al-Farabi Kazakh National University, Kazakhstan.

#### **MESSAGE FROM THE CONFERENCE CO-CHAIR BioTech 2019**



Professor Mark Smales Professor of Industrial Biotechnology Director of the Industrial Biotechnology University of Kent, United Kingdom



Dr. James Budge University of Kent United Kingdom

Dear Participants,

It is with great pleasure that we welcome you on behalf of the organizing committee to the the 4<sup>th</sup> International Conference on Bioscience and Biotechnology 2019 held in the beautiful city of Kuala Lumpur, Malaysia. This now established conference consists of a number of conference tracks that represent areas of biotechnology of interest and importance to the region. Within these the conference tracks there is a range of sessions and topics from biodrug discovery and manufacturing to bioremediation and from biological systems and models to nanomedicine. The full details of the wide and cross-disciplinary range of topics is detailed on the website and abstract handbook. The breath of topics covered reflects the fact that bioscience and biotechnology plays an important role in many facets of everyday life and offers the potential to solve local and global challenges that face us all.

The scientific sessions cover cutting edge new science in the areas outlined. The extensive program has been designed by the committee to give as many early career scientists the opportunity to presentation alongside the high profile and keynote speakers that we have attracted to the meeting. We thank all the speakers for agreeing to participate and those who submitted abstracts. The poster session also provides opportunities for authors to present their work and we encourage everyone to visit the posters and discuss the work with the authors.

The organizing committee would especially like to thank our sponsors. Their generosity helps support this conference. We would also like to thank the rest of the organizing committee for undertaking all their assigned tasks in a timely manner and working together so well.

Finally, the location, the scientific program, and the social events have been designed to encourage networking and enhance scientific discussion amongst participants. It is anticipated that the oral and poster presentations, delivered in this inspiring environment, will generate discussion, new ideas and the initiation of fruitful novel research projects and collaborations. Participants and their engagement make a scientific meeting, and it is up to us all to ensure this is a successful conference that we all reflect upon favorably. We thank you for participating in the conference and we wish you a successful and productive meeting in Kuala Lumpur.

#### **MESSAGE FROM THE CONFERENCE CO-CHAIR BioTech 2019**



It is my great pleasure to invite you all for the participation in 4<sup>th</sup> International Conference on Bioscience and Biotechnology (BioTech 2019), which is organised for the first time in Malaysia. As a co-chair of this conference, I am honoured to organise the conference with TIIKM for bringing together international scientists to exchange knowledge on the current progress of Bioscience and Biotechnology fields.

The previous meetings of BioTech have been well received by participants from Malaysia, and I believe that this is a good opportunity for promoting collaboration among the participants and delegates in Malaysia. With the theme of "Pursuing innovation in Bioscience and Biotechnology to solve local and global grand challenges", we hope the participants could share their strategies in tackling the challenges via the innovations made in the fields for the benefits of local and international participants.

I look forward to the exciting oral and poster presentation as well as the productive discussion. I wish you all have an enjoyable time in the conference and in Malaysia.

Dr. Edward Ooi Chien Wei Associate Professor Chemical Engineering Discipline School of Engineering, Monash University Malaysia

#### **KEYNOTE SPEECH**

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Anthony J. Clarke

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## **KEYNOTE SPEECH**





## THE IMPORTANCE OF TECHNOLOGICAL ADVANCES FOR ANTIBIOTIC DISCOVERY: IDENTIFICATION OF PEPTIDOGLYCAN *O*-ACETYLTRANSFERASES AS POTENTIAL NEW ANTIBIOTIC TARGETS IN BOTH GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

Anthony J. Clarke

University of Guelph, Canada

#### ABSTRACT

The O-acetylation of the essential bacterial cell wall polymer peptidoglycan was first discovered over 60 years ago, and it was shown soon after to occur in a large number of bacteria including many important human pathogens, such as Staphylococcus aureus, species of Enterococcus, Helicobacter pylori, Campylobacter jejuni, and Neisseria gonorrhoeae. Also, this modification to the C-6 position of N-acetylmuramoyl residues of peptidoglycan was recognized at that time to inhibit the action of muramidases (lysozymes) of innate immune systems in a concentration dependant manner, and it totally precludes the activity of the lytic transglycosylases, bacterial autolysins that are involved with the insertion of flagella, pili, and secretion/transport systems, as well as the general biosynthesis and turnover of the peptidoglycan sacculus. Thus, the enzymatic system involved in peptidoglycan Oacetylation was proposed to present a new target for antibiotic development. However, the complexities associated with studying integral membrane proteins that are produced in low copy number and function on a totally insoluble substrate, peptidoglycan, severely inhibited advances in our understanding until a number of technological advances in molecular biology and analytical biochemistry were made. In this presentation, I illustrate how these advances made over the past 30 years have facilitated our current search for new antibiotics that target this important bacterial function. Our discovery and subsequent biochemical, kinetic, and structural characterization of two distinct twocomponent systems for the O-acetylation of peptidoglycan in Gram-positive and Gramnegative bacteria, respectively, are presented. In Gram-negative bacteria, such as N. gonorrhoeae, an integral membrane protein, peptidoglycan O-acetyltransferase (Pat) A, is proposed to translocate acetate from cytoplasmic pools of acetyl-CoA through the cytoplasmic membrane to the periplasm for its transfer to peptidoglycan by PatB. With Gram-positive bacteria, such as S. aureus, a single protein, Oacetyltransferase (Oat), appears to be a fusion of PatA and PatB to catalyze both the translocation and transfer of acetate for peptidoglycan O-acetylation. This knowledge is now being used to permit the high throughput screening for inhibitors that may serve as leads to new antibiotics





# **ORAL PRESENTATIONS**





#### [01]

## MATRIX METALLO PROTEINASE INHIBITORS – A POTENTIAL DRUG TARGET AGAINST RHEUMATOID ARTHRITIS - AN *in vivo* AND *in silico* APPROACH

S. Bhagavathy<sup>1</sup>, N. Najeeb<sup>1</sup>, G. Priya<sup>1</sup>, L. Anitha L<sup>1</sup> and D. Shalini<sup>1</sup>

<sup>1</sup>PG & Research Department of Biochemistry, Mohamed Sathak College of Arts and Science, India

#### ABSTRACT

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally attacks flexible (synovial) joints. The pathology of the disease process often leads to the destruction of articular cartilage and ankylosis of the joints. The collagenases of the matrix metallo proteinase (MMP) family are key enzymes in mediating irreversible cartilage collagen loss in arthritis. Inhibition of these enzymes is, therefore, an important therapeutic target. New approaches to collagenase inhibition include active site inhibitors designed for specific enzymes. The present investigation was carried out to explore the drug from the medicinal plant Vitex negundo (V.negundo) and their interactions pertaining to RA is through in vivo and in silico approach. V.negundo ethanolic extract was subjected to the screening of phytochemical analysis. The Anti-inflammatory effect of ethanol extract from V.negundo leaf was determined by protein denaturation assay. In vivo anti collagenase activity was determined using Freund's complete adjuvant induced arthritis in rats treated with two doses (200 and 400 mg/kg) of ethanolic extract of V.negundo. Further, the molecular docking studies identified the interaction between collagenase and the bioactive compounds of V.negundo. The results confirm that the collagenase inhibitors interacted through active sites of collagenase. From the findings of our study it appears that it may be possible to maximize the anti-inflammatory effect by the selected medicinal plant V.negundo. This study will also be helpful to further pharmacological study for researchers and drug development in pharmaceutical industry.

Keywords: Rheumatoid Arthritis, Matrix Metalallo proteinases, Collegenases, Vitex negundo



#### [02]

#### ELUCIDATION ON THE ROLE OF SELENOPROTEINS IN WOUND HEALING

#### S. Hariharan and S. Dharmaraj

Department of Biochemistry, FASH, Karpagam Academy of Higher Education, India

#### ABSTRACT

Selenium is an essential Immunonutrient which has a crucial part in the human's metabolic activity. Selenium in the form of Selenocysteine, has integrated as selenoproteins in the human body which have a unique way of synthesis and coding. Selenoproteins are the regulatory antioxidants of thyroid hormones, oxidative stress, male fertility and inflammatory activities. Selenoproteins has been majorly classified as Glutathione peroxidases (GPxs), Iodothyronine deiodinases (DIOs), Thioredoxin reductases (TrxR) and other selenoproteins. Among the families, Glutathione peroxidases (GPxs) functions are free radical scavenging at the site of inflammation is well studied. Selenoproteins participate directly or indirectly in the mechanism of wound healing as an oxidative stress reducer. In the wound healing process, not many studies are detailed, apart from the functions of GPxs and the role of Selenoprotein-P (SEPP1), Selenoprotein-S (SEPS1) for the regulation of homeostasis and inflammation. In the present study, certain unresolved mysteries about selenoproteins participation in wound healing mechanism are discussed. Transport of selenoproteins to the wound area and also inflammatory site has been detailed. Through this understanding, the knowledge of selenium and selenoproteins in the human body mechanism can be better determined in the future.

Keywords: Selenium, Selenoproteins, Wound healing, Glutathione peroxidases



#### [03]

## DELINEATING THE EFFECT OF Cissus quadrangularis ON THE PRODUCTION OF CYTOKINES IN HUMAN OSTEOBLAST LIKE SaOS-2 CELLS

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#### ABSTRACT

The process of bone remodeling involves complex interactions between osteoblasts and osteoclasts. These cells produce cytokines that can affect osteoclast function and activity. In vitro and in vivo model systems have demonstrated that production of cytokines such as M-CSF, TNF- $\alpha$  and IL-1 $\beta$  by osteoblast increase osteoclastogenesis and production of OPG by osteoblast block osteoclastogenesis and favour osteoblastogenesis. Many of the hormones and herbal compounds have been shown to have influence on these cytokines while imparting their effect on bone. Even though, C. quadrangularis promoted the proliferation, differentiation and mineralization of osteoblasts, its direct effect on cytokines such as IL-1 $\beta$ , TNF- $\alpha$ , IL-4 and OPG secreted by human osteoblasts are not documented. Therefore, the present investigation is aimed to study the effect of C.quadrangularis on the secretion of IL-1 $\beta$ , TNF- $\alpha$  IL-4 and OPG by SaOS-2 cells in vitro. The levels of IL-1 $\beta$ , TNF- $\alpha$  and IL-4 were evaluated in the conditioned media of SaOS-2 cells after 48 h treatment with C. quadrangularis at 1  $\mu$ g/ml and 10  $\mu$ g/ml dose levels in vitro. The levels of IL-4 and TNF- $\alpha$  did not differ significantly in the conditioned medium after C. quadrangularis treatment. However, a significant decrease in the levels of IL-1 $\beta$  was observed after C. quadrangularis treatment. The decrease in the levels of IL-1 $\beta$  is 47 % and 55 % at 1µg/ml and 10µg/ml dose levels, respectively. These observations suggest a crucial role for OPG and IL-1 $\beta$  regulation by *C. quadrangularis*.

Keywords: Osteoporosis, Cytokines, C. quadrangularis, Osteoclast, Osteoblast



#### [04]

## GASTROPROTECTIVE EFFECTS OF FLAVOALKALOID, 8-(2"-PYRROLIDINONE-5"-YL)- QUERCETIN ISOLATED FROM Senecio candicans DC

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#### ABSTRACT

Peptic ulcer disease (PUD) encompassing gastric and duodenal ulcer is the most prevalent gastrointestinal disorder. Duodenal, gastric ulcers and gastric cancer are common and serious diseases all over the world. It is estimated that every year nearly four million people are affected worldwide due to peptic ulcer, which accounts to nearly 10% of world population with different aetiologies. Excessive stress, smoking, chronic alcohol intake, H. pylori bacterial infection and chronic usage of non-steroidal anti-inflammatory drugs are main causes of PUD. The flavonoidal alkaloids or flavo alkaloids build a quite special group of natural products, where the typical flavonoid backbone (two aromatic rings are combined with an oxygen heterocycle) is linked with a nitrogen containing moiety too. Such group of natural products exhibit interesting gastroprotective effects. Senecio candicans DC is an endemic shrub of the Western Ghats, The Nilgiris, India. The hot water decoction of S. candicans leaf is being traditionally practiced as a remedy for gastric ulcer. The gastroprotective activity of the extract has been scientifically validated and reported that the decrease in lipid peroxidation and subsequent oxidative damage or by free radical scavenging activity may be the underlying mechanism. Further, focusing on the free radical scavenging activity, a bioassay guided approach was adopted to isolate a flavoalkaloid, 8-(2"-pyrrolidinone-5"-yl)- quercetin from S. candicans by screening the in vitro antioxidant ability of different extractions and fractions. The isolated was characterized using different spectral studies. The compound was further evaluated for its gastroprotective effects in experimental animal models.

Keywords: Senecio, quercetin, flavoalkaloid, pyrrolidinone, gastroprotection



#### [05]

## GENOMIC DAMAGE PROFILING OF POLYCYSTIC OVARIAN SYNDROME USING INVASIVE AND NON INVASIVE BIOMARKERS

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#### ABSTRACT

Polycystic ovary syndrome (PCOS) is a common and perplexing endocrine disorder of women in their reproductive years, with a prevalence of up to 10%. Clinical expression of the syndrome varies but commonly includes menstrual cycle disturbance, hyperandrogenism (HA), Acne, Acanthosis nigaricans (AN), insulin resistance and obesity. PCOS is relatively common, especially in infertile women. The aim of this study was to investigate DNA damage using invasive and non invasive techniques in PCOS patients in South Indian cohort. 50 women with PCOS (Rotterdam criteria) and 30 controls were considered for study. Buccal assay (non invasive) and Cytokinesis-block micronucleus cytome assay (CBMN) (invasive) were carried out on all the women with PCOS to study genetic instability.More number of Binucleated cells, Micro nucleated cells in mono nucleated cells in patients are indicated, also presence of Brigde and Bud cells were prominently seen in patients compared to control samples. The presence of NBUDS, NPBs in PCOS patients indicates DNA damage and that can be used as biomarker for detection of genetic instability.

Keywords: PCOS, DNA damage, BMCyt assay, CBMN cyt assay



#### [06]

## ACE I/D GENE POLYMORPHISM AS MARKER OF PCOS DEVELOPMENT IN SOUTH INDIAN COHORT WOMEN

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#### ABSTRACT

Polycystic ovary syndrome (PCOS) is a common, complex endocrine/metabolic disease characterized by hyperandrogenism, ovulatory dysfunction, polycystic ovary (PCO) morphology, insulin resistance and obesity. The angiotensin-converting enzyme (ACE), a key enzyme in the renin-angiotensin system (RAS), can convert angiotensin I to angiotensin II, which is the main effector peptide of the system and also it's proved that the RAS may influence oocyte maturation, ovulation and steroidogenesis as well as formation of corpus luteum through complex interactions with other systems. The aim of this study was to determine whether ACE gene I/D polymorphism is a genetic marker of PCOS development and finding out the distribution of ACE gene I/D polymorphism genotypes and allele frequencies in South Indian cohort. 430 women with PCOS (Rotterdam criteria) and 300 controls were studied. PCR-RFLP technique was carried out on all the women with PCOS. The Genotyping distribution II, DD and ID among patients are 4.56%, 30.23% and 65.11% and whereas among the control group are 30%, 20% and 50%, The D allele frequency was indicated as 62.79 % and I allele was as 37.2 % in patients, whereas it was 45 % and 55 % respectively in the control group. As a result of our study we may affirm that angiotensin converting enzyme gene I/D Polymorphism ID genotype should be considered as a genetic marker in polycystic ovary syndrome development in this south Indian cohort study.

Keywords: PCOS, PCR, RFLP, Cohort, ACE Gene



#### [07]

## THE EFFECT OF Foeniculum vulgare ON POLYCYSTIC OVARIAN SYNDROME IN A RAT MODEL

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#### ABSTRACT

Polycystic Ovarian Syndrome (PCOS) is a complex endocrine disorder with associated metabolic abnormalities. There is a risk of developing Type II Diabetes, obesity and cardiovascular diseases in PCOS condition. The present study is aimed to determine the effect of aqueous extract of Foeniculum vulgare (FV) seeds on the hormonal, haematological, biochemical and histopathological parameters in letrazole induced PCOS rats. For PCOS induction 20 mature female Wistar rats were administered letrazole orally (50µg/day) for 5 weeks. After 5 weeks the rats were divided into 3 sub groups including PCOS induced group, PCOS treated with FV, PCOS treated with standard drug, metformin and 12 normal rats were separately divided into 2 groups with one group as control and another group treated with the seed extract of FV for 45 days. After the treatment period the serum hormone levels were measured using ELISA. PCOS induced groups showed elevated levels of LH and testosterone and reduced levels of FSH which is a major abnormality in PCOS condition. The levels of serum uric acid and creatinine were increased in PCOS induced group indicate the abnormalities in kidney functions. Treatment with the seed extract of FV reversed the changes in LH, FSH, testosterone, uric acid and creatinine in the serum of groups treated with FV and standard drug which indicates improved function. The level of cholesterol was also reduced in letrazole + FV treated group when compared to the letrazole treatment alone. The histopathological results of ovaries showed more number of follicles and blood vessel congestion in PCOS rats were restored to normal upon FV treatment. These changes were associated with micro-architectural changes such as mild inflammatory infiltrates in the tubules and blood vessel congestion. These changes demonstrate the beneficial effect of Foeniculum vulgare as a candidate drug to ameliorate symptoms associated with PCOS.

Keywords: Polycystic ovarian syndrome, Foeniculum vulgare, letrazole, metformin



#### [08]

## HAPLOTYPE ANALYSIS AND MOLECULAR MARKER DEVELOPMENT FOR BACTERIAL LEAF BLIGHT RESISTANCE GENE *xa13*

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#### ABSTRACT

The xa13 is a recessive gene that confers resistance against bacterial leaf blight in rice caused by Xanthomonas oryzae pv. oryzae. Assessment of genetic diversity is the initial step towards developing markers that are diagnostically linked to desired phenotypes. In a previous study, haplotypes and protein types of xa13 has been defined in a panel of 217 rice accessions. In the current study, the analysis was expanded to a genetically diverse rice panel, with the objective to detect novel haplotypes and polymorphisms that can be targeted to develop diagnostic molecular markers for the use in markerassisted selection of xa13. A 2,843 bp region of the xa13 (Oso8g0535200), in a panel of 2,913 rice accessions, retrieved from the 3K Rice Genomes Project, was aligned and assessed based on polymorphic sites. Three previously defined haplotypes were identified within the coding sequence of xa13, while no novel types were identified. The highest haplotype frequency observed was 64% (H3), and the lowest was 6% (H1). The haplotype diversity of the study panel was found to be moderately high (H = 0.4930). Considering a non-synonymous mutation found at the exon 5 (S2057), two previously defined protein types were identified in the current study. The previously used Xa13 marker assaying a 10 bp length polymorphism on the promoter was found not to be diagnostic for xa13. Hence, it is recommended to design a new marker flanking the SNP site at exon 5 to be assayed using temperature switch PCR.

Keywords: haplotype diversity, temperature switch PCR, xa13, bacterial leaf blight



#### [09]

## CHARACTERIZATION OF BACTERIAL LEAF BLIGHT RESISTANCE GENES Xa4 AND Xa21 IN RICE (Oryza sativa L.)

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#### ABSTRACT

Bacterial Leaf Blight (BLB) is one of the devastating diseases in rice (Oryza sativa L.). It is causes by Xanthomonas oryzae pv. oryzae. In rice, BLB disease resistance is conveyed by many QTL. Among them Xa4 and Xa21 mapped to chromosome 11 are two major genes conveying durable resistance. The Sri Lankan rice germplasm is uncharacterized with respect to alleles carried at these two major genes. In the current study, alleleic diversity of the Xa21 and Xa4 where characterized using intragenic markers ABUOP0001 and Xa4, and the association between the alleles and the BLB disease responses were evaluated for 84 rice accessions including traditional and newly improved Sri Lankan rice, and IRBB reference lines. The disease response ranged in a scale of one to nine, with one being most resistant and nine the most susceptible. Friedman test indicated significant (p = 0.002) variation in the BLB disease response among the 84 accessions tested. The traditional accessions Yakadawee and Suwadel was rated as the most susceptible with a BLB score of nine and eight, respectively. Apart from most IRBB lines, traditional accessions Devareddiri and Puwakmalata samba, and the newly improved accessions At 307 and Bg 352, were reported as the most tolerant to BLB. The accessions reported two alleles for Xa21 and three alleles for Xa4, of which one of the Xa4 alleles is a novel type. However, no significant correlation was reported between the BLB disease response and the alleles carried at Xa21 and Xa4.

Keywords: Bacterial leaf blight, Xa 4, Xa 21,



#### [10]

## PARTITIONING AND PRELIMINARY PURIFICATION OF BIOACTIVE COMPOUNDS FROM *Garcinia indica* USING 1-PROPANOL-MAGNESIUM SULPHATE AQUEOUS TWO-PHASE SYSTEM

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#### ABSTRACT

Garcinia indica fruit has known for the presence of bioactive compounds like Anthocyanins- a red colour pigments and antioxidant, Hydroxycitric acid- used as an anti-obesity ingredient, Garcinol and Isogarcinol- a yellow color fat-soluble pigment having high antioxidant property. An attempt has been made for simultaneous extraction of these compounds using 60% 1-propanol from fruit rinds of Garcinia indica. Aqueous two-phase extraction (ATPE) was employed for the partitioning and preliminary purification of these bioactive compounds with 1-propanol and magnesium sulphate system. The binodal curve was constructed at 303.15 K. The influences of concentration of salt and 1propanol, Tie line Length (TLL) were investigated to obtain the optimal partitioning and enrichment of garcinol and isogarcinol towards top-phase and anthocyanins and HCA in bottom phase. The system containing 25% w/w 1-propanol and 10 % w/w magnesium sulphate with no pH adjustment showed partitioning co-efficient about 30.984 and 13.048 for garcinol and isogarcinol in top-phase, 0.361 and 0.224 for anthocyanin and HCA in bottom phase respectively. The enrichment (yield) of bioactive compounds reached were 95.26%, 90.39 % for garcinol and isogarcinol, 84.31% and 90.59 % for anthocyanins and HCA respectively. The lower TLL values ranging 17.988 to 46.319 showed effective for simultaneous partitioning. The results of multi stage ATP with introducing fresh bottom and top phases to first step ATP showed over 90 % fractionation of garcinol and isogarcinol in top-phase and anthocyanin and HCA in bottom phase. The present work showed the effectiveness of ATPE and great potential of Garcinia indica to simultaneously extract and preliminary enrich these four bioactive components and simplify final purification step to isolate compounds with single process economically.

Keywords: Garcinia indica, anthocyanins, garcinol, isogarcinol, hydroxycitric acid



#### [11]

## PHARMACOLOGICAL POTENTIAL OF THE METHANOLIC EXTRACT OF Hypericum hookerianum FROM PALNI HILLS OF THE WESTERN GHATS, INDIA

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#### ABSTRACT

Oxidative stress affects the human body and causes ailments like acute and chronic inflammatory disorders, cancer, diabetes and arthritis. Hence, the present study seeks to determine the antioxidant, anti-inflammatory and anti-arthritic activities of methanolic extract of the leaves of Hypericum hookerianum of Palni Hills, Western Ghats, Tamil Nadu, South India. The various parts of H. hookerianum (Hypericaceae) have long been used traditionally to treat fever, inflammation, joint pain and mental disorders. The phytochemical analysis of methanolic extract of *H. hookerianum* is known to possess several physiologically active phytochemicals such as phenols, anthraquinones, flavonoids, triterpenoids, steroids, alkaloids etc. Since these bioactive constituents are of immense pharmacological value, we tested the antioxidant potential of the methanolic extract by various assays, including DPPH, FRAP and NO scavenging. The anti-inflammatory activity of the methanolic extract was assessed by HRBC (Human Red Blood Cell) membrane stabilization method and the anti-arthritic activity of the methanolic extract was assessed by the inhibition of protein denaturation method. The results showed that the methanolic extract of the leaves of H. hookerianum act as a probable radical scavenger against harmful damage instigated by the free radicals. The methanolic extract of Н. hookerianum exhibited remarkable antioxidant potential, anti-inflammatory activity and anti-arthritic activity.

Keywords: Medicinal plants, Western Ghats, Antioxidant activity, Anti-inflammatory activity, Antiarthritic activity, Phytochemistry



#### [12]

## GENETIC VARIABILITY AND PERFORMANCE OF ADVANCED MUTANTS OF FRENCH BEAN (Phaseolus vulgaris L.)

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#### ABSTRACT

French bean (Phaseolus vulgaris L.) having the diploid chromosome number of 22 belongs to the family Fabaceae is also known as snap bean, kidney bean, garden bean. It is an important protein sources in many developing countries including India. Beans are the "meat of the poor", contribute essential protein to the under nourished people. French bean is an important source of carbohydrate (61.4 %), proteins (17.5-28.5%) and mineral matter (3.2-5.0%). The experiment was laid out in a Randomised Block Design with two replications at plant spacing of 30 x 15 cm. The experimental block was well prepared and standard cultural, manurial and plant protection practices were followed to ensure a healthy crop growth. Five random sample plants were tagged in each plot and used for recording the observations of characters. Mean values for all characters were worked out. Thirty-five advanced mutant lines of french bean (Phaseolus vulgaris L.) along with one commercial check were evaluated in Randomized Complete Block Design (RCBD) with two replications evaluated during Kharif 20017. Phenotypic and genotypic coefficients of variation for most of the traits were found moderate to high except for protein content per cent. High heritability along with high genetic advance over mean (GAM) was observed for pod width (cm), followed by pod yield plot (kg) and number of pods per cluster indicating that these traits could be exploited for further improvement through selection procedures. Most of the vegetable traits recorded higher variability among the mutants.

Keywords: French bean, Variability, Heritability, Mutants



#### [13]

## NUTRITIONAL QUALITY OF BIOFORTIFIED WHEAT FLOUR AS INFLUENCED BY PHYTASE TREATMENTS

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#### ABSTRACT

Cereals are used as staple food almost all over the world as a source of nutrition and minerals. The bioavailability of micronutrients in cereals is usually low due to antinutritional factors such as phytate which form complex divalent cations and make them unavailable for intestinal absorption to monogastric animals. Phytase is the class of enzyme which hydrolyses phytate and its addition during processing reportedly enhances the bioavailability of micronutrients. In this study, three phytases i.e. Aspergillus aculeatus APF1 phytase, Penicillium oxalicum EUFR3 phytase and a commercial phytase from wheat were accessed for their effect on nutritional and antinutritional parameters in biofortified wheat derivatives. Analysis of various biofortified wheat derivatives was carried out for minerals (Inorganic phosphate, Fe and Zn) and antinutritional components (Phytic acid and Tannin) in comparison with parental wheat variety (PBW343+GPC B1+Lr24) as control. In all selected derivatives, iron content was enhanced to varying extent while the increased Zn level was comparatively low. The phytic acid and tannin level were observed in the range of 0.47-0.78% and 0.052-0.096%, respectively. The treatments with APF1, EUFR3 and Wheat phytases led to decreased phytic acid (3-68%) and tannin (5-53%) content over untreated samples with variation in their efficacy. The increased inorganic phosphate contents (3-145%) were measured over untreated sample after phytase treatments. Dialyzability of Mn, Fe and Zn was increased to different levels over untreated samples. The study reveals that phytases could be used to food applications to increase micronutrient dialyzability as well as to enhance nutritional quality.

Keywords: Phytase, Phytate, Tannin, Micronutrient dialyzability



#### [14]

#### PHYSICAL PROPERTIES OF ULTRASONICALLY TREATED CARROT JUICE

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#### ABSTRACT

Eco – friendly and innovative technologies are used in food processing for obtaining products with minimum modified organoleptic and sensorial characteristics. The purpose of this research was to determine physical properties of the high – intensity ultrasound treated carrot juice. The influence of the high – intensity ultrasound on absorbance, temperature, density and particle size distribution was observed under 6 and 10 minutes of treatment; 20, 60 and 100 % amplitude; 7 and 10 mm probe diameter. There were significant changes in physical properties made with lower amplitude values (20%), except in temperature. Processing time has been shown to be a most important parameter that leads to the most pronounced changes in the observed physical properties. Analogy of the output intensities obtained with 10 mm probe diameter of 7 mm, smaller diameter particles (0,05 – 0,5  $\mu$ m) are increased in number. The same particle size distribution is obtained at maximum amplitude indicating the possibility of minimum energy consumption with the goal to obtain a juice with acceptable physical properties and high stability.

Keywords: high intensity ultrasound, physical properties, particle size distribution, carrot juice


#### **B8**

#### [15]

## INFLUENCE OF NUTRIENTS ON GROWTH AND YIELD OF YARD LONG BEAN (Vigna anguiculta SUBSP. Sesquipedalis L.)

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#### ABSTRACT

Yard long bean also called as Chinese long bean, is a vigorous climbing annual plant, it is a subtropical, tropical plant and is widely grown in south eastern countries like Thailand and China for edible crisp, tender and delicious pods. Although, the crop comes up well in Indian region, but due to lack of production techniques it has not become very popular among the growers and is being cultivated only in a small scale. Therefore, the present study was under taken to work out the nutrition requirement of the crop, the experiment was conducted in *kharif* season during 2015 and 2016 and it consist of seven treatments with three replications and analysis done with RCBD design. The results revealed that among the treatments, there is significant increase in growth and yield parameters of yard long bean for different levels of nutrients when the crop grown durig kharif 2015-16. Based on the result of the study it is concluded that application of 108.5:87.5:122.5 kg NPK/ha ( $T_6$ ) recorded the maximum plant height (2.55 and 2.59 m, respectively), number of leaves (47.83 and 48.23, respectively), number of branches per plant (5.87 and 6.53, respectively), pod length (66.57 and 69.23 cm, respectively), number of pods per plant (37.75 and 38.30 respectively), pod yield (22.86 and 22.45 t/ha respectively) and highest benefit: cost ratio (4.13:1 and 4.01:1 respectively) of yard long bean followed by  $T_4$ -93:75:105 kg NPK/ha. Hence, these nutrient levels can be recommended for commercial cultivation of yard long bean during kharif season.

Keywords: Yard-lang-bean, Nutrition, Growth, Yield



**B9** 

#### [16]

## BIO-INTENSIVE MANAGEMENT OF LATE BLIGHT OF POTATO CAUSED BY Phytophthora infestans (MONT.)

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#### ABSRACT

Potato (Solanum tuberosum L.) is being suffering from more than ten important diseases. Among them, late blight incited by *Phytophthora infestans* is most severe disease in Karnataka. Due to use of non target fungicides, farmers were unable to achieve required management of these diseases. Hence, an attempt was made to identify the biointensive management practices were evaluated against late blight of potato. Observations on Per cent Disease Index for late blight and yield parameters were recorded. The results of *in vitro* application of different botanicals revealed that, Thyme (*Thymus vulgaris*) and clove (Syzygium aromaticum) found 100% inhibition at all the three concentrations (5, 10 and 15%) and these two treatments were significantly different from all the remaining treatments. Myrobolan (Terminalia chebula) and black pepper (Piper nigrum) was on par with each other at 5, 10 and 15% concentration with 91.41 and 90.0%, 97.66 and 99.17% and 100.0% inhibition respectively. Least inhibition per cent was observed in ginger (Ginger officinalis) at all the concentrations (22.5, 34.58 and 45.99% at 5, 10 and 15% respectively). Further, there was reduction in the PDI in treatment  $T_{10}$ (Iprovalicarb 5.5 % + Propineb 61.25 % WP @ 4 gm/l.) after the spray. Whereas, the least disease development was found in T<sub>7</sub> (Fenomidone 10% + Mancozeb 50 % @ 3 gm/L.) as compared to control whereas, after fourth spray, The least PDI (17.69%) in  $T_{10}$  (Iprovalicarb 5.5% + Propineb 61.25% WP @ 4 gm/l., followed by  $T_7$  (Fenomidone 10% + Mancozeb 50 % @ 3 gm/l.) (23.81% PDI) Whereas, highest PDI (90.8%) was observed in control.

Keywords: Late Blight, Phytophthora infestans, Potato, botanicals and fungicides



#### **B10**

#### [17]

## EFFECT OF PLANT GROWTH REGULATORS ON GROWTH, FLOWERING, QUALITY AND CORM MULTIPLICATION OF GLADIOLUS (*Gladiolus hybridus* L.)

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#### ABSTRACT

The present experiment entitled "Effect of plant growth regulators on growth, flowering quality and corm multiplication of gladiolus (Gladiolus hybridus L.)" was carried out during 2017-18 in the Department of Floriculture and Landscape Architecture, College of Horticulture, Bengaluru. The experiment was laid out in Randomized Completely Block Design (RCBD) and was planted by using the corms soaked for 24 hrs with different growth regulators at different concentrations viz., BAP (25, 50 and 75 ppm), GA<sub>3</sub> (50, 100 and 150 ppm) and NAA (50, 100 and 150 ppm) along with untreated control as treatments and was replicated thrice. Significant differences were observed among treatments with respect to growth, flowering quality and corm parameters. The results revealed that  $GA_3$  at 150 ppm recorded early sprouting of corms (9.00 days), significantly maximum sprouting percentage (83.91 %), maximum plant height (77.80 cm), stem girth (2.19 cm) and BAP at 75 ppm recorded maximum number of leaves per plant (12.76), leaf area (2173.45 cm<sup>2</sup>) and GA<sub>3</sub> at 150 ppm took minimum number of days for spike initiation (54.73 days) and also recorded significantly maximum spike length (68.10 cm), rachis length (53.97 cm), number of florets per spike (15.07), size of floret (10.80 cm), weight of spike (82.69 g) and vase life (14.03 days). The maximum number of spikes per plant (1.47), spikes per ha (2.45 lakh spikes), corms per ha (4.05 lakh Nos.) and cormels per ha (1.94 t/ha) were recorded in the treatment BAP at 75 ppm.

Keywords: Gladiolus hybridus, growth regulators, quality and corms



#### [18]

## EXPRESSION ANALYSIS OF MICRORNAS AND THEIR COGNATE GENES IN RESPONSE TO ABIOTIC STRESSES IN Arabidopsis thaliana

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#### ABSTRACT

Small RNAs including miRNAs have been leading a fundamental change in the understanding of complex biological mechanisms involved in plant responses to biotic and abiotic stresses. Abiotic stresses such as water stress, salt stress and heat stress remain to be challenging inters of signal perception and molecular events leading to tolerance in plants. Involvement of small RNAs and their cognates in modulating abiotic stress tolerance in plants have been implicated. Expression quantitation and in situ expression patterns of a set seven selected miRNAs was studied in Arabidopsis thaliana plants experiencing low and high temperature regimes, salt stress levels and water stress regimes separately were studied. The selected miRNAs were in situ hybridized with Locked Nucleic Acid (LNA)-modified oligonucleotide probes. Among the tested miRNAs, expression of miR161, miR168, miR171 and miR397a in leaf tissues and miR171 and miR397a in root tissues was recorded in control plants. Elevated expression of miR171 and miR397a was recorded in both tissue types of low temperature treated plants. A set of four miRNAs viz., miR171, miR395b, miR399e and miR399 showed their up regulation in both tissue types upon NaCl (300 mM) treatment. Expression of miR168 was recorded only in leaf tissues, and on the other hand, down regulation of miR397a was recorded in both tissue types in response to NaCl stress. The miRNA stem-loop RT-PCR assay indicated gradual increase in the expression of miR171 and miR397 with the highest of 4.28 and 3.49 fold changes in leaf tissues of A. thaliana plants experiencing low temperature stress and 6.5 and 6.3 fold up-regulation of miR171, 0.8 and 0.9 fold down-regulation of miR397a and 3.4-3.5 fold up-regulation of miR399 and miR399e in leaf and root tissues, respectively, at 24 hrs of exposure to salt stress. The RT-qPCR assay recorded reduced levels of miRNA target gene transcripts viz., SCL6 III, SCL6 IV, LAC2, and LAC17 in response to low temperature and SCL6 III, SCL6 IV, APS1, APS4 and AGO1 transcripts in response NaCl treatment in both tissue types of Arabidopsis thaliana plants. The study points at the possibility of modulating low & high temperature, salt, water stress tolerance in plants through the down regulation of specific cognate genes.

Keywords: microRNAs, in situ hybridization, Arabidopsis thaliana, qRT-PCR, abiotic stress



#### [19]

## BACTERIAL STRAINS FROM TANNERIES EFFLUENTS WITH MULTIPLE POTENTIALS: CHROMIUM AND Azo DYES REDUCTION ACTIVITIES

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#### ABSTRACT

Like heavy metal pollution the worldwide extensive use of synthetic dyes and their accumulation in environment due to difficulty in removal aerobically, is another industrial related threat to public health and wildlife. In the present study we isolated four bacterial strains with Nitroreductase, Chromium and Azo dyes reduction activities were isolated from tannery effluents in Sialkot, Punjab, Pakistan. On the basis of 16S rRNA these strains showed close relation to *Bacillus licheniformis* (PSA1 and PSA2), *Bacillus anthracis* (PSA3) and *Bacillaceae bacterium* (PSA4). These strains showed growth in the presence of hexavalent chromium ( $Cr^{6+}$ ) up to 100µg/ml. interestingly their growth seemed to be unaffected at 50µg  $Cr^{6+}$ /ml and highest Cr reduction was observed accordingly. The Azo dye named Methyl red was reduced more efficiently in the presence of glucose and yeast extract as compare to without glucose and yeast extract by the isolated bacterial strains. So, these bacterial isolates with multiple activities may be used as potent biotechnological tools for Leather and Textile industries waste bioremediation.



#### [20]

## UTILIZATION OF Aspergillus niger AND DUCKWEED FOR THE WASTEWATER TREATMENT OF NULLAH LAI

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#### ABSTRACT

Today clean water demands have increased, as rapid urbanization and industrialization has converted the fresh water resources into contaminated water's. To access safe drinking, urgent low-cost and well-effective approach is needed. Present study is an attempt to search solution of this problem. For this present concentration of heavy metals in water of Nullah Lai (rawalpindi-Islamabad) and their removal from selected samples is carried out. Thirty wastewater samples were collected from ten different sites of Nullah Lai: viz. NARC Colony, Ghori Town, Kahuta Road, Faizabad, Ali trust, Marir Hassan, Chaklala Scheme 3, Sawan etc. Heavy metals were analyzed by using Atomic Absorption Spectrometer and for bioremediation *Aspergillus niger*, and *Lemna minor* are used. Individual use of *Lemna minor* and *Aspergillus niger* was not proved efficient. Individually *Lemna minor* removed 54% Cadmium, 90.99% Chromium, 95% Lead, 37% Copper, 78% Nickel, 50.5% Zinc and 74% Arsenic. *Aspergillus niger* did not show efficient results comparatively except in case of zinc removal. It removed 40% cadmium, 38% chromium, 38% lead, 41% copper, 28% nickel, 60% zinc and 28% arsenic. Collective use of *A. niger* and duckweed removed about 80-100% heavy metals from wastewater. Therefore collective use of *A. niger* and duckweed should is recommended for removal of the heavy metals from waste water.

Keywords: Wastewater treatment, Bioremediation



#### [21]

## DIVERSITY, PHYLOGENY AND BIOSYNTHESIS OF Au NANOPARTICLE OF CULTURABLE ENDOPHYTIC FUNGI ASSOCIATED WITH Zingiber cassumunar ROXB.

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#### ABSTRACT

Zingiber cassumunar Roxb. locally known as 'Tekhao-yaikhu' in Manipur, India is an important ethno-medicinal plant of the various ethnic communities of the Northeastern region of India. The present study reported the diversity of culturable endophytic fungi associated with Z. cassumunar Roxb. and their usage in biosynthesis of gold (Au) nanoparticles. A total of 31 endophytic fungal isolates were obtained from 60 samples of healthy leaves and rhizomes and were grouped into ten taxonomic groups based upon their morphological characteristic. The leaf showed higher colonization frequency (73%) and isolation rate (0.73) of the endophytic fungi as compared to colonization frequency (30%)and isolation rate (0.30) of rhizome segments suggesting that the leaves of the plant are richer in fungal endophytes than the rhizomes. Collectotricum gloeosporioides was observed to be dominant fungal species with a colonization frequency of 36.6% and isolation rate of 0.37. Further, morphologically distinct fungal isolates were subjected for molecular phylogenetic analysis using nuclear ribosomal DNA sequences (ITS1, 5.8S and ITS2). Phylogenetic analysis of the fungal isolates showed four major clades: Sordariomycetes, (including two genera belonging to order Glomerallales); Dothideomycetes (including two genera belonging to two orders Pleosporales and Capnodiales); Eurotiomycetes (represented by Phialophora cyclaminis belonging to order Chaetothyriales) and Polyporales (represented by *Phanerochaete* sp.). The endophytes were tested for Au nanoparticles biosynthesis. Among the endophytic fungi, isolate Collectotrichum gloeosporioides ZCL1 was found to be promising for Au nanoparticles biosynthesis. Scanning confirmed the formation of Au nanoparticles of approximately 5-20 nm size. Collectotrichum gloeosporioides ZCL1 which could be further explored for the mass production of Au nanoparticles.

Keywords: Zingiber cassumunar, endophytic fungi, diversity, Au nanoparticle, biosynthesis



#### [22]

## OSTEOLOGICAL CHARACTERIZATION OF THE GENUS Puntius (TELEOSTEI: cyprinidae) RECORDED FROM SIX RIVER SYSTEMS OF SOUTHERN WESTERN GHATS, INDIA

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#### ABSTRACT

This paper provides information on the osteological characterization of the genus *Puntius* with respect to six river systems of Southern Western Ghats. Fishes were collected using cast net, dip net, gill net and drag net from various streams and rivers of Southern Western Ghats. Clearing and staining methods was carried out for osteological study. After clearing and double staining, the specimens were observed under a stereomicroscope and photographed using a digital camera. Twenty-nine morphometric and meristic osteology characters were taken. Principal component analysis and cluster analysis were performed to group the species and to detect the similarity between the species. Comparing all the species it was observed that the species were grouped into three groups. The first class has 10 species *P. mahecola, P. chola, P. bimaculatus, P. dorsalis, P. melanampyx, P. fasciatus, P. ticto, P. denisonii, P. sophore, P. conchonius*. The second class has 4 species; *P. filamentous, P. sarana spirulus, P. amphibious* and *P. ophicephalus*. The third class has only one species *P. carnaticus* well supporting the observations of Shantakumar & Vishwanath (2006).

Keywords: Chalakudy, Meristics, Morphometrics, Preethmoids, Skeleton, Skull



#### [23]

## SURFACE MODIFICATION OF BACTERIAL PLASTICS, POLY(3-HYDROXYBUTYRATE-CO-4-HYDROXYBUTYRATE) SCAFFOLDS FOR BIOMEDICAL APPLICATIONS

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#### ABSTRACT

Bacterial plastics or better known as polyhydroxyalkanoate (PHA) is synthesized as intracellular carbon and energy storage compounds. PHA has been in the forefront in many tissue engineering attempts. Among the different types of PHA employed, poly(3-hydroxybutyrate-co-4-hydroxybutyrate) [P(3HBco-4HB)] gained the most attention as a biocompatible and inert in-vivo degradation material. The surface of material for biomedical applications play a crucial role as it forms the interface between the scaffold and the cells. Polymers in general exhibits poor cell attachment capability due to the hydrophobic and lack of cell recognition sites thus, limiting their application. Here, several surface modifications were attempted in order to tailor-made efficient scaffolds for biomedical applications. Several modifications by covalent bonding such as aminolysis, porogen leaching technique, nanofiber fabrication using electrospinning as well as freeze-drying techniques were used to modify the surface architecture of the scaffolds. In vitro study carried out using fibroblast cells (V79) which showed an increase in cell proliferation of the various scaffolds fabricated. The goal of this study is to fabricate P(3HB-co-4HB) scaffolds with various surface topography aimed to address common problem faced in the field of biomedicine by achieving a surface with favorable characteristics that enhances cell attachment and maturation.

Keywords: Single, Paragraph, Summarizes, words indentation



#### [24]

### MORPHO-MOLECULAR APPROACH TOWARDS IDENTIFICATION OF Curvularia SPECIES

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#### ABSTRACT

*Curvularia* is a widespread air-borne facultative pathogen of soil and plants, which mostly survive as a saprophyte in tropical and sub-tropical regions. It is a dematiceous, filamentous fungus. *Curvularia* spp. are darkly pigmented fungi with spores (curved conidia) efficiently adapted for most aerial dissemination. The classification of *Curvularia* species based on morphology alone is difficult as genus has more or less similar characteristic in several species; an attempt has made to confirm the identity of species by integrating morphological and molecular characters. A set of 52 *Curvularia* isolates from Delhi-NCR region, ITCC, MTCC and NFCCI were collected and morphologically characterized and confirmed using ITS sequences from NCBI database as *C. aeria, C. affinis, C. australiensis, C. borreriae, C. catenulate, C. clavata, C. eragrostidis, C. geniculate, C. inaequalis, C. lunata, C. pallescens, C. prasadii, C. specifera, C. trifolli, C. tuberculata and C. verruculosa. Identity of these isolates was confirmed through NCBI data base using ITS region sequences. The identification percentage was found to be 90–100%.* 

Keywords: Curvularia, ITS region, dematiceous, Isolates



#### [25]

## DEGRADATION OF TRICLOSAN FROM DOMESTIC WASTEWATER BY BIOSURFACTANT PRODUCED FROM *Bacillus licheniformis*

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#### ABSTRACT

The purpose of the present study was to develop the HPLC method to determine the concentration of Triclosan from domestic wastewater and application of *Bacillus licheniformis* produced biosurfactant using crude sunflower oil for the removal of pollutant. The study obtained the significant  $R^2$  value of 0.9989 using acetonitrile and methanol as a mobile phase at 0.3 ml/min using HPLC. The sample extraction by solid-phase extractor using methanol showed successful extraction of triclosan from domestic wastewater. The application of biosurfactant obtained the maximum removal of triclosan for 1:1 ratio of wastewater and biosurfactant with contact period of 16 hours compared to 2:1 with contact period of 2 hours.

Keywords: Triclosan, HPLC, Solid-Phase Extractor, Biosurfactant, Bacillus licheniformis



#### [26]

## EFFECT OF GREEN SYNTHESIZED COPPER NANOPARTICLES IN COMPARISON WITH COPPER SALT ON THE DYEABILITY OF COTTON FABRIC TOWARDS SULPHUR DYE

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#### ABSTRACT

Improvement in color strength and washing fastness of the sulphur dyed fabrics is a challenge. This is generally caused by improper color dissolution, color precipitation, poor solubility of the dyes, poor and insufficient washing. This limits their use on materials where good wash fastness is required. To achieve the goal of good fastness properties and color strength different procedures were employed to fix the impregnation of dye on the fabric. Due to this scientists diverted their focus to modify the fabric surface and act as cross linking agents due to penetration in the dye molecules. The emerging nano technology plays an important role because of their small particle size and ultimately improves serviceability of the material. Therefore the present study was focused on assessing the effect of Copper salt as a mordant in combination with green synthesized copper nano particles using Conocarpus *Erectus* leaves on the dyeability, color strength and fastness properties of the cotton fabrics using single shade of Diresul Indiblue RDT sulphur dye. Dyeing was carried out by continuous method. Among the fabric samples, few were treated first with copper acetate mordant of two different concentrations 10% and 20% and dyed and few were treated with green synthesized copper nanoparticles and dyed and few were dyed with copper nanoparticles. Copper acetate and copper nano particles were applied using pad-dry-cure method. All the samples were subjected to various laboratory tests to evaluate the effect of treatments on tensile strength, color strength and color fastness properties using the standard ISO procedures. SEM analysis was carried out to identify the size of copper nanoparticles. SEM analysis of untreated and copper nanoparticle treated samples was done to visualize their effect on the fiber's surface. SEM analysis of copper nanoparticles indicated that they were in dispersed cluster form with a size range of 30-70 nm. SEM analysis of fabric had shown even distribution of copper nanoparticles on fabric surface. Prescribed treatment have shown improvement of sulphur dyed fabric in terms of Fabric Strength, Color strength (K/S), and Fastness; washing, light and rubbing. Higher concentration of copper acetate mordant has shown better results. The samples treated with copper nanoparticles and dyed later have shown good color strength (K/S) and improved color fastness properties than their counterparts. These results were helpful in implementing where textiles are dyed with sulphur dyes.

Keywords: Dyeing, Sulphur dye, Nanoparticles, Spectrophotometer, SEM, X-Ray diffraction



#### [27]

## SYNTHESIS AND NEMATICIDAL ACTIVITY OF DERIVATIVES OF OLEANOLIC ACID

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#### ABSTRACT

Four acyl derivatives of oleanolic acid (1a-1d) have been prepared by converting the C3- hydroxyl group to acyl derivatives respectively. These are characterized by <sup>1</sup>H-NMR, UV and IR spectroscopy. Compounds 1b, 1c and 1d are reported for the first time and compound 1a have been reported earlier. Parent compound along with derivatives 1a-1d were evaluated for their nematicidal activity against root knot nematode *Meloidogyne incognita*, in which parent compound was found to be most active whereas compound 1b exhibited 60% mortality after 72 h at 1% conc. among other derivatives, 1a and 1c showed 52% mortality and 1d was less active.

Keywords: Oleanolic acid, nematicidal activity, Meloidogyne incognita



#### [28]

## THE EFFECT OF PHENOLIC ACTIVE FRACTION OF Ficus deltoidea VAR. Kunstleri (KING) CORNER ON FATTY ACID-INDUCED INSULIN RESISTANCE CELL MODELS

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#### ABSTRACT

Type 2 diabetes mellitus (T2DM) is the commonest type of diabetes mellitus and characterized by the insulin resistance. Many literatures showed that insulin resistance in T2DM and obesity is due to oxidative stress. Most of the traditional medicinal plants that are claimed useful in treating diabetes having antioxidant activity and believe to be beneficial in preventing the oxidative stress. The study tried to relate the relationship between antioxidant and insulin resistance. The study was conducted by determining the effect of Ficus deltoidea phenolics fraction that having the strongest antioxidant activity on glucose uptake of the myotubes and adipocytes in insulin resistance condition. The study consists of sequential extraction of the F. deltoidea and followed by fractionation using DPPH guided activity, phytochemicals analysis of the strongest antioxidant activity fraction by UPLC-QTOF-MS/MS and the fraction was used for the glucose uptake activity. The DPPH assay result showed methanol extracts and F1 fraction (ethyl acetate fraction) was the strongest antioxidant active fraction. For the cell culture, palmitate was able to induce insulin resistance in C2C12 myotubes but not in 3T3-L1 adipocytes. None of the strength of the fraction able to improve the insulin resistance. 10 µg/mL and 100  $\mu$ g/mL of ethyl acetate fraction reduced glucose uptake in both C2C12 myotubes and adipocytes. Further investigation is needed to be done on the other possible mode of action by F. deltoidea in reconciling its anti-diabetic claims. The finding implies that not all antioxidants rich medicinal plants can reverse the insulin resistance and their beneficial effects on T2DM need detail elaboration.

Keywords: Ficus deltoidea, T2DM, C2C12, 3T3-L1, antioxidant, insulin resistance



[29]

## *DMD* GENE MUTATION IDENTIFICATION IN DUCHENNE MUSCULAR DYSTROPHY PATIENTS OF QUETTA, BALOCHISTAN, PAKISTAN

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<sup>2</sup>Department of Pediatrics, Combined Military Hospital, Quetta Cantt, Pakistan

#### ABSTRACT

Duchenne muscular dystrophy (DMD) is the most common inherited muscular disorder in children. It is caused by mutations in DMD gene which encodes dystrophin protein. Mutation analysis has been challenging in DMD gene due to its large size (2.4Mb with 79 exons). The present study aims to identify the mutations in distal hot spot regions of DMD gene in families affected with DMD of Balochistan. 2 families were identified from different areas of Balochistan. Patients were clinically diagnosed by group of expert Pediatricians. After approval from Institutional ethical committee and informed consent of patients', venous blood samples were drawn from affected and healthy individuals of both families. DNA extraction was performed using inorganic DNA extraction method. Polymerase Chain Reaction (PCR) was performed for the 11 distal hot spot regions of DMD gene using specific primers, followed by gel electrophoresis. PCR products were further analyzed by DNA sequencing. Results of PCR indicated no exons deletions in family 1while in family 2 exons 48-50 were found deleted. DNA sequencing has not revealed any mutations in hot spot regions of both families. The results conclude that deletion mutation was observed in one affected individual of family 2 while further studies may require for confirmation of the mutations in proximal hot spot regions of other family.

Keywords: DMD, Mutation, Hot spot regions, Balochistan, Pakistan



#### [30]

## β-AMYRIN INDUCES ROS MEDIATED APOPTOSIS THROUGH p38 MAPK AND JNK PATHWAYS AND FACILITATES CASPASES ACTIVATION IN HeLa CELLS

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#### ABSTRACT

β-Amyrin, a well-known triterpenoids showed solid cytotoxic effect on breast cancer cell line. However its effects on cervical cancer, a second most common leading disease in women was not yet studied. The present study investigated the β-Amyrin destined HeLa cells to apoptosis by activating ROS and p38MAPK pathway. The antiproliferative effect was measured by the MTT assay. Genotoxic effects were studied by using micronucleus assay. Total ROS, NO and Caspase 3 level were determined by spectrofluorimeter and colorimeter. Protein expression was analyzed using immunoblotting. β-Amyrin  $(10-200 \ \mu\text{M})$  and Cisplatin  $(0.01-100 \ \mu\text{M})$  had an inhibitory effect on the proliferation of cancer cells in a dose-dependent manner, with the IC50 values at 100  $\mu$ M and 10  $\mu$ M for  $\beta$ -Amyrin and Cisplatin, respectively. Western blot analysis revealed expressions of apoptotic pathway related proteins (Bcl-2, caspase-3, caspase-9, phospho-p38 MAPK, and phospho-JNK, GADD456) were done in all groups. More interestingly, genotoxic effects were observed after treatment with  $\beta$ -Amyrin as well as with Cisplatin. However, β-Amyrin upregulates phospho-p38 MAPK, phospho-JNK and GADD45β on HeLa cells, increased phospho-JNK directly activate the caspases and decreased Bcl-2 in HeLa cells. These results conclude that  $\beta$ -Amyrin induced the apoptosis through ROS mediated mechanism by activating p38 MAPK and JNK through transcriptional factor GADD45B. In turn, activated JNK directly activates caspase-9 and caspase-3 activation thereby destined HeLa cells to programmed cell death.

Keywords: β-Amyrin, Cervical cancer, Apoptosis, Micronucleus, p38MAPK, JNK



#### [31]

## SUSCEPTIBILITY OF RAW-BLUE<sup>TM</sup> ISG CELL CULTURES TO Chequa iflavirus AND ATHTAB BUNYA-LIKE VIRUS ISOLATED FROM REDCLAW CRAYFISH (Cherax quadricarinatus)

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#### ABSTRACT

The lack of an established crustacean cell line is one reason why researchers have looked extensively for systems to understand the interaction between host cells and crustacean viruses. Mouse macrophage interferon reporter cells a.k.a. RAW-BlueTM ISG cells was evaluated to find out the susceptibility to two RNA viruses (Chequa iflavirus and Athtab bunya-like virus). Six T75 flasks were used to culture the cells (5x106 cells/ml) at 37°C until reach 70 % mono-layer. Virus inoculate (1ml) were added onto the surface of the cells in the three flasks (labeled as infected) while DMEM (1ml) were added onto the surface of cells in the other three flasks (labeled as control). After the inoculation, cells were incubated at 30°C. Cell samples were collected on 2,4, and 7dpi. Cells were subjected to HE staining and to RT-PCR. Two set of primers were used in the RT-PCR: 104F-CTCCTTCTGGGTGCGTTTA- / 104R-ATACTCTGGCGCATGCTCTCand 207F-GATCCGCAGAATACGAGGG-/ 207R-ACAACTGTCTGGCTATGGC-. The results showed that HE staining failed to show the signs of vacuoles (CPE), in both cell groups. RT-PCR results demonstrate that both C. iflavirus and bunya likevirus can infect RAW-Blue<sup>TM</sup> ISG cells and can maintain their survival in the cells until 7dpi. Nevertheless, possible the stronger amplicons during 2 and 4dpi, showed in amplification of Athab bunya-like virus are due to the viruses immediate early (IE) gene expression, RNA-dependent RNA polymerase on which the RT-PCR is based is turned on -mRNA + viral genome, but mRNA of RNA polymerase expression shuts down after 4pi and other genes then transcribed. It is important to evaluate RAW-Blue<sup>™</sup> ISG cell cultures for studying nuclear location signals (NLS)s of C. iflavirus & Athtab bunya-like virus.

Keywords: RAW-BLUE<sup>TM</sup> ISG, Chequa iflavirus, Athtab bunya-like virus, crayfish



#### [32]

### INVASION OF Aristobia reticulator (VOET) (COLEOPTERA: cerambycidae) ON LITCHI PLANTATIONS IN INDIA

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India

#### ABSTRACT

Recently, litchi plants in the North Eastern region of India were severely damaged by a stem borer, Aristobia reticulator (Voet) and it was first time noticed in India in litchi. The beetles were observed in the field from mid June to August and found to cause damage in young as well as old plantations of litchi. Newly hatched grubs first start feeding on sub cortically and then entered in sapwood. Grubs continued to move down the branches, feeding and ejecting frasses and excreta by making tunnels. During the survey 382 plants were observed in Arunachal Pradesh of the age group of 4 to 20 years, of which 338 plants (88.48%) were found damaged by the litchi borer. The grubs cause maximum damage in saplings and branches of litchi plantations in later instars, however, 21.98 percent saplings and branches were killed by excessive tunneling in the sapwood. The eggs were laid near the fork of the branches or saplings, which are 15.8 to 21.8 mm in diameter. The average width of the mature larval tunnel was 6.2 to 6.8 mm whereas the average breadth was 13.8 to 15.2 mm. The larvae were found to move downward and the average length of larval tunnel ranged from 121.7 to 143.3 cm. Pupal cells were found just beneath the bark and its average width ranged from 11.6 to 13.1 mm with an average breadth from 28.2 to 31.1 mm. The average diameter of the branches or stem in which the pupation occurred and exit holes were found ranged from 39.3 to 69.6 mm. The average diameter of the exit holes ranged from 11.13 to 12.14 mm. The larval tunnels were longer in old age plantations as compared to young plants.

Keywords: Aristobia reticulator, litchi, stem borer, biology, long-horned beetle



#### [33]

#### **MORPHO-MOLECULAR APPROACH**

#### TOWARDS IDENTIFICATION OF CURVULARIA SPECIES

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#### ABSTRACT

Curvularia is a widespread air-borne facultative pathogen of soil and plants, which mostly survive as a saprophyte in tropical and sub-tropical regions. It is a dematiceous, filamentous fungus. Curvularia spp. are darkly pigmented fungi with spores (curved conidia) efficiently adapted for most aerial dissemination. The classification of Curvularia species based on morphology alone is difficult as genus has more or less similar characteristic in several species; an attempt has made to confirm the identity of species by integrating morphological and molecular characters. A set of 52 Curvularia isolates from Delhi-NCR region, ITCC, MTCC and NFCCI were collected and morphologically characterized and confirmed using ITS sequences from NCBI database as C. aeria, C. affinis, C. australiensis, C. borreriae, C. catenulate, C. clavata, C. eragrostidis, C. geniculate, C. inaequalis, C. lunata, C. pallescens, C. prasadii, C. specifera, C. trifolli, C. tuberculata and C. verruculosa. Identity of these isolates was confirmed through NCBI data base using ITS region sequences. The identification percentage was found to be 90–100%.

Keywords: Curvularia, ITS region, dematiceous, Isolates



**V1** 

#### [34]

## STRUCTURAL CHARACTERIZATION OF NPAS4-ARNT DIMERIZATION THROUGH COMPUTATIONAL SIMULATION

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#### Pakistan

#### ABSTRACT

Neuronal PAS Domain Protein 4 (Npas4) is an activity dependent transcription factor harboring basic helix-loop-helix (bHLH)-PAS domain, mediating the expression of target genes involved in neurotransmission. NPAS4 crucially regulates response to various excitatory stimuli and has a role in GABAergic neuronal synapse development. Functionally, NPAS4 as a transcription factor dimerizes with the ARNT protein to serve as complete transcription factor and start the transcription of downstream genes. However, NPAS4 dimerization characteristics with ARNT has not been studied so far. Hence the current study aimed to identify the interaction pattern of NPAS4-ARNT complex through computational docking via HADDOCK. The interaction pattern were determined through pdbSum. The electrostatic surface calculations were performed through APBS plugins in PyMOL. The stability of interactions were determined through MD simulation. The results indicated that PASB domain of NPAS4 is involved in interactions with the PAS B domain of ARNT. A toll of 136 structures generated by HADDOCK were further grouped into 14 clusters. The cluster with the minimum energy value of -82.6 KJ/mol was then further selected for interaction analysis. The results showed that there is one salt bridge, 12 H-bonding interactions and 156 non-bonded contacts between two proteins. The important interactions among two proteins are Asp224:NPAS4 and Gln421:ARNT, Asp229:NPAS4 and Ser442:ARNT, Glu232:NPAS4 and Thr361:ARNT, Phe240, Glu241:NPAS4, and Arg440:ARNT. The MD simulation also showed the stability of these interactions. The electrostatic potential of these two proteins revealed the binding interface of NPAS4 and ARNT to be neutral hence favoring hydrophobic interactions. The findings can help elucidate Npas4 role in interacting with other neuronal proteins involved in neuronal signaling. Moreover, the interaction findings provide useful comparative insight with other bHLH proteins.

Keywords: Npas4, bHLH proteins, Dimerization, Neurotransmission, Molecular simulation



# POSTER PRESENTATIONS





#### [35]

## HEALTH RISK OF INFECTIOUS DISEASES ASSOCIATED WITH MARINE PRODUCTS - MICROBIOLOGICAL QUALITY AND FOODBORNE PATHOGENS IN PROCESSED SEAFOOD PRODUCTS

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#### ABSTRACT

Since marine environments and raw materials of processed seafood are habitats of human infectious pathogens, biological risk management based on background information regarding microbiological quality and safety of processed seafood are needed. Although salted seafood (SS) and ready-to-eat seafood (RTES) are categorized into processed seafood, there is a lack of severe heat treatment not only during manufacturing processes but also before consumptions. Thus, the evaluation of microbiological quality and safety of processed seafood is essential to prevent foodborne diseases. The aim of this study is microbiological analysis for hygienic indicator bacteria [total coliforms (TC), fecal coliforms (FC)] and foodborne pathogens [Vibrio spp. (Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio cholerae), Escherichia coli, Staphylococcus aureus] of 193 samples (SS, n = 90; RTES, n = 103). Physicochemical analysis (pH, salinity) of samples was also conducted. Foodborne pathogens were rarely detected (V. parahaemolyticus, 1.6%, n = 3; E. coli, 3.1%, n = 6; S. aureus, 0.5%, n = 1). The detection rate of TC (28.9%, n = 46) was higher than FC (0.6%, n = 1). More TC level was observed (p <0.05) in RTES (2.6  $\pm$  1.2 log CFU/g) than SS (1.9  $\pm$  0.7 log CFU/g). Since the salinity of SS (18.0  $\pm$ 4.4%) was significantly higher than RTES (5.3  $\pm$  5.0%) (p <0.05), salinity was expected as a determinant factor of TC levels. Whereas pH was not different (p > 0.05) between SS and RTES. We reveal the presence of foodborne and hygienic indicator bacteria in both SS and RTES even though there is no intervention against pathogens before consumption. These results highlight the needs of nonthermal decontamination strategies considering the distinct manufacturing processes of SS and RTES.

Keywords: Processed seafood, infectious diseases, total coliform, raw material, salinity, non-thermal decontamination strategies



#### [36]

## BIOLOGICAL METABOLISM OF *Bacillus* STRAINS IN RECONSTITUTED INFANT FORMULA: UNRECOGNIZED SOURCE OF NITRITE AS A HEALTH RISK

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#### ABSTRACT

Mishandling of reconstituted infant formula (RIF), which is represented by the long-term storage in bottle warmers or under room temperature has been regarded as the prerequisite for the propagation of pathogens. Thus, the elimination of pathogens during the reconstitution of infant formula at high temperatures (70-80°C) has been adopted as an intervention method against microbiological health risks of infants. However, although major spore-forming bacteria (SFB) can endure heat-stresses derived from the reconstitution, there is lack of information regarding the impact of biological metabolisms and bacterial growth of SFB in RIF on infantile health risks. Our previous research showed that SFB could convert nitrate into nitrite, the cause of infantile methemoglobinemia, under food processing conditions but nitrite metabolism with the perspective to RIF is understudied. In this study, nitrite metabolism of 133 SFB isolated from work-in-process and end-products of infant formula was analyzed in RIF (w 100 ppm KNO<sub>3</sub>) under storage conditions (30, 40°C). Since four strains of Bacillus (strain ID: FHS-PPBM 449, 481, 236, 237) showed relatively rapid onset of metabolism (within 4 h), the effect of key determinant factors [e.g., substrate concentration (100, 10 ppm KNO<sub>3</sub>), bacterial population (2–3, 1–2 log CFU/ml)] to metabolic activities and bacterial growth was examined. At 40°C, all isolates could produce nitrite more rapid than 30°C and strain FHS-PPBM 449 even could produce nitrite as much as the excessive amount of ADI (0.07 mg kg<sup>-1</sup> bw day<sup>-1</sup>) in 4 h of storage under 40°C. Our findings highlight the needs on the biotechnological basis for the risk management through the selective detection of nitrite-producing SFB and the manipulation of RIF handling conditions to prevent bacterial nitrite metabolisms.

Keywords: biological metabolite, nitrite metabolism, bacterial growth, powdered infant formula, sporeforming bacteria, *Bacillus* 



#### [37]

## SYNERGISTIC BACTERICIDAL EFFECTS OF BENZOYL DERIVATIVES IN COMBINATION WITH CAPRIC ACID AGAINST Staphylococcus aureus

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#### ABSTRACT

Staphylococcus aureus is a leading opportunistic human pathogen that causes a wide variety of infections. The aim of this study was to investigate the synergistic bactericidal effects of benzoyl derivatives (benzoic acid [BA], 4-hydroxybenzoic acid [HA], and β-resorcylic acid [β-RA]) and capric acid (CAP) against S. aureus and to identify the underlying mechanisms. S. aureus was treated with benzoyl derivatives (1, 3, and 5 mM), capric acid (0.1, 0.15, 0.2 mM), or different combinations of both materials. Membrane damage and efflux pump activity were also examined by flow cytometry. Individual treatment of each material showed low bactericidal effects ( $< 1.5 \log$ ); however, combined treatment resulted in synergistic bactericidal effects. For example, complete reduction of S. aureus (> 7.3 log reduction, not detected) was obtained by the treatment of 5.0 mM  $\beta$ -RA plus  $\geq 0.15$  mM CAP. Though flow cytometry analysis of bacteria treated with CAP showed evidence of membrane disruption, the cell damage was recoverable. In contrast, cells exposed to combined treatment showed complete membrane disintegration and cell death (max. 98.4%). ß-RA and CAP also showed effective inactivation activity against efflux pump. The mechanisms underlying the bactericidal effects of combined treatment with benzoyl derivatives and CAP may involve the membrane disruption and decreased efflux pump activity, which then facilitates the entry of the antimicrobials into the cytoplasm. The main advantage of this technique is that we used only consumer-preferred natural borne antimicrobials and a very small amount of materials is needed based on the synergistic effects. This technique could be used in public health, medical centers, and the food industry.

Keywords: *Staphylococcus aureus*, natural borne antimicrobials, benzoyl derivatives, capric acid, synergistic effect



#### [38]

## LabChip BASED MULTIPLEX DIAGNOSIS SYSTEM FOR THE RAPID DETECTION OF Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, AND Candida albicans IN COSMETIC PRODUCTS

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#### ABSTRACT

Since the microbial contamination of cosmetic products can result in the spoilage of products and occurrence of health risk to the consumer by the skin infection, efficient and accurate detection methods is needed for the assessment of microbiological safety and quality. However, standard detection methods provided from representative institutions governing microbiological issues of cosmetic products (e.g., WHO, US-FDA, EP, etc.) is based on the conventional culture method which requires long-term incubation and different experimental conditions for target microbes. In this study, microfluidic LabChip-based diagnosis system for representative microorganisms which have been linked to the microbiological risk of cosmetic products (Candida albicans, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus) was developed to overcome current limitations from not only conventional method but also rapid detection technologies (e.g., complexity of protocol, high-cost device and/or supplies, lack of portability). Thermocycling device equipped with dual heating-blocks optimized to plastic-chips for rapid thermal conduction of samples in microfluidicchannels was designed to accelerate reactions. To unify the experimental conditions regardless of target microbe, incubation in tryptic soy broth under 37°C was adopted as enrichment procedure and detection results using developed system could be obtained within *ca*. 30 minutes for all target microorganisms. Detection performance was evaluated by specificity tests using 33 microorganisms including bacteria, fungi, yeast, and virus. Cross-reactivity tests for gDNA of target bacterial species showed that there was no interference-factor causing false-positive results. The sensitivity of the developed technology was estimated by acceptable Ct value (within 36) in low concentration (2 log CFU/ml). This study presents a novel approach for the rapid molecular diagnosis which can provide both convenience and accuracy for multiplex detection of target microorganisms in cosmetic products.

Keywords: rapid detection technology, molecular diagnosis, representative microorganisms, cosmetic products



[39]

## PREVALENCE AND KEY GENES GOVERNING GASTROENTERITIS-RELATED PATHOGENICITY OF Aeromonas hydrophila ISOLATED FROM MARINE FISH IN SOUTH KOREA

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#### ABSTRACT

**P5** 

Few studies have investigated the prevalence of Aeromonas hydrophila, an opportunistic pathogen mainly found from aquatic environments, in various marine fish and pathogenicity of the isolates. To build groundwork for the management of foodborne A. hydrophila, identification and analysis on virulence genotypes of the isolates from marine fish (n = 200; raw anchovies, dry anchovies, flatfish,seabass, Korean rockfish, frozen tuna) were conducted. Fish fillets of each sample were used for the analysis except for anchovies which were used as whole fish. Tryptic soy broth with ampicillin and starch ampicillin agar were used for the selection of the target bacteria. Identification of isolates was performed through 16s rRNA analysis; among 200 fish samples, A. hydrophila were isolated from 57 samples (28.5%). Flatfish showed the highest detection rate (50.9%), whereas none of the dry anchovies was positive. Tunas showed second-to-lowest prevalence (3.3%), possibly indicating the inhibition of bacteria during drying or freezing processes. Putative virulence factors associated with gastroenteritis, aerolysin (aer), heat-stable/labile enterotoxins (ast, alt), were investigated for each isolate with single or multiplex PCR. Total 38 out of 57 isolates carried the virulence gene(s) (66.7%), and flatfish showed the highest frequency of the virulent strains (41.5%). Among eight categories of virulence genotypes, most frequently observed was an avirulent type (n = 19), followed by a type carrying *aer* solely (n = 19) 10), or all three genes (n = 9). Considering the lack of information on the prevalence and genetic features of virulent A. hydrophila in South Korea, isolates newly obtained in this study can be used to analyze the potential health risk of foodborne A. hydrophila with perspective to the regional characteristics.

Keywords: Aeromonas hydrophila, marine fish, prevalence, virulence factor, pathogenicity, PCR



#### [40]

## IDENTIFICATION AND GENETIC CHARACTERIZATION OF PATHOGENIC Vibrio SPP. (Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio cholerae) FROM RAW READY-TO-EAT SEAFOOD

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#### ABSTRACT

Vibrio spp. (V. parahaemolyticus, VP; V. vulnificus, VV; V. cholerae, VC) is a causative agent of gastrointestinal diseases, wound infection, and septicemia by the human infection via consumption or contact of raw seafood. Detection of pathogenic Vibrio spp. in major sources of contamination is the primary strategy for the preventative intervention against infectious diseases. This study aims to perform genetic identification on both taxonomical and virulent features for 310 raw RTE seafood samples (raw fish, n = 154; shellfish, n = 102; cephalopod, n = 34; tunicates, n = 20). Vibrio spp. were quantitatively detected only from raw fish consumed with its skin (gizzard shad, conger, pomfret) (VP:  $2.9 \pm 0.9 \log$  CFU/g; VV:  $2.8 \pm 0.9 \log$  CFU/g; VC:  $1.0 \log$  CFU/g), and those samples also showed higher detection rate (29.2%) from qualitative analysis (raw fish consumed without its skin: 1.9%; shellfish: 5.9%; cephalopod: 5.9%; tunicates: 10.0%). In terms of toxin genes, vcgC and vcgE were detected in VV isolates as 76.5% (n = 17). Toxigenicity of VP (*tdh*) was determined in few isolates (2%, n = 51) whereas no toxin gene was observed from VC isolates (i.e., ctx, O1rfb, O139rfb). Overall results showed that Vibrio spp. possessing toxin genes were mainly isolated from raw fish with its skin. This might be due to (i) the fish skin exposed to Vibrio spp. in aquatic environments and (ii) the absence of sterilization process for skin during the handling of raw fish. This study provides fundamental information on toxigenic Vibrio spp. from raw RTE seafood and highlights the necessity for the additional pathogen control measures specified for the products consumed without the removal of fish skin.



#### [41]

## PATHOGENIC Arcobacter SPP. WITH TOXIN GENES FROM SHELLFISH IN SOUTH KOREA -DETERMINATION OF PUTATIVE VIRULENCE BY THE CHARACTERIZATION OF GENOTYPES

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#### ABSTRACT

Arcobacter butzleri, A. cryaerophilus, and A. skirowii have been considered as potential human enteropathogen. Several studies have isolated Arcobacter spp. from various foods, but there is a lack of comprehensive information regarding the detection of virulence genes of Arcobacter spp. isolated from shellfish. This study investigated the prevalence of Arcobacter spp. from shellfish samples (81 refrigerated oyster, 9 frozen oysters, 5 mussels, and 5 clams) purchased during winter in 2018 in South Korea. Two isolates were collected from CCM agar inoculated with enriched culture using Arcobacter selective broth, and genetic characterization for those presumptive isolates (taxonomical identification, virulent genotypes) was conducted by PCR. Nine-putative virulent genes specific for Arcobacter spp. (cadF, ciaB, cj1349, hecA, hecB, mviN, pldA, irgA, and tlyA) were analyzed to categorize the genotype of each strain. Twenty-seven strains isolated from 15 samples (refrigerated oysters, clams, and mussels) were identified as Arcobacter spp. (A. cryaerophilus, 81.5%, n = 22; A. butzleri, 18.5%, n = 5). Analysis of the virulence genes showed that the mviN gene was detected from most of the isolates (96.3%, n = 26). All A. butzleri isolates (n = 5) showed an identical genotype consisted of *ciaB*, *mviN*, *pldA*, and *tylA*. In the case of *A*. *cryaerophilus* (n = 22), *mviN* was detected from 21 isolates (95.5%) and 13 isolates harbored *ciaB* (59.1%). To our knowledge, this is the first study to investigate the Arcobacter spp. from shellfish in South Korea, and overall results regarding the prevalence with genetic virulent factors highlight the needs on novel perception to pathogenic Arcobacter spp. as a causative agent of outbreaks linked to shellfish.

Keywords: Arcobacter spp., shellfish, potential emerging pathogen, virulence factor, toxin gene



#### [42]

## CULTIVATION OF CYANOBACTERIA IN DOMESTIC WASTEWATER FOR BIODIESEL PRODUCTION

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#### ABSTRACT

Currently, the cultivation and use of biomass of various grains and oilseeds is mainly considered as renewable energy sources. Bioethanol and biodiesel are produced from these sources. Biodiesel is usually produced from oilseed crops, such as rapeseed, soybean, sunflower, and palm trees, etc. The industrial crops used today for the production of biofuels displace food and feed crops from the field and due to this food products are becoming more expensive. Cyanobacteria in energy output is significantly superior to palm and rapeseed oil which are usually used for the production of biodiesel. Presently, the two critical problems affecting the world: the lack of freshwater and the energy crisis. Cultivation of cyanobacteria in wastewater could be promising approach for the production of biodiesel. This integration is cost-effective and environmentally friendly technology for the sustainable production of biofuels, since a huge amount of water and nutrients in wastewater can be reused by cyanobacteria for growth. During comparative analysis of cyanobacteria strains, strains characterized by the greatest amount of C14 fatty acids, Cyanobacterium sp. B-1200 and Cyanobacterium aponium IPPAS B-1201 were selected. Such fatty acids composition is considered to be rare for cyanobacterium and especially 14:0 and  $\triangle 9$ -14:1 FA are the most popular compounds for the production of biodiesel. In this work, collection of strains cyanobacterium sp. IPPAS B-1200 and Cyanobacterium aponium IPPAS B-1201, strain Anabaena variabilis R-I-5, isolated from rice fields of Baghlan region (Afghanistan), were cultivated on various media: 1) wastewater from the sewage treatment plant of Almaty city; 2) wastewater with a nutrient medium in a 1:1 ratio; and 3) BG-11 nutrient medium. The cultivation of the strains was carried out for 14 days. It was shown that investigated cultures characterized by intensive growth on wastewater with nutrient medium in 1:1 ratio. For investigation of ability of researched strains of cyanobacterium to bioremediation, the physical and chemical composition of wastewater before and after cultivation of cyanobacetrium was analysed. During the research work, it was detected that wastewater after biological treatment is considered as more suitable medium for cyanobacterium sp. IPPAS B-1200 Cyanobacetrium aponium IPPAS B-1201 and Anabaena variabilis R-I-5 cyanobacteria culture growth.

Thus cyanobacteria can be used in two directions: biomass production for sustainable biodiesel production and wastewater bioremediation.

Keywords: cyanobacteria, biodiesel, wastewater

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#### [43]

## PHYSIOLOGICAL AND GENOMIC INSIGHTS INTO THE NITRITE-PRODUCING Geobacillus stearothermophilus IN THE PROCESSING LINES OF POWDERED INFANT FORMULA

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#### ABSTRACT

Major issues regarding the microbiological safety of powdered infant formula (PIF) has focused on the foodborne pathogens and heat-treatments of processing steps followed by the control of post-processing contamination are expected to ensure the product safety. However, highly heat-resistant sporeformers surviving under processing conditions of PIF still remain as a challenge in PIF industries. Especially bacterial growth of thermophilic sporeformers in post-sterilization steps of processing lines can result in the production of nitrite, a bacterial metabolite which can result in infantile fatal diseases (e.g., methemoglobinemia) through the oral ingestion. To control and prevent the potential risk of nitrite, physiological and genetic analysis of the bacterial nitrite-producers are suggested: 1) examination on the bacterial community of PIF processing lines to find out the source of bacterial contamination and the impact of manufacturing processes on microbial composition, 2) identification and characterization of nitrite-producing sporeformers in processing lines and end-product of PIF, 3) whole genome sequencing coupled with bioinformatics tools to establish genetic markers for the identification and quantification of nitrite-producers. Based on the overall results, representative thermophile Geobacillus stearothermophilus was suggested as the major nitrite-producer. Genomic basis of nitrite-producing G. stearothermophilus (NPG) facilitates direct detection of NPGs from raw materials and processing steps, surveillance based on the specific quantification of nitrite-producers, and the inhibition of metabolism by manipulating the processing conditions. In conclusion, a paradigm shift of nitrite into microbiological risk in PIF processing reveals the underrecognized source of nitrite and provides background information for the risk management against the microbial source of nitrite in PIF.

Keywords: *Geobacillus stearothermophilus*, Whole genome sequencing, powdered infant formula, nitrite metabolism, comparative genomics, thermophilic sporeformer







# VIRTUAL PRESENTATIONS





#### [44]

## THE IDENTIFICATION OF GENETICS FACTOR OF NON-SYNDROMIC OROFACIAL CLEFT IN THE SOUTHEAST ASIAN POPULATION: A SYSTEMATIC REVIEW

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#### ABSTRACT

The aim of this study was to identify the genetics factor involved in the formation of orofacial cleft on the Southeast Asian population. An electronic search was conducted using the databases PubMed and Google Scholar (from 1980 to 2017). The design of this study include the search strategies used in the preliminary stage, the inclusion/exclusion criteria, and data extraction from studies reporting orofacial cleft gene/s involved in the prevalence of the incidents on the Southeast Asian population. There were two stages of the screening process; the first stage was selection of the titles and abstracts of the relevant articles from both databases; in the second stage, the articles were read in full for a final selection of the studies, with the inclusion and exclusion criteria being checked. Quality assessments of publication bias were performed using the Newcastle-Ottawa Scale (NOS). Five genes from 6 articles were identified after the second screening stage, namely TGFA, IRF6, MSX1, CDH1 and ARHGAP29. CDH1, ARHGAP29 and 4 other genes from first screening stage, namely VAX1, PAX7, ABCA4 and BMP4, were excluded in the final screening stage. Based on the final screening, MSX1 showed up in more than one article and the result suggested MSX1 as one of the main gene candidates on the prevalence of orofacial cleft disorders in the Southeast Asian.

Keywords: gene, orofacial cleft, Southeast Asian, electronic search, the Newcastle-Ottawa Scale (NOS)



#### [45]

## FREEZING AND FREEZE-DRYING OF STRAWBERRIES WITH AN ADDITIONAL EFFECT OF MICRO-VIBRATIONS

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#### ABSTRACT

Our research focuses on the formation of ice crystals and evaluating of the structure preservation frozen and freeze-dried strawberries. Strawberries were frozen in two ways. One-half of strawberries were frozen at -30°C under conditions of convective heat exchange. The other half of strawberries were frozen under the same conditions with an additional effect on the strawberries of micro-vibrations created in the air of the freezer according to a specific program. A digital frequency synthesizer that generates 250 W/m3 electromagnetic field rectangular pulse packets in the frequency bands of 2,500 kHz to 5,000 kHz creates micro-vibrations. The microstructure of strawberries, the number of cells that have retained their structure and firmness were determined in frozen strawberries. The strawberries retained 25-30% of the cell structure of their total number during traditional freezing, and 65-70% of the cell structure when frozen under micro-vibration. The data of the penetration and shear stress showed that the strawberries frozen under micro-vibration conditions were 10-15% stronger. Then researched strawberries were vacuum freeze-dried. The primary drying temperature was 30+1 °C below zero and at the secondary drying the temperature was 38 - 40 °C. The microstructure and firmness of strawberries were researched in dried samples also. Freeze-dried strawberries frozen under microvibration had small and evenly distributed capillaries and their firmness was 8-10% higher than freezedried strawberries frozen by the traditional method. Thus, freezing strawberries with the additional effect of micro-vibration has a positive effect on the firmness of both frozen strawberries and freezedried strawberries.

Keywords: vacuum freeze drying, strawberry, micro-vibration, microstructure, firmness



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