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Keynote

Beyond 2021 : Building Sustainable Science

Raha Abdul Rahim[a,b] *

[a]*Chancellery, Universiti Teknikal Malaysia Melaka,*
[b]*Faculty of Biotechnology and Biomolecular Science, Universiti Putra Malaysia*

*Corresponding author email: raha@utem.edu.my

Abstract
Economic viability, environmental protection and social equity have been identified as the three pillars of sustainability. Science is critical to tackle complex challenges for humanity such as the impacts of climate change, biodiversity loss, food security, pollution and poverty reduction; as it lays the foundation for new approaches and solutions. How can science best fulfil these commitments? How can we create dynamic connections between knowledge and action? Sustainability science promotes cross-disciplinary approaches to advance the understanding of human-environment interactions and systems, and how these interactions affect the challenge of sustainability (Nada Al-Nashif, UNESCO's Assistant Director-General for Social and Human Sciences).

A fundamental challenge facing science educators at the Higher Education Institutions is how to develop and support the implementation of project-based, technology-rich science curriculum and demand-driven research connectivity that is consistent with international calls for a "new approach" to science education. In this presentation, I will highlight some initiatives promoted by relevant ministries to elevate the importance of interrelationship and interdisciplinary factors and taking into account these factors when seeking solution strategies. The role of biotechnology and molecular biology in advocating sustainable science will also be discussed. Lastly, we will address the need of educators to ingrain the right values and technical know-hows to the future generation.

Keywords: Sustainable science, interdisciplinary and cross-disciplinary, demand-driven research.

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Keynote

The Triumphs and Vicissitudes of Biotechnologies on Malaria Control

Georges Snounou[a]*

[a]*CEA-Université Paris Sud 11-INSERM U1184, Immunology of Viral Infections and Autoimmune Diseases (IMVA-HB) IDMIT Department, IBFJ, DRF, Fontenay-aux-Roses, France*

*Corresponding author email: gsnounou@gmail.com

Abstract
The disease we now call malaria had been recorded by all civilisations throughout human history. Then as now, malaria significantly impacts development and exacts a severe public health burden. A confluence of biotechnological advances in the 1970's has propelled a search for a vaccine against the highly adaptable and diverse parasites, which still remains elusive. Nonetheless, the elaboration of novel advanced biotechnological tools over the recent years are giving researchers the opportunity to probe hitherto inaccessible mechanisms of the parasites' life cycle. In this lecture, I wish to present an overview of the strategies adopted for malaria research and for control programmes, and to discuss how such biotechnological advances contributed to shape these strategies, and the consequences that this has had in the past and could potentially have in future.

Keywords: Biotechnology, History, Malaria, Parasitology, Research

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Plenary

It's Not in Our DNA: Non-Transformative Gene Silencing in Orchids

Jennifer Ann Harikrishna[a,b]*, Purabi Mazumdar[b], Rofina Yasmin Othman [a,b]

[a]*Institute of Biological Sciences, Faculty of Science, University of Malaya., Kuala Lumpur, Malaysia*
[b]*Centre for Research in Biotechnology for Agriculture (CEBAR), University of Malaya, Kuala Lumpur, Malaysia*

*Corresponding author email: jennihari@um.edu.my

Abstract

Introduction: The uncovering of the mechanism of RNA-mediated gene silencing led to a precise technology for application in crop protection and targeted modifications in plants. RNA silencing though the direct application of double stranded RNA (dsRNA), a non-transgenic approach using ectopically applied dsRNA, or RNA sprays, has been demonstrated to provide effective crop protection and to be a powerful and rapid reverse genetic tool to study gene function and for validation of target sequences for gene silencing. **Methods:** dsRNAs targeting orchid virus and orchid endogenous mRNAs were synthesised in an RNase deficient strain of *E. coli*, and used for RNA silencing by direct application of dsRNA to orchid plants and flowers. **Results:** Application of dsRNA based on the coat protein of Cymbidium mosaic virus (CymMV), a major pathogen affecting commercial orchid production operations and hobbyists, effectively protected tissue cultured orchid plantlets from the virus and so has potential for use to protect plantlets during transplantation and pruning. In separate experiments, the use of dsRNA to silence endogenous plant genes to determine gene function and for potential use in the modification of flower phenotype were explored. Experiments with dsRNAs based on R2R3 MYB transcription factor mRNA sequences from orchids showed that these were able to alter phenotypes of orchid flower cell shape and anthocyanin content. **Conclusion:** Non-transformative dsRNA-mediated gene silencing provides a useful tool for crop protection and for fundamental studies of gene function without the expense, time or need to introduce new DNA or alter plant DNA.

Keywords: dsRNA, flower, gene silencing, MYB, orchid

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Plenary

Functional Nanodomains of Cardiomyocytes in Heart Failure

Julia Gorelik*

National Heart and Lung Institute, Imperial College London, W12 0NN, London, United Kingdom

*Corresponding author email: j.gorelik@imperial.ac.uk

Abstract

Introduction: Heart failure (HF) is a major contributor to the cardiovascular disease burden and impacts significantly to global health expenditure. A host of pathological remodelling changes are consistently observed in HF patients; that includes the alterations in beta adrenergic receptors (βARs), ion channels, in particular L-type calcium channels (LTCCs), Ca^{2+}-handling proteins, and proteins mediating cell-cell coupling. In HF, a progressive loss of T-tubules results in molecular remodelling and in the development of triggers of arrhythmia (early and delayed after-depolarizations, EADs and DADs). In cardiac myocytes, receptors are coupled to effector molecules and ion channels in nanodomains. **Methods:** Using novel cutting edge methodology including scanning ion conductance microscopy (SICM), scanning patch clamp technique , SICM/FRET combination we uniquely resolved surface topography of the live myocyte at nanoscale and then activate adrenoceptors in precise locations identified, with either imaging of cAMP changes in the vicinity or ion channel recording. **Results:** Particularly we focused on the T-tubules and the caveolae and on the relationship between β-Adrenoceptors and L-type Ca^{2+} channels. We studied how the disturbance of these microdomains affects cell function in ventricular myocytes from a rat model of infarct and from human patients with several myopathies. Further, we attempted to restore the normality of nanodomain organisation to indirectly influence the calcium channels and improve contraction. **Conclusion:** This work gives an understanding of how signalling operates at nanoscale resolution in living cells. This will translate to new methods of restoring proper organisation of nanodomains for treatment of failing hearts.

Keywords: Cardiomyocytes, heart failure, ion channels, beta-adrenoreceptors

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Plenary

Mechanism of Functional Homeostasis in Bacterial Complex System

Fatma Azwani Abdul Aziz[a], Kenshi Suzuki[b], Abd Rahaman Jabir Mohd Din[c,d], Masanori Honjo[d], Koki Amano[e], Yosuke Tashiro[d,e], Hiroyuki Futamata[d,e,f]*

[a] *Laboratory of Food crops, Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor D.E., Malaysia*
[b]*Microbial Ecotechnology, Department of Biotechnology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 113-8654, Japan*
[c]*Innovation Centre in Agritechnology for Advanced Bioprocess, UTM Pagoh Research Center, 84300 Johor, Malaysia*
[d]*Graduate School of Science and Technology, Shizuoka University, Hamamatsu, Hamamatsu 432-8561, Japan;*
[e]*Department of Applied Chemistry and Biochemical Engineering, Graduate School of Engineering, Shizuoka University, 432-8561, Hamamatsu, Japan;*
[f]*Research Institution of Green Science and Technology, Shizuoka University, Shizuoka 422-8529, Japan*

*Corresponding author email: futamata.hiroyuki@shizuoka.ac.jp

Abstract
Introduction: Controlling microbial communities is vital for sustainable agriculture, human health care, wastewater treatment and bioremediation. However, we do not know yet why/how engineered microbial communities and whole microbial systems have been maintained functionally. **Methods:** Synthetic bacterial communities (SBCs) were constructed with phenol-degrading bacteria and phenol as sole carbon source under chemostat conditions to unveil the mechanism of functional homeostasis because microbial ecosystems are too complex to analyze the mechanism. While some SBCs collapsed, an SBC maintained phenol degrading activity although phenol-supply stoppage was set in several times. Population dynamics of bacterial strains were monitored by real time-quantitative PCR (qPCR) targeting gene encoding large subunit of phenol hydroxylase in the SBC. Kinetic parameters for phenol of the SBC were tracked by an oxygen electrode. Transcriptional levels of phenol hydroxylase and catechol dioxygenase of bacterial strains were analyzed by reverse transcription-qPCR (RT-qPCR). **Results:** The kinetic parameters for phenol of the SBC changed before and after the phenol-supply stoppage, which suggests a change in functional roles of strains in the SBC. The RT-qPCR analyses revealed that all strains shared phenol and survived independently before the phenol-supply stoppage. After the stoppage, a strain would incur the cost for degradation of phenol and catechol, whereas other strains seemed to be cheaters using metabolites, indicating the development of the metabolic network. **Conclusion:** These results indicated that it is important for the management and redesign of microbial communities to understand the flexibility of metabolism of bacterial communities.

Keywords: Microbial ecosystem, Complex system, Bacterial community, Homeostasis

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Plenary

Active Edible Coatings/Films for Perishable Foods Preservation: Potential, Present Limits and Future Prospects Illustrated by Some Case Studies

Pascal Degraeve*, Nadia Oulahal

BioDyMIA (Bioengineering and Microbial Dynamics at Food Interfaces) Université Lyon 1- ISARA Lyon research unit, IUT Lyon 1, technopole Alimentec, 01000 Bourg en Bresse, France

*Corresponding author email: pascal.degraeve@univ-lyon1.fr

Besides limiting drying and oxidation of the superficial zones of perishable foods due to their gas barriers properties, edible coatings/films can be suitable carriers of antimicrobial and/or antioxidant agents. The possibilities to add antimicrobial and/or antioxidant biomolecules (e. g. lysozyme, nisin, plant phenolic) and even, although more rarely, to add living bioprotective lactic acid bacteria in coatings/films have thus been considered in order to extend the shelf life and/or improve the safety of perishable foods. The potential advantage of using active coatings/films to deliver such agents over their direct spraying on the surface of perishable foods lies in their capacity to improve the stability of active agents before their application and/or to control their release from coatings/films to the superficial zone of foods following their application. In this context, the formulation of coatings/film-forming suspensions can be defined to design edible coatings/films matrices with a structure favoring (i) the preservation of the activity of active biomolecules or of the viability and metabolic activity of bioprotective lactic acid bacteria and/or (ii) the controlled release of active molecules/metabolites.
This will be illustrated by two case-studies:

- the possibility to change the properties of gelatin films and the kinetics of release of lysozyme added in these films by enzymatic crosslinking with microbial transglutaminase
- the possibilities offered by alginate-caseinate aqueous two-phase systems to prepare coatings with a microstructure maintaining both the viability and antagonistic activity against *Listeria* spp. strains of bioprotective lactic acid bacteria.

Keywords: edible coatings, edible films, antimicrobial activity, food preservation, controlled release

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Plenary

Chitosan Hydrogel Niomaterial from Fish Scales for Topical Wound Healing Application

Lai Yin Yee[a], Thirukanthan C.S.[d], Suvik Assaw[c,d], Mohd Effendy Abd Wahid[b,d,e]*

[a]*Department of Policy Planning and Coordination, Ministry of Foreign Affairs, 62602 Putrajaya*
[b]*Faculty of Fisheries and Food Science, University Malaysia Terengganu, 21300 Kuala Nerus, Terengganu.*
[c]*Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21300 Kuala Nerus, Terengganu*
[d]*Institute of Marine Biotechnology, University Malaysia Terengganu, 21300 Kuala Nerus, Terengganu*
[e]*Institute of Tropical Aquaculture and Fisheries, University Malaysia Terengganu, 21300 Kuala Nerus, Terengganu*

*Corresponding author email: effendy@umt.edu.my

Abstract
It was estimated that the fish processing industry generates between 18 and 30 million tons of fish wastes annually, of which 4% are fish scales. Hence a study was conducted to extract chitin from tilapia fish scales for chitosan hydrogel from the sequence of chemical processes involving demineralization, deproteinization, and deacetylation. The product was applied as wound healing gel and evaluated using full-thickness wounded skin with 8 mm diameter in Swiss albino mice model. Eighteen clinically healthy mice were divided into two groups; Group 1 as Control Group without any treatment, and Group 2 as Treatment Group that was applied with chitosan hydrogel treatment once daily. Observation and measurement of wound contraction were recorded daily for 21 days. Three mice from each group were euthanized, and the skin was dissected for histology evaluation of the wounded skin on days 7, 14, and 21 post wounding. The wounded skins treated with chitosan hydrogel showed better reconstruction healing than the control group. They generally attained full wound closure and over 95% of collagen density within two weeks post wounding. The absence of pathological abnormalities in the organs obtained by necropsy indicates its biocompatibility with no side effects to the hosts. All parameters observed were significant ($p < 0.05$) in comparison to the control group. The accelerated wound healing shown with higher collagen deposition, short epithelialization, and rapid wound closure suggests a significant positive effect of chitosan hydrogel to be an effective wound healing agent.

Keywords: fish scales, chitin, chitosan hydrogel, Swiss albino mice, wound healing

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Plenary

Understanding Alzheimer's Disease through Yeast Studies

Ian Macreadie*, Sudip Dhakal

School of Science, RMIT University, Bundoora, Victoria, Australia

*Corresponding author email: ian.macreadie@rmit.edu.au

Abstract

Introduction: Alzheimer's Disease (AD), the leading cause of dementia, is age-related and associated with a neurotoxic 42 amino acid peptide, amyloid beta (Aβ) that is a proteolytic product of a natural human protein called APP. Aβ levels increase with age and are observed in AD patients as brain plaque composed of amyloid fibrils. **Methods:** Yeast, the simplest eukaryote, can be used to study Aβ, to explore ways to remove it and to find agents that may increase or reduce its toxicity. Yeast were engineered to express native Aβ, or Aβ fused to green fluorescent protein (GFP) to aid its visualisation. **Results:** Aβ oligomers, but not fibrils are toxic to yeast (and neuronal cells). Simvastatin, the common drug prescribed for lowering cholesterol levels, is the best drug to prevent AD (and Parkinson's Disease). In yeast simvastatin, reduces Aβ levels, suggesting it may act through increasing a protein clearance mechanism. Some compounds like tyramine and aluminium increase the toxicity of Aβ possibly accounting for the sporadic appearance of AD. **Conclusion:** Yeast represents a convenient model for the development and testing of compounds that may have utility in prevention of Alzheimer's Disease.

Keywords: Alzheimer's Disease, amyloid beta, model organism, toxic peptide, yeast biotechnology

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Invited

Moving GenomeSelect™ to the Field – Highs and Lows

David Ross Appleton*, Chee Keng Teh, Harikrishna Kulaveerasingam

Sime Darby Plantation Technology Centre, 1st Floor, Block B, UPM-MTDC Technology Centre III, Lebuh Silikon, UPM, Serdang, Selangor 43400, Malaysia

*Corresponding author email: david.ross.appleton@simedarbyplantation.com

Abstract

Introduction: After seven years of research, starting from a single oil palm genome sequence, Sime Darby Plantation planted the first commercial-scale field with GenomeSelect™ palms in 2016. Since then, we have observed commercial yield data and developed ways to scale up planting to satisfy all seed requirements in Sime Darby Plantation. This talk will present the highs and lows of the journey to realising commercial value from genomics tools in this very important crop. **Methods:** Following 2nd gen sequencing of a reference oil palm, GWAS methods were applied to test populations of commercial palms in order to develop genomic selection models that can be applied to commercial seed production. The first planting was split into two locations; coastal and inland to determine possible environment effects. **Results:** Coastal yield of GenomeSelect™ palms has been encouraging while inland yields indicate significant impact of rainfall and water supply on yields. Controlled trials confirm the positive impact of genomic selection models, which have now been applied in parental selection for commercial seed scale up. **Conclusion:** the application of genomic selection techniques for commercial oil palm seed production has shown significant promise and will certainly be improved based on the large amount of data now available.

Keywords: Oil palm, genomic selection, crop improvement

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Invited

Application of Antarctic Microorganisms in Diesel Bioremediation Process

Siti Aqlima Ahmad [a,b,c,*]

[a]*Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[b]*Center for Research and Antarctic Environmental Monitoring (CIMAA), Universidad de Magallanes, Av-da. Avda. Bulnes, Punta Arenas, Regiòn de Magallanes y Antàrctica Chilena 01855, Chile*
[c]*National Antarctic Research Center, B303 Level 3, Block B, IPS Building, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.*

*Corresponding author email: aqlima@upm.edu.my

Abstract
Antarctica offers perennially cold, sub-zero temperatures due to the extreme climate of the continent. The presence of recalcitrant petrogenic hydrocarbons in Antarctica is generally associated with extreme conditions that impede the natural attenuation of hydrocarbon components. These pollutants have caused major perturbations in the Antarctic ecosystem, suggesting that conventional bioremediation approaches are largely ineffective at present. The purpose of this study is to evaluate the hydrocarbon-degrading ability of marine and soil bacteria consortia to assimilate diesel fuel as the sole carbon source, using statistical optimisation of growth conditions and diesel degradation. Concurrently, the obtained data were implemented in a scale-up bioreactor design study, with additional investigation on the effects of soil washing duration, agitation speed, soil to water ratio, surfactant concentration and aeration mode. In this study, the bacterial community isolated from soil successfully degraded up to 95% of a 1.75% v/v diesel, while the marine bacterial community have ability to degrade up to 80% of 1% v/v diesel. A scale-up study using the fabricated soil washer and bioreactor *in situ* Antarctic settings showed that optimised conditions for soil washing were at 40 rpm soil agitation, 3 h washing time, 1:5 soil to water ratio and 1% w/v SDS. The data obtained suggest that the Antarctic bacterial community has a potential for use in low-temperature bioremediation applications of diesel pollution.

Keywords: Antarctic, Bioremediation, Diesel

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Invited

Omics Technologies:- Elucidating Relationships between Microbes and Cancer and Future Applications

Pei Pei Chong*

School of Biosciences, Faculty of Health and Medical Sciences, Taylor's University.

*Corresponding author email: peipei.chong@taylors.edu.my

Abstract
Cancer is known as a disorder with multi-factorial causes. Throughout the last few decades, different microbes have been shown to have causal links to carcinogenesis. Some microbes are direct carcinogens through the expression of viral oncogenes whereas others are indirect carcinogens through toxins accumulation or chronic inflammation. Through next-generation sequencing technologies and transcriptomic studies, researchers were able to pinpoint the specific genes whose aberrant expression leads to multiple mutations. Through a combination of NGS, gene knockdowns and immunofluorescence studies, we found that HPV infection could induce APOBEC3B upregulation which in turn leads to mutations in various genes in breast carcinoma. We postulate that HPV infection and estrogen receptor expression together with APOBEC3B exists in a tri-partite relationship which promotes early-stage breast carcinogenesis. Recently, the gut microbiota has been shown to affect local immunity, systemic immunity, as well as microbe-specific immune programming, and dysbiosis of microbiota is associated to various cancers. An important achievement in cancer treatment in the last decade has been the introduction of immune checkpoint blockade or (ICB) immunotherapy, including anti-CTLA-4 and anti-PD-1/PD-L1 inhibitor drugs. Interestingly, but not surprisingly, gut microbes were found to be able to profoundly influence the potency of immunotherapy and some chemotherapies with immunestimulatory functions. Using NGS, certain species of gut microbiota were shown to be associated with poor response to cancer immunotherapy, while other species were related to good prognosis. These findings have important implications for not only cancer immunotherapy but also development of microbiota-based therapies as an adjunctive treatment in the future.

Keywords: Cancer, Microbiota, Immunotherapy, NGS, Omic Technologies

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Invited

Single Cell Proteomic Analysis of COVID-19 Patient Samples by Mass Cytometry

James Badger Wing*

Laboratory of Human Immunology (Single Cell Immunology), Immunology Frontier Research Centre (IFReC), Osaka University, Osaka, Japan

*Corresponding author email: jbwing@ifrec.osaka-u.ac.jp

Abstract
Introduction: The devastating outbreak of SARS-CoV2 and the associated COVID-19 has had a severe impact on the global community. While recent advances in vaccination have given some hope of respite the proven ability of the virus to mutate and potentially generate vaccine resistant strains indicates that there can be no relaxation of our urgent efforts to better understand the effects of this disease. A critical part of this effort is to understand the changes to the immune system of infected patients since both viral clearance and most symptoms of the disease are mediated by the immune system. Recent advances in single cell technologies, such as mass cytometry (also known as CyTOF) have revealed high levels of heterogeneity among immune cells. **Methods:** Mass cytometry was used to assess the single cell proteome of millions of cells from the blood of patients with COVID-19. **Results:** Wide ranging changes to a variety of cell populations occur. Simultaneous assessment of changes to both common populations and such as classical monocytes, and rare sub-populations of FOXP3 expressing regulatory T-cells and T-follicular helper cells was observed **Conclusion:** Simultaneous assessment of wide ranging cell populations may indicate how they interact during COVID-19 and suggest that differences between regulatory T-cell subsets among moderate, severe, and critical patient groups may be a factor in pathogenesis.

Keywords: Mass cytometry, Immunology, Regulatory T-cells, COVID-19.

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Invited

Beneficial Endophytic Actinomycetes

Arinthip Thamchaipenet[a,b,*]

[a]*Department of Genetics, Faculty of Science, Kasetsart University, Bangkok, Thailand*
[b]*Omics Center for Agriculture, Bioresources, Food, and Health, Kasetsart University (OmiKU), Bangkok, Thailand*

*Corresponding author email: arinthip.t@ku.ac.th

Abstract
Introduction: Endophytic actinomycetes residing *in planta* provide plant growth promoting (PGP) benefit by enhancement of growth and protection plants from abiotic and biotec stresses. **Methods:** Endophytic actinomycetes were isolated from agricultural, economical, and medicinal plants in Thailand. Their PGP traits were determined and demonstrated to promote growth and stress tolerance in crops. **Results:** A new genus and seven new species of endophytic actinomycetes were validated. Novel bioactive compounds were identified. Many of them possess PGP traits including phytohormone and siderophore secretion, phosphate solubilisation, ACC-deaminase, chitinase, and anti-microbial production. These traits were proven to promote plant growth, protect plants from phytopathogens, and increase plant stress tolerance *in planta*. Molecular plant-actinomycete interaction using transcriptome analysis towards salinity stress have been demonstrated. **Conclusion:** Extensive understanding of the interaction of endophytic actinomycetes with plants will sustainably enable application of these endophytes as added value biofertilizer and biocontrol agents for green agriculture in the future.

Keywords: actinomycete, endophyte, new species, PGP, stress

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Oral

Attenuation of *Macrobrachium rosenbergii* Nodavirus Through Codon-Deoptimization of Virus Functional Genome

Siti Noor Fatimah Ismail[a], Hui Yee Chee[b], Syarul Nataqain Baharum[a], Chen Fei Low[a]*

[a]*Institute of Systems Biology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia*
[b]*Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

*Corresponding author email: low@ukm.edu.my

Abstract

Introduction: White tail disease (WTD) in the giant freshwater prawn, *Macrobrachium rosenbergii* has been affecting the aquaculture production of the species in several countries, which include Taiwan, China, India, Thailand, Australia and Malaysia. WTD affects larval, post-larval and early juvenile stages of *M. rosenbergii*, which could cause up to 100% mortality of the infected prawn. The disease is caused by *Macrobrachium rosenbergii* nodavirus (MrNV), a non-enveloped virus containing bipartite positive sense single stranded RNA genome. To-date, there is no effective prophylactic and therapeutic agent available to control its outbreak. **Methods:** This study evaluated the attenuation of MrNV through genome-wide multiple point mutation, which deoptimized the codon usage of the MrNV RNA2. A total number of 125 synonymous codon substitutions were introduced into the viral RNA2. Viral genes (wild-type RNA1, RNA2 and mutant RNA2) were *de novo* synthesized and harboured by pUC57 plasmid. Viral genes were expressed through *in vitro* transcription using T7 promoter, which were subsequently transfected into confluent Sf9 cells. Then, the pathogenicity of these infectious clones in the transfected cells were compared. **Results:** Cytopathogenic effects recorded in Sf9 cells transfected with the wild-type MrNV clones include cytoplasmic swelling and aggregation of the infected cells into clumps of various sizes. While cells transfected with mutant MrNV clones observed insignificant cytoplasmic swelling and aggregation. **Conclusion:** Results indicated that codon-deoptimization of viral RNA2 attenuated the MrNV. Further analysis is currently being conducted to evaluate its potential as live-attenuated viral vaccine against WTD in *M. rosenbergii*.

Keywords: *Macrobrachium rosenbergii* nodavirus, reverse genetics, viral vaccine, codon deoptimization

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Transcriptomic Analysis of *Chlorella sorokiniana* for Pigment Production under Moderate Light Intensity

Siti Nor Ani Azaman[a,*], Darren C. J. Wong[b], Sheau Wei Tan[c] , Fatimah M. Yusoff[d], Swee Keong Yeap[e]

[a]*Aquatic Animal Health and Therapeutics Laboratory (AquaHealth), Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*
[b]*Ecology and Evolution, Research School of Biology, The Australian National University, Canberra, ACT 2600, Australia*
[c]*Laboratory of Vaccine and Biomolecules (VacBio), Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*
[d]*Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*
[e]*China-ASEAN College of Marine Sciences, Xiamen University Malaysia, Sepang, Selangor, Malaysia*

*Corresponding author email: s_norani@upm.edu.my

Abstract

Introduction: *Chlorella* can produce an unusually wide range of metabolites under various nutrient availability, carbon source, and light availability. Manipulation of light intensity could induce the formation of secondary metabolites such as pigments, and carotenoids in *Chlorella*. Besides, other metabolic pathways also affected; such as lipids, starch, carbohydrate and isoprenoids biosynthesis pathways. This study was carried out to gain knowledge about how these biosynthetic pathways could be regulated in response to moderate light intensity. **Methods:** De Novo transcriptome profiling of *C. sorokiniana* growth under moderate light intensity was conducted to study the effect of physiological condition towards genetic changes. **Results:** The moderate light intensity used to induce pigment formation caused a total of 937 genes were upregulated, and 1124 genes were downregulated. Genes that were upregulated involved in carotenoids production (specifically lutein biosynthesis), fatty acid biosynthesis, TAG accumulation, and the majority of the carbon fixation pathways. Conversely, starch biosynthesis, sucrose biosynthesis, and isoprenoid biosynthesis were downregulated. **Conclusion:** Novel insights into the pathways that link the enhanced production of pigments in C. s*orokiniana* grown under moderate light intensity is presented.

Keywords: *Chlorella sorokiniana*, *de-novo* transcriptome profiling, metabolic pathways, pigments

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Growth Promotion of Sweet Potato by IAA-Producing Rhizobacteria

Farzana Yasmin*, Asita Elengoe

Department of Biotechnology, Faculty of Science Lincoln University College, 47301 Petaling Jaya, Selangor, Malaysia

* Corresponding author email: farzanayasmin@lincoln.edu.my

Abstract

Introduction: Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria that living on the surface of roots, providing plant growth regulators or phytohormone such as indole-3-acetic acid to improve plants growth and fixed nitogen to plants. This could increase crop productivity and lower N fertiliser input This study was conducted to determine the effect of IAA-producing rhizobacteria isolates on growth of sweetpotato. **Methods:** A pot experiment was conducted in the glasshouse of Universiti Putra Malaysia. Vine cuttings of sweet potato was grown in two kg of sterilized sandy soil The bacterial treatments consisted of : i) Control, ii) SP1 iii) SP2 and iv) *Azospirillum* sp. SP7. An inoculum concentration of 10^9 cfu/ml was applied into the respective pots. Each pot was inoculated at planting and two weeks after planting with five ml inoculum/pot/application. Plants were harvested at 30 days after planting. **Results:**. The results showed that inoculation with IAA producing rhizobacteria significantly improved the early growth of sweet potato plants. Plants inoculation with SP1 and SP2 increased growth (tops and roots), nutrient uptake (N, P, K, Ca and Mg) of the sweet potato plants. bacterial population and IAA concentration in soil. **Conclusion:** This study indicated that the bacterial strains have the potential plant growth promoters and may be used as bioenhancers.

Keywords: Sweet potato, Plant growth promoting rhizobacteria,Indole-3-acetic acid, *Azospirillum* sp., Bioenhancers

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GCMS-based Metabolomics and Effect of Fatty Acids Dietary Supplementation on the Survival and Innate Immunity Response of Hybrid Grouper Against Vibriosis

Maya Erna Natnan[a], Chen Fei Low[a], Chou Min Chong[b], Syarul Nataqain Baharum[a,*]

[a]*Institute of Systems Biology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor D.E., Malaysia*
[b]*Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor D.E., Malaysia*

*Corresponding author email: nataqain@ukm.edu.my

Abstract

Introduction: Grouper is one of the high market value marine fish, that well received in Malaysia. However, due to it easy exposure to infectious diseases such as bacteria, the mass mortality of this fish culture poses a major problem in aquaculture industry. **Methods:** Metabolite profiles from survived control and challenged fingerlings against *Vibrio vulnificus* infection were determined by gas chromatography-mass spectrometry (GCMS). Based on GCMS analysis, several metabolites from fatty acid group showed significant roles regarding differential metabolites between the control and challenged fingerlings. Hence, the fish feed dietary was prepared with the addition of these fatty acids to determine the effectiveness of fatty acid as an immunostimulants on survival, growth, and innate immune response of hybrid grouper (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*) fingerlings against vibriosis infection. **Results:** Several metabolites from fatty acid group such as oleic acid, stearic acid, palmitic acid and behenic acid had been identified to be highly abundant in survived *E. fuscoguttatus* during the preliminary experimental infection against *V. vulnificus*. After six weeks of feeding experiment, fingerlings fed with oleic acid showed the highest specific growth rate (SGR) compared to other dietary. Moreover, after challenged with *V. vulnificus*, fingerlings with oleic acid dietary observed the highest survival rate (60.7%) as compared to control (42.9%) ($p<0.05$). Whereas, in immunology assay; lysozyme activity, respiratory burst activity and phagocytic activity of fingerlings fed with oleic acid showed highly significant differences ($p<0.05$) compared to fingerlings fed with control dietary. **Conclusion:** The finding suggested that oleic acid as modulator used in fish dietary elevated the survival and innate immune response of hybrid grouper which give a potent effect in fish disease resistant.

Keywords: fatty acid, metabolites, immune response, hybrid grouper, vibriosis

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CRISPR-Mediated Generation of *In Vitro* Breast Cancer Models to Study *P53* Gain of Function Mutation

Asmaa Y. I. Abuhamad[a,b], Mohamad Aimanuddin Mohtar[b], Asilah Ahmad Tajudin[c], Amir Syahir Amir Hamzah[d,e*], Saiful Effendi Syafruddin[b,*]

[a]*Department of Cell and Molecular biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia,*
[b]*UKM Medical Molecular Biology Institute (UMBI), Universiti Kebangsaan Malaysia, Cheras 56000, Kuala Lumpur, Malaysia,*
[c]*Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia.*
[d]*Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia.*
[e]*UPM-MAKNA Cancer Research Laboratory, Institute of Bioscience, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia.*

*Corresponding author email: effendisy@ppukm.ukm.edu.my; amirsyahir@upm.edu.my

Introduction: Breast cancer (BC) is the second leading cause of cancer-related deaths among women. Aggressive BC is commonly linked to *P53* gene mutations. The predominant *P53* mutation is missense that can result in either loss of functions or acquisition of novel pro-oncogenic functions (gain-of-function mutation). Establishing robust in vitro models to interrogate mutated *P53* molecular properties and functions is essential. In this study, we embarked on generating three different in-vitro BC models by using CRISPR-mediated gene editing tool. **Methods:** Using the T47D BC cell line that carries the L194F gain-of-function mutation, we targeted P53 in the pooled cells population by stably expressing the Cas9 and P53-targeting sgRNA using the lentiviral transduction approach. The P53 targeting efficiency was assessed using Western blotting. The second model was generated by transiently transfecting the T47D cells with plasmid that encodes for the Cas9 and P53-targeting sgRNA, followed by single cell isolation, clonal expansion, and screening for complete P53 knockout using Western blotting. For the third model, we employed new CRISPR variant called Prime Editing to revert T47D L194F mutation. **Results/Conclusions:** We have successfully established the first and second in-vitro BC models that can be subsequently utilized to study P53 gain-of-function mutations molecular properties and roles in BC. Nonetheless, for the 3rd model, we are still screening for edited single cell-derived clones. We found that the efficiency of Prime Editing technology is very low. Given the fact that this technology was just recently developed, there are definitely plenty of rooms for improvisations and further developments.

Keywords: Breast cancer, Gene editing, Mutp53, CRISPR, Prime Editing

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Tocotrienol-Rich Fraction Lowers the Ratio of Phospho NF-ϰB to NF-ϰB In Retinal Tissue of Diabetic Rats

Muhammad Zulfiqah Sadikan[a], Nurul Alimah Abdul Nasir[a,*], Igor Iezhitsa[b], Renu Agarwal[b]

[a] *Centre for Neuroscience Research (NeuRon), Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh, Selangor, 47000, Malaysia.*
[b] *School of Medicine, International Medical University, Bukit Jalil, 57000 Kuala Lumpur, Malaysia.*

*Corresponding author email: nurulalimah@uitm.edu.my

Abstract
Background and Aim: Diabetic retinopathy (DR) is the second commonest microvascular complication in diabetes mellitus. It is characterized by chronic inflammation associated with increased transcription factor, nuclear factor kappa B (NF-ϰB), expression. Palm oil-derived tocotrienol-rich fraction (TRF), a potent antioxidant and anti-inflammatory substance, may provide protection against DR development. Therefore, we investigated the effect of TRF on retinal NF-ϰB and phospho-NF-ϰB expression in rats with streptozotocin (STZ)-induced DR. **Methodology:** Sprague-Dawley rats weighing 200-250 grams were grouped into normal control rats (NC) and diabetic rats. NC was intraperitoneally injected with citrate buffer, whereas diabetic rats received intraperitoneally streptozotocin (55 mg/kg body weight). STZ-treated rats with blood glucose of more than 16 mmol/L were considered diabetic. Diabetic rats were further subgrouped into diabetic-control (DC) and diabetic-treated (DT) groups. N and DC group received vehicle treatment, whereas DT received TRF treatment (100 mg/kg body weight) via oral gavage daily for 12 weeks. At the end of experimental period, rats were euthanized, and retinal tissues were collected for measurement of NF-ϰB p65 and phospho-NF-ϰB p65 (Ser536) using enzyme-linked immunosorbent assay (ELISA). Results: Greater phospho-NF-ϰB and ratio of phospho- to total NF-ϰB were detected in DC compared to NC (1.85-fold, $p<0.001$ and 1.58-fold, $p<0.05$ respectively), whereas lower expression of the same parameters was observed in DT compared to DC (1.65-fold, $p<0.01$ and 1.57-fold, $p<0.05$ respectively). Total NF-ϰB expression was comparable in all groups. **Conclusion:** Oral TRF may provide protective effect against DR by reducing the activation of retinal transcription factor (NF-ϰB) in streptozotocin-induced diabetic rat.

Keywords: Diabetic Retinopathy, Tocotrienol-Rich Fraction, Streptozotocin, NF-ϰB and Inflammation.

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Oral

In Silico-SELEX Reveals PRDBDapt17 as the Potential Aptamer Against Progesterone Receptor DNA Binding Domain

Tholasi Nadhan Navien, Thean-Hock Tang, Marimuthu Citartan*

Advanced Medical & Dental Institute (AMDI), Universiti Sains Malaysia, Bertam, 13200 Kepala Batas, Penang, Malaysia

*Corresponding author email: citartan@usm.my

Abstract

Introduction: Aptamers are single-stranded DNA or RNA oligonucleotides generated by SELEX that exhibit high binding affinity and specificity against a wide variety of target molecules based on their unique tertiary structural conformation. The tediousness and rigor associated with certain steps of the conventional SELEX intensify the efforts to adopt *in silico*-SELEX in developing DNA aptamers. That said, we report an *in silico* selection of DNA aptamer to the DNA binding domain of progesterone receptor (PR) using ssDNA sequences derived from human progesterone response elements (PREs). PRDBD plays a vital role in the progression of breast cancer, which makes it a promising diagnostic biomarker and potential therapeutic target. **Methods:** Sixty-four different near-native ssDNA analogs of the corresponding PRE sequences were subjected to secondary and tertiary structural determination. Docking of the ssDNA sequences was carried out against PRDBD using PatchDock. The sequence with the highest binding score was selected as the candidate and further subjected to *in vitro* validation using ELASA. **Results:** Among the candidates, we selected the ssDNA sequence (PRDBDapt17; 5'- AGAACAGCGTGTTCT -3'), which showed the highest docking scores of 11334 as a promising PRDBD binding aptamer. The PRDBDapt17 revealed a higher binding affinity towards PRDBD in ELASA. **Conclusion:** In conclusion, PRDBDapt17 is identified as the potential aptamer using the *in silico*-based aptamer selection.

Keywords: *in silico*, aptamer, progesterone receptor DNA binding domain, docking, ELASA

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Ultrasensitive and Label-free Optical Nanobiosensor for miRNA Detection in Breast Cancer

Zheng Wei Wong, and Siu Yee New*

School of Pharmacy, Faculty of Science and Engineering, University of Nottingham Malaysia, Semenyih, 43500 Malaysia

* Corresponding author email: SiuYee.New@nottingham.edu.my

Abstract

Introduction: Breast cancer accounts for 30% of all female cancers and 15% of all cancer-related mortalities. Early detection is known to improve the prognosis and overall survival-rate of breast cancer. Hence, extensive research has been focused on microRNAs (miRNAs) as diagnostic and prognostic biomarkers, for their regulatory role in post-transcriptional gene expression. In breast cancer patients, the expression of miRNA-155 is commonly upregulated as compared to healthy individuals. Herein, we present a nanobiosensor to detect miRNA-155, comprising hybridization chain reaction (HCR) and DNA-stabilized silver nanoclusters (AgNCs), that serve as an enzyme-free amplification strategy and label-free fluorescent detection probes, respectively. **Methods:** Under mild conditions, DNA probes were mixed with miRNA-155 to initiate HCR, followed by the addition of reduced silver salt to form fluorescent AgNCs. Gel electrophoresis was performed to validate the performance of HCR, and a spectrofluorometer was used to measure the fluorescence emission from AgNCs. **Results:** The HCR-AgNCs nanobiosensor showed high selectivity towards the miRNA-155, with capabilities to detect single-base mismatch. Furthermore, the HCR-AgNCs nanobiosensor displayed high sensitivity with a wide linear range between 100 fM and 10 nM, and a LOD of 7 fM. In real sample analysis, the nanobiosensor exhibited exceptional reproducibility and stability with human serum samples. **Conclusion:** In comparison to current breast cancer detectors, the HCR-AgNCs nanobiosensor displayed relatively better performance at a miniscule fraction of cost, effort and time required. Furthermore, the highly-responsive HCR-AgNCs nanobiosensor potentially offers a non-invasive and safe approach towards the clinical detection miRNA-155 and point-of-care early diagnosis of breast cancer.

Keywords: Biosensor, Breast Cancer, Hybridization Chain Reaction, MicroRNA, Silver Nanoclusters

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Bacterial Diversity Analysis in Mangrove Ecosystem of Johor Using Minion Sequencing

Roshan Mascarenhas*, Szer Min Wong, Nicola Lai, Jia Yee Ho, Jeyanthy Eswaran

Newcastle University Medicine Malaysia, No 1, Jalan Sarjana 1, Educity, Iskandar Puteri 79200, Johor, Malaysia

*Corresponding author email: roshan.mascarenhas@newcastle.edu.my

Abstract

Introduction: Mangrove sediments provide rich habitat for diverse bacterial population predominantly members of phyla Proteobacteria, Firmicutes and Actinobacteria. Actinobacteria are predominantly Gram-positive bacteria that make up largest bacterial phyla. Due to their adaptation to challenging habitats they produce abundant bioactive compounds like antibiotics, cytotoxins and enzymes. The increase in pollution of water bodies, emerging antibiotic resistance and loss of habitat makes it important to study bacterial diversity in less explored ecosystems of different geographical areas. Mangrove ecosystem in Johor has witnessed disturbance due to rapid development. Therefore, we sought to measure bacterial abundance, diversity using molecular approach. **Methods:** We sequenced bacterial 16S rRNA gene to identify taxonomical groups using Oxford Nanopore Technologies MinION sequencing. Samples were collected from Mangrove sediments from upstream and downstream from Sungai Melayu, Pulau Kukup locations. Samples were barcoded to allow multiplexing in the same sequencing run and sequencing data was analysed using EPI2ME software. **Results:** Recorded abundant Proteobacteria such as *Erythrobacter litoralis, Erythrobacter vulgaris, Sulfurovum aggregans, Paracoccus marcusii* and Actinobacteria such as *Ilumatobacter fluminis, Ilumatobacter nonamiensis* with variable diversity. *Bacteroidetes* like *Flavobacterium haoranii* was detected in less abundance. Upstream vs downstream analysis of bacterial diversity shows low Proteobacteria and high Actinobacteria in downstream suggesting influence of human activity, pollution in shaping the microbial flora. **Conclusion:** Human activity such as aquaculture, release of household wastewater and agricultural waste poses higher selection pressure and thus some Actinobacteria could have enriched due to their ability to produce bioactive compounds against xenobiotics but pose threat to their diversity.

Keywords: Actinobacteria, MinION-sequencing, mangrove sediments, water quality

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Eco-friendly Silver Nanoparticles (AgNPs) Fabricated by Green Synthesis Using Marine Polychaete, *Marphysa moribidii*

Wan Iryani Wan Ismail[a,b]*, Nur Syakirah Rabihah Rosman[a,b], Noor Aniza Harun[c], Izwandy Idris[d]

[a]*Cell Signalling and Biotechnology Research Group (CeSBTech), Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*
[b]*Biological Security and Sustainability (BioSeS) Research Group, Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*
[c]*Advanced NanoMaterials (ANOMA) Research Group, Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*
[d]*South China Sea Repository and Reference Centre, Institute of Oceanography and Environment (INOS), Universiti Malaysia Terengganu, Terengganu, Malaysia*

*Corresponding author email: waniryani@umt.edu.my

Abstract

Introduction: Silver nanoparticles (AgNPs) have received significant attention because of their antimicrobial activity. However, current methods are expensive and have detrimental effects on the environment. Biological methods e.g., using marine polychaete (*Marphysa moribidii*), which are abundantly found in Malaysian seashore may offer the alternative solution. Therefore, we aim to synthesize and optimize AgNPs production from the polychaete and measure their antibacterial ability. **Methods:** The synthesis process was initiated by adding the aqueous polychaete extract with silver nitrate ($AgNO_3$). Optimization procedures e.g., polychaete size, $AgNO_3$ concentration, pH and temperature were conducted to increase the AgNP stability, yield, and characteristics. The color changes were observed visually and through surface Plasmon resonance (SPR) using UV-Vis spectroscopy. The AgNP formation validation was performed using scanning and transmission electron microscopy (SEM and TEM), Fourier transform infrared (FTIR), and X-ray diffraction (XRD). The antibacterial assessment was carried out using the optimized AgNPs on several bacteria. **Results:** Optimized conditions for obtaining high yield and stable synthesized AgNPs were polychaetes with 6-8 mm of body width, 1 mM $AgNO_3$ with polychaete crude extract of pH 9, preheated at 90 °C for 15 minutes before incubation at 30 °C (150 rpm) for 24 hours, and stored at 4 °C for long-term stability. The analyses were confirmed by an analysis of color variations from pinkish to yellowish-brown, as well as the appearance of SPR bands at 398–400 nm using ultraviolet-visible spectroscopy. The AgNPs were characterized by SEM, TEM, FTIR, and XRD and showed a significant effect ($p < 0.05$) in inhibiting the bacteria. **Conclusion:** The eco-friendly biosynthesized and optimized AgNPs with antibacterial activity were successfully synthesized and characterized in the study.

Keywords: eco-friendly, green synthesis, marine polychaete, nanobiotechnology, optimization, silver nanoparticles

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Preliminary Synthesis of P(3HB-3HV) Using Glycerin Pitch as Main Carbon Source from Cupriavidus malaysiensis USMAA1020

Rozina Kakar, Amirul A. A.*

School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

*Corresponding author email: amirul@usm.my

Abstract

Introduction: Deposition of petrochemical plastic waste in the environment is an evolving challenge in today's world. Researchers have established utterly eco-friendly plastics such as Polyhydroxyalkanoate (PHAs) to discover alternative products. PHA extracted from bacterial cells show commercial properties like polypropylene. Today, the industrialized world is exceptionally dependent on non-renewable fuel sources for commercial procedures and manufacturing structural materials such as plastics, adhesives, coatings, and foams. The present obstacles are the substitute of standard plastics from a non-sustainable source with an eco-friendly resource. Moreover, petrochemical plastics have not just decomposed in nature for many decades, also produced toxins via the destruction process. *Cupriavidus malaysiensis* USMAA1020, a Gram-negative bacterium able to produce biodegradable polymer P(3HB-co-3HV). **Methods:** In this study glycerin pitch was used as carbon source. The biosynthesis of P(3HB-co-3HV) was performed into 50 ml mineral medium in 250 ml Erlenmeyer flasks whereas inoculum (0.10 g/l) was added. The incubation period for this shake flask fermentation was 48 hours at 200 rpm. The presence of 3HB and 3HV was confirmed by the gas chromatography technique following with freeze-dryer and methanonolysis. Experiments performed on different parameters include concentrations of glycerin pitch, 1-pentanol, ammonium sulfate, and different precursors with 1-pentanol: Besides, different oleic acid concentrations were used as a secondary carbon source to enhance the production of PHA. **Results:** It was found that among various mixtures, 2% glycerin pitch, 1.1 g/l ammonium sulphate,1-pentanol 0.06 wt%c and oleic 0.5 wt %C yields PHA content 65%, 3HB (95 mol %), 3HV (4 mol %), RCDW (4 g/l), and PHA concentration (6 g/l.) respectively. **Conclusion:** This study found that glycerin pitch, a byproduct of the biodiesel industry, is a low-cost carbon source capable of producing copolymer.

Keywords: PHA, biodegradable plastics, glycerin pitch, biowastes, polymer P(3HB-co-3HV).

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Bioprospecting and Molecular Identification of Waste Transformer Oil-degrading Bacteria for Bioplastic Production

Shehu Idris[a,b], Rashidah Abdul-Rahim[a], Amirul A.A.[a]*

[a]*School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia*
[b]*Department of Microbiology, Faculty of Science, Kaduna State University, P.M.B 2339, Kaduna, Nigeria*

*Corresponding author email: amirul@usm.my

Abstract

Introduction: One of the major impediments to commercialization of biodegradable plastic is the high cost of substrate. Consequently, there is a continuous search for effective microorganisms and cheaper carbon substrates to reduce the high production cost of these ecofriendly materials. **Methods:** In this study, transformer oil-degrading bacteria were isolated from the environmental samples comprising soil, wastewater, and sediment. The isolation was accomplished using mineral salt medium (MSM) supplemented with 1% waste transformer oil as the sole carbon source. The isolates were screened for polyhydroxyalkanoates (PHA) production using Nile red staining and fluorescence Microscopic techniques. Accumulation of PHA granules were confirmed using Transmission electron microscopy. The oil degradation analysis was accomplished using solvent extraction and gravimetric methods. Bacterial identification was achieved using 16S DNA sequence homology according an established protocol. **Results:** A total of sixty-two (62) transformer oil-degrading bacteria were isolated from the different samples. Sixteen 16(26%) isolates showed a positive result when subjected to qualitative test using Nile red fluorescence microscopy. The bacteria identified belong to four different taxonomic genera of *Acinetobacter*, *Bacillus*, *Proteus,* and *Serratia*. The percentage oil degradation observed among the different isolates ranged between 19.58% to 57.51%. Gas chromatographic analysis of the polymer revealed the presence of medium chain length polyhydroxyalkanoate (mcl-PHA) monomers. **Conclusion:** The findings of this work have further highlighted the diversity of the bacteria capable of utilizing waste stream such as waste transformer oil. Consequently, the isolates can be explored as agents of converting waste transformer oil into bioplastics.

Keywords: Bioprospecting, transformer oil, bioplastic, polyhydroxyalkanoates.

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Oral

Targeting Double Genes in Multiplex PCR: an Innovation in PCR Technology for the Authentication of Food Products

M. A. Motalib Hossain[a]*, Syed Muhammad Kamal Uddin[a], Mohd Rafie Johan[a]

[a]*Nanotechnology and Catalysis Research Centre (NANOCAT), University of Malaya, 50603 Kuala Lumpur, Malaysia.*

*Corresponding author email: motalib_123@yahoo.com

Abstract
Nowadays, consumers' concern about the authenticity of food products is increasing due to the occurrence of food adulteration scandals worldwide. Specifically, food adulteration is of serious religious, health and economic concern. Consequently, researchers have paid more attention to the development of polymerase chain reaction (PCR) based methods for food authentication. However, existing PCR assays are mainly based on a single and a long DNA marker which often breaks down under food processing treatments. Instead of targeting a single gene, double genes targeting short-amplicon length PCR assay would be more reliable because where two different targets are present if one target is broken down due to heat treatment the second target will complement the missing target. It is highly unlikely that both targets would be broken down under food processing. We, for the first time, developed double gene targeting short-amplicon length multiplex PCR assay for discriminating bovine, buffalo and porcine materials in a single assay platform. Mitochondrial cytb and ND5 genes were targeted and all targets were amplified from raw, boiled, autoclaved and microwaved cooked meat under pure and mixed matrices. The detection limit was 0.02 ng DNA under pure states and 0.1% meat in admixtures. Screening of Malaysian meatball products revealed all beef products were buffalo positive in which 35% were totally replaced. In contrast, all pork products were found uncontaminated from beef and buffalo. Thus, this novel assay demonstrated sufficient merits to be used by regulatory bodies for food authentication in any samples even under degraded conditions.

Keywords: Multiplex PCR, Double gene-targets, DNA degradation, Food products, Authentication

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Oral

Production of Spray Dried Sea Cucumber (*Cucumaria frondosa*) By-products Hydrolysate Powder

Lee Sin Chang[a]*, Nur' Aliah Daud[a], Nurul Nadia Muhammad[a], Abdul Salam Babji[b,c], Chin Hoi Chan[d], Chze Chong Tey[e], Seng Joe Lim[a,b]*

[a]*Department of Food Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 4300 UKM Bangi, Selangor D.E., Malaysia*
[b]*Innovative Centre for Confectionery Technology (MANIS), Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia*
[c]*Centre for Innovative and Technology Transfer (INOVASI-UKM), Chancellery, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia*
[d]*Agridon Technologies Sdn. Bhd., Lot 2745-D, Jalan Industri 12, Kampung Baru Sungai Buloh, 47000, Selangor, Malaysia*
[e]*Harmony Marine Products Sdn. Bhd., PLO 210, Jalan Besi, Kawasan Perindustrian Pasir Gudang, 81700, Johor, Malaysia*

*Corresponding author email: joe@ukm.edu.my; leesin@ukm.edu.my

Abstract

Introduction: Sea cucumber is one of the valuable marine animals which is an important nutritious food source. However, approximately 50% biomass (by weight) in the form of visceral are discarded as waste during the processing of sea cucumber, causing an environmental threat. **Methods:** This study was aimed to transform these sea cucumber (*Cucumaria frondosa*) by-products (SC_{byp}) into value-added product. Two enzymes (alkaline protease and exopeptidase) were used to hydrolyse the SC_{byp} and release protein hydrolysate and the characteristics of the spray-dried protein hydrolysate powder were examined. **Results:** The production of SC_{byp} hydrolysate powder was feasible, in which the powder that was treated with maltodextrin ($SCh_{byp}MD$) exhibited better stability than powder without the addition of maltodextrin (SCh_{byp}), with the former possessing significantly ($p \leq 0.05$) lower moisture content (5.50±0.60%dw) and water activity (0.25±0.02). The $SCh_{byp}MD$ also demonstrated significantly ($p \leq 0.05$) lower pH (5.33±0.06), higher total soluble solids (5.07±0.12°Brix) and water absorption index (82.33±2.20%dw) and brighter (L^*=47.51±1.86) than SC_{byp} and SCh_{byp}. Interestingly, the hydrolysates contained all the 9 essential amino acids, corresponding to 39.81% and 36.66% of total amino acids in SCh_{byp} and $SCh_{byp}MD$, respectively. Thermal profiles indicated that melting point of $SCh_{byp}MD$ was higher (114.9°C) than that of SCh_{byp} (94.2°C), due to the protective effect of maltodextrin. **Conclusion:** Converting waste into product not only preserve the environment but also could be potentially profitable to the industry as it can be applied as functional ingredients, animal feed or in nutraceutical product.

Keywords: Enzyme, Hydrolysate, Marine, Powder, Protease

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Nutritive Bambara Groundnut Powdered Drink Mix: Consumer Acceptability, Physicochemical Characterization, and Storage Stability

Wen-Chian Tan, Choon-Hui Tan*

Department of Food Science with Nutrition, Facultyof Applied Sciences, UCSI University, 56000 Cheras, Kuala Lumpur, Malaysia.

*Corresponding author email: tanch@ucsiuniversity.edu.my

Abstract

Introduction: Underutilized crops that have increased resilience to climate change and inherent good nutritional composition are highlighted among scientists in recent years. Therefore, this study aimed to develop and characterize a nutritive powdered drink mix using Bambara groundnut (BGN) that is drought-tolerant crop. **Methods**: BGN powder was blended with soy powder at 0% (0B100S), 10% (10B90S), 20% (20B80S) and 30% (30B70S). Consumer acceptability of this BGN powder was assessed using a 9-point hedonic liking scale. The physicochemical properties (pH, color measurement, proximate analysis, protein and fiber analyses) of the product were determined. The storage stability of the product was determined by enumerating the fungal and bacteria upon storage six months under ambient room temperature. **Results**: Sample 10B90S was the most preferred sample with the highest mean values for aroma (6.22±1.28), flavor (6.92±1.31), grittiness (6.78±1.31), and overall acceptability (6.98±1.15). The concentration of total dietary fiber increased significantly as the BGN percentage in the beverage increased. The insoluble, soluble, and total dietary fiber of sample 10B90S were 11.23±0.024%, 5.31±0.031%, and 16.53±0.043%, respectively. The samples' water activity and microbial enumeration did not have significant ($p>0.05$) change after six months of storage. **Conclusion**: In conclusion, BGN blended with soy powder (10B90S) was acceptable by consumers and could be marketed as a functional food that has improved nutritional values.

Keywords: Bambara Groundnut, Physicochemical, Hedonic liking scale, Storage stability, Proximate analysis

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Detection of Animal Species in Food Chain by Multiplex PCR-Restriction Fragment Length Polymorphism (RFLP) Assay

Syed Muhammad Kamal Uddin*, M. A. Motalib Hossain, Zaira Zaman Chowdhury, Mohd Rafie Johan

Nanotechnology and Catalysis Research Centre (NANOCAT), University of Malaya, 50603 Kuala Lumpur, Malaysia.

*Corresponding author email: syedmdkamaluddin@gmail.com

Abstract

Introduction: The adulteration or misrepresentation of food products is commonplace in the society. Detection of meat species in food products is important for economic, religious and medical perspectives. Among the DNA-based methods, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) techniques are especially applicable in meat speciation because they utilize the sequence variations existing within a selected region of target DNA and allow very closely related species' differentiation with the use of selected restriction enzymes. It offers PCR products authentication through the analysis of restriction-digested products. **Methods:** We developed a simple multiplex PCR-restriction fragment length polymorphism (PCR-RFLP) assay using species-specific primer sets to authenticate highly consumed species such as beef, buffalo, chicken, duck, goat, sheep and pork simultaneously in food products. Short-length amplicons were used targeting mitochondrial cytochrome b (cytb) and NADH dehydrogenase subunit 5 (ND5) genes. Species-specificity was assured through cross-amplification reaction with 25 non-target species. PCR products authentication was performed by digesting with three restriction enzymes namely *Fat*I, *Bfa*I, and *HPY*188I. **Results:** Distinctive fingerprints were obtained for each of the seven targets. The assay could detect as low as 0.5% (w/w) meat in admixed states and food products and it retained its stability under heat treatments of boiling and autoclaving. A market survey on burger and frankfurter revealed a rampant substitution of beef with buffalo while chicken and porcine products were found pure. **Conclusion:** The developed assay could be conveniently used for differential detection of animal species as raw states as well as in meat products.

Keywords: PCR-RFLP, Meat products, Authentication, detection, Short target

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Elucidation of Anthocyanin Biosynthesis Pathway in *Cosmos caudatus* Kunth.

Darvien Gunasekaran, Hamidun Bunawan*

Institute of Systems Biology, Universiti Kebangsaan Malaysia, Bangi 43600, Selangor, Malaysia

*Corresponding author email: hamidun.bunawan@ukm.edu.my

Abstract

Introduction: *Cosmos caudatus* Kunth. or "King's salad", contains high nutritional compounds. Although widely consumed for its medicinal value, information on phytochemical contents and their biosynthesis in the species is scarce. Among the exciting compounds are the anthocyanins that possess a dual role; an antioxidant and natural colorant. **Methods:** In this study, transcriptomic approaches with Illumina Hiseq, metabolomic analysis by Liquid chromatography-mass spectrometry (LC-MS), and anatomy description using light microscopy were used to identify the anthocyanin biosynthesis pathway in different tissues (leaf, stem, flower and root) of *C. caudatus*. **Results:** The transcriptomic analysis revealed nine main structural genes encoding enzymes in the anthocyanin biosynthetic pathway and the genes encoding the transcription factors relevant to the pathway. A total of 11 anthocyanins of cyanidin, pelargonidin, and delphinidin derivatives that are significantly abundant in the species were identified, correlating with the anthocyanin mainstream gene pathway. The occurrence of anthocyanin was further validated by light microscopy. Anthocyanin pigments in *C. caudatus* were detected at the epidermal layer of the leaf, stem, flower, and cortex of stem and root. **Conclusion:** A complete anthocyanin biosynthetic pathway in *C. caudatus* was elucidated using transcriptomics, metabolomics, and anatomical approaches in this study. To our knowledge, this is the first work that has delineated the complete anthocyanin biosynthetic pathway in Malaysia's underutilized plant, *C. caudatus* Kunth. This study correlated multi-omics data that will help to integrate systems biology and synthetic biology for a detailed understanding of the molecular mechanism and characterization of the anthocyanin biosynthesis using heterologous expression studies.

Keywords: *Cosmos caudatus* Kunth.; next-generation sequencing; liquid chromatography; anthocyanin; anatomy

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Cold Gas Spray Hydroxyapatite Coatings with Thermally Grown Interlayer Titanium Oxide on Ti-6Al-4V

Norarney Ahmad[a]*, Norehan Mokhtar[a], Siti Noor Fazliah Mohd Noor[a], Suzianti Iskandar Vijaya[a], Zuhailawati Hussain[b]

[a] *Craniofacial and Biomaterial Sciences Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Kepala Batas, Pulau Pinang, Malaysia*
[b] *School of Material and Mineral Resources Engineering, Engineering Campus, Universiti Sains Malaysia, 14300 Nibong Tebal, Pulau Pinang, Malaysia*

*Corresponding author email: norarney@student.usm.my

Abstract

Introduction: Bioactivity and biocompatibility are important features in improving osseointegration for successful implants placement. Titanium alloy lacks bioactivity and Hydroxyapatite (HA) coating through modified cold gas spraying at room temperature has shown to improve bioactivity and enhance osseointegration. **Objectives:** This study aims to investigate, the morphology, and phase composition of HA coated Ti-6Al-4V with thermally grown titanium oxide as interlayer oxide. **Methods:** The Ti-6Al-4V wafer was oxidized in atmospheric conditioned at different temperature for thermally grown titanium oxide interlayer resulting in wavy surfaces prior to HA coating. The coating was produced by modified cold spray method which used low temperature processing. The thermally grown titanium oxide and coating was characterized by field emission scanning electron microscopy (FESEM) and X-ray diffraction. **Results:** The thermally grown titanium oxide layer maintain its morphology at different temperature. The mixed oxide phase showed reducing wavy structures as the temperature increases with occurrence of rutile and anatase peaks. There is no change in HA phases after coating formation by cold gas spray. The hardness value (HV) increased with increasing the oxidation temperature. **Conclusion:** The thermally grown titanium oxide at 550°C has shown the highest hardness value which prevent the severe crack on the morphology of coated HA.

Keywords: Ti-6Al-4V, Hydroxyapatite, Cold Gas Spray

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Morindone from *Morinda citrifolia* as Potential Antiproliferative Agent on Colorectal Cancer Cell Lines

Cheok Wui Chee[a], Najihah Mohd Hashim[b,c], Nurshamimi Nor Rashid[a,c]*

[a]*Department of Molecular Medicine, Faculty of Medicine, Universiti Malaya, 50603 Kuala Lumpur, Malaysia*
[b]*Faculty of Pharmacy, Universiti Malaya, 50603 Kuala Lumpur, Malaysia*
[c]*Centre for Natural Products Research and Drug Discovery (CENAR), Universiti Malaya, 50603 Kuala Lumpur, Malaysia*

*Corresponding author email: nurshamimi@um.edu.my

Abstract

Introduction: Colorectal cancer (CRC), ranked the third most prevalent cancer worldwide is associated with p53 and KRAS mutation. Crucial role of phytochemical compounds as chemoprevention for CRC is definite, as there is strong correlation between dietary factors and colorectal risk. Anthraquinone compounds which are found in abundance in *Morinda citrifolia* demonstrate various pharmacological properties. **Methods:** Three CRC cell lines, HCT116, LS174T, HT29 cells and normal colon cell line, CCD841 CoN cells were treated with eight anthraquinone compounds obtained from the roots of *Morinda citrifolia* at various concentrations. The cell cytotoxicity and antiproliferation effect of anthraquinones were determined by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) analysis and xCELLigence Real Time Cell Analyzer (RTCA), respectively. Cell cycle analysis was performed with flow cytometry. The gene expression level of p53 and KRAS gene following anthraquinone treatment was evaluated with RT-qPCR and RNA sequencing. **Results:** Based on the MTT analysis, damnacanthal and morindone exhibited significant antiproliferative effects in HCT116 and HT29 cells. The real time impedance measurement showed that the proliferation rate of HCT116 and HT29 cells decreased in dose- and time-dependent manner following treatment with damnacanthal and morindone. Damnacanthal induces G1 cells accumulation in HCT116 cells while morindone induces G1 cells accumulation in HT29 cells. Both RT-qPCR and RNA sequencing data reported that morindone downregulates the p53 and KRAS gene expression in HT29 and HCT116 cells, respectively. **Conclusion:** Therefore, morindone may act as a competitive antiproliferative agent in treating CRC.

Keywords: *Morinda citrifolia*, morindone, anthraquinone, colorectal cancer, antiproliferative activity.

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Tocotrienol-rich Fraction (TRF) Improves Gross Liver Morphology and Liver Histological Changes in Non-alcoholic Fatty Liver Disease (NAFLD) Mice Model

Mohd Danial Mohd Efendy Goon[a,b], Sharaniza Abd Rahim[a], Musalmah Mazlan[a], Siti Hamimah Sheikh Abdul Kadir[a,c]*

[a]*Department of Biochemistry and Molecular Medicine, Faculty of Medicine, Universiti Teknologi MARA (UiTM), Selangor D.E., Malaysia*
[b]*Institute of Medical Molecular Biotechnology (IMMB), Faculty of Medicine, Universiti Teknologi MARA (UiTM), Selangor D.E., Malaysia*
[c]*Institute of Pathology, Laboratory and Forensic Medicine (IPPerForM), Faculty of Medicine, Universiti Teknologi MARA (UiTM), Selangor D.E., Malaysia*

*Corresponding author email: sitih587@uitm.edu.my

Abstract

Introduction: Non-alcoholic fatty liver disease (NAFLD) is a major cause of liver disease worldwide with an estimated prevalence around 37% in Malaysia. It encompasses a spectrum of manifestations strongly associated with the presence of oxidative stress and excessive fat accumulation in the liver. Palm tocotrienol-rich fraction (TRF) has reported as a potential agent in ameliorating the liver of NAFLD subjects. Therefore, this study aimed to determine the effect of TRF supplementation on NAFLD parameters of animal model **Methods:** Twenty-one B6.Cg-LepOb/J (*ob/ob*) male mice were randomly divided into three groups comprised of high-fat diet (HFD; 60% kcal from fat) only, HFD with vehicle palm-kernel oil (PKO; 200mg/kg/day), HFD with TRF (TRF; 200 mg/kg/day) for six weeks. Body weight and food intake were measured each week. After six weeks, mice were euthanized and abdominal circumference and random blood glucose were measured. Liver span were measured prior to being assessed grossly and histologically using haematoxylin and eosin (H&E) and Masson Trichrome staining. **Results:** No differences were observed on abdominal circumference and random blood glucose reading in all groups ($p>0.05$). TRF group recorded with smallest liver span compared to other groups ($p<0.05$). Based on gross liver analysis, TRF groups were less steatotic compared to HFD only and PKO. NAFLD activity score (NAS) was less in TRF group with lower severity of steatosis, lobular inflammation and fibrosis compared to HFD and PKO. **Conclusion:** TRF exhibited profound histological improvement in *ob/ob* mice in comparison with HFD only group.

Keywords: non-alcoholic fatty liver disease, liver steatosis, knockout mice, high-fat diet, tocotrienols-rich fraction,

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Generation of DNA Aptamers against Envelope 2 (E2) Protein of Chikungunya Virus by *in vitro* Systematic Evolution of Ligands for Exponential Enrichment (SELEX) for Diagnostic Application

Andrew Anna[a,b], Sum Magdline-Sia-Henry[c], Marimuthu Citartan[a], Thean-Hock Tang[a]*

[a] *Advanced Medical and Dental Institute, Universiti Sains Malaysia (USM), 13200 Kepala batas, Pulau Pinang.*
[b] *Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak (UNIMAS), 94300 Kota Samarahan, Sarawak.*
[c] *Institute of Health and Community Medicine, Universiti Malaysia Sarawak (UNIMAS), 94300 Kota Samarahan, Sarawak.*

*Corresponding author email: tangth@usm.my

Abstract

Introduction: Chikungunya virus (CHIKV) causes febrile illnesses in human and these cases have rapidly expanded across the globe in recent years. The current antibody-based tests for CHIKV such as ELISA have a variety of limitations associated with the molecules such as batch-to-batch variation, high cost and less stable. Aptamers are single-stranded DNA or RNA that have high affinity and specificity against a wide variety of target molecules. Compared to antibodies, aptamers are cheaper, produced *in vitro*, no batch-to-batch variations and thus serve as a good molecular recognition element for the development of diagnostic tests for CHIKV. **Methods:** Cloning, expression and purification of the recombinant CHIKV E2 was carried out and its identity was verified with western blot analysis. The purified protein was subjected to 9 SELEX cycles, the resulting nucleic acid pools were cloned and sent for sequencing. The secondary structure of the aptamer was predicted using Mfold web server and the performance of the aptamer was determined by enzyme-linked aptamer assay (ELAA). **Result:** The 24kDa recombinant E2 proteins were successfully cloned and purified. The protein was reactive against anti-CHIKV positive sera and anti-CHIKV polyclonal antibody with no cross reactivity with anti-dengue positive pool sera. Sequencing result revealed there were 6 potential candidates of DNA aptamers. DNA aptamer candidate with the highest frequency (61.9%) showed two loops in their predicted secondary structures. ELAA analysis revealed a binding affinity (Kd) of 177.5 nM and limit of detection was 3.3 nM. **Conclusion:** DNA aptamers were successfully generated and it has great potential as a feasible tool in CHIKV detection.

Keywords: Chikungunya virus, E2 protein, DNA aptamer, SELEX

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Expression of Specific Biomarkers toward Microfold Cells Differentiation in 3D Culture of Caco-2 and Raji B Cells

Mizanurfakhri Ghazali[a, b], Sharaniza Ab-Rahim[a], Lakshmi Selvaratnam[c], Mudiana Muhamad[a]*

[a]*Department of Biochemistry and Molecular Medicine, Faculty of Medicine, Universiti Teknologi MARA, Cawangan Selangor, Kampus Sungai Buloh, 47000 Sungai Buloh, Selangor, Malaysia.*
[b]*Institute Medical and Molecular Biotechnology (IMMB), Faculty of Medicine, Universiti Teknologi MARA, Cawangan Selangor, Kampus Sungai Buloh, 47000 Sungai Buloh, Selangor, Malaysia.*
[c] *Dept. of Biomedical Sciences (Anatomy), School of Medicine and Health Sciences, Monash University Malaysia, Jalan Lagoon Selatan, 47500 Bandar Sunway, Selangor D.E., Malaysia*

*Corresponding author email: mudiana_muhamad@uitm.edu.my

Abstract

Introduction: Microfold cells (M cells) are specialized epithelial cells found in follicle-associated epithelia (FAE) of the Peyer's patches, involved during transcytosis of enterocytes pathogen invasion. Differentiation of M cells induces expression of receptors which is recognized by specific M cells markers including alpha 5, beta 1 and Sialyl Lewis A antigen (CA19-9). However, studies on the expression of these markers in 3-dimensional culture system are lacking. Hence, this study aims to investigate the expression of the markers in two types of 3D culture model. **Methods:** The M cells differentiation were carried out in Transwell plate and in alginate hydrogel beads, whereby Caco-2 cells were seeded for 14 days prior to co-culture with Raji B cells. Polarity of the cell monolayer was measured as the transepithelial electric resistance (TEER). At post-21 days of co-culture, total cellular proteins were extracted using RIPA buffer (1X) for Western Blot analysis. **Results:** The TEER value of 270 Ωcm^2 referred to the highly polarised monolayer. The alpha 5 expression was highest in Transwell compared to monolayer however its expression in alginate beads was lower than monolayer culture. Meanwhile, beta 1 expression was reduced in both 3D co-culture systems when compared to monoculture. Interestingly, CA19-9 was not expressed in both monoculture and 3D culture system. **Conclusion:** Both types of 3D culture systems were supportive towards the M cells differentiation, indicated by presence of alpha 5 and beta 1 integrins although at different level of expression. Thus, both integrin proteins are suitable as markers for M cells.

Keywords: microfold cells, 3D system, CA19-9, alpha 5 integrin, beta 1 integrin.

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Phage Enzybiotics Secreted from *Lactococcus lactis* as Potential Antimicrobials

Carumathy Chandran[a], Raha Abdul Rahim[b,c], Erin Swee Hua Lim[d], Khatijah Yusoff[a,c], Adelene Ai-Lian Song[a,c]*

[a]*Department of Microbiology, Faculty of Biotechnology and Biomolecular Science. Universiti Putra Malaysia, 43400, UPM Serdang, Selangor*
[b]*Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences. Universiti Putra Malaysia, 43400, UPM Serdang, Selangor*
[c]*Institute of Bioscience, Universiti Putra Malaysia, 43400, UPM Serdang, Selangor*
[d]*Health Sciences Division, Abu Dhabi Women's College, Higher Colleges of Technology, 41012 Abu Dhabi, United Arab Emirates.*

*Corresponding author email: adelene@upm.edu.my

Abstract

Introduction: Phage therapy is a potential alternative solution to the global antimicrobial resistance problem currently plaguing mankind. Bacteriophages are specific viruses which can lyse bacteria cells through the production of lysin enzymes that breaks down specific bonds in the peptidoglycan of the target bacteria. These lysins, also known as enzybiotics, can be broadly categorized into virion associated peptidoglycan hydrolases (VAPGH) or endolysins. The former locally degrade the bacteria cells during entry of the phage while the latter lyse the cells during release of new progenies at the end of the replication cycle. *Staphylococcus aureus* is an opportunistic pathogen which causes diseases ranging from skin infections to life-threatening meningitis and bacteremia. It is further complicated by emergence of drug resistant *S. aureus*, especially Methicillin Resistant *S. aureus* (MRSA). **Methodology:** In this study, lysins from the staphylococcal Phage 88 was cloned and expressed in *Lactococcus lactis*. *L. lactis*, a generally regarded as safe (GRAS) bacteria have been extensively used for decades in the food industry but more recently has become a model for genetic engineering in lactic acid bacteria. **Results:** The recombinant lysins secreted from *L. lactis* showed lysis activity on plate assays as well as inhibition of *S. aureus* growth in turbidity reduction assay. Additionally, the cell free extracts of the recombinant *L. lactis* was able to inhibit the growth of *S. aureus* by up to 4-log reductions. **Conclusion:** This study implies that the incorporation of recombinant probiotics secreting phage lysins in food starter cultures or cosmetic products may garner added value by inhibiting undesirable pathogens.

Keywords: phage therapy, endolysins, VAPGH, *Lactococcus lactis,* MRSA, antibiotic resistance

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Mutation on Newcastle Disease Virus V protein, Yes or No?

May Ling Tham[a], Khatijah Yusoff[a,b,d], Siti Sarah Othman[c], Suet Lin Chia[a,d]*

[a]*Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[b]*Malaysia Genome Institute, National Institute of Biotechnology Malaysia, Jalan Bangi, 43000 Kajang, Selangor, Malaysia*
[c]*Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[d]*UPM-MAKNA Cancer Research Laboratory, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

*Corresponding author email: suetlin@upm.edu.my

Abstract

Introduction: Newcastle disease virus (NDV) is a negative, single-stranded RNA virus which targets specifically towards avian species causing severe economic losses during an outbreak. The V protein was identified as one of the virulence factors of NDV that antagonises the host innate immunity for successfully viral replication in the host cells. However, detailed investigation of recombinant NDV expressing mutated V protein is scarce. Therefore, V protein was chosen as the factor to study the relation of mutation and pathogenicity. **Methods:** Using overlapping PCR, site-directed mutagenesis was carried out to introduce four premature-stop codon at the V reading frame respectively. The virus was then rescued, propagated in embryonated chicken eggs followed by pathogenicity analysis. **Results:** Only three out of four mutant viruses were successfully rescued. However, the substituted thymine was mutated into cytosine in mutant virus. Therefore, the stop codon was substituted with other amino acids and the V protein was no longer truncated. As a result, pathogenicity of the mutant viruses was not reduced due to substitution of the desired mutation into another nucleotide. **Conclusion:** It appears that an intact V protein is important for viral replication and pathogenicity. This study explored the possibility of V protein mutation in NDV through exploiting genetic engineering and warrants a further investigation on modifying a mutation for successful virus replication.

Keywords: *Paramyxoviridae*, Newcastle disease virus, V protein, C terminal, pathogenicity

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Oral

Recombinant Cell Penetrating Peptides and Intrabodies Targeting Inner Membrane-Bound Mutated KRAS Antigen

Jeremy Jeack Ceen Ng[a], Larsen Alessandro[a], Zhi Xuan Low[a], Jung Yin Fong[a], Audrey Onn Yi Au Yong[b], Minn-E Ng[c], Michelle Yee Mun Teo[a], Hok Chai Yam[a], Lionel Lian Aun In[a]*

[a]*Department of Biotechnology, Faculty of Applied Sciences, UCSI University, No 1, Jalan Menara Gading, UCSI Heights, 56000 Cheras, Kuala Lumpur, Malaysia*
b Department of Life Sciences, Imperial College London, United Kingdom.
c School of Biological Sciences, The University of Edinburgh, Scotland.

*Corresponding author email: lionelin@ucsiuniversity.edu.my

Abstract

Introduction: KRAS is an effector GTPase molecule responsible for signal transduction from ligand-bound epidermal growth factor receptor (EGFR) to the nucleus. The occurrence of specific somatic point mutations in the KRAS gene impairs its ability to regulate signal transduction, and are considered early drivers of carcinogenesis. Targeting this antigen however poses a problem as KRAS proteins are localized along a cell's inner membrane and are not easily accessible. In order to overcome this obstacle, two concepts using (i) cell penetrating peptides (CPPs) and (ii) adenoviruses were introduced. In this study, two different internalization mechanisms, namely endocytosis and viral transduction, were applied for the delivery of anti-mutant KRAS single-chain variable fragment (scFv). **Methods:** The fusion of scFv with an enhanced green fluorescence protein (eGFP) and Antennapedia-PTD (Antp), a CPP, was achieved through SOE-PCR. The first Antp-scFv-eGFP construct was successfully expressed in E. coli (BL21) at a concentration of 0.085 mg/ml. The second scFv-eGFP construct was cloned into an adenoviral vector and recombinant adenoviral particles were harvested from HEK293 cells. Both Antp-scFv-eGFP and adenoviral scFv-eGFP constructs were treated on SW480 and HeLa cells. **Results:** Mutant KRAS scFv binding efficiency between both approaches were compared based on eGFP localization and intensity, where the intrabodies approach was found to be exhibit a higher (3 folds) signal intensity compared to the former. **Conclusion:** In conclusion, the intrabodies approach was evidently concluded to be a more reliable method as its construction was quicker, easier reproducibility, and the intrabodies produced contains a higher green fluorescence intensity.

Keywords: KRAS antigen, inner membrane, cell penetrating peptides, intrabodies, eGFP localization and intensity

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Poster

In Silico Characterization of UGT76G1 Protein in *Stevia rebaudiana* Bertoni Accessions MS007

Nor Iswani Mokthar[a], Afiqah Rahmatullah Khan[b], Zarina Zainuddin[b], Tamil Chelvan Meenakshi Sundram[b], Nurul Hidayah Samsulrizal[b]*

[a]*Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, The National University of Malaysia, 43600 UKM Bangi, Selangor, Malaysia*
[b]*Department of Plant Science, Kulliyyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia*

*Corresponding author email: hidayahsamsulrizal@iium.edu.my

Abstract
Stevia rebaudiana is a member of the Asteraceae family which is being consumed as an artificial sweetener. Stevia has been identified to be safe for human consumption and has been used as an alternative sweetener for diabetic and obese patients. This study used an *in silico* analysis of the transcriptome dataset of *Stevia rebaudiana* accession MS007 to describe the structure and gene content involved in the production of UGT76G1 protein. Homologous search using BLASTp resulting high similarity to UDP-glycosyltransferase 76G1-like *Helianthus annuus* (ID: XP_021973845.1) as the highest percentage of identity. Protein family search using InterPro showed the presence and entry of IPR002213 available at positions 89 to 246. The phylogenetic tree was constructed by selecting 14 out of 100 protein sequences from BLASTp using MEGA X software. The phylogenetic tree analysis showed the same protein family which is Asteraceae. The protein structure prediction of primary, secondary and tertiary of UGT76G1 protein was computed using ProtParam ExPASy, PSIPRED and Phyre2. The tertiary structure prediction of UGT76G1 protein scored 100.0% confidence by the single highest scoring template and coverage of 98% and the dimension of the model is (Å) of X: 52.453, Y: 61.270, and Z: 48.102. The findings of this study will lead to a deeper understanding of the characteristics of UDP-glycosyltransferase 76G1 (*S. rebaudiana* MS007) and enhance target recognition mechanisms, resulting in a better understanding of protein-protein interaction in *Stevia rebaudiana* MS007.

Keywords: Stevia, UGT76G1, phylogenetic, structure prediction.

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Poster

Investigation of the Antibacterial Potentials of the Black Soldier Fly (*Hermetia illucens*) Larvae

An Nie Tee[a], Shun Kai Yang[a], Siew Yoong Leong[b], Eddy Seong Guan Cheah[a]*

[a]*Department of Biological Science, Faculty of Science, Universiti Tunku Abdul Rahman, 31900 Kampar, Perak, Malaysia*
[b]*Department of PetroChemical Engineering, Faculty of Engineering and Green Technology, Universiti Tunku Abdul Rahman, 31900 Kampar, Perak, Malaysia*

*Corresponding author email: cheahsg@utar.edu.my

Abstract

Introduction: As a sustainable approach of waste management, the black soldier fly larvae (BSFL) are commonly used for bioconversion of organic waste. The ability to survive in nutrient-rich environment heavily colonized by microorganisms suggests that BSFL have antimicrobial potentials. This study aimed to investigate the potential antibacterial strategies of BSFL, which could be mediated by their microbiota or endogenous chemical compounds. **Methods:** Both the internal and surface larval microbiota were isolated and identified via the 16S rDNA sequencing. The antibacterial activities of the larval isolates were then evaluated via the cross-streak test. The larvae were also subjected to sequential solvent extraction and the inhibitory activities of the resulting crude extracts tested via the resazurin microplate assay (REMA). **Results:** The identities of the larval isolates were *Providencia rettgeri, Escherichia coli, Morganella morganii, Bacillus cereus, Alcaligenes faecalis, Staphylococcus* spp., *Staphylococcus sciuri, Brachybacterium* sp. and *Corynebacterium casei*, where the former five were internal bacteria and the latter four were surface bacteria. In the cross-streak test, only *B. cereus* yielded an inhibition zone of about 14 mm against MRSA. The growth of MRSA in REMA was inhibited by the chloroform, hexane and methanol extracts; the latter two were also inhibitory against *E. faecalis*. The growth of *S. flexneri* was inhibited by the ethanol (3.75 mg/mL) and methanol (1.875 mg/mL) extracts; the latter was also active against *S. aureus*. All the inhibitions observed were at 7.5 mg/mL, unless otherwise stated. **Conclusion:** The findings of this study suggest that BSFL are potential source of antimicrobial agents.

Keywords: Black soldier fly larvae, *Hermetia illucens*, Antibacterial activity, Larval microbiota, Larval crude extracts

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Poster

In Silico Identification and Characterization of Kaurene Synthase Protein in *Stevia rebaudiana* MS007

Nur Fathiah Rosilan[a], Nor Adilah A.Rani[b], Mohammad Amirul Samsulrizal[b], Zarina Zainuddin[b], Nurul Hidayah Samsulrizal[b], Tamil Chelvan Meenakshi Sundram[b]*

[1]*Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, The National University of Malaysia, 43600 UKM Bangi, Selangor, Malaysia*
[2]*Department of Plant Science, Kulliyyah of Science, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia*

*Corresponding author email: tamil@iium.edu.my

Abstract
Stevia rebaudiana (Sr), belonging to Asteraceae family is a plant native to Paraguay. It is currently being used as a healthier alternative for sugar. *Sr* produces steviol glycosides (SGs), a group of secondary metabolite compounds that is responsible for its sweetening taste. SGs act as sweetener due to the presence of two major compounds, Stevioside and Rebaudioside A. Biosynthesis of these compounds involves enzymes such as geranylgeranyl pyrophosphate (GPPS), copalyl diphosphate synthase (CPPS), kaurene synthase (KS) and kaurene oxidase (KO) in the pathway. In this study, the identification and characterization of *Stevia rebaudiana* MS007 kaurene synthase (*Sr*KS) were done by *in silico* analysis of the transcriptomic dataset. Homology search from BLASTx resulting in *Sr*KSfrom query Cluster-31069.42907 (*Sr* MS007) of transcriptomic dataset shows the highest similarity percentage identity (99.62%). ExPasy tools were used to translate the nucleotide sequence into protein sequence. The protein domain is predicted by protein domain search analysis using Interpro and shows IPR005630 (terpene synthase metal-binding domain) available at positions 454 to 719 and IPR001906 (terpene-synthase-N-terminal-domain) at position 222 to 411 as the domains. In constructing the phylogenetic analysis tree, multiple sequence alignment was initially done using MUSCLE and MEGA-X was used as phylogenetic tree analysis tools. Cluster-31069.42907 shows the relationship between the ancestors, based on the bootstrap value. Bootstrap value of *Helianthus annuus* and *Stevia rebaudiana* is 100% as both the sequences are from the Asteraceae family. This study contributes to a deeper understanding of *S. rebaudiana* MS007 Kaurene synthase through *in silico* analysis.

Keywords: Stevia rebaudiana, Kaurene synthase, Steviol glycosides, Rebaudioside A, Stevioside

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Poster

Antifungal and Antimicrobial Activity of *Solanum nigrum* and *Solanum torvum* Mediated Silver Nanoparticles

Melannie Roberts Samy Muthu, Kanchana Appalasamy , Abirami Sanniraj, Sumitha Samuggam, Suresh V. Chinni*

Department of Biotechnology, Faculty of Science, AIMST University Malaysia, 08100 Bedong Kedah., Malaysia

*Corresponding author email: cvsureshgupta@gmail.com

Abstract

Introduction: *Solanum nigrum* known called as European black nightshade is native for few countries such as America, Australia and South Africa. It is a common herbal plant which is indigenous to India. *Solanum torvum* commonly known as pea eggplant. The herbs of Solanum species have found to be effective against numerous pathogenic fungi and bacteria infection. **Methods**: This study focused on the evaluation of antimicrobial and antifungal activity of silver nanoparticles (AgNPs) by using leaf extract. Several pathological bacterial strains and fungal strains such as *Candida* species were used to study the antimicrobial and antifungal activity of *Solanum sp.* **Results:** The zone of inhibition obtained by disc diffusion method and dilution method indicates the inhibition of microbial and fungal growth. The synthesized *Solanum nigrum* and *Solanum torvum* based silver nanoparticles were found to have antimicrobial and antifungal properties. **Conclusion:** These results indicate the potential applications of the medicinal plants to the field of nanotechnology.

Keywords: *Solanum nigrum*, *Solanum torvum*, silver nanoparticles, antimicrobial, antifungal

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Poster

The Effectiveness of Red Palm Oil in Preventing Alzheimer's Disease Induced in Rat

Siti Norliyana Che Mat Zubaidi [b]*, Shariff Halim[a], Zamzarina Mohd Kasini[a], Nur Ezza Fazleen[a], Vigneswary Thiruselvam[b], Vilaasyini Rajagopal[b]

[a]*Neuroscience Research Group, International Medical School, Management & Science University, University Drive, Off Persiaran Olahraga, 40100 Shah Alam, Selangor, Malaysia*
[b]*Bachelor in Medical Sciences, International Medical School, Management & Science University, University Drive, Off Persiaran Olahraga, 40100 Shah Alam, Selangor, Malaysia*

*Corresponding author email: drhalim_shariff@msu.edu.my

Introduction: Alzheimer's disease is a chronic irreversible neurodegenerative brain disorder. In a brain of Alzheimer's disease patient, there is disposition of senile plaque in the extracellular space of the brain and neurofibrillary tangles (NFT) within the intracellular space of neuron. One of the causes that lead to these formation is high level of ROS within the brain that finally induce and increase the formation of NFT and senile plaque. Hence, this study was carried out to determine the effectiveness of red palm oil (RPO) in preventing the Alzheimer's disease induced in rat as RPO is one of the natural sources that rich with anti-oxidant. **Methods:** Forty male Sprague-Dawley rats were divided into 5 groups (n=8) that comprised of two groups that were administered by RPO daily (200 and 400 mg/kg), induced by D-Galactose only (negative control), donepezil administration (0.25 mg/kg) (positive control) and normal saline (control). This pre-treatment groups were given RPO through oral gavage. Y-maze spontaneous alternation test was done every week to evaluate the spatial working memory of the rats in exploring new area. After 21 days, the blood sample was collected to measure the level of GSH, MDA, SOD, TAC and dopamine through Elisa test kit. **Results:** Results showed low level of MDA and high level of dopamine, GSH, SOD and TAC in rats treated with RPO and donepezil compared to the rats that were induced with D-Galactose only after 21 days of pre-treatment ($p<0.05$). On the other hand, the group of rats that were treated with RPO and donepezil also showed significant improvement in exploring new area ($p<0.05$). **Conclusions:** Red palm oil has showed significant evidence of anti-oxidant effect in attenuating the oxidative stress in Alzheimer's disease by lowering down the level of MDA and improving the spatial memory of the rats.

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Poster

Review: CRISPR-Cas9 as a Cancer Therapy

Ali Adil Salim*

Department of Pathological Analysis, Faculty of Health Medical Techniques, University of AL-Furat AL-Awsat Technical, 54001, Najaf, Iraq

*Corresponding author email: aliadil41994@gmail.com

Abstract
Cancer is a hereditary disorder caused by a series of genetic and epigenetic errors. It is one of the leading causes of death worldwide and still a major social and economic challenge. According to data, about 8.5 million people die each year as a result of cancer's lethality, and cancer rates are expected to rise by 50% in the next ten years, resulting in around 15 million deaths. There are many forms of cancer such as single or several gene alterations, chromosomal abnormalities, and translocations. Although, several approaches have been used to diagnose cancer, but they are still insufficient in cancer combating. Thus, scientists are investigating a variety of emerging methods for early cancer detection. Therefore, one of the most recent, simple, and effective technologies that has been used during the last few years in gene editing and cancer therapy is; Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-associated protein-9 (Cas9), a unique RNA domain-containing endonuclease-based genome engineering technology. In simple, CRISPR/Cas9 have been developed from a bacterial defense mechanism against virus infection. Recently, this technique showed its effectivity in the treatment of cancer and gene editing. CRISPR/Cas9 use the homology-directed repair and non-homologous end joining pathways to edit the genome. Moreover, CRISPR/Cas9 can also be used to quickly engineer immune cells and oncolytic viruses for cancer immunotherapy. In general, this paper reviewed this influential mechanism and its components. Also, this paper shows the pros and cons of this mechanism in details. Specifically, in this paper, we highlight the possible uses and recent developments of CRISPR/Cas9 in cancer treatment, as well as the difficulties that might be faced during clinical studies. In this regard, we hope to contribute to optimizing work CRISPR/Cas9 as well as focusing on showing the possible future directions of this technology.

Keywords: CRISP, Cancer, Modern Technology, Cas9, Therapy.

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Poster

Elucidating the Effectiveness of *Elaeis Guineensis* on Sex Hormone in PCOS Induced Rats

Vigneswary Thiruselvam*

International Medical School, Management and Science University, 47150 Puchong, Selangor

*Corresponding author email: vigneswarythiruselvam@gmail.com

Abstract

Introduction: Elaeis Guineensis is commonly known as palm oil which is known to have many medicinal uses as they are rich in nutritional values. Several studies have reported that the nutritional content of the palm oil showed a positive effect on improving PCOS. Therefore the current study is intended to identify the mechanism of Elaeis Guineensis on improving induced PCOS in rats. **Methods:** Forty Sprague Dawley rats were divided into 5 experimental groups with 8 rats in each group. The drugs and the palm oil were given to the rats orally for 28 days. The rats were given 1mg/kg of Letrozole diluted with 0.9% NaCl to induce PCOS (4 groups). Negative control group were not given any induction or treatment. Positive control group were given 100mg/kg of Metformin dissolved in 0.9% NaCl followed by letrozole. Group 1 and Group 2 were given 200mg/kg and 400mg/kg of palm oil respectively followed by letrozole. Group 3 were only given Letrozole. The Estrous cycle of the rats were monitored throughout the research. At the end of the research period the rats were sacrificed and the blood samples were collected to evaluate the serum level of sex hormones such as LH, FSH, Estrogen, Porgestrone and testosterone using ELISA kit. The ovaries were removed to study on the histopathology of PCOS. **Results:** LH, testosterone and Estrogen level in group 2 (400mg/kg) showed a significant decrease compared to other groups ($p<0.05$). FSH and Progesterone level in group 2 (400mg/kg) increased significantly ($p<0.05$). The number of cyst decreased and the number of primary follicle, antral follicle, graafian follicle and corpus luteum were increased in group 2 (400mg/kg). **Conclusion:** From this study it is evident that Elaeis Guineensis has the ability to improve the level of sex hormones and tissue symptoms of PCOS.

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Poster

Over-Expression and Purification of *Acinetobacter baumannii* Hfq Protein in BL 21 Cells.

Kanchana Appalasamy[a], Akram Hossain KM[a], Sumitha Samuggam[a], Sridevi Viswanathan[b], Suresh V. Chinni[a]*

[a]*Department of Biotechnology, Faculty of Science, AIMST University, 08100 Bedong Kedah., Malaysia*
[b]*Unit of Biochemistry, Faculty of Medicine, AIMST University, 08100, Bedong, Kedah, Malaysia*

*Corresponding author email: v_suresh@aimst.edu.my

Abstract

Introduction: *Acinetobacter baumannii* is a coccobacillary gram-negative bacteria that survives well in hospital environment. It readily infects human by silencing immune responses. The strain is often multidrug resistant; thus, new strategies are required to combat the pathogen. Recently, non-protein coding RNAs emerged as a mediator of bacterial virulence regulation and performing their function by binding to RNA chaperone Hfq protein, forming ribonucleoprotein complex. In this study, we would like to establish a method to identify novel npcRNAs by sequencing cDNA library of Hfq protein bound RNA of *A. baumannii*. **Methods:** The Hfq gene from *A. baumannii* was amplified and cloned into pET-28b+ vector. Ligated mixture was transformed into TOP-10 cells and this transformed bacterial colony was screened by antibacterial (Kanamycin) selection and then further confirmed using PCR technique. The recombinant plasmid was transformed into *E. coli* BL21 and induced with IPTG for Hfq over-expression. **Results:** The over expressed Hfq protein was purified using Ni-NTA affinity chromatography. A highly purified Hfq protein was obtained by elucidation and confirmed by SDS PAGE analysis. The purified Hfq protein were mixed together with npcRNA of *A.baumannii* to identify all the possible npcRNA binding to Hfq protein and sent it for sequencing. **Conclusion:** We had successfully purified Hfq protein from *A. baumannii*. The identification and characterization studies will be carried out by RNA binding study for the selection of the npcRNA that binds to the protein.

Keywords: *Acinetobacter baumannii,* Hfq, npcRNA, over-expression, protein

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Poster

Transcriptome Analysis of *S.* Typhi Treated with Durian Waste (rind) Mediated Silver Nanoparticles

Sumitha Samuggam[a], Savithri Shanmugam[a], Ravichandran Veerasamy[b], Suresh V. Chinni[a,*]

[a]*Department of Biotechnology, Faculty of Applied Sciences, AIMST University, 08100 Bedong, Kedah, Malaysia.*
[b]*Pharmaceutical Chemistry Unit, Faculty of Pharmacy, AIMST University, 08100 Bedong, Kedah, Malaysia.*

*Corresponding author email: cvsureshgupta@gmail.com

Abstract

Introduction: *Durio zibethinus* is a climacteric, seasonal tropical fruit of Southeast Asia which has high nutritional value and rich bioactive properties. Many research have been done in exploring the potential food value of the edible and non-edible parts of durian. Yet, during durian season, a huge amount of durian waste in the form of seeds and rind or shell were disposed which leads to environmental pollution. In the present research, we aimed to study the transcriptome of *S.* Typhi treated with durian waste (rind) mediated silver nanoparticles. **Methods:** The synthesized silver nanoparticles were incubated with *S.* Typhi and the total RNA was extracted. These total RNA were sequenced via Illumina HiSeq 2000 platform. The fastQ format transcriptome sequences were analysed using bioinformatics software tools such as Trimmomatic and Bowtie2. Un-annotated intergenic regions were screened for the possible novel ncRNA candidates using Artemis. Differential expression of exponential phase and synthesized durian waste (rind) mediated silver nanoparticles in *S. T*yphi transcriptomes were analysed using HTSeq and DESeq software. **Results:** A total of 4290 coding genes, 1533 and 2131 showed significantly up and down regulations, respectively. The metabolic pathways of selected significantly differential expression mRNAs were studied. **Conclusion:** This preliminary transcriptome analysis data reveals interesting insights in gene regulation of *S.* Typhi treated with durian rind silver nanoparticles.

Keywords: silver nanoparticles, transcriptome, *S.* Typhi, *Durio zibethinus*

Acknowledgement: We would like to acknowledge Ministry of Higher Education for financing this work under Fundamental Research Grant Scheme (FRGS) FRGS/1/2015/SKK08/AIMST/02/1

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Poster

Renal Protective Effects of *Trigonella Foenum-Graecum* Seeds on Morphine Withdrawal Rats - A Review

Sutha Sharminni Krishnan[b]*, Ashok Kumar Jeppu[a], Shariff Halim[a], Thur Sina Alkesah[c]

[a]*Neuroscience Research Group, International Medical School, Management & Science University, University Drive, Off Persiaran Olahraga, 40100 Shah Alam, Selangor, Malaysia*
[b]*Bachelor in Medical Sciences, International Medical School, Management & Science University, University Drive, Off Persiaran Olahraga, 40100 Shah Alam, Selangor, Malaysia*
[c]*Postgraduate Candidates, International Medical School, Management & Science University, University Drive, Off Persiaran Olahraga, 40100 Shah Alam, Selangor, Malaysia*

*Corresponding author email: ssharminni@gmail.com

Abstract
Morphine is an opioid drug, that widely used for treating chronic pain caused by post-operations and cancer. It is considered as gold standard opioid therapy because it has the potential to exhibit analgesic property. However, prolonged morphine administration can trigger induce tolerance to its analgesic effect. Thus, a higher dose of morphine is needed in order to overcome the tolerance and attain the same pharmacological effect. But, over time, morphine withdrawal can occur, if the amount of opioid drug intake is not sufficient to get the desired pharmacological effect or when changed to the use of other opioid drug therapy. Simultaneously, there are several adverse effects when morphine is abused. This is because a high dose of morphine administration is capable of triggering the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Thereby, it can lead to a condition called oxidative stress that can induce damage to the cells. Since, the liver performs its role in drug metabolism and the kidney in detoxification, both these organs can be highly affected due to morphine administration. On the other hand, the plant called fenugreek, which has the scientific name *Trigonella-Foenum Graceum*, is well known for its antioxidative properties. The presence of polysaccharides, flavonoids, polyphenols along with minerals such as calcium, zinc and magnesium, primarily support the role of fenugreek seeds as an antioxidant. Hence fenugreek seed extract, may have a renal protective effects on the morphine withdrawal rats.

Keywords: Morphine, Fenugreek, Oxidative stress, *Trigonella-Foenum Graceum,* kidney

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Isolation and Characterisation of Probiotic Bacteria from Livestock and Kimchi and Assessment of Their Antibacterial Potentials

Annie Mei Tin Low, Chong Lay Khoo, Yi Wen Lim, Yun Ru Tan, Yee Han Chan, Gabriel Kallang Chun Leng Jong, Eddy Seong Guan Cheah*

Department of Biological Science, Faculty of Science, Universiti Tunku Abdul Rahman, 31900 Kampar, Perak, Malaysia

*Corresponding author email: cheahsg@utar.edu.my

Abstract

Introduction: Probiotics are microorganisms that are not harmful to the consumers but rather, they confer health benefits. They are mainly bacteria and are usually found in the animal gastrointestinal tracts and fermented foods. The aim of this study was to isolate and characterise potential probiotics from several of these sources. **Methods:** The samples tested include chicken intestines and gizzards, pig intestines, cow intestines and faeces, and kimchi, which were homogenised in saline and then cultured on the de man, Rogosa and Sharpe (MRS) agar. The resulting bacterial isolates were assessed for their antibacterial potentials and then identified by the 16S rDNA sequencing. **Results:** Among the isolates obtained include *Lactobacillus pentosus*, *Lactobacillus reuteri*, *Lactobacillus fermentum*, *Leuconostoc mesenteroides*, *Lactobacillus sakei*, and *Lactobacillus plantarum*. The former three were from livestock sources while the rest were from kimchi. In the well diffusion assay, the culture filtrates of *L. pentosus*, *L. plantarum*, *L. mesenteroides*, and *L. fermentum* were shown to be inhibitory against laboratory strains of *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Shigella* sp. Some of these were also inhibitory against the bacteria isolated from the milk of a goat with mastitis. Their inhibitory effects diminished post-neutralisation, thus suggesting the role of the organic acids produced in these. The probiotic isolates were preliminarily shown to be able to tolerate 0.3% bile but only two were acid-tolerant (pH 2). **Conclusion:** The outcomes from this study may contribute to development of novel probiotic consortia that can be commercialised for use in animal feed in livestock farming.

Keywords: Probiotics, *Lactobacillus*, Livestock, Kimchi, Antibacterial activities

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Poster

Antagonistic and Inhibitory Potentials of some Natural β-Carboline Alkaloids against 5-HT1A Homology Receptor as Potent Antidepressants

Yusuf Oloruntoyin Ayipo[a,b], Sani Najib Yahaya[a,c], Waleed A. Alananzeh[a], Mohd Nizam Mordi[a]*

[a] *Centre for Drug Research, Universiti Sains Malaysia, USM 11800, Pulau Pinang, Malaysia.*
[b] *Department of Chemistry, Kwara State University, P. M. B. 1530, Malete, Ilorin, Nigeria.*
[c] *Department of Pharmaceutical and Medicinal Chemistry, Bayero University, Kano, Nigeria.*

*Corresponding author email: mnizam@usm.my

Abstract

Introduction: Serotonin antagonists and reuptake inhibitors (SARIs) are atypical antidepressants with pharmacological application on major depressive disorder, and oftentimes, co-administered with selective serotonin reuptake inhibitors (SSRIs) to mitigate SSRI-induced aftereffects such as insomnia and sexual dysfunction. Only few of this important drug class are available and not without serious side effects including priapism and hyponatremia symptoms. Natural β-carboline alkaloids are reported with spectrum of neuropharmacology against several psychiatric disorders including anxiety and depression. However, their potentials as SARIs remain underexplored. This study is aimed at identifying potent SARIs among 55 β-carboline alkaloids from natural products. **Methods:** Maestro Glide docking, Swiss ADMET and VEGA ToxRead mutagenic simulations. **Results:** Selectively, monoacetylarenarine B, arenarine B, cordysinin E, ingenine E and eudistomidin A show strong binding affinity as docking scores, -5.713, -5.865,-6.204, -5.815 and -6.011 kcal/mol respectively compared to the positive controls, fluoxetine, trazodone and WAY100635 with -6.126, -5.980 and -5.196 kcal/mol respectively. The negative controls, albendazole and metforming display lowest scores, -3.835 and -1.601 kcal/mol respectively, thereby validating the docking protocols. The selected compounds occupy similar binding pockets of 5-HT1A homology receptor and interact with pharmacologically essential residues in the receptor active site, Asp 116, Asn 386 and Phe 362 referenced to the antidepressant standards. The phytochemicals, from *Arenaria kansuensis, Psilocybe* mushrooms, *Acanthostrongylophora ingens, Annona foetida* and *Eudistoma* spp., possess good ADMET, druggability and mutagenicity parameters ideal of lead-like candidates. **Conclusion:** The study models a faster, cheaper and environmental friendly approach to identify potent SARIs from natural β-carboline alkaloids for further experimental studies.

Keywords: 5-HT1A receptor, molecular docking, β-carboline alkaloid, natural products, antidepressant

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Poster

Phytochemical Composition and Antioxidant Potential of Methanolic Extract of Filamentous *Anabaena* sp. and Unicellular *Microcystis* sp.

Reem Abdulsalam Dawood Al-Nedawe[a], Zetty Norhana Balia Yusof [a,b,c]*

[a]*Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.*
[b]*Aquatic Animal Health and Therapeutics Laboratory (AquaHealth), Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.*
[c]*Bioprocessing and Biomanufacturing Research Center, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.*

*Corresponding author email: zettynorhana@upm.edu.my

Abstract

Introduction: Cyanobacteria (blue-green algae) are a diverse group of photosynthetic, prokaryotic micro-organisms found in fresh and marine waters. They produce a diversity of secondary metabolites having potential activities such as antibacterial, anticancer, antiviral, antioxidant, and other pharmacologically active compounds. Phytonutrients and pigments present in cyanobacteria have been shown to act as antioxidants, which facilitate the formation of the body's defence mechanism against free radical damage to cells. **Methods:** In this study, methanolic extract of *Anabaena* sp. and *Microcystis* sp., which are indigenous Malaysian cyanobacteria species was evaluated for their total phenolic contents (TPC), total flavonoid contents (TFC), carotenoids, and antioxidant activity. TPC, TFC and carotenoids were analysed spectrophotometrically while 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays were carried out to determine the antioxidant activity. **Results:** the results showed that the methanolic extract of *Anabaena* sp. contains higher TPC (43.05±7.2 mg GAE/g) and TFC (99.83±3.56 mg QE/g) while *Microcystis* sp. methanolic extract showed higher amount of carotenoids (9.890 µg/g) and higher antioxidant potential through DPPH assay (54.04±0.347 %). **Conclusion:** The present study revealed that *Anabaena* sp. and *Microcystis* sp. are excellent sources of natural products representing both filamentous and unicellular classes of cyanobacteria respectively which could be explored for various purposes specifically for human health and well-being.

Keywords: *Anabaena* sp., *Microcystis* sp., phytochemical, antioxidant

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Poster

Suppressing Cinnamate-4-hydroxylase (C4H) Gene Using RNA and CRISPR Interference Systems Increases the Expression of Flavonoid-related Genes in Ginger and Tobacco

Chou Khai Soong Karlson, Boon Chin Tan*

Centre for Research in Biotechnology for Agriculture (CEBAR), University of Malaya, 50603 Kuala Lumpur, Malaysia

*Corresponding author email: boonchin@um.edu.my

Abstract

Introduction: *Boesenbergia rotunda*, commonly known as fingerroot ginger, produces valuable compounds, including panduratin A, pinostrobin, and pinocembrin. However, the low abundance of these compounds in nature limits their industrial applications. Cinnamate-4-hydroxylase (C4H) is a vital enzyme located in the first branching point in the flavonoid biosynthetic pathway. The re-direction of the metabolic flux after silencing the branching pathway may enhance flavonoid production in plants. This study demonstrated how C4H silencing affects flavonoid-related genes in *B. rotunda* and *Nicotiana tabacum* cell suspension cultures. **Methods:** The partial cDNA of *C4H* isolated from *B. rotunda* was cloned into an RNAi vector. To construct a CRISPRi vector, sgRNA sequences targeting the promoter region of *C4H* were designed. Both constructed vectors were introduced into the established *B. rotun*da and *N. tabacum* cell suspension cultures. The putative transformants were analysed using PCR. The expression of *C4H* and flavonoid-related genes were determined using qPCR. **Results:** RNAi and CRISPRi silencing vectors targeting *C4H* were successfully constructed and introduced into *B. rotun*da and *N. tabacum* cell suspension cultures. About 50% of *B. rotun*da and *N. tabacum* regenerants were transformed. Both RNAi and CRISPRi vectors effectively reduced *C4H* expression. **Conclusion:** This study showed that C4H is a key enzyme in modulating flavonoid production.

Keywords: Metabolic Engineering, Flavonoids, CRISPRi, Gene silencing, Cell suspension culture

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Poster

Generation of Superfolder Green Fluorescent Protein Variants as Non-invasive Reporters in Plants

Wai Keat Toh, Xin Yen Tor, Yuh Leng Teo, Pek Chin Loh, Hann Ling Wong*

Department of Biological Science, Universiti Tunku Abdul Rahman (UTAR), Kampar Campus, Jalan Universiti, Bandar Barat, 31900, Kampar, Perak, Malaysia.

*Corresponding author email: hlwong@utar.edu.my

Abstract

Introduction: Green fluorescent protein (GFP) is the most widely used non-invasive reporter proteins to-date. However, the interference of plant auto-fluorescent compounds with GFP fluorescence when excited at 480 nm, remains a major complication in plant research. Hence, improvements in expression efficiency and fluorescent intensity are required for GFP to be a useful marker in intact plants. Recently, superfolder GFP (sfGFP) has been developed as a robust folding reporter. **Methods:** To enhance the performance of sfGFP as a reporter protein in plants, site-directed mutagenesis and random mutagenesis were performed. The engineered *sfGFP* variants were cloned, expressed, and subsequently purified. Purified sfGFP variants were then subjected to fluorescence spectroscopy to identify their respective excitation and emission spectra. Lastly, the engineered *sfGFP* variants were separately cloned into a plant expression vector and *Agrobacterium*-mediated transient transformation of *Nicotiana benthamiana* leaves was performed. **Results:** Through site-directed mutagenesis at S65 and/or T203, a total of three sfGFP variants, namely sfGFPuv, sfYFP and sfYFPuv were generated. Next, using yellow fluorescent proteins (YFPs) Venus and EYFP as references, *sfYFPuv* was subjected to further site-directed mutagenesis to generate *esfYFP*. Moreover, using *sfYFPuv* as template, an exceptional brighter variant, *esfYFPuv* was obtained through random mutagenesis. A novel point mutation which caused the exceptional brightness in *esfYFPuv* was revealed through DNA sequencing. **Conclusions:** A total of five sfGFP variants, namely sfGFPuv, sfYFP, sfYFPuv, esfYFP and esfYFPuv were constructed and were proven to be functional *in planta*. Lastly, a novel mutation that may enhance GFP brightness under UV excitation was discovered.

Keywords: *Agrobacterium*-mediated transient transformation, fluorescent protein, reporter gene, sfGFP

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Poster

Tandem Mass Tag-Based Quantitative Proteomics Analysis Reveals Prospective Drought Response Mechanism in *Pandanus amaryllifolius*

Muhammad Asyaf Amnan[a], Wan Mohd Wan Kamaruddin[b], Fiqri Dizar Khaidizar[a], Boon Chin Tan[a]*

[a] *Centre for Research in Biotechnology for Agriculture (CEBAR), University Malaya, 50603 UM, Kuala Lumpur, Malaysia*
[b] *Institute of Systems Biology (INBIOSIS) Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan, Malaysia*

*Corresponding author email: boonchin@um.edu.my

Abstract

Introduction: Drought is one of the major threats to the agricultural sector and food security. Drastic changes in morphology and molecular mechanisms are crucial for plant survival under unfavourable environments. *Pandanus amaryllifolius*, a moderate drought-tolerant plant, is well known for its ability to survive in prolonged low-level soil moisture conditions. Understanding the molecular regulation of drought stress signalling in this plant is important to help guide the rational design of crop plants to counter these environmental challenges. This study aimed to determine the morpho-biochemical and protein changes of *P. amaryllifolius* in response to drought stress. **Methods:** *P. amaryllifolius* plants were subjected to drought treatment by withholding water for 4, 7, 10, and 14 days. The 7-day drought-treated plants were then re-watered. Morphological changes, such as plant height, relative water content (RWC), relative electrolyte leakage (REL), and antioxidant enzyme activities, including superoxide dismutase, peroxidases, ascorbate peroxidase, catalase, glutathione reductase, proline, and malondialdehyde (MDA), were recorded. Total protein of the leaf samples was extracted, labelled with tandem mass tags reagent, and analysed by LCMS/MS. **Results:** Drought stress significantly affected the morpho-biochemical of plants. The RWC and height for drought-treated plants were significantly reduced, while REL, antioxidant enzymes activities, proline, and MDA were significantly increased. Of the 1,415 identified proteins, 134 proteins were significantly changed in each treatment comparison. These proteins are predominantly involved in carbohydrate metabolism, metabolism of cofactors and vitamins and genetic information processing. **Conclusion:** This study provides an important and valuable drought-responsive proteome dataset for the future crop improvement programme.

Keywords: Drought stress, antioxidants, liquid chromatography mass spectroscopy, tandem mass tags, pandan

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Poster

Proteomic Analysis of Giant Freshwater Prawn, *Macrobrachium rosenbergii* Eyestalks throughout Different Molting Stages

Amy Raihanah Husain[a], Kenneth Francis Rodrigues[b], Syarul Nataqain Baharum[a], Benjamin Yii Chung Lau[c], Wan Mohd Aizat Wan Kamaruddin[a], Chen Fei Low[a]*

[a]*Institute of Systems Biology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia*
[b]*Biotechnology Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia*
[c]*Proteomics and Metabolomics (PROMET) Group, Malaysian Palm Oil Board, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia*

*Corresponding author email: low@ukm.edu.my

Abstract

Introduction: Giant freshwater prawn or *Macrobrachium rosenbergii* is a native species of Malaysian freshwaters. This species has been farmed for commercial purposes in Malaysia since the 80s, nevertheless, our national production for the past years were not impressive. It is said that poor growth performance such as growth retardation and heterogenous growth among individuals in the same proximity are the setbacks for the industry to obtain maximum production for the year. Since molting is the principle of growing for this species, comprehensive understanding of molting cycle mechanism on molecular level is important. **Methods:** In this study, differentially expressed proteins (DEPs) throughout different molting stages of *M. rosenbergii* were studied using high-throughput proteomic approach. Three molting stages of post-, inter- and pre-molt were involved with three biological replicates for each, making it in total of nine samples. **Results:** Overall, a total of 369 proteins were identified from the samples and 17 proteins were significantly up- or down- regulated from all nine samples. DEPs in pre-molt are hypothesized to be assisting the ecdysis to happen, thus, focuses on these proteins are crucial. Out of 17 DEPs, 6 DEPs were found in pre-molt stage namely ribosomal protein S3A, crustacean calcium-binding protein 23-like, elongation factor 1-gamma-like, putative clotting protein, uncharacterized protein LOC119585594 and Histone 2A. **Conclusion:** Here, we presented the proteins that could potentially help in speeding up the molting cycles in *M.rosenbergii*. These findings are advantageous to the species farming industry as they eventually may perhaps will be useful in growth strategy planning.

Keywords: Giant freshwater prawn, molting, ecdysis, eyestalks, proteomic

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Poster

Metabolite Profiling of 50% Ethanolic Extraction of *Clinacanthus nutans* by Using GC-MS and LC-MS/MS and Cytotoxicity Analysis of Succinic Acid, Betulinic Acid and Artemisnin on J774.2 Macrophage Cell Line

Ravi Kumar Kalaichelvam[a], Vuanghao Lim[b], Ida Shazrina Ismail[a]*

[a]*Regenerative Medicine Cluster, Institut Perubatan dan Pergigian Termaju (IPPT), Universiti Sains Malaysia (USM), 13200, Bertam, Pulau Pinang, Malaysia*
[b]*Integrative Medicine Cluster, Institut Perubatan dan Pergigian Termaju (IPPT), Universiti Sains Malaysia (USM), 13200, Bertam, Pulau Pinang, Malaysia.*

*Corresponding author email: idashazrina@usm.my

Abstract

Introduction : *Clinacanthus nutans* (*Burm f.*) *Lindau* (*C. nutans*) or commonly known as belalai gajah or Sabah snake grass, is a well-known traditional medicine in South East Asia. It consists of abundant phytomedicinal properties such as anti-viral, anti-bacterial and anti-inflammation. This work aimed to profile the phytochemical compounds in 50% ethanolic extracts of *C.nutans* and to identify the cytotoxic effect of 3 selected compounds on J774.2 macrophage cell. **Methods:** Metabolite profiling of the extracted *C.nutans* was carried out using GC-MS and LC-MS/MS analysis. MTT assay was carried out to measure the cytotoxicity of the 3 compounds in J774.2 at concentrations ranging from 1- 100 uM at 24-, 48- and 72- h of incubation**. Results:** More than 100 compounds were profiled by both chromatography analysis, identifying an abundance in artemisnin, betulinic acid and succinic acid. Based on the MTT assay, artemisnin and betulinic acid caused a significant decrease ($p < 0.05$) in J774.2 cell viability at a concentration and time-dependent manner. However, in succinic acid, at concentrations of 1uM and 5uM there were significant ($p < 0.05$) increases in J774.2 cell viability in a time-dependent manner until 48 h. However, a decrease in cell viability was observed at 72 h for all concentration. Nevertheless, all the concentrations of succinic acid at 24-, 48- and 72 h incubation time exhibited a > 80% cell viability.**Conclusion:** This study provides informative data on the existing metabolites in 50% ethanolic extracts of *C.nutans* and showed cytotoxicity effect of the compounds on J774.2 macrophage cell.

Keywords: *Clinacanthus nutans*; anti-proliferative; succinic acid; artemisnin; betulinic acid

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Poster

Physical and Chemical Preventive Countermeasures of Disinfectants Used during the SARS-CoV-2 Pandemic: Implications on Toxicity and Resistance Development

Sani Yahaya Najib[a,b]*, Yusuf Oloruntoyin Ayipo[a,c], Abubakar Sha'aban[d,e], Mohammed Mustapha[d,e], Mohd Nizam Mordi[a]

[a]*Centre for Drug Research, Universiti Sains Malaysia, USM 11800, Pulau Pinang, Malaysia*
[b]*Department of Pharmaceutical and Medicinal Chemistry, Bayero University Kano, PMB 3011, Kano Nigeria.*
[c] *Department of Chemistry, Kwara State University, Malete, PMB 1530, Ilorin, Nigeria*
[d]*School of Pharmaceutical Sciences, Universiti Sains Malaysia, USM 11800, Pulau Pinang, Malaysia*
[e]*Departmnet of Clinical and Administrative Pharmacy, Ahmadu Bello University, Zaria*

Corresponding author email: nysani.phc@buk.edu.ng

Abstract
The novel human coronavirus (CoV) 2019 known as COVID-19, similar to previous CoVs infections outbreaks has posed a serious and an unprecedented challenge on the entire health care system in the world, that needs aggressive preventive and easily accessible measures, policies for effective regular disinfection. Rampant use of disinfectants may pose toxicity to human, environmental hazards and, in some cases, decrease effectiveness and development of resistance due to other ingredients in the disinfectant agents. This review comprehensively highlights the effects of physical and chemical countermeasures and their related potential toxicity on human and environment. The study reveals that physical inactivation especially the effects of temperature, humidity and light mostly ultraviolet-C (UV-C), have significantly demonstrated proven efficacy in reducing the spread of CoV infections. Similarly, chemical countermeasures especially alcohol- and iodine-based disinfecting agents have shown potentials inhibition against the survival of the viruses and other pathogenic micro-organisms on surfaces. Large number of disinfectants were reported to contain corrosive chemicals that are toxic to humans especially children and destroy the environment due to unhealthy accumulation and pollution, and other additional ingredients having potentials to develop resistance and decrease effectiveness of the disinfectants. This review sumarizes the imporatnce of physical and chemical preventive countermeasures currently in use against CoV infections for further modifications and translational study to design improved disinfecting agents.

Keywords: SARS-COV-2, disinfectants, toxicity, resistance, physical & chemical agents, viral inactivation,

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Effects of Trans-Resveratrol and RU-615 on Human Trabecular Meshwork Cells Morphology and Viability

Amy Suzana Abu Bakar[a,b]*, Noor Fahitah Abu Hanipah[a,b], Renu Agarwal[c], Igor Iezhitsa[c], Norhafiza Razali[a,b]*

[a]*Department of Pharmacology, Faculty of Medicine, Universiti Teknologi MARA (UiTM), Sungai Buloh Campus, 47000 Sungai Buloh, Selangor, Malaysia*
[b]*Institute of Medical Molecular Biotechnology (IMMB), Universiti Teknologi MARA (UiTM), Sungai Buloh Campus, 47000 Sungai Buloh, Selangor, Malaysia*
[c]*School of Medicine, International Medical University (IMU), Bukit Jalil, 57000 Kuala Lumpur, Malaysia*

*Corresponding author email: norhafiza8409@uitm.edu.my; amysuzanaaa2611@gmail.com

Abstract

Introduction: Steroid-induced ocular hypertension (SIOH) is a close representation of primary open-angle glaucoma with elevated intraocular pressure (IOP). Human trabecular meshwork cells (HTMC) regulate IOP by maintaining the drainage of the aqueous humour. Trans-resveratrol (TR) is a polyphenolic compound with potent anti-oxidant and anti-inflammatory effects, whereas RU-615 belongs to benzimidazole group, a class of heterocyclic, aromatic organic compounds that have anti-microbial and anti-hypertensive activities. Both agents were shown to reduce the IOP of SIOH rats, however, their mechanisms are yet to be investigated. The use of HTMCs is important in studying IOP lowering effects of potential antiglaucoma agents. Therefore it is important to ensure the concentrations chosen are safe to use on HTMCs. **Methods**: Passage 5 primary HTMCs were divided into 7 groups and treated with resveratrol and RU-615 with or without dexamethasone (Dexa) for 7 days. These cells were then subjected to morphological assessment daily followed by MTS assay on day-7 of treatment. **Results**: Vehicle treated HTMCs were generally wide, large and flat with spindle-like shape. Treatment with TR and RU-615 with or without Dexa for 7 days did not alter much of the morphology, however the shape of HTMCs treated with TR 12.5 μM alone showed more elongated appearance. The cell viability revealed no significant differences ($p \geq 0.05$) between the vehicle-treated group and those treated with TR and RU-615 with or without Dexa. **Conclusion**: Treatment of HTMC with TR 12.5 μM and RU-615 0.1 mM with or without Dexa 100 nM are safe and non-toxic to the HTMCs viability.

Keywords: Cell viability, Resveratrol, RU-615, Human Trabecular Meshwork Cells, MTS assay

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Poster

The Toxicity Level of Raw Titanium Alloy Sample (Ti-6Al-4V) on Human Periodontal Ligament Fibroblast

Mohammad Idham Abd Hamid*, Suzianti Iskandar Vijaya, Norehan Mokhtar, Siti Noor Fazliah Mohd Noor , Norarney Ahmad.

Craniofacial and Biomaterial Sciences Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam 13200 Kepala Batas, Pulau Pinang, Malaysia

*Corresponding author email: idhamhamid@student.usm.my

Abstract

Introduction: Titanium alloy is one of the biomaterials that has been widely used for medical implant especially in orthodontics and orthopaedics.It is important to know the toxic dose of titanium before developing any implants. The unknown toxicity level of titanium implants might trigger the host inflammatory response leading to failure of osseointegration, causing implant rejection. Therefore, the purpose of this study was to determine titanium toxicity level using raw titanium alloy sample (Ti-6Al-4V) on human periodontal ligament fibroblast cell (hPDLFc). **Methods:** Conditioned medium (CM) was prepared by immersing 2g raw Ti-6Al-4V in 20 ml DMEM medium for 1000mg/ml initial concentration of CM solution. hPDLFc were exposed to different concentrations of raw Ti-6Al-4V (1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.9, 1.95 mg/ml) for 24 h and the cell viability were assessed by Alamar Blue assay. **Results**: After 24 h, Ti-6Al-4V sample at concentrations 125, 62.5, 31.25, 15.63, 7.81, 3.9, and 1.95mg/ml showed increased hPDLFc cell viability. 122% cell viability which was the highest, was observed in cells treated with 125mg/ml Ti-6Al-4V. While at 250, 500, and 1000mg/ml Ti-6Al-4V concentrations, the cell viability was reduced and the lowest cell viability was observed at 1000mg/ml which was -81%. **Conclusion**: The different concentrations of raw Ti-6Al-4V affected the hPDLFc viability. The toxic level of raw Ti-6Al-4V based on this experiment is 250mg/ml. Ti-6Al-4V content in implants should be at a non-toxic level to ensure successful implant placement.

Keywords: Human periodontal ligament fibroblast cell, Ti-6Al-4V

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Poster

Effect of Spironolactone Towards Proliferation of Metastatic Osteosarcoma Cells HOS-143B

Farhana Khamarudin[a,b], Mudiana Muhamad[a], Mohd Johari I[a], Wan Nor Izzah WMZ[a], Sharaniza Ab-Rahim[a]*

[a]*Department of Biochemistry and Molecular Medicine, Universiti Teknologi MARA, Cawangan Selangor, Kampus Sungai Buloh, Sungai Buloh, Selangor, Malaysia*
[b]*Institute for Medical Molecular Biotechnology, Universiti Teknologi MARA, Cawangan Selangor, Sungai Buloh Campus, Sungai Buloh, Selangor, Malaysia.*

*Corresponding author email: sharaniza_abrahim@uitm.edu.my

Abstract

Introduction: Osteosarcoma (OS) is a primary malignant bone tumour affecting mostly children and adolescents. Previous study reported its ability in inducing the expression of Natural Killer Group 2 member D ligand (NKG2DL) in cancer cells, suggesting possible role towards cancer immune reactions. Thus, the aim of this study is to investigate the effect of SPIR towards proliferation of normal osteoblast cells (hFOB) and highly metastatic OS cells (HOS-143B). **Methods:** Both hFOB and HOS-143B cells were treated with SPIR at different concentrations ranging from 5 to 40 μM. Cytotoxicity level of SPIR was determined at post- 24, 48 and 72 hours using the Cell Proliferation Assay. **Results:** SPIR exerted its effect in a dose-dependent manner for the hFOB cells at all incubation time, with viable cell percentage ranging from 95% to 35% following the dose increment. Meanwhile, at post- 24 hours and 48 hours, the SPIR exerted effect in HOS-143B was independent of the doses, indicated by the similar pattern of high percentage (85% and above) of viable cells at all doses. **Conclusion:** SPIR exerted a more consistent effect towards the normal cells compared with the cancer cells. However, despite being dose independent, SPIR exerted low cytotoxicity towards HOS-143B indicated by the high percentage of viable cells at all doses with the optimum reaction time of 48 hours. In conclusion, SPIR exerted an overall positive effect towards proliferation of both normal osteoblast and OS cancer cells when used below its cytotoxic doses.

Keywords: osteosarcoma, spironolactone, cell proliferation assay, immune cells, natural killer (NK) cell ligand

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Poster

Genome-wide SNP Analysis of the Evolutionary Multidrug Resistant *Mycobacterium tuberculosis* (MDR-TB) in Malaysia

Hui Wen Teh[a]*, Hazrina Yusof Hamdani[a], Marimuthu Citartan[a], Norzuliana Zainal Abidin[b], Lay Kek Teh[b], Mohd Zaki Salleh[b], Thean Hock Tang[a]*

[a] *Universiti Sains Malaysia (USM), Bertam 13300 Kepala Batas, Pulau Pinang, Malaysia*
[b] *Universiti Teknologi MARA (UiTM), Puncak Alam, 42300 Kuala Selangor, Selangor*

*Corresponding author email: huiwenteh@student.usm.my

Abstract

Introduction: Tuberculosis (TB) control has been a constant battle, and this is beleaguered by the increasing cases of multidrug-resistant tuberculosis (MDR-TB). Understanding the underlying factors of MDR-TB would augment our understanding and could potentially pave the trajectory towards an effective treatment. In this study, we have identified mutations potentially associated with drug resistance in MDR-TB using Next-Generation Sequencing (NGS) approach. **Methods:** A total of 24 available genomes sequences of clinical strain of *Mycobacterium tuberculosis* (MTB) were subjected to NGS sequence analysis. The obtained variants were filtered by using the existing protein-coding genes in MTB, obtained from NCBI. Selected candidates were singled out and subjected to protein structural prediction analysis. **Results:** Using H37Rv genome as the reference, overall sequences ranging from 4.2Mb to 4.3Mb were acquired. A deletion was detected for heparin-binding hemagglutinin (HBHA) and forkhead-associated (FHA) domain (FHAA), respectively. Both protein-coding genes that contains a deleted segment were consistently present in most of the isolates. These deleted segments may potentially change the structural conformation of the proteins. The deletion in HBHA protein is speculated to cause a reduction in the adherence of MTB to epithelial cell, hence inhibit autophagy and facilitate apoptosis in macrophage that promotes MTB infection. Deletion in the segment in FHAA protein, may led to potential downregulation of cell growth, causing the cell wall to be impermeable to the antibiotics thus inducing antibiotic resistance. **Conclusion:** These findings suggest that the mutation in both HBHA and FHAA proteins may confer antibiotic resistance.

Keywords: *Mycobacterium tuberculosis* (MTB), multidrug resistant, next generation sequencing (NGS), protein structure prediction

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Poster

Investigation of Cytotoxins from Malaysian Cobras (*Naja sumatrana* and *Naja kaouthia*) as A Potential Antineoplastic Agent

Ho Phin Chong[a], Kae Yi Tan[b]*, Choo Hock Tan[a]

[a] *Venom Research and Toxicology Lab, Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur*
[b] *Protein and Interactomics Lab, Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur*

*Corresponding author email: kytan_kae@um.edu.my

Abstract

Introduction: Cancer is a leading cause of death worldwide. The pursuit of novel antineoplastic agents has reinvigorated interest in natural proteins and peptides for target-specific and effective treatments of cancer. With this, cobra venoms are one of the promising natural resources with antineoplastics potential. Here, we investigated the antineoplastic potential of cobra venom and their purified cytotoxins of two Malaysian cobras, *Naja sumatrana* (equatorial spitting cobra) and *Naja kaouthia* (monocled cobra) on three types of cancer and non-cancerous cell lines, i.e., breast (MCF-7 and 184B5), A549, NL20 (lung), PC-3 and RWPE-1 (prostate). **Methods:** The cytotoxins were purified via sequential fractionation using ion-exchange chromatography, followed by C18 reverse-phase high-performance liquid chromatography (HPLC). The protein homogeneity was validated with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and identified through liquid chromatography-tandem mass spectrometry (LCMS/MS). The cytotoxicity was determined with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and the half-maximal inhibitory concentration (IC_{50}) of the cytotoxins towards cell lines were determined. **Results:** The cytotoxins exhibited cell-type specificity, by showing a more potent cytotoxicity against A549 (IC_{50} <1.5 μg/ml) and PC-3 (IC_{50} = 3–4 μg/ml) compared to MCF-7 (IC_{50} = 9–12 μg/ml). In comparison, these cytotoxins also demonstrate different selectivity towards cancer and non-cancer cell lines, with the most promising selectivity observed in the lung cell line (Selective Index > 2). However, the cytotoxins were not selective in breast and prostate cell lines (SI < 2). **Conclusion:** The findings suggest that the cytotoxins of both Malaysian cobras may serve potential as a prospective antineoplastic candidate despite the need for further studies to address its limitations and challenges.

Keywords: snake venom, cobra cytotoxin, anticancer

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Poster

BPIFB1 Role in the Regulation of EMT Associated Genes in the Gastric Cancer Cells

Anis Suraya Mohd Jamil[a], Zeti Rahayu A. Karim[c], Siti Hamimah Sheikh Abdul Kadir[d], Maslinda Musa[a,b]*

[a] *School of Biology, Faculty of Applied Sciences, University Technology MARA, Selangor, Section 2, 40450, Shah Alma, Selangor, Malaysia,*
[b]*Institute of Science (IOS), Level 3, Block C, Kompleks Inspirasi, Universiti Teknologi MARA 40450 Shah Alam, Selangor Darul Ehsan, Malaysia,*
[c]*Department of Surgery, Faculty of Medicine, Universiti Teknologi MARA, Selangor, 47000, Sungai Buloh, Selangor Malaysia,*
[d]*Department of Biochemistry and Molecular Medicine, Faculty of Medicine, Universiti Teknologi MARA, Selangor, 47000, Sungai Buloh, Selangor, Malaysia*

*Corresponding author email: maslinda64@uitm.edu.my

Abstract

Introduction: Bacterial/Permeability-Increasing Fold Containing Family B Member 1 (BPIFB1), is a member of the BPI-fold family of protein differentially expressed in various types of cancers. Overexpression of BPIFB1 protein has been reported to down-regulate the expression of E-cadherin (CDH1). CDH1 is an epithelial marker regulating the epithelial to mesenchymal transition (EMT) of cancer cells, hallmark of invasion and metastasis stage of cancer. The loss of CDH1 expression leads to EMT and alter the expression of mesenchymal markers such as CDH2 and VIMENTIN as well as other EMT associated genes such as Snail 1 (SNAI1). This study aims to investigate the effect of BPIFB1 overexpression in the mesenchymal marker gene expression *in-vitro* in GC cells that have been previously transfected to overexpressed BPIFB1 expression. **Methods:** Three different GC cell lines were used in this study, AGS, HGC-27 and MKN-45 cells. Expression analysis was carried out via qRT-PCR to determine target genes expression and comparative expression analysis was done using the transfected and non-transfected GC cells with normalization to the standard reference gene *β-ACTIN* (*ACTB*). **Results:** The results showed that overexpression of BPIFB1 leads to the differential expression in the EMT associated genes in the AGS, HGC27 and MKN45 GC cells. **Conclusion**: The data generated from this study can be used to provide more insight on the role of BPIFB1 protein as the transcriptional regulator for the EMT process in GC cells.

Keywords: Bacterial/Permeability-Increasing Fold Containing Family B Member 1 (BPIFB1), Gastric cancer (GC), Epithelial Cadherin (CDH1), Snail 2 (SNAI2), Epithelial-to-mesenchymal transition (EMT).

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Poster

Cell-Free Supernatants from Probiotics Inhibit Colon Cancer Cells proliferation

Ifeoma Julieth Dikeocha[a], Abdelkodose Mohammed Al-Kabsi[a], Salasawati Hussin[a], Mohammed Abdullah Alshawsh[b]*

[a]*Faculty of Medicine, University of Cyberjaya, Persiaran Bestari 63000 Cyberjaya, Selangor Darul Ehsan, Malaysia.*
[b]*Department of Pharmacology, Faculty of Medicine, University of Malaya, Kuala Lumpur 50603, Malaysia*

*Corresponding author email: alshaweshmam@um.edu.my

Abstract

Introduction: Probiotics are live microorganisms which when administered in adequate amounts provide health benefits to the consumer. Patients with colorectal cancer benefits from the consumption of probiotics as it helps to reduce colorectal cancer spread and progression through various mechanisms. The cytotoxic effect of the cell free supernatant of Propionibacterium freudenreichii and Faecalibacterium prausnitzii was investigated in vitro using HCT116 colon cancer cell line. **Methods:** MTT cell viability assay was used to check the cytotoxicity and inhibitory effects of Cell free supernatant of P. freudenreichii and F. prausnitzii against human colorectal cancer cells (HCT116). The cells were seeded at 5000 cells/well overnight after which they were exposed to both probiotic supernatants singly and as a cocktail at different concentrations. and 5-FU standard chemotherapeutic drug at different concentrations then incubated at 37oC and 5% CO2 for 12, 24, 48 and 72h. Afterwards, 10µl MTT solution was added to the wells and reincubated for 3 hours. 100µl of DMSO was used to lyses the cells to expose the formazan crystal formed and the absorbance was measured at 570 nm. **Results:** Cell free supernatant of P. freudenreichii and F. prausnitzii exhibited cytotoxic effects on HCT116 cells in a time dependent and dose dependent manner. The cells that were treated with the highest concentration of the CFS had the lowest viability rates across all the time points. **Conclusion:** The result gotten from this study shows that probiotics are efficient against colorectal cancer cells even when no live cells are present.

Keywords: Probiotics; Colorectal cancer; *Propionibacterium freudenreichii; Faecalibacterium prausnitzii*; MTT assay

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Poster

Antibiotic Resistance Patterns of WHO Priority Pathogens Isolated from Acute Pharyngitis Patients in Private Primary Care Clinics

Christina Shook Cheng Chong[a], Zhuang Mian Bo[a], Wei Keat Tan[a], Benedict Lim Heng Sim[b], Christopher Kwok Chong Lee[b], Renee Lay Hong Lim[a], Crystale Siew Ying Lim[a]*

[a]*Department of Biotechnology, Faculty of Applied Sciences, UCSI University, No1, Jalan Menara Gading, UCSI Heights, Taman Connaught, 56000 Cheras, Kuala Lumpur, Malaysia*
[b]*Department of Medicine, Hospital Sungai Buloh, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia*

*Corresponding author email: crystalelim@ucsiuniversity.edu.my

Abstract

Introduction: The World Health Organization (WHO) has developed a global priority pathogen list (PPL) with 12 antibiotic-resistant bacteria. Acute pharyngitis (AP) is a common reason for primary care consultation and antibiotics are often prescribed by general practitioners (GPs) for AP patients in Malaysia. However, there is limited primary care antibiotic resistance surveillance data available in Malaysia. Therefore, this study aims to investigate the prevalence and antibiotic resistance profiles of WHO global priority pathogens specifically towards last-resort drugs in AP patients in the community. **Methods:** A cohort of 205 AP patients visiting private primary care clinics in Klang Valley, Malaysia was selected. Throat swabs were taken and bacterial identification were done using Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). Disc diffusion or broth microdilution were performed on 237 PPL isolates found in 153 AP patients following standard guidelines. **Results:** *Staphylococcus aureus* (n=89), *Streptococcus pneumoniae* (n=62), *K. pneumoniae* (n=49), *Acinetobacter baumannii* (n=7), *Pseudomonas aeruginosa* (n=6) and *Enterobacter* spp. (n=24) were found among the WHO PPL. Results showed 23 out of 62 *S. pneumoniae* isolates were penicillin-non-susceptible and more than half of *S. aureus* isolates were penicillin-resistant (n=52/89) although penicillin V has been recommended for AP management in Malaysia. Alarmingly, all 5 community-acquired methicillin-resistant *S. aureus* (MRSA) throat isolates found were also vancomycin-resistant *S. aureus* (VRSA), indicating the spread of VRSA in the Malaysian community. **Conclusion:** In conclusion, this study demonstrates that these pathogens are acquiring trends for resistance towards last-resort antibiotics, therefore, these data obtained can be useful to improve antibiotic stewardship in Malaysia. (250 words)

Keywords: Acute pharyngitis, antibiotic resistance, community-acquired, private primary care, WHO global priority pathogens list

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Poster

HMGCR Expression in Relation to HER2 Status of Breast Cancer

Rosmaizan Drahman[a], Siti Norasikin Mohd Nafi[a,b]* , Wan Faiziah Wan Abdul Rahman[a,b]

[a]*Department of Pathology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kota Bharu, Kelantan, Malaysia*
[b]*Department of Pathology, Universiti Sains Malaysia, Health Campus, 16150 Kota Bharu, Kelantan, Malaysia*

*Corresponding author email: snmn@usm.my

Abstract

Introduction: 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) is the rate-limiting enzyme in cholesterol biosynthesis pathway that has been associated with cancer development. The association between HMGCR and HER2 has been established, however, the role of HMGCR in HER2 positive breast cancer progression remains unknown. This study aims to evaluate the HMGCR expression in relation to HER2 status of breast cancer. **Methods:** Cell lines MDA-MB-453, MDA-MB-361, MDA-MB-231 and MCF-7 were grown in DMEM medium with 4% FBS and 1% antibiotic. The cells were subcultured in petri dishes and protein were extracted upon reaching confluency. Western blotting was performed on the protein extracts targeting HMGCR protein at 98 kDa. In addition, tissue block samples of breast cancer patients diagnosed in Hospital USM were selected for IHC staining of HMGCR. Besides, the HMGCR mRNA levels were also studied using patient samples with IHC scored HER2 0, HER2 1+, HER2 2+ and HER2 3+. **Results:** High basal protein expression of HMGCR was observed in MDA-MB-453 (HER2 moderate overexpression) compared to the other breast cancer cell lines. Moreover, the percentage of IHC HER2 3+ samples with high protein expression of HMGCR was 91.7 % (33 out of 36 cases), which was higher compared to 69.4% (25 out of 36 cases) of high HMGCR expression in IHC HER2 0 & 1+ samples. qPCR analysis showed the mRNA level of HMGCR increased in IHC HER2 3+ breast cancer samples as compared to the other HER2 breast cancer subtypes. **Conclusion:** Overall, increased HMGCR protein and mRNA expression was seen in HER2 overexpression breast cancer. Further study should be carried out to examine the molecular interactions between HMGCR and HER2 positive breast cancer in relation to tumour progression of HER2 positive breast cancer.

Keywords: HMGCR, HER2 status, breast cancer, cholesterol biosynthesis

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Poster

Proteomic Analysis Reveals Protective Role of Melatonin in Tumour-Prone *Drosophila melanogaster lethal giant larvae* Mutants

Kar-Cheng Wong[a], Jaime Jacqueline Jayapalan[a,b]*, Perumal Subramanian[c], Puteri Shafinaz Abdul-Rahman[a,b]*

[a]*Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[b]*University of Malaya Centre for Proteomics Research (UMCPR), University of Malaya, 50603 Kuala Lumpur, Malaysia*
[c]*Department of Biochemistry and Biotechnology, Annamalai University, Chidambaram, 608 002 Tamil Nadu, India*

Corresponding author email: jaime_jacklyn@um.edu.my; terisar@um.edu.my

Abstract

Introduction: *Lethal giant larvae* (*lgl*) is the first neoplastic tumour suppressor gene identified in *Drosophila melanogaster* and it encodes protein that regulates cell polarity and epithelial integrity. The aberrant expression of human homolog of *lgl, Hugl,* is associated with the progression of multiple cancers, including colorectal cancer. However, molecular characterisation of *lgl* mutant flies is still lacking. In this study, the proteome profile of intestinal tissues of *lgl* mutant flies and the effects of melatonin treatment were investigated. **Methods:** *Drosophila melanogaster* flies were cultured and categorised into three groups: (i) wild type; (ii) *lgl* mutant; and (iii) *lgl* mutant treated with melatonin. Total protein isolated from the intestinal tissues was subjected to two-dimensional gel electrophoresis and silver-staining. The gel images were analysed using Progenesis SameSpots software. Protein spots of interest were digested for mass-spectrometry analyses and subsequently identified using MASCOT search engine against *Drosophila* entries in the Swiss-Prot database. **Results:** A total of 40 protein spots were detected with significant differences ($p < 0.01$) in abundance between the groups of flies. Among these, upregulation of eukaryotic initiation factor 4A and actin-79B, and downregulation of glutathione S-transferase S1 were observed in *lgl* mutants. Interestingly, the reversal changes in these proteins were observed in those flies under melatonin treatment. **Conclusion:** The results indicate changes in protein expression in *lgl* mutant flies and normalisation upon treatment with melatonin, thus suggesting a protective effect of melatonin in tumour-prone *Drosophila melanogaster lgl* mutant.

Keywords: *Drosophila melanogaster*, *lethal giant larvae*, intestine, proteomics, melatonin

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Poster

Evaluation of Antidiabetic Activity of Methanolic Extract of *Coccinia grandis* (voight) and *Spondias mombin* (Linn) Leaves in Streptozotocin Induced Diabetic Sprague Dawley Rats.

Ramachawolran Gobinath[a], Subramani Parasuraman[b], Subramaniam Sreeramanan[c,d], Suresh V. Chinni[a]*

[a]*Department of Biotechnology, Faculty of Applied Sciences, AIMST University, Bedong 08100, Kedah, Malaysia*
[b]*Department of Pharmacology, Faculty of Pharmacy, AIMST University, Bedong 08100, Kedah, Malaysia*
[c]*Department of Industrial Biotechnology, Universiti Sains Malaysia, 11800 Georgetown, Penang, Malaysia*
[4]*Department of Bioprocess Engineering, Universiti Malaysia Perlis, 02600 Arau, Perlis, Malaysia*

*Corresponding author email: cvsureshgupta@gmail.com

Abstract

Introduction: Medicinal plants are well documented for treating diabetes mellitus. However, scientific evidence on antidiabetic activity of *Coccinia grandis* and *Spondias mombin* leaves from Malaysian origin have not been significantly reported so far. Present study is to evaluate the antidiabetic potential of methanolic extract of *Coccinia grandis* (MECG) and *Spondias mombin* (MESM). **Methods:** Antidiabetic activity of MECG and MESM was evaluated in streptozotocin induced diabetic rats by administering glibenclamide (20 mg/kg) as standard drug, (125, 250 and 500 mg/kg) individually and combined doses (125 mg/kg + 125 mg/kg, 125 mg/kg + 250 mg/kg, and 125 mg/kg + 500 mg/kg) for 28 days. **Results:** Qualitative analysis of MECG and MESM showed the presence of reducing sugar, tannins, alkaloids, phenols and flavonoids and HPLC analysis indicated presence of quercetin and rutin. Blood glucose, serum cholesterol triglycerides, low density lipoproteins (LDL), very low-density lipoproteins (VLDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), were significantly ($p<0.05$) decreased while, high density lipoproteins (HDL) were increased in MECG, MESM and glibenclamide treated rats compared to diabetic control and combined doses treated rats. Furthermore, histopathological study of pancreas in MECG, MESM and glibenclamide treated rats showed significant regeneration of β-cells of islets of Langarhans compared to diabetic control and combined doses treated rats. No changes observed in combined dose treated rats. **Conclusion:** This study provided evidences for significant antidiabetic activity of MECG and MESM.

Keywords: *Coccinia grandis*, *Spondias mombin*, antidiabetic activity, type 2 diabetes, histopathology.

Acknowledgement: We would like to thank Ministry of Higher Learning Malaysia for financing this research work under the Fundamental Research Grant Scheme, FRGS/1/2018/STG03/AIMST/02/1.

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Poster

Changes in Inflammatory Biomarker Vcam-1 and Insulin Levels During Progression of Premature Atherosclerosis in Rats

Maleeka Abdullah Hilmy[a], Muhammad Fakhruddin Irfan[a], Zulhabri.Othman[a]*

[a]*School of Graduate Studies, Post-Graduate Centre, Management & Science University, 40100, Shah Alam, Selangor, Malaysia*

*Corresponding author email: zulhabri_othman@msu.edu.my

Abstract

Introduction: Atherosclerosis is an inflammatory disease which can cause various systemic complications. Obesity is a traditional biomarker and one of the major risk factors contributing to atherosclerosis. The study aims to assess changes in inflammatory marker serum VCAM-1 and metabolism marker serum insulin levels during progression of premature atherosclerosis alongside traditional markers. **Methods:** 10 healthy Sprague Dawley rats were randomly divided into a high fat diet group (n=5) and a normal diet group (n=5). Animals were observed for a period of 16 weeks. BMI, blood lipid profiling and fasting blood glucose levels were assessed weekly as traditional biomarkers. Systemic markers serum VCAM-1 and insulin levels were determined by ELISA. HOMA-IR index was calculated and histological analysis of aorta was used to measure intima media thickness. **Results:** The study found that there was a significant difference in BMI $t(8)=7.483$, $p<0.001$, LDL-C $t(8)=8.551$, $p<0.001$, HDL-C $t(8)=-14.213$, $p<0.001$, $p<0.001$, AI $t(8)=14.803$, $p<0.001$, VCAM-1 $t(8)=9.506$, $p<0.001$ and fasting blood glucose levels $t(8)=10.450$, $p<0.001$ between groups. Strong positive correlations were observed for VCAM-1 with body weight ($r=0.97$, $p<0.001$) and atherogenic index ($r=0.94$, $p<0.001$). HDL levels had strong negative correlations with VCAM-1 ($r=-0.93$, $p<0.001$). A strong positive correlation was observed between LDL levels and HOMA-IR index ($r=0.81$, $p=0.005$), IMT and VCAM-1 ($r=0.87$, $p=0.001$). **Conclusion:** Inflammatory marker VCAM-1 showed significant increase among the HFD. The increase in serum insulin in the HFD was not significant. Therefore, VCAM-1 can pose as a potential systemic biomarker alongside traditional biomarkers for better prediction of premature atherosclerosis progression.

Keywords: Biomarker, Atherosclerosis, VCAM-1, Insulin, Obesity, ELISA

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Poster

Investigation of Upper Respiratory Carriage of Bacterial Pathogens and Their Antibiotic Susceptibility Profile in the Kinta Valley, Perak, Malaysia

Hing Huat Ong[a], Lee Quen Phoon[b], Stuart C. Clarke[c], Eddy Seong Guan Cheah[a]*

[a]*Department of Biological Science, Faculty of Science, Universiti Tunku Abdul Rahman (UTAR), Kampar Campus, 31900 Kampar, Perak, Malaysia.*
[b]*Department of Allied Health Sciences, Faculty of Science, Universiti Tunku Abdul Rahman (UTAR), Kampar Campus, 31900 Kampar, Perak, Malaysia.*
[c]*Faculty of Medicine, University of Southampton, Southampton SO17 1BJ, United Kingdom.*

*Corresponding author email: cheahsg@utar.edu.my

Abstract

Introduction: Some normal microbiota in the human upper respiratory tract can be potentially pathogenic when they overgrow or translocate; the latter could lead to pneumonia, septicaemia and meningitis. The occurrence of these pathogens can be investigated via the respiratory carriage studies, which are limited in Malaysia and therefore, their prevalence remains poorly understood. This study aimed to investigate the upper respiratory carriage of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* among the Kinta Valley population. **Methods:** Nasal, oropharyngeal, and nasopharyngeal swabs (n = 563 samples) from 236 subjects aged 2-90 years were collected. Detection of the fastidious target bacteria (*S. pneumoniae*, *H. influenzae*, and *N. meningitidis*) was by multiplex PCR on the swab DNA extracts. The presence of non-fastidious targets (*S. aureus*, *K. pneumoniae*, and *P. aeruginosa*) was assessed by culture on selective media followed by their respective species-specific PCR assays. **Results:** The carriage rates of *S. pneumoniae*, *S. aureus*, *H. influenzae*, *K. pneumoniae*, *N. meningitidis* and *P. aeruginosa* were 44%, 33%, 16%, 11%, 1.3% and 0.8%, respectively. In the Kirby-Bauer assay, 86% and 14% of the *S. aureus* isolates obtained were resistant to penicillin and tetracycline, respectively. Among the *S. pneumoniae* isolates, 54% and 42% were resistant to trimethoprim/sulphamethoxazole and tetracycline, respectively. **Conclusion:** The carriage rate for *S. pneumoniae* is the highest among the study population. The recent inclusion of the pneumococcal conjugate vaccine in the National Immunisation Programme has the potential to reduce this carriage in the community.

Keywords: Bacterial carriage, Upper respiratory tract, *Streptococcus pneumoniae*, Antibiotic susceptibility, Epidemiology

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Poster

The Anti-Proliferative Role of 15,16-Dihydrotanshinone I (DHTS) Extracted from *Salvia miltiorrhiza* in Autosomal Dominant Polycystic Kidney Disease (ADPKD).

Rhubaniya Mahendran[a]*, Soo Kun Lim[b], Kien Chai Ong[a], Kek Heng Chua[a], Hwa Chia Chai[a]

[a]*Department of Biomedical Science, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia.*
[b]*Division of Nephrology, Department of Medicine, University Malaya Medical Centre, 50603 Kuala Lumpur, Malaysia.*

*Corresponding author email: rhuban29@gmail.com

Abstract

Introduction: Autosomal polycystic kidney disease (ADPKD) is a kidney disorder caused by mutations in polycystin proteins resulting in cystic formation in kidney. Tolvaptan, a vasopressin-2-receptor (V2R) antagonist, is the only FDA approved drug currently for ADPKD but it demonstrates serious side effects such as hepatotoxicity. Dihydrotanshinone I (DHTS) extracted from *Salvia miltiorrhiza* has been shown previously to stop proliferation in cancers. Given that ADPKD also involves cell proliferation, the present study aims to repurpose this natural compound for ADPKD treatment. **Methods:** The cell viability of ADPKD cells (WT 9-12), normal kidney cells (HK2), and hepatocellular carcinoma cells, (HepG2, positive control cell line) treated with various concentration of DHTS, metformin and tolvaptan (positive control drugs) was assessed using crystal violet viability assay on day 3 and day 6 of treatment. The cells were treated daily (Model 1), every two days (Model 2), and every three days (Model 3). The DHTS concentration(s) that significantly reduced the WT 9-12 cell viability with minimal inhibitory effect on HK2 cells (≤50%) will be further analyzed with sulforhodamine B (SRB) cytotoxic assay and real time cell analyser (RTCA). **Results:** 5μM ($p<0.05$) and 10μM ($p<0.001$) DHTS treatment from Model 2 significantly inhibited the WT 9-12 viability in the preliminary studies with minimal cytotoxic effects on HK2. (SRB assay and RTCA results will be obtained before conference) **Conclusion:** DHTS showed promising anti-proliferation effects on ADPKD cell in this study. Although the side effects of DHTS is yet to be determined, this natural compound can be gentler alternative than tolvaptan for ADPKD treatment.

Keywords: ADPKD, treatment, DHTS, proliferation

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Poster

Tocotrienol Rich Fraction (TRF) Upregulates Farnesoid X Receptor (FXR) and Modulates FXR Target Genes in the Heart of High Fat Diet-Fed Mice

Nur Aliah Natasha Md Shahrulnizam[a], Siti Hamimah Sheikh Abdul Kadir[a,b], Effendi Ibrahim[a]*, Sharaniza Abd Rahim[a], Mohd Danial Mohd Effendy Goon[a], Musalmah Mazlan[a]

[a]*Faculty of Medicine, Universiti Teknologi MARA, Cawangan Selangor, 47000, Sungai Buloh, Selangor, Malaysia*
[b]*Institute for Pathology, Laboratory and Forensic Medicine (I-PPerForM), Universiti Teknologi MARA, Cawangan Selangor, 47000, Sungai Buloh, Selangor, Malaysia*

*Corresponding author email: effendi953@uitm.edu.my

Abstract

Introduction: Cardiovascular Disease (CVD) is the number one cause of mortality due to non-communicable disease and high fat diet as one of the major risk factors for CVD development. FXR have been found to be expressed in the heart and is reported to exert anti-inflammatory and anti-antheroslerotic effects following its activation thus enhances cardiac remodeling and reduction in blood pressure. Tocotrienol-rich fractions (TRF) possess cardioprotective effects such as anti-atherosclerosis, anti-inflammatory, antioxidant activities, anti-thromboembolic and anti-adipogenic. However, it is not known whether TRF activates FXR and its target genes in high fat diet (HFD) animal model. **Methods:** A total of 21 Leptin deficient Jax male mice were randomly divided into HFD only (control), HFD with PKO (vehicle control) and HFD with TRF (TRF supplementation). After 6 weeks of treatment, heart tissues were extracted for real time polymerase chain reaction (qRT-PCR) to measure *fxr, shp, stat3, sod1, sod2* and *gpx1* expression from all animals. **Results:** Our findings demonstrated a significant increase in *fxr* expression of HFD+TRF and HFD+PKO group respectively by 3.18±0.61 folds and 10.0±2.05 folds as compared to control. A significantly reduced expression of *stat3* in both HFD+PKO (0.49±0.048) and HFD+TRF (0.53±0.053) groups, while increased *gpx1* expression was observed in HFD+TRF group (1.789±0.164 fold). No significant differences were noted in *shp, sod1* and *sod2* expression between HFD+TRF and control. **Conclusion:** Our results suggest that in HFD mice, the cardioprotective effect of TRF could be mediated by *fxr* signalling pathway through downregulation of inflammatory gene (*stat3*) and not through *shp* dependent-target genes.

Keywords: Tocotrienol-Rich Fraction, High fat diet, Nuclear receptor FXR

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Poster

Evaluation of Antioxidant and Antidiabetic Potential of Medicinal Weeds, *Mimosa pudica*

Nor Saffana Baharuddin[a], Azzreena Mohamad Azzeme[b], Zuraida Ab Rahman[c], Mohd Ezuan Khayat[b] & Mohamad Zulfazli Mohd Sobri[a]*.

[a]*Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Science, Universiti Putra Malaysia, 43400 Seri Kembangan, Selangor, Malaysia.*
[b]*Department of Biochemistry, Faculty of Biotechnology and Biomolecular Science, Universiti Putra Malaysia, 43400 Seri Kembangan, Selangor, Malaysia.*
[c]*Biotechnology Research Centre, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia.*

*Corresponding author email: zulfazli@upm.edu.my

Abstract

Introduction: *Mimosa pudica* is one of the common weeds used in ayurvedic medicine in treating diabetes. Preliminary screenings in previous studies found this species to possess bioactive compounds with potential antidiabetic effects due to its antioxidative content. This study aimed to optimize the solvent extraction parameters of *M. pudica* using response surface methodology in order to enrich the accumulation of antioxidants in the extracts. This study also examined the effects of *M. pudica* extract on the cell viability and glucose uptake ability in 3T3-L1 adipocyte cell line. **Methods:** A series of 17 runs were performed in solvent extraction of dried *M. pudica* aerial parts by manipulating three factors; extraction duration, water bath temperature and ethanol concentration. Then, the pre-adipocyte cell line was exposed to *M. pudica* extract at different concentrations of between 0.05 - 0.80 mg/ml to assess its cell viability level. 2-NBDG glucose analogue was used to measure the glucose uptake ability of matured adipocyte cells treated with *M. pudica* extract. **Results:** The results indicated highest ethanol concentration used as solvent had the greatest impact on the accumulation of antioxidant compounds in the extract. The highest TPC and TFC value recorded were 94.015 mg GAE and 1212.63 mg QE using 100% ethanol, three-fold and five-fold respectively higher as compared to using 50% ethanol. The lowest IC50 value recorded was 19.92 μg/ml using 100% ethanol as compared to 975.02 μg/ml using 50% ethanol. The optimized extraction factors value for duration, temperature and solvent concentration were 82 minutes, 40°C and 100% ethanol respectively. In the cell viability assay, all extract concentrations treatment recorded viability level of above 50%. Extracts at concentration of 0.05 and 0.10 mg/ml were used to proceed in glucose uptake assay using 2-NBDG analogue. The results showed 2-NBDG uptake in cells treated with 0.10 mg/ml extract combined with insulin were more than 200% as compared to control group. **Conclusion:** These initial findings suggest that future research could fruitfully explore the compound and mechanism involved in *M. pudica* which could benefit in the treatment of diabetes.

Keywords: *Mimosa pudica*, antioxidant, antidiabetic, adipocyte, medicinal weeds.

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Poster

Determining Structural Stability Of Glucose-6-Phosphate Dehydrogenase Deficiency Variants Using Molecular Dynamics Simulation

Naveen Eugene Louis[a], Muaawia Ahmed Hamza[b,c], Nurriza Ab Latif[a], Mona Awad Alonazi[d], Arjumand Warsy[e], Syazwani Itri Amran[a]*

[a]*Bioscience Department, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor., Malaysia*
[b]*Faculty of Medicine, King Fahad Medical City, Riyadh, Kingdom of Saudi Arabia*
[c]*Research Center, King Fahad Medical City, Riyadh, Kingdom of Saudi Arabia*
[d]*Department of Biochemistry, Science College, King Saud University, Riyadh, Kingdom of Saudi Arabia*
[e]*Central Laboratory, Center Female Center for Scientific and Medical Studies, King Saud University, Riyadh, Kingdom of Saudi Arabia*

*Corresponding author email: syazwaniitri@utm.my

Abstract

Introduction: Glucose-6-phosphate dehydrogenase is responsible for replenishing the NADPH supply in erythrocytes, which protect against free radical induced oxidate stress. G6PD deficiency is the most common enzyme deficiency disorder. More than 186 variants are linked with G6PD deficiency by decreasing the activity or stability of the enzyme. G6PD dimerization is responsible for structural stability and catalytic efficiency. Despite geostatistical studies highlighting the incidence of many variants in Southeast Asia (SEA), there is still a gap in knowledge for these variants in a structural and a biochemical context. The structural-functional relationship of G6PD variants were unraveled by employing molecular dynamics simulation (MDS) to validate existing biochemical data. **Methods:** A complete G6PD dimer was constructed by using AutoDock 4.2 and AutoDock Vina to dock G6PD ligands, hence creating a complete dimer. The MDS package Gromacs 2018.1 was used to study the protein. SEA variants V291M (Viangchan), L128P (Vanua Lava) and H32R (Gaohe) were chosen and created by in silico site-directed mutagenesis using PyMOL. Trajectory analyses were performed to understand the structural changes exhibited by the mutants. **Results:** H32R was loosely packed which indicates poor catalytic activity, and all three mutants had higher number of hydrogen bonds towards their substrates and cofactors. **Conclusion:** Biochemical studies have shown that H32R exhibits a 90% reduction in catalytic activity and variants with low catalytic activity have greater affinities to their substrate and cofactors which is a compensatory mechanism. This establishes a structural and functional link that was formed by validating *in silico* and *in vitro* findings.

Keywords: Glucose-6-phosphate Dehydrogenase Deficiency, Computational Biology, Molecular Docking Simulation, Molecular Dynamics Simulation, Dimerization

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Poster

Perfluoorooctane Sulfonate (PFOS) Exposure Affects Beating Rate and Brain Natriuretic Peptide (BNP) Expression of Cardiomyocytes

Siti Suhana Abdullah Soheimi[a], Effendi Ibrahim[b], Amirah Abdul Rahman[b], Normala Abdul Latip[c], Siti Hamimah Sheikh Abdul Kadir[b,d]*

[a]*Institute of Medical Molecular Biotechnology (IMMB), Faculty of Medicine, Universiti Teknologi MARA, Cawangan Selangor, 47000 Sungai Buloh, Selangor, Malaysia*

[b]*Faculty of Medicine, Universiti Teknologi MARA, Cawangan Selangor, 47000 Sungai Buloh, Selangor, Malaysia*

[c] *Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Faculty of Pharmacy, Universiti Teknologi Mara, Cawangan Selangor, 42300 Puncak Alam, Malaysia*

[d]*Institute for Pathology, Laboratory and Forensic Medicine (I-PPerForM), Universiti Teknologi MARA, Cawangan Selangor, 47000, Sungai Buloh, Selangor, Malaysia*

*Corresponding author email: sitih587@uitm.edu.my

Abstract

Introduction: Perfluoorooctane Sulfonate (PFOS) is non-biodegradable synthetic compounds widely used in manufacturing household products. Exposure to PFOS demonstrated to increase the risk of cardiovascular diseases. This study aimed to investigate the effects of PFOS exposure on cardiomyocytes beating rate and cardiac markers. **Methods:** Primary neonatal cardiomyocytes cultured were exposed to PFOS with concentration from 10 to 125 μM to determine its effective concentration (EC_{50}). Cells were cultured and divided into unexposed (control), PFOS-exposed, Endothelin-1-exposed and combination of PFOS with Endothelin-1-exposed. All cells were subjected for beating rate assessment assay and stained for cardiac marker F-actin and hypertrophy marker (Brain natriuretic peptide, BNP). **Results:** In this study, EC_{50} was determined at 50 μM in 48-hour treatment ($n=4$, $p<0.05$). PFOS-exposed groups (60 $\pm$ 2 beats/min) showed significant increase in beating rate compared control (43 $\pm$ 3 beats/min); ($n=6$, $p<0.001$). Reduction in F-action expression were indicated in PFOS-exposed (21 $\pm$ 4 % intensity) and Endothelin-1-exposed (25 $\pm$ 2 % intensity) compared to control (44 + 7 % intensity); ($n=4$, $p<0.001$). Meanwhile, increased in BNP expression were indicated in PFOS-exposed (16 $\pm$ 2 % intensity) and Endothelin-1-exposed group (15 $\pm$ 3 % intensity) compared to control (0.4 $\pm$ 0.1 % intensity); ($n=4$, $p<0.05$). **Conclusion**: Our findings indicate that PFOS may affect the normal function of cardiomyocytes and lead to the development of cardiac hypertrophy.

Keywords: Perfluoroalkyl compound, Perfluorooctane sulfonate, Peralkyl substance, Hypertrophy, Cardiovascular Disease

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Downstream Effects of Berberine Treatment in Colorectal Cancer Cell Line, HCT 116

Muhammad Azizan Samad[a], Mohd Zuwairi Saiman[a,b], Nazia Abdul Majid[a], Saiful Anuar Karsani[a] and Jamilah Syafawati Yaacob[a,b]*

[a]*Institute of Biological Sciences, Faculty of Science, Universiti Malaya, Kuala Lumpur 50603, Malaysia*
[b]*Centre for Research in Biotechnology for Agriculture (CEBAR), Faculty of Science, Universiti Malaya, Kuala Lumpur 50603, Malaysia*

*Corresponding author email: jamilahsyafawati@um.edu.my

Abstract

Introduction: Colorectal cancer (CRC) is one of the most common cancer which is associated with the increment of telomerase expression and activity. Telomerase is the enzyme that functions to maintain the length of telomere which contributes to the unlimited proliferative potential in cancer cells. **Methods:** HCT 116 cell cycle distribution was analysed at 24, 48 and 72 hours of culture. Screening of telomerase inhibitors (boldine, silymarin and berberine) on HCT 116 was done to determine the compound with the lowest concentration that caused 50% inhibition (IC_{50}). TeloTAGGG PCR ELISA was used to determine the telomerase activity. Protein levels were determined by western blot, while RNA levels as well as the relative telomere length were determined by Real Time-PCR. **Results:** The highest S phase percentage occurred at 48 hours. It was revealed that berberine had the lowest IC_{50}. Berberine decreased the telomerase activity in HCT 116 by simultaneous downregulation of *TERT* and *TERC* levels, which resulted in a decrease of the TERT protein level subsequently caused telomere attrition. Berberine induced G_0/G_1 arrest through upregulation of cyclin D1 (CCND1) and downregulation of cyclin-dependent kinase 4 (CDK4) protein and RNA levels, and its effect on the cell cycle was time-dependent. Hydrogen peroxide concentration increased in berberine-treated HCT 116 due to the increment of superoxide dismutase (*SOD*), and decrement of catalase (*CAT*) levels subsequently caused damage to the nuclei. **Conclusion:** In summary, our research has shown the downstream effects of berberine in HCT 116, in which it has inhibited the proliferative ability of the cells.

Keywords: colorectal cancer, HCT 116, berberine, telomerase, telomere

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Poster

The Association of Small Dense LDL Cholesterol Level with Clinical Risk Factors of Metabolic Syndrome Subjects in Selangor

Nur Atiqah Auni Razali[a]*, Nur Amirah Shibraumalisi[b], Zaliha Ismail[c], Thuhairah Hasrah Abdul Rahman[a]*

[a]*Department of Pathology. Faculty of Medicine, UiTM Sg Buloh. Jalan Hospital, 47000, Sg. Buloh, Selangor*
[b]*Department of Primary Care Medicine, Faculty of Medicine, UiTM Selayang. Jalan Prima Selayang 7, 68100, Batu Caves, Selangor*
[c]*Department of Public Health Medicine, Faculty of Medicine, UiTM Sg Buloh. Jalan Hospital, 47000, Sg. Buloh, Selangor*

*Corresponding author email: thuhairah@uitm.edu.my, auniirazalii@gmail.com

Abstract

Introduction: Low density lipoproteins (LDL-c) has been shown to be strongly associated with Metabolic Syndrome (MetS). MetS is strongly associated with atherosclerosis of which small dense LDL-c (sdLDL-c) has been shown to be the atherogenic lipid in the formation of atherosclerotic plaques. However, the determination of sdLDL-c among these subjects have not been widely studied. The aim of this study is to determine the correlation between sdLDL-c in MetS and risk factors of MetS. **Methods:** Fifty-five MetS subjects diagnosed by Joint Interim Statement criteria 2009 were recruited from Primary Care Clinic UiTM Selayang from July 2020 until January 2021. Demographic data and anthropometric measurements were collected and blood samples were analyzed for direct LDL-c concentration on an automated platform c501 and applied to the sdLDL equation by Srisawasdi to derive sdLDL-c level. **Results**: The mean sdLDL-c level among MetS subjects were (1.93±0.77 mmol/L) which was well above the sdLDL cut-off (<1.27 mmol/L). sdLDL-c showed positive correlation with triglycerides (TG) ($r=0.53$, $p<0.01$) and systolic blood pressure (BP) ($r=0.33$, $p<0.05$). Multiple Linear Regression analysis showed that only TG and systolic BP are the significant factors (R square=0.395, $p<0.05$) where 39.5% of the variations in sdLDL-c level can be explained by TG and systolic BP. There were no significant correlations between sdLDL-c and high denstity lipoproteins, fasting blood sugar and waist circumference. **Conclusion:** sdLDL-c is elevated among subjects with MetS, having positive correlation with TG and systolic BP. This suggests that sdLDL can potentially be a useful marker in assessing risk for atherogenesis.

Keyword: sdLDL-c, Metabolic syndrome, direct LDL, lipid profile, automated platform

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Poster

Extraction of Tannin and Antioxidant Activities from *Musa acuminata* cv. Cavendish Peel by Using Various Solvent

Nur Arnisah Ishak[a], Azhari Samsu Baharuddin[a] and Nor Asma Ab Razak[b]*

[a]*Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor D.E., Malaysia*
[b]*Laboratory Molecular Medicine, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor D.E., Malaysia*

*Corresponding author email: norasmarazak@upm.edu.my

Abstract

Introduction: Banana (*Musa* spp.) is consumed fruit and among the highest hectarage fruit crops in Malaysia based on Crop Statistic Report, Department of Agriculture Malaysia 2016. Cavendish cultivars is the most commercially important banana in worldwide and highly potential for industrial export due to their nutrient and shelf life. Rejected banana peel from industry processing which are not equivalent to the standard are cheap and unlimited beneficial content. The usage of synthetic antioxidant contains harmful ingredient and effect human health in long period consumption. Thus, it is important to find an alternative ingredient such as tannin compound to replace it. **Materials and method:** This study investigates the different solvent: aqueous, methanol, ethanol, acetone and mixture of solvent with water for extraction to evaluate tannin, phenolic, flavonoid and antioxidant activity from unripe and ripe Cavendish banana peel. **Result:** Aqueous extract in unripe peel had the highest tannin content (172.30 mg TAE/g sample) compared to the ripe peel (135.090 mg TAE/g sample). Meanwhile, the total content of phenolic (209.154 mg GAE/g sample) and flavonoid (44.111 mg RE/g sample) were higher compare to other solvents. In addition, the antioxidant potential by DPPH and ABTS assay are comparable to other solvents. **Discussion:** Unripe peel of Cavendish banana contain high tannin and phenolic compound than ripe peel from aqueous extract. Aqueous extraction by using water is compromise approach that are eco-friendly nature, effective, cheap, safe and avoiding the toxic solvent. **Conclusion:** High antioxidant compound and activity could be obtained from unripe banana peel by using aqueous extraction.

Keywords: tannin, aqueous, antioxidant, Cavendish, *Musa acuminata,*

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Poster

Establishment of Reference Interval for Haematological Parameter among Afghan Population

Sherzay Najia[a,b], Siti Hamimah Sheikh Abdul Kadir[a,c]*, Noor Shafina Mohd Nor[a,c]

[a]*Institute for Pathology, Laboratory and Forensic Medicine (I-PPerForM), Universiti Teknologi MARA, Cawangan Selangor, 47000,Sungai Buloh, Selangor, Malaysia*
[b]*Department of Biocehemistry, Faculty of Pharmacy, Kabul University, Karte Sakhi, Kabul, Afghanistan*
[c]*Faculty of Medicine, Universiti Teknologi MARA, 47000 Sungai Buloh, Selangor, Malaysia*

*Corresponding author email:sitih587@uitm.edu.my

Abstract

Introduction: The reference interval of haematological parameters is widely affected by race, gender, age and environmental factors. The reference interval used for haematological parameters in Afghanistan are usually the one that are estimated for western population. Therefore, this study was conducted to establish the reference values of haematological parameters for healthy adult males and females among the residents of Kabul city, and to compare these values with mostly used values in local laboratories in Kabul city. **Methods**: The research was designed as a cross sectional study and the samples were collected as per non-random sampling method. Blood samples were collected from the students and employees of Kabul University, Afghanistan. In this study, 166 males and 125 females, aged 18 to 45 years old were included. **Results**: Reference range for haemoglobin was determined between 13.5 g/dl to 18g/dl for male and 11 to 17 g/dl for female, for RBC the reference range was established 4.5 to 7 million per microliter for male and 4 to 6 million per microliter for female. For haematocrit the reference range was established from 36 to 56% and 30 to 54% for female. For MCV the reference range was 73 to 91 fl for male and 64.5 to 94 fl for female, MCH was 24.5 to 35.5 pg for male and 23 to 33 pg for female and MCHC was determined, 32.5 to 38g/l for male and 32.5 to 37.5g/l for female. **Conclusion**: Reference interval for haematological parameters for Afghan population are different from the western parameters.

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Poster

Differential Lipocalin-1 Levels in Keratoconus in a Malaysian Cohort

Jun Bin Ng[a], Jaime Jacqueline Jayapalan[b], Kien Chai Ong[a], Rozaida Yuen Ying Poh[a]*

[a]*Department of Biomedical Science, Faculty of Medicine, University of Malaya, 50603 Lembah Pantai, Kuala Lumpur, Malaysia*
[b]*Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Lembah Pantai, Kuala Lumpur, Malaysia*

*Corresponding author email: rozaiday@um.edu.my

Abstract

Introduction: Keratoconus is an eye disorder characterised by corneal thinning. It is suggested that degenerative processes, inflammatory mediators and biochemical signalling are involved in the pathogenesis of keratoconus. Lipocalin-1 (LCN1) is a major protein component in tears which functions as an extracellular lipid binding protein in tears. Interestingly, LCN1 has been implicated in the prevention of dry eyes and removal of harmful lipophilic molecules. This study aims to identify differentially expressed proteins in Malaysian keratoconus patients and controls. **Methods:** Tear samples were collected from keratoconus patients (n = 7) and normal controls (n = 8). Proteins were separated using two-dimensional gel electrophoresis (2DE) and were detected using modified glutaraldehyde silver stain. Differently expressed protein levels were detected based on maximum fold change of > 1.5 and p-value of < 0.05 using Progenesis. False discovery rate was used to verify for any false-positive readings from the data analysis. Differently expressed protein spots were isolated and identified by MALDI-ToF/ToF. **Results:** Of the 600 protein spots detected, eleven spots were differentially expressed ($p < 0.05$). Three of those were identified as LCN1 and immunoglobulin J chain. LCN1 was lower in keratoconus patients compared to healthy controls ($p < 0.05$). **Conclusion:** The differential protein expression of LCN1 suggested its importance in maintaining homeostasis and viscosity of tears. The protein profiling may be used to screen for keratoconus.

Keywords: Keratoconus, Lipocalin 1, Tears, Proteomics, Malaysia

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Poster

Deciphering the Evolutionary Divergence of Bleg1_2478 B3 Metallo-β-Lactamase from Structural Perspective

Shaw Xian Au[a,b], Noor Dina Muhd Noor[a,c], Hiroyoshi Matsumura[d], Raja Noor Zaliha Raja Abdul Rahman[a,e], Yahaya M. Normi[a,b]*

[a]*Enzyme and Microbial Technology (EMTech) Research Center, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.*
[b]*Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.*
[c]*Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.*
[d]*College of Life Sciences, Ritsumeikan University, Noji-Higashi, Kusatsu 525-8577, Japan.*
[e]*Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.*

*Corresponding author's e-mail: normi_yahaya@upm.edu.my

Abstract

Introduction: Metallo-β-lactamase (MBL) is a hydrolytic enzyme that deactivates β-lactam antibiotics predominantly used in combating bacterial infections. Previously, a hypothetical protein, Bleg1_2437 (currently named as Bleg1_2478) with properties similar to B3 subclass MBL was found to be evolutionary divergent. With recently solved Bleg1_2478 crystal structure, its key structural and binding features with ampicillin compared to a model B3 subclass MBL would be able to shed light on its evolutionary divergence. This is addressed in the present study through *in silico* molecular docking approach. **Methods:** Crystal structures of Bleg1_2478 and L1 B3 MBL from *Stenotrophomonas maltophilia* (PDB ID: 1SML, chain A) were docked with ampicillin using AutoDock4.2.6 software. The key interactions and structural features involved in substrate binding were analysed. **Results:** Results showed that long loop structures at the N- and C-terminals of Bleg1_2478 and L1 active sites played a key role in interacting with the aromatic side chain and penam ring of ampicillin respectively, stabilised mainly by hydrophobic interaction contributed by amino acids at the N-terminal region. The "door", "ceiling" and "floor" architectures observed in L1 MBL were present in Bleg1_2478 and are postulated to be important in substrate accommodation at the active site. However, Bleg1_2478 does not possess certain interactions observed in L1 MBL-ampicillin complex at certain domains. **Conclusion:** Based on these results, Bleg1_2478 may have further differences in active site structure and key interactions than other closely related members of MBL superfamily. Further comparison on their structures may be able to yield more details on its evolutionary divergence.

Keywords: Bleg1_2478, metallo-β-lactamase, substrate binding, molecular docking

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Tocotrienol-Rich Fraction (TRF) Upregulates Antioxidant Genes of Human Endometrial Stromal Fibroblast Cells of Endometriosis

Nur Amira Md Amin[a,b,c], Siti Hamimah Sheikh Abdul Kadir[a,b,c]*, Akmal Hisyam Arshad[d], Norhaslinda Abdul Aziz[e], Nurul Alimah Abdul Nasir[f], Normala Abd Latip[g]

[a]*Institute for Pathology, Laboratory and Forensic Medicine (I-PPerForM), Universiti Teknologi MARA, Cawangan Selangor, 47000, Sungai Buloh, Selangor, Malaysia*
[b]*Institute of Medical Molecular Biotechnology (IMMB), Faculty of Medicine, Universiti Teknologi MARA, Cawangan Selangor, 47000 Sungai Buloh, Selangor Malaysia*
[c]*Department of Biochemistry, Faculty of Medicine, Universiti Teknologi MARA, 47000 Sungai Buloh, Selangor, Malaysia*
[d]*Department of Obstetrics and Gynecology, Faculty of Medicine, Universiti Teknologi MARA, 47000 Sungai Buloh, Selangor, Malaysia*
[e]*Department of Obstetrics and Gynecology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Cheras, 56000, Kuala Lumpur, Malaysia*
[f]*Department of Pharmacology, Faculty of Medicine, Universiti Teknologi MARA, 47000 Sungai Buloh, Selangor, Malaysia*
[g]*Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Faculty of Pharmacy, Universiti Teknologi MARA, 42300 Puncak Alam, Cawangan Selangor, Selangor, Malaysia*

*Corresponding author email: sitih587@uitm.edu.my

Abstract

Introduction: Endometriosis is a common gynecological inflammatory disease defined by the presence of endometrial-like glands and stroma throughout the pelvic cavity. It is often associated with pain and infertility as 30 to 50% of infertile women had been diagnosed with endometriosis. Tocotrienol-rich fraction (TRF) possesses potent antioxidant and anti-inflammatory activities. Therefore, the objective of this study was to investigate the effects of TRF on the cell viability and antioxidant genes of endometrial stromal fibroblast (eSF) cells. **Methods:** eSF cells were isolated from the endometrium of patients with endometriosis (PEC). PECs were cultured and incubated with 25, 50 and 75µg/ml of TRF (LCT carrier) or ETRF (MCT carrier) for 48 hours. Cells were then subjected for MTS assay after incubation period. The differential in antioxidant genes expression were measured by Real-time PCR (qRT-PCR). **Results:** There are no significant differences in the cell viability for 25ug/ml TRF-treated and 25ug/ml ETRF-treated cells in comparison to untreated cells. However, 50 and 75µg/ml of both TRF supplementation showed significant reduction in cell viability up to 10%. Hence, 50 and 75ug/ml supplementation of TRF and ETRF may affect PEC cells viability. SOD1 and SOD2 were significantly upregulated in TRF-treated cells (2.0 ± 0.40 and 1.7 ± 0.11, accordingly). While no significant changes on SOD genes expression were observed in other groups. **Conclusion:** TRF at a concentration of 25ug/ml may activate the expression of antioxidant genes and alleviate the oxidative stress in PEC.

Keywords: Tocotrienol-Rich Fraction, Tocotrienols, Oxidative stress, Endometrial Stromal Fibroblast Cells, Endometriosis

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Poster

Preliminary Evaluation of Optimised PCR-based Assay for the Detection of Pathogenic Familial Hypercholesterolaemia Gene Variants among Asian Population

Norhidayah Rosman[a,b], Hapizah Mohd Nawawi[a,b], Alyaa Al-Khateeb[a,b], Yung-An Chua[a], Ang-Lim Chua[b]*

[a]*Institute of Pathology, Laboratory, and Forensic Medicine (I-PPerForM), Faculty of Medicine, Universiti Teknologi MARA, 47000, Sungai Buloh, Selangor D.E., Malaysia.*
[b]*Faculty of Medicine, Universiti Teknologi MARA, 47000, Sungai Buloh, Selangor D.E., Malaysia.*

*Corresponding author email: anglim@uitm.edu.my

Abstract

Introduction: Familial hypercholesterolaemia (FH) is an inherited disorder characterised by elevation low-density lipoprotein cholesterol levels, which leads to premature coronary artery disease (PCAD). Identification of pathogenic FH variants via genetic confirmation, may prompt early initiation of statins, therefore preventing PCAD. Current method for molecular confirmation of FH is next-generation sequencing (NGS), which is costly and time-consuming. In this study, an optimised tetra-amplification refractory mutation system (T-ARMS) PCR assay was developed and evaluated as a viable alternative to genotype pathogenic FH variants of low-density lipoprotein receptor *(LDLR)* and apolipoprotein B *(APOB)* genes among Asian populations. **Methods:** The T-ARMS PCR assay diagnostic evaluation was performed using 16 NGS-confirmed patients' DNA with pathogenic FH variants, and 17 normal patients' DNA. This assay was designed to detect 12 pathogenic *LDLR* and *APOB* gene variants, at a standardised annealing temperature of 64.6°C and analysed using 1.5% agarose gel electrophoresis. **Results:** The preliminary evaluation of the PCR assay revealed 100% for both sensitivity and specificity. The assay was also able to distinguish between homozygous and heterozygous pathogenic variants amongst the tested samples, which were consistent with the earlier NGS results. **Conclusion:** The promising results of the initial diagnostic evaluation of the optimised T-ARMS PCR implied its potential application as a cheaper and more accessible alternative FH genetic confirmation method, which is suitable to be used among Asian population. Future studies on engaging a larger sample size and multi-centre testing should present a clearer picture of its diagnostic performance.

Keywords: Familial hypercholesterolaemia, pathogenic variant, *LDLR, APOB*, T–ARMS PCR.

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Poster

Global Gene Expression of Obese Patients with Knee Osteoarthritis

Chiaw-Lee Chiew[a], Sik-Loo Tan[a]*, Seow-Hui Teo[b], Azlina Amir Abbas [a,b], Tunku Kamarul Zaman[a,b]*

[a]Tissue Engineering Group (TEG), National Orthopaedic Center of Excellence for Research & Learning (NOCERAL), Department of Orthopaedic Surgery, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur
[b] Sport Team, NOCERAL, Department of Orthopaedic Surgery, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur

*Corresponding author email: tansikloo@gmail.com, tkzrea@gmail.com

Abstract

Introduction: The development and progression of knee osteoarthritis (OA) are closely related to obesity. Limited evidence on focusing the gene expression profile of OA SDFs in obesity. The objectives of this study were to characterize the genes and pathways of OA synovium derived fibroblasts (SDFs) in non-obese and obese subjects (BMI>27.5kg/m^2). **Methods:** Synovium were collected from subjects who underwent arthroplasty and arthroscopy procedures (MREC reference no: 20164-2398) and grouped as non-OA and non-obese (G1, n=3), non-OA and obese(G2, n=3), OA and non-obese(G3, n= 3) and; OA and obese(G4, n=3). SDFs were isolated from the OA and non-OA synovium with the enzymatic digestion method. Total RNA extracted from SDFs were used for Clariom™ S Pico Assay. **Results:** A total 97 gene was upregulated in OA obese SDFs compared to non-OA SDF. The top 10 genes upregulated in OA obese SDFs were PLA2G2A, FGL2, CRIP1, KRBOX1, CGNL1, HPSE, PRRG4, IL13RA2, EPB41L4A and OMG. Among these genes, PLA2G2A and FGL2 are involved in systemic inflammation. Immunofluorescence staining demonstrated a positive expression of VCAM1 in OA SDFs, which may play a role in the infiltration of inflammatory cells in OA SM. Pathway analysis based on the upregulated genes in G4 showed upregulation of protein-protein interactions in podocytes, VEGFA/VEGFR2 signalling pathways. **Conclusion:** Our study identified a spectrum of genes up- and down-regulated in G3 and G4. The pathways identified would allow us to elucidate further the specific cell adhesion molecules that facilitate inflammatory cells infiltration in OA synovium.

Keywords: Osteoarthritis, Obesity, Gene expression, Inflammation

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Poster

Interaction between *In Utero* BPA Exposure and Postnatal Trans Fat Diet on Small Intestine Function of Male Sprague Dawley Rats

Sarah Zulkifli[a], Noor Shafina Mohd Nor[b,c]*, Siti Hamimah Sheikh Abdul Kadir[c,d]

[a]*Institute of Medical Molecular Biotechnology, Faculty of Medicine, 47000 Universiti Teknologi MARA (UiTM) Kampus Sungai Buloh, Selangor, Malaysia*
[b]*Department of Paediatrics, Faculty of Medicine, 47000 Universiti Teknologi MARA (UiTM) Kampus Sungai Buloh, Selangor, Malaysia*
[c]*Institute for Pathology, Laboratory and Forensic Medicine (I-PPerForM), Faculty of Medicine, 47000 Universiti Teknologi MARA (UiTM) Kampus Sungai Buloh, Selangor, Malaysia*
[d]*Department of Biochemistry and Molecular Medicine, Faculty of Medicine, 47000 Universiti Teknologi MARA (UiTM) Kampus Sungai Buloh, Selangor, Malaysia*

*Corresponding author email: drshafina@uitm.edu.my

Abstract

Introduction: Bisphenol A (BPA) level has been associated with higher body mass index children and adults, as well as gut inflammation. Studies reported conflicting findings on BPA exposure impact on the gastrointestinal function and the risk of obesity. Thus, this study aimed to investigate whether in utero BPA exposure and postnatal TFD poses synergistic effects on the small intestine morphology and increases the risk of obesity. **Methods:** Twelve pregnant Sprague Dawley rats were divided into two groups: vehicle control (P80) and BPA-exposed (BPA; 5 mg/kg/day) until end of gestation. Twenty-four male offsprings from the pregnant rats were weaned off at postnatal week (PNW) 3 and were fed with either normal diet (ND) or 25%kcal TFD from PNW3–13; grouped into P80-ND, P80-TFD, BPA-ND and BPA-TFD groups. Body weight (BW), WC, water and food intake of offsprings were measured weekly from PNW3 to PNW13. **Results:** Increase in BW and WC were observed as offsprings developed from weanlings (PNW3) to adults (PNW13), with significant differences in BW observed between P80-TFD vs BPA-ND from PNW11 (470.7 ± 13.1 vs 431.2 ± 11.1 g; $p<0.05$) to PNW13 (521.5 ± 15.1 vs 469.8 ± 13.9 g; $p<0.01$). Additionally, P80-ND consistently showed significantly higher food and water intake than BPA-TFD from PNW7–12. However, for histology, no significant differences were observed in villi length and width, and crypt length between all groups. **Conclusion:** Our findings suggest that prenatal BPA with and without postnatal TFD may have no significant effects on the small intestinal architecture which could lead to obesity development.

Keywords: *in utero* BPA exposure, postnatal trans fat diet, obesity, small intestinal function

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Poster

Natural Quinones Induce ROS-Mediated Apoptosis and Inhibit Cell Migration in PANC-1 Human Pancreatic Cancer Cell Line

Prasad Narayanan[a]*, Reyhaneh Farghadani[b], Shaik Nyamathulla[a,d,]*, Jayakumar Rajarajeswaran[c]
R. Thirugnanasampandan[e], Gunasekaran Bhuwaneswari[f]

[a]*Department of Pharmaceutical Technology, Faculty of Pharmacy, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.*
[b]*Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, Selangor Darul Ehsan 47500, Malaysia.*
[c]*Department of Molecular Medicine, Faculty of Medicine, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.*
[d]*Centre for Natural Products Research and Drug Discovery (CENAR), Universiti Malaya, 50603 Kuala Lumpur, Malaysia.*
[e]*PG and Research Department of Botany, Kongunadu Arts and Science College, Coimbatore-641029, Tamil Nadu, India.*
[f]*PG and Research Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore-641029, Tamil Nadu, India.*

*Corresponding author email: prasad1906@siswa.um.edu.my; nyamathullask@um.edu.my

Abstract

Introduction: Pancreatic cancer is a malignancy with poor prognosis and high mortality rates worldwide. Thymoquinone, plumbagin, and juglone, are *natural quinones, widely reported for promising anticancer effect on different cancer cells.* However, the cytotoxic activity and their anti-metastatic effects are unknown against human pancreatic cancer cell line (PANC-1). **Methods:** Cytotoxic activity of quinones was evaluated on PANC-1 cells through MTT cell viability assay. Next, apoptosis was assessed through morphological changes, AO/EB staining and further confirmed by Annexin-V-FITC staining assay. ROS generation was quantified by DCFDA fluorescent probes. Anti-migratory effect was studied by wound healing (scratch) assay and MMP-9 assay. **Results:** Cell viability significantly decreased in a dose-dependent manner with the IC_{50} value of 5.72, 5.74 and 22.22 μM respectively for juglone, plumbagin and thymoquinone after 24-hour treatment. *Typical apoptotic morphology was demonstrated in treated PANC-1 cells compared to control.* Flow cytometry analysis following Annexin V-FITC and PI staining demonstrated the potency of quinones to induce early and late apoptosis in treated cells. ROS accumulation confirmed the involvement of the mitochondrial intrinsic pathway. Wound healing potential of all three quinones were demonstrated by inhibiting cell migration in a time-dependent manner. Only juglone dose-dependently reduced MMP-9 expression in treated PANC-1 cells, which was in accordance with its effect on cell migration whereas plubagin and thymoquinone showed no effect. **Conclusion:** In conclusion, thymoquinone, plumbagin, and juglone significantly inhibited cell growth and induced ROS-mediated apoptosis in PANC-1 cells. In addition, they could be potent anti-metastatic agents due to their anti-migratory effect against PANC-1 cells.

Keywords: pancreatic cancer, quinones, cell viability, apoptosis, anti-metastatic

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Poster

Cytotoxicity effects of Allicin towards human oral squamous cell carcinoma (OSCC) cell lines, ORL-48 and SCC-15

Farrah Hazwani, Indah Mohd Amin*, Mohd Aizat Abdul Rahim

Faculty of Dentistry, UiTM Sungai Buloh Campus, Jalan Hospital, 47000 Sungai Buloh, Selangor.

*Corresponding author email: indahma@uitm.edu.my

Abstract

Introduction: Oral squamous cell carcinoma (OSCC) has been a contributing factor to 90% to 95% of oral malignancies. The 5-year survival rate for patients with oral cancer has been stagnant at 50% for decades. Despite many advances in treatment and prognosis, the main solution for oral cancer is chemotherapy and surgery. The genotoxic drug, cisplatin has been introduced as an alternative for the treatment. However, cisplatin has been studied to give out repercussions such as nausea, diarrhea, pain and vomiting. Therefore, a more reliable anticancer agent has to be discovered. Allicin (diallylthiosulfinate) has been known for decades for its antioxidant, antimicrobial, antifungal and anti-inflammatory activities. Serving as an anticancer agent is also allicin's most prominent advantage and has been studied in numerous types of cancer cells. In a dose-dependent manner, allicin has been studied to inhibit proliferation on cancer cells. Unfortunately, not many studies reported on oral cancer cell lines. Hence, this paper will provide an overview of the research framework to investigate cytotoxicity effects of allicin in oral cancer cell lines, ORL-48 and SCC-15 in comparison with cisplatin. **Methods**: Pure compound allicin at final concentration of 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml was diluted and used in the experiment. The in vitro cytotoxicity test was determined by WST-1 assay. **Results** and **conclusion**: The expected findings may reveal the cytotoxicity effects of allicin towards oral cancer cell lines, ORL-48 and SCC-15.

Keywords: oral cancer, allicin, OSCC, cytotoxicity, cisplatin.

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Poster

Evaluation of Cadmium Treatment on ATP Level, Cell Membrane Integrity and Cell Viability in *Caco-2* Cell Line

Fu Hou Wong [a,b],Chiat Yin Ng [a,b], Nur Adania[a], Palanirajan Vijayarajkumar[c], Farahnaz Amini[a]*

[a] *School of Healthy Aging, Medical Aesthetics, Regenerative Medicine, Faculty of Medicine and Health Sciences, UCSI University, Kuala Lumpur, Malaysia*
[b] *Faculty of Applied Science, UCSI University, Kuala Lumpur, Malaysia*
[c] *Department of Pharmaceutical Technology, UCSI University, Kuala Lumpur, Malaysia.*

*Corresponding author email: farahnaz@ucsiuniversity.edu.my

Abstract

Introduction: Human exposure to heavy metals via food, water, and air pollution may partially explain the higher incidence of non-communicable diseases. Cadmium is one of the most hazardous heavy metals for human health. This study aimed to evaluate cell viability, ATP level and cell membrane integrity in Caco-2 treated with cadmium. **Methods:** Caco-2 cells were cultured in DMEM(5 mM galactose) and treated with different cadmium concentration (0.25, 1, 2.5, 5, 7.5, 10 and 20) μg/ml for 24, 48, 72 and 192 hours. ATP level, cell viability and membrane integrity were measured using CellTiter-Glo Luminescent Cell Viability and Mitochondria ToxGlo Assay. **Results:** ATP level decreased once cadmium concentration was increased (48.6% - 6.9% at 24 hours; 21.09% - 1.09% at 48 hours; 15.76% - 0.47% at 72 hours; and 13.3% - 0.63% at 96 hours). Cell viability test indicated IC_{50} of 171 uM, 100.52 uM, 69.66 uM and 72.86 uM for 24, 48, 72, and 96 hours, respectively. Protease level decreased once cadmium concentration was increased (62.5% to 40.1% at 24 hours; 80.86% to 62.14% at 48 hours; 85.44% to 66.71% at 72 hours; and 69.97% to 57.22% at 96 hours). **Conclusion:** The results showed that regardless of cadmium concentration, ATP level decreased with longer exposure time. After cadmium exposure in Caco-2, ATP level decreased in a dose- and exposure time-dependent manner. A higher concentration of cadmium indicates the lower activity of protease released from the membrane-compromised cell. Cadmium demonstrated a biphasic dose-response phenomenon characterized by low-dose stimulation and high-dose inhibition.

Keywords: Cadmium, Caco-2, ATP, membrane integrity, protease level

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Poster

Apoptotic Mechanisms of Tocotrienol Isomers towards Oral Squamous Carcinoma Cell Lines, ORL-48.

Atiqa Syazwani Ridzuan, Khor Goot Heah, Indah Mohd Amin*

Faculty of Dentistry UiTM Campus Sg Buloh, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia

*Corresponding author email: indahma@uitm.edu.my

Abstract

Introduction: Tocotrienol is a subgroup of Vitamin E and it is divided into four isomers known as alpha-, beta-, gamma-, and delta-tocotrienol. These compounds can be found in nature where their major source is palm oil. Recent studies showed that tocotrienol is effective in inducing apoptosis to other cancer cell lines such as cervix (HeLa cell line), lung (A549 cell line), brain (U87MG cell line) and neuroblastoma (SH-SY5Y cell line). This paper aims to investigate the effectiveness of tocotrienol isomers in inducing cell death of oral squamous carcinoma cell lines, ORL-48. **Methods:** The pure compounds of γ- and δ-tocotrienols at the concentration of 50 μM, 75 μM, 100 μM, 125 μM and 150 μM were used in the experiment. The *in vitro* cytotoxicity of tocotrienol isomers on ORL-48 was determined by using MTS Cell Proliferation Colorimetric Assay. The cell death mechanism of the isomers on ORL-48 was analysed by using TUNEL Assay. **Results and Conclusion:** The expected findings may reveal the toxicity of tocotrienol isomers on ORL-48, and the mode of cell death induced by the isomers on ORL-48. A review of relevant literature on anti-cancer properties of tocotrienol isomers also be presented in this paper.

Keywords: Vitamin E, tocotrienols, apoptosis, oral squamous carcinoma cell lines

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Poster

Modulation of Small Interfering RNA in Newcastle Disease Virus Persistently Infected Colorectal Cancer Cells

Lee-Chin Chan[a], Adelene Ai-Lian Song[a], Khatijah Yusoff[a,b], Suet-Lin Chia[a,b]*

[a]*Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor D.E., Malaysia*
[b]*UPM-MAKNA Cancer Research Laboratory, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor D.E., Malaysia*

*Corresponding author email: suetlin@upm.edu.my

Abstract
Introduction: Newcastle disease virus (NDV) is an oncolytic virus that shows promising results in various cancer cell lines both *in vitro* and *in vivo*. During the course of infection, some cancer cells develop resistance to NDV infection while permitting the virus to replicate within the cells. Mechanism involved is yet to be elucidated. **Methods:** In this study, we aimed to develop several persistently NDV-infected (PI) colorectal cancer cell lines, including HCT116, RKO and LoVo to study the mechanism in the persistence of infection. These cancer cell lines were infected with recombinant NDV expressing green fluorescent protein, (rAF-GFP). After three rounds of infections, the NDV-resistance subpopulation in each cell lines emerged. **Results:** GFP expression of these PI cells were confirmed by flow cytometry. Virus released from these PI cells are infectious and able to form plaques on cancer cells. Annexin V/propidium iodide assay revealed that the PI cells elevated in level of apoptotic markers. Upregulation of pro-survival Bcl-2 proteins that detected in PI cells suggested that these proteins expression promoted cellular viability under viral persistent infection. Small RNA will be used to knockdown the elevated proteins to enhance the NDV therapeutic effect. **Conclusion:** This study describes the potential key players that contribute to PI development of NDV in colorectal cancer cells.

Keywords: Newcastle disease virus, colorectal cancer, persistent infection

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Poster

Expression of Dominant AMPK Gamma 1 Subunit Gene in Post-mortem Obese Human Perihepatic Adipose Tissue

Norainfarahin Zainal Aznam[a,b], Ruzi Hamimi Razali[b], Thuhairah Hasrah Abdul Rahman[b], Zaliha Ismail[b], Siew Sheue Feng[c], Aletza Mohd. Ismail[b], Mansharan Kaur Chainchel Singh[a,b,c]*

[a]*Institute of Pathology, Laboratory and Forensic Medicine (I-PPerForM), Universiti Teknologi MARA, Sungai Buloh, Selangor, Malaysia.*
[b]*Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh, Selangor, Malaysia.*
[c]*National Institute of Forensic Medicine (IPFN), Hospital Kuala Lumpur*

*Corresponding author email: mansharan@uitm.edu.my

Abstract

Introduction: Adenosine monophosphate (AMP)-activated protein kinase (AMPK), regulator of energy balance, is a heterotrimeric complex consisting of α catalytic subunit and β and γ regulatory subunits. Theoretically, 12 possible complexes have been formed exhibiting differential tissue specificity, in muscle three complexes were identified (α2β2γ1, α2β2γ3, and α1β2γ1) whilst one in liver (α1βγ1). The aim of this study is to determine the dominant AMPK subunit genes present in perihepatic adipose tissue. **Methods:** Perihepatic adipose tissue was collected from post-mortem cases (32 lean; 33 obese) at the National Institute of Forensic Medicine (IPFN), Hospital Kuala Lumpur. Total RNA was extracted from perihepatic adipose tissues with its quantity and integrity determined by bioanalyzer. Gene expressions of all AMPK subunits were studied by real-time polymerase chain reaction using SYBR-green as a fluorophore detection. Then, comparative $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression of each target gene; with the expression levels of all genes normalized to that of GAPDH, RPLP0 and HPRT1. Mean differences were evaluated by Mann-Whitney test for two-sample comparison using SPSS 26.0 (SPSS Inc., USA). **Results:** There were no significant difference of α and β subunits gene expressions in both lean and obese individuals. For gamma subunits, gene expressions of γ2- and γ3-subunits were lower whilst only γ1-subunit was significantly higher in obese ($U = 367$, $p = .034$) compared to lean individuals. **Conclusion:** Results show that the dominant AMPK subunit genes present in perihepatic adipose tissue among obese individuals is γ1. This suggests that γ1-subunit present in perihepatic adipose tissue is specific to obesity.

Keywords: AMPK, gamma subunit, adipose tissue, post-mortem, human

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Poster

Glycosylation Inhibitory Effect of 1-DNJ Towards Extracellular Matrix Proteins Expression in Osteosarcoma Cells

Zulaika Roslan[a,b], Mudiana Muhamad[a], Lakshmi Selvaratnam[c], Sharaniza Ab-Rahim[a]*

[a] *Department of Biochemistry and Molecular Medicine, Faculty of Medicine, Universiti Teknologi MARA, Cawangan Selangor, Kampus Sungai Buloh, 47000 Sungai Buloh, Selangor, Malaysia.*
[b]*Institute for Medical and Molecular Biotechnology (IMMB), Faculty of Medicine, Universiti Teknologi MARA, Cawangan Selangor, Kampus Sungai Buloh, 47000 Sungai Buloh, Selangor, Malaysia.*
[c]*Dept. of Biomedical Sciences (Anatomy), School of Medicine and Health Sciences, Monash University Malaysia, Jalan Lagoon Selatan, 47500 Bandar Sunway, Selangor D.E., Malaysia.*

*Corresponding author email: sharaniza_abrahim@uitm.edu.my

Abstract

Introduction: Rapid progression of cancer cells towards metastasis particularly in highly metastatic osteosarcoma is mainly attributable to cell migration involving interactions between cell-cell and cell-extracellular matrix (ECM). ECM proteins are regulated by glycosylation of integrins of which when aberrant, increases the rate of cancer cells migration and invasion. Inhibition of the aberrant glycosylation by 1-Deoxynojirimycin (1-DNJ) towards alpha-glucosidase reduced migration rate of melanoma cells. Thus, this study aims to investigate the glycosylation inhibitory effect of 1-DNJ towards ECM proteins osteosarcoma cells. **Methods:** Two types of osteosarcoma cells, MG63 and HOS143B were treated with 1-DNJ at concentration of 0.5mM. At post- 24 hours treatment, total cellular protein was extracted for Western blot (WB) analysis while scratch assay was performed on another set of treated cells. **Results:** WB result showed high level of expression of ECM proteins; collagen type II (COL II), fibronectin (FN), and vitronectin (VN) in 1-DNJ treated cells of both types compared to the control (non-treated). Scratch assay results revealed higher rate of cell migration in both MG63 and HOS143B treated cells compared with control. **Conclusion:** Results showed that inhibition with 1-DNJ intensified the effects of aberrant glycosylation in osteosarcoma cancer cells, as evidenced by increased ECM protein expression and cell migration rate. Nonetheless, osteosarcoma metastasis involves various cellular microenvironment factors which further investigation to elucidate the underlying mechanism between aberrant glycosylation and cancer cell progression. Various external factors may also involve in migration process hence further research is required to fully understand 1-DNJ's mechanism in OS cancer progression.

Keywords: 1-Deoxynojirimycin, Extracellular Matrix Proteins, cell migration, osteosarcoma cells, aberrant glycosylation

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Poster

Effect of hsa-miR-3131 Transfection Upon Cell Viability in Imatinib Sensitive and Imatinib Resistance Chronic Myeloid Leukemia Cells

Syarifah Faezah Syed Mohamad*, Marjanu Hikmah Elias

Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia, Persiaran Ilmu, 71800 Bandar Baru Nilai, Negeri Sembilan, Malaysia.

*Corresponding email address: faezahalyahya@gmail.com

Abstract

Introduction: Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by the Philadelphia chromosome and the BCR-ABL fusion gene. Despite tyrosine kinase inhibitors (TKIs) wide application, TKIs resistance occurred in nearly 20-30% of chronic myeloid leukemia (CML) patients. Identification of new molecular therapeutic target to overcome TKI resistance is in demand. MicroRNAs (miRNAs) are small, noncoding, single-stranded RNAs that consist of 18 to 25 nucleotides that participate in various cellular processes by posttranscriptional regulating gene expression. microRNA has demonstrated potential as a biomarker for cancer diagnosis, prognosis, and therapeutic targets. In present study, our aim was to determine cell proliferation and viability of K562-s and K562-r cells transfected with hsa-miR-3131. **Methods**: Imatinib IC_{50} for 24h and 48h in both cells were determined. miRNA targeting 3'UTR of ABL1 was verified using DIANA TOOLS, the micro-T CDS. **Results**: In-silico analysis shows that miR-3131 has a strong binding conformity with 3'UTR of ABL1 with the miTG score of 0.97. Hence, mir-3131 was selected to be transfected with both cells. The study included four groups which are the (hsa-miR-3131), (Imatinib), (hsa-miR-3131 & Imatinib), (no transfection control) and (miR-1 as the positive control). All groups were transfected and incubated for 24h, 48h and 72h for cell proliferation using MTS assay. Results of IC_{50} for K562-s and K562-r cell for 24h were 45.3 μM and 60.5 μM, while for 48h IC_{50} were 2.38 μM and 23.47 μM, respectively. Hsa-mir-3131 transfected into K562-s was able to inhibit cell proliferation at 72h with the lowest cell viability of 78%. Whilst the K562-r cell proliferation was highly inhibited when transfected with hsa-miR3131 during 48h with 83% cell viability. However, hsa-miR-3131 transfected K562-s cells did show an increase of inhibition of 8.9% with Imatinib addition compared when treated with Imatinib alone during 24h. **Conclusion**: Although hsa-mir-3131 may retain certain effect towards the CML cell proliferation, it is highly suggested to determine its molecular mechanism underlying CML cells proliferation.

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Poster

GAS5 Associated CeRNA Network in Lower Grade Glioma

Jesminder Kaur Jaswant Singh*, Anwar Norazit, Nurain Salehan, Kamariah Ibrahim

Department of Biomedical Science, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

*Corresponding author email: 17042064@siswa.um.edu.my

Abstract

Introduction: Grade II and grade III lower grade gliomas (LGG) consist of astrocytic, oligo-astrocytic and oligodendroglia. Although LGG has a better survival rate compared to patients with glioblastoma multiforme (Grade IV glioma), it is a fatal disease of young adults because grade II lesions can eventually progress to high-grade glioma (Grade III and IV). Long noncoding RNA GAS5 has been identified as a tumour suppressor gene located at chromosome 1q25 with low levels of GAS5 expression associated with poor overall survival in cancers. GAS5 associated regulatory axis can be targeted as potential therapeutic targets or biomarkers in lower grade glioma. **Methods:** In this study, we grouped the TCGA-LGG dataset into high (n=125 samples) and low level (n=128 samples) of GAS5 expression, and using the bioinformatics methods, GAS5 associated competing endogenous RNA (ceRNA) network was constructed to identify GAS5 regulatory axis in lower grade glioma. **Results:** Gene set enrichment analysis showed low GAS5 group has high enrichment score in the epithelial mesenchymal transition (EMT) gene set. ceRNA network including genes from EMT gene set was established. EMP3, TNFRSF11B and TIMP3 genes were found to be upregulated in low GAS5 expression group. **Conclusion:** By analysing TCGA-LGG dataset**,** we identified EMP3, TNFRSF11B and TIMP3 are negatively regulated by GAS5. The ceRNA network can further be evaluated as potential therapeutic targets or potential biomarkers in LGG.

Keywords: Glioma, lncRNA, bioinformatics

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Poster

Synthesis and Cytotoxic Effects of Novel Paclitaxel (PTX)-loaded PLGA(Poly (lactic-co-glycolic acid) Nanoparticle Bio-conjugate with EGFR Monoclonal Antibody for Triple Negative Breast Cancer

Shalini Krishnan[a]*, Shahrul Bariyah Sahul Hamid[a], Varatharajan[b]

[a]*Oncological & Radiological Sciences Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Penang, Malaysia*
[b]*Department of Pharmacology, Faculty of Pharmacy, AIMST University, Kedah.*

*Corresponding author email: shalinikrishnan31@gmail.com

Abstract

Introduction: Triple negative breast cancer (TNBC) is categorized as an aggressive subtype of breast cancer that expressed EGFR receptor. Currently, Paclitaxel is a standard chemotherapy drug that is used in treatment. Delivery of this drug can be improved for targeted therapy by binding to EGFR receptor. **Objective:** The aim of this study was to develop an efficient drug delivery system for specifically targeting the TNBC cells without affecting healthy cells by formulating the Paclitaxel (PTX)-loaded PLGA (Poly (lactic-co-glycolic acid) nanoparticle bio-conjugate with EGFR monoclonal antibody (mAb). **Methods**: The nanoparticles were synthesized by the using double emulsification solvent evaporation method. The integrity of mAb was confirmed by performing SDS page. Subsequently. cytotoxicity of the PTX-loaded PLGA nanoparticles was tested in TNBC cell line (MDA-MB-231) with MTT assay. **Results and Discussion**: The sizes of the PTX-loaded PLGA nanoparticles were approximately 120 nm. The SDS PAGE analysis indicated that the mAb integrity remained the same after conjugation with nanoparticles. The cytotoxicity activity was confirmed by using MDA 231 cell line with MTT Assay with an inhibitory concentration (IC_{50}) of 2 mg. Results indicated that the and antibody conjugated nanoparticles showed best specific targeting of the EGFR receptor in MDA 231 cells. **Conclusion:** Current study suggest that the anti-EGFR anchored PTX loaded nanoparticle may have the ability to target the TNBC cells and could potentially improve the therapeutic action of PTX.

Keywords: Triple negative breast cancer (TNBC), PLGA Nanoparticles, EGFR monoclonal antibodies

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Poster

Hyperglycaemia Disrupts FKN and RANK-RANKL-OPG System in a Pregnant Rat Model

Noor Eliza Hashim[a]*, Mardhiah Tahir[a], Intan Suhana Zulkafli[a], and Rasheeda M. Zamin[a]

[a]*Department of Anatomy, Faculty of Medicine, University of Malaya, Kuala Lumpur*

*Corresponding author email: nooreliza@um.edu.my

Abstract

Introduction: Diabetic pregnancy is linked to heightened inflammatory state, which is the root cause for various complications. Fractalkine (FKN) and RANK-RANKL-OPG system are inflammatory markers associated with angiogenesis and vascular calcification. This study aims to examine the effects of hyperglycaemia on pregnancy outcomes and its association with the levels these markers in maternal serum and placental tissue. **Methods:** Pregnant female *Sprague-Dawley* rats were injected with 45mg/kg body weight of Streptozotocin on gestational day (GD) 7 to induce hyperglycaemia. The control group was injected with the vehicle. The pregnancies were monitored until experimental endpoints at GD15 and GD21. Pregnancy outcomes were documented. Placenta and maternal plasma were collected for protein levels and mRNA expressions of FKN, RANK, RANKL and OPG. **Results:** Hyperglycaemia resulted in a significant reduction of maternal daily body weight despite an increased in daily food consumption. There was also an increase in foetal resorption and a reduction in foetoplacental weight ratio in the hyperglycaemic group. There was a significant increase in circulating FKN, RANKL and OPG together with increased placental FKN levels. OPG mRNA expression was also upregulated in the placental tissue. **Conclusion:** Maternal hyperglycaemia alters systemic and local FKN and RANK/RANKL/OPG system. These inflammatory markers are known to cause vascular remodeling and calcification in the peripheral artery disease. Disruption of these markers in maternal hyperglycaemia could be responsible for the adverse pregnancy outcomes observed in this study. Further investigation need to be done to evaluate the possibility of advanced aging and microcalcification in the placenta**.**

Keywords: Hyperglycaemia, FKN, RANK-RANKL-OPG, placenta, rat

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Poster

Exopolysaccharide (EPS) from *Bifidobacteria pseudocatenalatum* Induced Apoptosis Cell Death in Caco-2 Cells

Husna Zulkipli[a,b], Hifa Nazirah Mohammed Yaziz[b], Maslinda Musa[b], Siti Hamimah Sheikh Abdul Kadir[c] , Khalilah Abdul Khalil[b]*

[a]*Faculty of Applied Sciences, Universiti Teknologi MARA, Cawangan Perak, Kampus Tapah, Tapah Road, 35400 Perak, Malaysia*
[b]*Department of Biomolecular Sciences, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia*
[c]*Institute for Medical Molecular Biotechnology, Universiti Teknologi MARA, Cawangan Selangor, Sungai Buloh Campus, Sungai Buloh, Selangor, Malaysia*

*Corresponding author email: khali552@uitm.edu.my

Abstract

Introduction: Exopolysaccharide (EPS) is one of the bacterial components, predominantly studied in lactic acid bacteria (LAB). Its crucial roles are evidenced in probiotic activity including survival, adhesion, and anti-tumor/cancer effect. Several studies have also reported that LAB EPS exhibited highest cytotoxicity effect and mitigated colorectal cancer (CRC) cells proliferation when compared to other bacterial components. Besides LAB, another probiotic known as Bifidobacteria also inhabit the human colon and produce EPS likewise. However, the EPS has not been well explored. Thus, the aim of this study is to investigate its potential specifically in susceptibility to CRC treatment. **Methods**: *Bifidobacteria pseudocatenalatum* was cultured in rice wastewater medium. EPS was extracted and purified. Caco-2 cells were treated with EPS at concentrations 1, 5 and 10 mg/ml. Cytoxocity level was determined at 24- and 48-hour time point using MTS assay. Apoptosis analysis were performed using Annexin V and cell cycle assay. 30 µM Rapamycin was used as positive control. **Results**: EPS was able to inhibit Caco-2 cells proliferation in dose- and temporal-dependent manner. With viable cells at 44%, 5 mg/ml EPS at 48-hour time point was used for further experiments. The EPS-treated Caco-2 cells has highlighted an increase in early and late apoptosis induced cell death as compared to negative control ($P<0.001$). In addition, EPS also increased sub G2/M population of Caco-2 cells ($P<0.001$). **Conclusion**: These fundamental findings revealed potential anticancer properties of EPS through apoptosis in CRC. EPS is part of probiotics which naturally inhabits human colon; thus, the results may support the application of EPS as prospective therapeutic in the treatment of CRC in the future.

Keywords: Exopolysaccharide, Bifidobacteria, Colorectal cancer, Caco-2 cells, Apoptosis

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Poster

Optimization of Milk Fermentation Incorporated with Recombinant *Lactococcus lactis* NZ3900

Yu Hsuan How[a], Michelle Yee Mun Teo[b], Lionel Lian Aun In[b], Liew Phing Pui[a]*

[a]*Department of Food Science with Nutrition, Faculty of Applied Sciences, UCSI University, 1, UCSI Heights, Jalan Puncak Menara Gading, Taman Connaught, 56000 Cheras, Wilayah Persekutuan Kuala Lumpur, Malaysia*
[b]*Department of Biotechnology, Faculty of Applied Sciences, UCSI University, 1, UCSI Heights, Jalan Puncak Menara Gading, Taman Connaught, 56000 Cheras, Wilayah Persekutuan Kuala Lumpur, Malaysia*

*Corresponding author email: puilp@ucsiuniversity.edu.my

Abstract

Introduction: *Lactococcus lactis* NZ3900 is a common food-grade recombinant strain used for protein expression and a potential delivery carrier for orally-administrated vaccines. However, the lactococcal strain could be susceptible to gastrointestinal digestion. Hence, milk could serve as a protective matrix and nutrient source for the recombinant *L. lactis*. This study aimed to optimize the formulation and fermentation condition of *L. lactis* NZ3900-fermented milk to achieve high viability and low syneresis. **Methods:** The milk was fermented using a recombinant *Lactococcus lactis* NZ3900 strain expressing a cancer peptide vaccine. The formulation and condition of milk fermentation were optimized using the Box-Behnken design. The effect of initial inoculum concentration (5, 10, 15% v/v), fermentation time (18, 24, 30 h), and fermentation temperature (23, 30, 37°C) on viability and syneresis of the *L. lactis*-fermented milk was investigated. Three commercial fermented milk served as control. **Results:** The predicted responses of maximum viability (9.56 $\log_{10}$ CFU/mL) and minimum syneresis (59.6%) for *L. lactis* NZ3900-fermented milk was obtained by 11.2% (v/v) initial inoculum concentration, 30 h fermentation time, and 27 °C fermentation temperature. The viability of the *L. lactis* NZ3900 in fermented milk displayed higher as compared to all three of the commercial fermented milk. Furthermore, the syneresis of the *L. lactis* NZ3900 falls within the range of commercial fermented milk. **Conclusion:** This study demonstrated that milk acts as a good carrier for food-grade recombinant *L. lactis* NZ3900 and the *L. lactis* NZ3900-fermented milk has the potential to be developed as an orally-administrated approach for vaccine delivery.

Keywords: Fermentation, *Lactococcus lactis*, milk, recombinant vector, oral vaccine.

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Poster

Determination of Total Phenolic Content and Antibacterial Activities of Malaysian Traditional Plants against Selected Pathogenic Bacteria

Ibrahim Abuga[a,c] Shaida Fariza Sulaiman[b] Ridhwan Abdul Wahab[c]
Mohammad Syaiful Bahari Abdull Rasad [c]*

[a] *Faculty of Life Science, Department of Plant Science and Biotechnology Kebbi State University of Science and Technology, Aliero, Nigeria.*
[b]*School of Pharmaceutical Sciences, Universiti Sains Malaysia, Malaysia.*
[c]*Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, Malaysia.*

*Corresponding author email: syaiful@iium.edu.my

Abstract

Introduction: Malaysia has vast species of medicinal plants that are traditionally used to combat diseases, thus, it has been counted as one of the 12 megadiverse nations with high rate of endemism. Many researches have been conducted on Malaysia medicinal plants as antibacterial agents. However, lack of scientific studies to prove the effectiveness of these plant materials is of great concern. **Methods:** The in vitro antibacterial activities of 5 Malaysian medicinal methanolic crude extracts were determined using broth dilution method against six different strains of pathogenic bacteria; *Escherichia coli* 0157:H7, *Escherichia coli* (ATCC 25922), *Listeria monocytogenes* (ATCC 19115), *Salmonella paratyphi* (ATCC 9150), *Salmonella typhi* (ATCC 14028), *Staphylococcus aureus* (ATCC 700699). Total phenolic content (TPC) was determined using Folin-Ciocalteu's (FC) method. The results were expressed as microgram of gallic acid equivalents per milligram extract (μg GAE/mg extract). **Results:** Most of the extracts exhibited antibacterial activities against two or more tested bacteria with the lowest minimum inhibitory concentration (MIC) value of 31.25 μg/ml obtained from the bark extract of *Cinnamomum verum* against *Listeria monocytogenes*. The total phenolic content (TPC) of each extract was calculated using the gallic acid calibration curve. Bark extract of *Cinnamomum verum* was observed to possess the highest total phenolic content of 437.03 ± 3.59 μg GAE/mg. **Conclusion:** Our findings suggest the possibility of using the bark extract of *Cinnamomum verum* as a new source of natural antibiotics in future.

Keywords: Methanolic crude extracts, antibacterial screening, Total phenolic content. Malaysian traditional plants.

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Poster

Isolation and Characterization of Prenyltransferase Isomers from *Boesenbergia rotunda*

Alicia Jia Huey Ng[a], Nur Kusaira Khairul Ikram[a]*, Boon Chin Tan[b]*

[a] *Institute of Biological Science, Faculty of Science, University of Malaya, 50603, Kuala Lumpur, Malaysia*
[b] *Centre for Research in Biotechnology for Agriculture (CEBAR), University of Malaya, 50603, Kuala Lumpur, Malaysia*

*Corresponding author email: nkusaira@um.edu.my; boonchin@um.edu.my

Abstract

Introduction: Prenylated flavonoids exhibit beneficial effects on human health. Nevertheless, the low abundance of prenylated flavonoids in nature has limited their applications. Prenyltransferase (PT), an enzyme involved in the prenylation process is crucial in catalysing prenylflavonoids. To date, only a few flavonoid-related PT genes have been identified probably due to the membrane-bound nature of PTs. Hence, this study aimed to isolate and characterise PTs from a medicinal ginger, *Boesenbergia rotunda* (designated as *BrPT1*). **Methods:** The full-length cDNA of *BrPT1* isolated from *B. rotunda* using PCR was characterized by determining its molecular weight (MW) and isoelectric point (p*I*) using Compute MW/pI (https://web.expasy.org/compute_pi/). The deduced amino acid sequence of BrPT1 was aligned with other PTs using ClusterW before constructing a phylogenetic tree using the neighbourhood-joining method with a bootstrap value of 1000 using the Molecular Evolutionary Genetics Analysis version 10 (MEGAX). Conserved motif sequences were analysed using Multiple Em for Motif Elicitation (MEME) (https://meme-suite.org/meme/tools/meme), whereas transmembrane domains were predicted using TMHMM (http://www.cbs.dtu.dk/services/TMHMM/). **Results:** The isolated cDNA of *BrPT1* (1,197 bp) encodes a predicted 398 amino acid residues with a molecular weight of 44.6 kDa and p*I* of 9.86. BrPT1 has 9 transmembrane domains. Phylogenetic and motif sequence analysis showed that BrPT1 belongs to the HG family, sharing homologies with homogentisate PTs involved in tocopherol biosynthesis. **Conclusion:** The isolated *BrPT1* is a prenyltransferase localised in the plastid, predicted to be involved in the biosynthesis of tocopherol.

Keywords: Prenyltransferase, flavonoids, ginger, biotechnology

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Poster

Identification and Characterization of Novel non-protein coding RNAs (npcRNAs) associated with Global Transcriptional Regulator, Hfq in Pathogenic Bacteria *S. typhi*

Krusnamurthy Kishonthani[a], Sumitha Samuggam[a], Gokul Shankar[b], Suresh V. Chinni[a]*

[a]*Faculty of Applied Science, AIMST University, Semeling, Bedong, Kedah, Malaysia*
[b]*Faculty of Medicine, AIMST University, 08100, Bedong, Kedah, Malaysia*

*Corresponding author email: v_suresh@aimst.edu.my

Abstract
Introduction: Most of cellular pathways involve in bacterial pathophysiology is regulator by non-protein coding RNAs (npcRNAs). In Gram negative bacteria the binding of Hfq protein and npcRNA facilitates its binding to the targeted mRNAs. Hfq gene is conserved in a wide variety of bacteria and it involved in many cellular functions such as stress adaptation and regulation of gene expression. **Methods**: The Hfq gene of *S*. Typhi was amplified and recombined in pET28b+ to be transformed into TOP10 cells.The bacterial with recombinant plasmid was screened by antibacterial selection and confirmed by sequencing. The recombinant plasmid was transformed into *E. coli* BL21 and induced the expression with IPTG. The over expressed Hfq protein was purified using Ni-NTA affinity chromatography. The purified Hfq protein will bind with *S*. Typhi npcRNAs to identify all possible Hfq protein binding npcRNAs. **Results:** Hfq gene had been successfully cloned into pET-28b(+) vector and transformed into TOP10 and BL21. the binding assay between total RNA and purified Hfq protein were carried out and the bound RNA were precipitated and sent for sequencing. **Conclusion**: The Hfq protein of *S*. Typhi was purified successfully and the Hfq bound RNAs were sent for Illumina Sequencing to identify the npcRNAs which is regulated by Hfq protein.

Keywords: *Salmonella enterica* serovar Typhi (*S*. Typhi), non-protein coding RNA, Hfq protein, over-expression.

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Poster

Knocking-out the Non-protein Coding RNA Gene (PmiR-137) in *Proteus mirabilis* to Understand Its Role in Pathogenesis

Abirami Sanniraj[a], Lee Su Yin[a], Eugene Boon Beng Ong[b], Suresh V. Chinni[a]*

[a]*Faculty of Applied Science, AIMST University, Semeling, Bedong, Kedah, Malaysia*
[b]*Institute for Research in Molecular Medicine, Universiti Sains, Malaysia, 11800 Pulau Pinang, Malaysia*

*Corresponding author email: v_suresh@aimst.edu.my

Abstract

Introduction: *Proteus mirabilis* is a Gram-negative, facultatively anaerobic rod-shaped bacterium, best known for its form of multicellular surface mobility termed swarming. *P. mirabilis* causes symptomatic urinary tract infections (UTI) such as cystitis and pyelonephritis, and also catheter-associated urinary tract infections (CAUTI). Our recent study revealed 240 Hfq-associated novel non-protein coding RNAs (npcRNAs) in *P. mirabilis*. One npcRNA of interest is PmiR-137, which is predicted to regulate virulence by association with the mRNA of fimbriae and flagella proteins. **Methods:** Plasmid pKD46 was transformed into *P. mirabilis* and screened using ampicillin marker. The deletion DNA fragment with kanamycin resistance gene, FRT sites and 100 bps of homologous sequence from the flanking regions of PmiR-137 gene was amplified by PCR and transformed into *P. mirabilis* with pKD46. To detect PmiR-137 mutants, the transformed colonies were selected on kanamycin plate and verified by PCR using FRT-specific primers. **Results:** The plasmid with lambda red-recombinase gene, pKD46 was successfully transformed into *P. mirabilis*. The deletion fragment was also successfully transformed into *P. mirabilis* with pKD46. Screening of colonies with FRT-specific primers resulted the PCR products with desired length. **Conclusion:** We obtained the PmiR-137 knock-out strain of *P. mirabilis*. The knock-out of the npcRNA gene will be further confirmed by sequencing analysis and the mutant strain will be used for functional characterization of npcRNA PmiR137.

Keywords: *Proteus mirabilis,* non-protein coding RNA, knock-out, lambda red-recombinase.

Acknowledgement: We would like to acknowledge Ministry of Higher Education for financing this work under Fundamental Research Grant Scheme (FRGS) FRGS/1/2018/STG03/AIMST/02/1

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Poster

Identification of Cucumber-Associated Fungus by Using Recombinase Polymerase Amplification (RPA) Method

Hawa Najibah Rasni, Maryam Rehan, Nazariyah Yahaya*

Department of Food Biotechnology, Faculty of Science and Technology, Universiti Sains Islam Malaysia, 71800 USIM Nilai, Negeri Sembilan D.K., Malaysia

*Corresponding author email: nazariyah@usim.edu.my

Abstract

Introduction: Agricultural crops often suffer from various diseases which causes great loss to the economy. A simple and rapid identification method of phytopathogen for an early warning and quick response is very important to aid in the disease management process. In this study, rapid identification method for species-specific diagnosis of fungi causing severe leaf blight in *Cucumis sativus* was established using recombinase polymerase amplification (RPA). **Methods:** *Fusarium incarnatum* and *Aspergillus japonicus* were isolated and identified from diseased leaves of *C. sativus*. The fungus was subjected to pathogenicity test to prove Koch's postulate and species-specific RPA primers for *F. incarnatum* and *A. japonicus* were designed based on sequence divergence within translation elongation factor 1 alpha (TEF-1a) and calmodulin (CaM) gene. **Results:** With incubation temperature of 37℃, fragments of TEF-1a and CaM gene were successfully amplified within 20 minutes. **Conclusion:** This method allows for rapid and sensitive detection of *F. equiseti and A. japonicus*, and will be useful in the disease management and rapid detection to prevent further spread of this pathogen.

Keywords: *Cucumis sativus*, pathogenic fungi, molecular diagnosis, recombinase polymerase amplification (RPA)

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Poster

The Effects of Extraction Time and Temperature on Phenolic Compounds and Antioxidant Capacity of Malaysian Trigona Propolis Aqueous Extract Using Response Surface Methodology

Abdullah Hagar[a], Nurlisa Fatihah Abd Rani[a], Idris Adewale Ahmed[b], Norazida Ramli[c], Abbe Maleyki Mhd Jalil[d], Muhammad Ibrahim[a]*

[a] *Department of Nutrition Sciences, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, Jalan Istana Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia*
[b] *Centre for Natural Products Research and Drug Discovery (CENAR), Level 3, Research Management & Innovation Complex, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[c] *Department of Biomedical Science, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia, Jalan Istana, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia*
[d] *School of Nutrition and Dietetics, Universiti Sultan Zainal Abidin | UniSZA , Kuala Terengganu, Terengganu, Malaysia*

*Corresponding author email: abumaisarah@iium.edu.my

Abstract

Introduction: Propolis has been used in many applications such as foods additive, natural preservatives, biopharmaceuticals, and cosmetics products, due to its phytochemicals. **Methods:** The effect of extraction time and temperature on total phenolic content, total flavonoid content, and antioxidant activity were investigated for Trigona propolis aqueous extract using central composite design, response surface methodology. The influence of extraction temperature (X1:30–60°C) and time (X2:24–72 hours) on total phenolic content (Y1), total flavonoid content (Y2), and antioxidant capacity (DPPH (Y3), ABTS (Y4), and FRAP (Y5) were analysed. **Results:** The experimental results were satisfactorily fitted into a second-order polynomial model with regard to total phenolic content (R^2=0.9461, p=0.0003), total flavonoid content (R^2=0.9110, p=0.0015), DPPH (R^2=0.9482, p< 0.0001), ABTS (R^2=0.9663, p<0.0001), and FRAP (R^2=0.9058, p= 0.0018). The best extraction temperature and time were 43.75 ºC and 52.85 hours. The predicted outcomes for total phenolic content, total flavonoid content, DPPH, ABTS, and FRAP were 104.30 mg GAE /100g, 6.95 mg QE/g, 3.24 mMTE/g, 2.59 mMTE/g, and 4.34 mMTE/g. The experimental values were close to the predicted values [100.41± 2.74 mg GAE /100g, 6.74± 0.08 mg QE/g, 3.17±0.08 mMTE/g, 2.76±0.14 mMTE/g, and 4.60±0.14 mMTE/g, respectively. **Conclusion:** The optimized parameters for the antioxidant potential of aqueous propolis extract can be incorporated in food additives, beverages, cosmetics, and pharmacological applications.

Keywords: Phenolic Content, Flavonoid Content, Antioxidant, Trigona Propolis, Response Surface Methodology.

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Poster

Application of Mesenchymal Stem Cell-Derived Secretome to Promote Full-Thickness Wound Healing- An in Vivo Study

Jia Xian Law[a]*, Mohd Yusof Mahidin[b], Zahra Rashidbenam[a], Min Hwei Ng[a], Geok Chin Tan[c], Mohd Yazid Bajuri[b]

[a]*Centre for Tissue Engineering and Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, 56000, Kuala Lumpur, Malaysia*
[b]*Department of Orthopedic & Traumatology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, 56000, Kuala Lumpur, Malaysia*
[c]*Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, 56000, Kuala Lumpur, Malaysia*

*Corresponding author email: lawjx@ukm.edu.my

Abstract

Introduction: Diabetes mellitus (DM) is one of the most prevalent non-communicable diseases worldwide and one of the most common complications of DM is diabetic wound ulcer (DFU). Stem cells have been reported to promote wound healing via secretion of paracrine factors. In this study, we examined the role of adipose tissue-derived mesenchymal stem cell (ADSC) secretome on wound healing using a diabetic rat model induced with streptozotocin. **Methods:** Full-thickness wounds were created at the dorsum part 2 weeks after DM induction. The wounds were treated with ADSC secretome at day 0, 3 and 7, whilst the control group received DMEM medium. Gross examination was performed at day 4, 7 and 14, and the animals were euthanized at day 14 to collect the skin biopsy for histological and immunohistochemical (IHC) analysis. **Results:** Faster wound closure was observed in the animals treated with ADSC secretome ($p < 0.05$). Hematoxylin and eosin staining revealed thicker epidermal layer in the secretome group ($p < 0.05$) and Masson's trichrome staining showed less intense collagen deposition in the secretome group. The secretome group has higher mean histological score at 10 ± 1.73 compared to the score of 8.33 ± 1.52 in the control group, indicating more mature tissue architecture. IHC staining revealed higher expression of alpha-smooth muscle actin in the control group, indicating presence of more myofibroblasts that responsible for wound matrix deposition. **Conclusion:** ADSC secretome enhances the healing of DM wounds and this could be a new therapeutic strategy to treat DFU in the future.

Keywords: diabetes, wound healing, mesenchymal stem cells, secretome

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Poster

Measurement of Fatty Acid Beta-Oxidation Target Protein using In-Cell Enzyme-linked Immunosorbent Assay (ELISA) in Human Fibroblast

Rosnani Mohamed[a,d]*, Nur Jannaim Muhamad[a], Izyan Mohd Idris[a], Fatimah Diana Amin Nordin[a], Affandi Omar[a], Anasufiza Habib[b], Noornatisha Salleh[b], Leong Huey Yin[c], Nazia Abdul Majid[d], Julaina Abdul Jalil[a]

[a]*Inborn Errors of Metabolism and Genetic Unit, Nutrition Metabolism Cardiovascular Research Centre, National Institutes of Health, Shah Alam, 40170, Selangor, Malaysia.*
[b]*Biochemistry Unit, Specialized Diagnostic Centre, Institute for Medical Research, Jalan Pahang, 50588, Kuala Lumpur, Malaysia.*
[c]*Department of Genetic, Hospital Kuala Lumpur, Jalan Pahang, 50586, Kuala Lumpur, Malaysia*
[d]*Institute of Biological Sciences, Faculty of Sciences, University of Malaya, 50603, Kuala Lumpur, Malaysia*

*Corresponding author email: rosnani.mohamed@moh.gov.my

Abstract

Introduction: Fatty acid beta-oxidation (FAO) pathway plays an important role in energy homoeostasis particularly in liver, heart and skeletal muscle. The most studied enzymes in this pathway were ACADM, ACADVL and HADHA. Measuring FAO enzyme activities are tedious, hence, we aimed to evaluate an alternative method using In-Cell ELISA. **Methods:** A commercial ATCC® fibroblast and 20 normal fibroblasts were used for precision study and determination of reference range. In-Cell ELISA method was used to quantify target proteins (ACADM, ACADVL and HADHA) in cultured cells. Human fibroblasts were cultured and seeded at ~40,000 cells per well on amine coated plate for overnight. The cells were fixed with 4% paraformaldehyde and permeabilized with blocking buffer, before incubated with primary and HRP labelled secondary antibodies. TMB peroxidase substrate was added into the plate and the reaction was stopped by adding hydrochloric acid solution after 30 min incubation. The absorbance was measured using a TECAN® spectrophotometer at 450 nm. The target proteins were normalized to cell amount, measured by Janus Green whole cell stain. **Results:** The coefficient variation of the intra- and inter-assays were less than 10% for all proteins. The reference range of ACADM, ACADVL and HADHA proteins in fibroblast cell were 2.201±0.463, 4.784±1.455 and 4.195±0.718 (n=20), respectively. From 6 suspected positive FAO disorder patients analysed by ELISA, two of them showed low level of ACADM protein and confirmed by western blot analysis. **Conclusion:** In-Cell ELISA is a precise and specific alternative method to measure level of FAO proteins in human fibroblast.

Keywords: Fatty acid oxidation, human fibroblast, ELISA

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Poster

Pyrogallol Induces Antimicrobial Effect and Cell Membrane Disruption on Methicillin Resistant *Staphylococcus aureus* (MRSA)

Yik-Ling Chew[a]*, Chairunnisa Arasi[b], Joo-Kheng Goh[b],

[a]*Faculty of Pharmaceutical Sciences, UCSI University, 56000, Kuala Lumpur, Malaysia.*
[b] *School of Science, Monash University Malaysia, 47500, Subang Jaya, Selangor Darul Ehsan, Malaysia.*

*Corresponding author email: chew.yikling@gmail.com

Abstract

Introduction: Pyrogallol is present naturally in numerous plants and it is also an important functional group in many polyphenol compounds. However, the antibacterial activity, efficacy and mechanism of pyrogallol towards MRSA strains had not been studied so far. **Methods:** Microbroth dilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Time-kill kinetic assay was adopted to determine the killing pattern of pyrogallol towards MRSA. The antibacterial mechanism was determined using scanning electron microscopy (SEM), Fourier Transform-Infrared (FT-IR) spectroscopy and crystal violet assay. **Results:** Pyrogallol exhibited strong antibacterial activity against MRSA with MIC and MBC 15.6 μg/mL. Pyrogallol could inhibit the exponential growth of MRSA and kill the bacterial cells at higher concentrations. Pyrogallol was found targeting the cell membrane fatty acids, proteins/peptides, polysaccharides/carbohydrates and peptidoglycan of cell walls in the antibacterial mechanism. This has been confirmed through SEM, FT-IR spectroscopy and crystal violet assay. **Conclusion:** This study suggested that pyrogallol has the potential to be used for inhibiting multidrug-resistant bacteria.

Keywords: Pyrogallol, Methicillin resistant Staphylococcus aureus, Antibacterial, Mechanism, Cell membrane

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Poster

Cervical Cancer and Next Generation Sequencing (NGS): A Systematic Review

Nazefah Abdul Hamid*, Marjanu Hikmah Elias, Noraziah Nordin

Department of Basic Medical Sciences I, Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia, 71800, Nilai, N. Sembilan

*Corresponding author email: nazefah@usim.edu.my

Abstract

Introduction: Cervical cancer is one of the most common types of cancer frequently reported in women worldwide. It is ranked as the second most occurring cancer in women worldwide. Next Generation Sequencing (NGS) is one of the emerging technologies that can to contribute towards precision medicine. In this study, we aim to determine the use of NGS using cervical cancer research. **Methods:** This systematic review was done using PubMed as the database of searching. Keywords used is 'cervical cancer' AND 'Next Generation Sequencing'. The search limits were languages (English), dates (year from 2005 until 2021) and types of articles (original articles). **Results:** A total of 63 articles were found to fit the criteria. From the list, further inclusion criteria were used including original articles, using human samples and not on genotyping of the viruses. A total of 7 studies met the inclusion criteria and the full text was obtained for further analyses. The studies used CIN1, CIN2, CIN3 and cervical carcinoma obtained either as fresh tissues or FFPE. From these studies, four studies reported on CpG methylation, while only one study reported on the genes that are expressed, miRNA and mutations, respectively. **Conclusion:** As a conclusion, the data obtained from this systematic review is hoped to help us to understand the molecular mechanisms underlying progression in cervical cancer.

Keywords: cervical cancer, genes, methylation, Next Generation Sequencing

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Poster

Antimicrobial Effect of *Quercus infectoria* and *Acorus calamus* in Wound Model

Sharmanee Thiagarajah[a]*, Effiong P. E.[b], Siyumbwa S. N. [b], Eugenie Sin Sing Tan[a], Chung Keat Tan[a], Farahnaz Amini[a], Normina Bustami[a], Edmond Siah Chye Ng[a]

[a]*Faculty of Medicine and Health Sciences, UCSI University, No.1, Jalan Menara Gading, UCSI Heights, Cheras, 56000, Kuala Lumpur*
[b]*Faculty of Applied Sciences, UCSI University, No.1, Jalan Menara Gading, UCSI Heights, Cheras, 56000, Kuala Lumpur.*

*Corresponding author email: sharmanee@ucsiuniversity.edu.my; sharmanee18@yahoo.com

Abstract

Introduction: Wound infection is a global health concern especially in the tropical regions. It is crucial to ensure none or minimal bacterial growth for speedy recovery as well as to reduce risk of scaring. This research aimed to evaluate antimicrobial effect of plant-based extracts of *Quercus infectoria* and *Acorus calamus* in standard bacterial strains and to assess their efficacy for wound healing. **Methods:** The antimicrobial activity of plant-based extracts *Q. infectoria* and *A. calamus* against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were assessed using minimum inhibitory concentration resazurin assay. Six wounds were created using biopsy puncture on each Sprague Dawley (SD) rats and these wounds were treated using plant-based extracts. Wounds on five SD rats were assessed on second, fourth, seventh, twelfth and fifteenth day by measuring the progression of wound healing. **Results:** Both plant-based extracts showed a minimum inhibitory concentration of less than 1.0 mg/ml on all three bacterial strains. *A. calamus* showed significant wound healing ($p<0.05$) on the fourth day, while *Q. infectoria*, showed significant wound healing ($p<0.05$) on twelfth day. **Conclusion:** Methanolic extract of *Q. infectoria* and *A. calamus* demonstrated good wound healing properties. It exhibited potential to be developed into medicament for wound treatment and care.

Keywords: *Quercus infectoria, Acorus calamus*; Antimicrobial effect, Wound model

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Poster

Detection of Nuclear & Surface Marker of Pluripotency in Human Induced Pluripotent Stem Cells by Immunofluorescent Microscopy

Nur Jannaim Muhamad, Rosnani Mohamed, Fatimah Diana Amin Nordin, Julaina Abd Jalil, Izyan Mohd Idris*

IEM and Genetics Unit, Nutritional, Metabolic and Cardiovascular Research Centre (NMCRC), Institute for Medical Research (IMR), National Institute of Health (NIH) Malaysia. Jalan Setia Murni U13/52, Seksyen U13 Setia Alam, 40170 Shah Alam, Selangor.

*Corresponding author email: izyan.idris@moh.gov.my

Abstract

Introduction: Characterisation of pluripotent stem cells by its nuclear and surface markers is required to ensure high quality stem cells for downstream applications as well as to define & demonstrate its pluripotency. Immunofluorescent microscopy is used to detect and visualize the expression protein markers of pluripotency via antigen-antibody reaction. The aim of the study is to develop a method for visualisation of pluripotency in induced Pluripotent Stem Cells (iPSC) using immunofluorescent detection. **Methods:** Protocols carried out on generated (iPSC) colonies derived from neonatal foreskin fibroblasts (BJ)(ATCC) grown in feeder-free system. iPSC colonies are grown in chamber slides. The cells were fixed with 4% paraformaldehyde, permeabilised with 0.1% Triton X-100 and blocked with 1% horse serum. Colonies were incubated in combination of antibodies specific for nuclear (Oct4, Nanog and Sox2) and surface (Tra-1-60, Tra-1-81 and SSEA4) markers of pluripotency overnight. Then, colonies were incubated in secondary antibodies (DyLight 549, DyLight 488) for 1 hour before counter staining with DAPI for nuclear visualisation. Fluorescent signals were visualised using a ZEISS Axio Imager 2 microscope. Images were analysed using ImageJ software. **Results:** Nuclear transcription factors Oct4, Nanog and Sox2 and surface markers namely keratan sulfate antigen, Tra-1-60 and Tra-1-81 and glycolipid antigens SSEA4 were expressed in the BJ derived iPSC. Permeabilization of cells may disrupt certain detection of protein located extracellularly. **Conclusion:** We have developed a method for visualisation of pluripotency in iPSC by detection of fluorescent expression using specific combinations of nuclear & surface markers in single analysis.

Keywords: iPSC; Pluripotency; nuclear marker; surface marker

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Poster

In vitro Screening of Selected Ulam for the Inhibition of CYP450 3A4 Activity

Sumathi Govindasamy[a], Nurdiana Jamil[a], Wan Rosalina Wan Rosli[a]*

[a]*Faculty of Pharmacy, University of Cyberjaya, Cyberjaya, Selangor D.E., Malaysia*

*Corresponding author email: rosalina@cyberjaya.edu.my

Abstract

Introduction: CYP3A4 is estimated to be involved in the metabolism of 50% of all available drugs and is a major enzyme in drug response by contributing 40% to 45% of all phase I metabolism. CYP3A4 has been shown to have many herb and food interactions that include St John's wort and grapefruit. Malaysia has a special group of traditional vegetables called ulam that may be cosumed raw or cooked. Ulam is gaining popularity as people are moving toward healthier lifestyle by increasing vegetable intake. However, information on the inhibitory activities of ulam toward the activity of CYP3A4 is lacking. Therefore, the aim of this study was to investigate the inhibition of CYP3A4 activity in ulam to determine the food-drug interaction of ulam if they are administered concomitantly with drugs metabolised by CYP3A4. **Methods:** Four types of ulam namely Cosmos caudatus ("ulam raja"), Melicope ptelefolia ("tenggek burung"), Centella asiatica ("pegaga") and Oenanthe javanica ("selom") investigated. Each plant methanol extract were tested on the CYP3A4 enzyme activity with ketoconazole as controls. The abilities of these extracts to inhibit human CYP3A4 enzyme activity were analysed using P450-Glo™ CYP3A4 Assay (Luciferin-PPXE) DMSO Tolerant Assay. **Results:** Centella asiatica showed the most potent inhibitory activity against CYP3A4 with IC50 value of 23.99 μg mL-1, followed by Melicope ptelefolia, Cosmos caudatus and Oenanthe javanica with IC50 value of 34.67, 52.48 and 107.15μg mL-1, respectively. **Conclusion:** The findings suggest that Centella asiatica, Melicope ptelefolia and Cosmos caudatus may contribute to food-drug interactions if they are administered concomitantly with drugs metabolised by CYP3A4.

Keywords: ulam, luminescent assay, CYP 3A4, inhibition, food-drug interaction

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Poster

Cytokilling Effect of NK-92 Cells on Nasopharyngeal Carcinoma (NPC) Cell Lines

Fatin Nur Asyiqin Abd Talib, Norazlin Abdul Aziz, Susan Ling Ling Hoe, Marini Marzuki*

Molecular Pathology Unit, Cancer Research Centre, Institute for Medical Research, National Institutes of Health, Ministry of Health Malaysia, 40170 Shah Alam, Selangor, Malaysia

*corresponding author email: marini.marzuki@moh.gov.my

Abstract

Introduction: Nasopharyngeal carcinoma (NPC) is a head and neck cancer typically associated with the Epstein-Barr virus (EBV). It is the fifth most common cancer in Malaysia, affecting males at a 3:1 ratio to females (2012-2016). Natural killer (NK) cells can kill virally-infected cells including cancer cells without prior sensitization, unlike cytotoxic T cells. **Methods:** The optimisation of *in vitro* growth curves and co-culture media of HK1, NPC43 and C666-1 (target cells) and NK-92 cell line (effector) were independently assessed using xCELLigence real-time cell adherence (RTCA) system for 168h. Cell viability and growth were measured as impedance, and expressed as cell index (CI) values. Cytotoxicity was measured by co-culturing target with differing effector cell concentrations for 72h, after initial target cell adhesion for 24h. 50% killing time (KT50) of target cells was determined to evaluate the efficient killing time of NK-92 on target cells. An imaging assay using GFP-transfected NPC cell lines was used to reflect the cytokilling effect of NK92. **Results:** Different optimal seeding densities were recorded in NPC cell lines ($2.5 – 3.5 \times 10^4$ cells/well). The cell growth, morphology and adhesion of control NPC cell lines were unaffected in the co-culture media throughout 72h (co-culture treatment length). As target:effector (T:E) ratios increased, there was a decrease in the KT50 values and number of GFP cells in the targets. **Conclusion:** HK1, NPC43 and C666-1 cells can be effectively killed with NK-92 and its cytokilling can be monitored real-time by RTCA system.

Keywords: nasopharyngeal carcinoma, natural killer cells, xCELLigence, co-culture, cytokilling

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Poster

Cytotoxic Effect of Lapatinib on T84 Human Colonic Epithelial Cell Line

Wan Nor I'zzah Wan Mohamad Zain[a]*, Raja Nurfirzanah Syaza Raja Sharin[a], Siti Shuhada Mokhtar[a], Jesmine Khan[a], Sharaniza Ab. Rahim[a], Mudiana Muhammad[a]

[a]*Faculty of Medicine, Universiti Teknologi Mara, Kampus Sungai Buloh, Jalan Hospital, 47000 Sungai Buloh, Selangor, Malaysia*

*Corresponding author email: wnizzah@uitm.edu.my

Abstract

Introduction: Lapatinib, an ErbB1/ErbB2 tyrosine kinase inhibitor (TKI) is effective in breast cancer treatment but is associated with diarrhoea. ErbB1 inhibition by lapatinib may interfere with the normal functioning in the intestine. The mechanism of lapatinib-induced diarrhoea remains unclear. This study aimed to determine the cytotoxic effect of lapatinib on T84 colonic epithelial cells and to evaluate the relationship between ErbB1 and tight junction protein zona occluden-1 (ZO-1) expressions and sensitivity to growth inhibition by lapatinib. **Methods:** T84 human colonic epithelial cell line was cultured using DMEM-F12 supplemented with 10% foetal bovine serum, 1% antibiotic-antimycotic and 2 mM L-glutamine in a 37°C incubator with 5% CO_2. The half maximal inhibitory concentration (IC_{50}) of lapatinib on T84 cells was evaluated via MTS assay. Regulation of lapatinib on ErbB1 and ZO-1 expression in T84 cells was determined via real-time PCR and immunofluorescence staining. **Results:** MTS results showed 50% inhibition by lapatinib on T84 cell growth at 26.48±1.64μM. Lapatinib downregulates ErbB1 and ZO-1 mRNA expressions in T84 cells. Decrease ErbB1 and ZO-1 staining intensities were also observed in lapatinib-treated cells. **Conclusion:** Lapatinib exhibited cytotoxic effect on T84 cell line that expressed ErbB1 and tight junction protein ZO-1. The cytotoxic effect on intestinal cells may explain ErbB1 TKI-induced diarrhoea in patients administered with the drug. Nevertheless, further investigations are required to confirm the findings.

Keywords: Lapatinib, ErbB1, ZO-1, T84 colonic epithelial cell line, diarrhoea

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Poster

Drug Repurposing for COVID-19 and Understanding the Molecular Action Pathways of SARS-CoV-2

Idris Adewale Ahmed*

Centre for Natural Products Research and Drug Discovery (CENAR), Level 3, Research Management & Innovation Complex, University of Malaya, 50603 Kuala Lumpur

*Corresponding author email: idrisahmed@um.edu.my; ahmedris1400@gmail.com

Abstract

Introduction: The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) which is responsible for the coronavirus disease 2019 (COVID-19) has become the greatest global public health crisis of the century with a profound impact on the global economy, politics, educational and health institutions as well as social changes. The pandemic has caused has so far affected more than 110 million individuals and more than 2.4 million confirmed deaths globally. Despite the recent presence of a few approved vaccines, more infectious variants and mutants of the virus are being discovered. Thus, the war against this pandemic is far from being won. Hence, developing a good therapeutic drug is of immense importance. **Methods:** Existing literature on molecular action pathways of SARS-CoV-2 infection were reviewed. The data on COVID-19 were collated from various resources and databases such as PubMed, Science Direct, Wiley, Springer, Taylor and Francis, Scopus, Inflibnet, Google, and Google Scholar. **Results:** Considering the multiple molecular signaling of action and replication of the COVID-19 virus, targeting multiple prospective targets seems reasonable. The repurposing of existing drugs will also shorten the time of drug discovery because the binding of SARS-CoV-2 viral particles to the host cell receptors is the key to its spread and pathogenesis. **Conclusion:** The development of strategies to control or prevent the binding thereby disabling the entry of the virus to the cells could be of great help towards combating the disease.

Keywords: COVID-19; Drug repurposing; Molecular Pathways; Therapeutics.

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Poster

Knowledge, Attitude and Perception towards Colorectal Cancer among Public.

Petricia Pawel[a], Sharmanee Thiagarajah[a], Sheiladevi Sukumaran[a]*

[a]*School of Optometry, Faculty of Medicine and Health Science, UCSI University, 56000 Kuala Lumpur, Wilayah Persekutuan., Malaysia*

*Corresponding author email:sheiladevis@ucsiuniversity.edu.my; clasheila@yahoo.com.sg

Abstract
In 2019, it was estimated that about 43,837 new patients were diagnosed to have colorectal cancer in Malaysia. Colorectal Cancer (CRC) is the second highest type of cancer among Malaysian. Overall 5years survival rate for colorectal cancer in our country is only 53% compared to United Kingdom, United States of America, and Singapore. The aim of this study was to analyse the association between sociodemographic and level of knowledge, awareness and perception towards CRC among Malaysian. This was a cross sectional survey with questionnaire divided into five major parts of health status, lifestyle, knowledge, awareness and perception. A total of 384 participants took part in this study. Predominant participants based on demographic factors were male (64.3%), aged 30-39 (31.5%), Malay ethnicity (45.1%), single (47.7%) and has a degree qualification (19.5%). The finding of this study showed that there were association between knowledge, attitude and perception that corelate with sociodemographic on CRC among public. The variables level of education ($P \leq .000$)*** was highly significant and correlated with knowledge on CRC, with higher percentage of the degree qualification, 25% respondents. The survey showed the variables age ($P \leq .004$)** and work status ($P = .036$)* were significant and correlated with attitude on CRC. There were moderate significant association among religion and marital status which correlated with attitude or perception toward CRC. However, there is still a need to increase public awareness and knowledge to detect and treat CRC in early stage.

Keywords: Colorectal Neoplasms, Colorectal Cancer, Patient Medication Knowledge, Attitude to Health, Perception

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Poster

Nisin Induces Apoptosis of MG63 Osteosarcoma Cells while Protecting Non-neoplastic Cells

Muhammad Fairuz Azmi[a]*, Alyaa Al Khateeb[b,d], Gabriele Anisah Froemming[c], Effat Omar[d,e], Sharaniza Ab Rahim[b]

[a]*Department of Anatomy, Faculty of Medicine, Universiti Teknologi MARA, Jalan Hospital, 47000 Sungai Buloh, Selangor D.E., Malaysia*
[b]*Department of Biochemistry, Faculty of Medicine, Universiti Teknologi MARA, Jalan Hospital, 47000 Sungai Buloh, Selangor D.E., Malaysia*
[c]*Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak*
[d]*Institute of Pathology, Laboratory and Forensic Medicine (I-PPerForM), Faculty of Medicine, Universiti Teknologi MARA, Jalan Hospital, 47000 Sungai Buloh, Selangor D.E., Malaysia*
[e]*Department of Pathology, Faculty of Medicine, Universiti Teknologi MARA, Jalan Hospital, 47000 Sungai Buloh, Selangor D.E., Malaysia*

*Corresponding author email: fairuz.azmi83@gmail.com

Abstract

Introduction: Osteosarcoma is a primary bone tumour typically treated with neo-adjuvant chemotherapy, with its inherent adverse effects on normal cells. Hence targeted therapy is an area of concentrated research effort. Nisin, a polycyclic bacteriocin, has been shown to exhibit anti-cancer properties on epithelial malignancies by inducing apoptosis and inhibiting proliferation while sparing non-cancerous cells. Data on the effects of nisin on osteosarcoma cells is still lacking. This study aimed to investigate the effect of Nisin on osteosarcoma vs. non-neoplastic osteoblast cells. **Methods:** MG63 (osteosarcoma cells) and hfOB1.19 (human foetal osteoblast cells) were cultured at 37°C, 95% humidity and 5% CO_2. They were divided into control, nisin (562μg/ml), doxorubicin at (500ng/ml) and nisin+doxorubicin treatment groups. The cell morphology, viability, apoptosis (Annexin V-FITC assay) and cell cycle arrest were recorded. **Results:** At 24-hour post treatment, nisin caused death of 50% MG63 cells, but spares 80% of the hfOB1.19 cells. For nisin-treated MG63 cells, 43.5% were in early and only 2.03% in late apoptosis. Doxorubicin caused approximately 34% cell death of both cancer and osteoblast cells which increased to 60% when combined with nisin. Nisin caused increased arrest at G2/M phase of the cell cycle (33.7% ± 1.6%) compared to control (25.9% ± 3.3%), $p<0.05$. However, doxorubicin exhibited greater effect (47.7% ± 4.3%). Microscopy confirmed more apoptotic bodies among MG63 cells treated with nisin, compared to hfOB1.19cells. **Conclusion:** Nisin leads to increase apoptosis and cell death in osteosarcoma cells whilst sparing normal cells. This effect was enhanced when administered with doxorubicin.

Keywords: osteosarcoma, nisin, apoptosis, cell cycle arrest

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Poster

Air Pollution Skin Effects and Hospital Admissions for Eczema in Kuala Lumpur, Malaysia

Thavin Kumar Mathana Sundram[a,b], Eugenie Sin Sing Tan[a], Shiau Chuen Cheah [a], Hwee San Lim[c], Marjan Sadat Seghayat[d], Normina Ahmad Bustami[a], Chung Keat Tan[a]*

[a]*Faculty of Medicine and Health Sciences, UCSI University, 56000 Kuala Lumpur, Malaysia*
[b]*Faculty of Applied Sciences, UCSI University, 56000 Cheras, Kuala Lumpur, Malaysia*
[c]*School of Physics, Universiti Sains Malaysia, Pulau Pinang, Malaysia*
[d]*Faculty of Medicine, Bioscience &Nursing, MAHSA University 42610 Jenjarom, Selangor, Malaysia*

*Corresponding Author Email: cktan@ucsiuniversity.edu.my

Abstract

Introduction: Adverse health effects due to air pollution have become a major global public health concern over the decades. Epidemiological studies are suggestive of $PM_{2.5}$'s adverse effect on human skin; however, the findings remain limited. This study aims to compare the association of ambient air $PM_{2.5}$ with skin dryness and itchiness among indoor and outdoor workers and also association between $PM_{2.5}$ concentration and hospital admission for eczema cases. **Methods:** A 2 cycle - 3 months cohort follow-up study was conducted involving 440 respondents being categorized as indoor and outdoor workers with 220 respondents on each arm. Weekly self-reporting questionnaire on skin dryness and itchiness using Visual Analogue Scale (VAS) were conducted. Demographic information was collected at recruitment stage. A DustTrakTM Aerosol Monitor Model 8520 was used to obtain $PM_{2.5}$ concentrations. Daily hospital eczema admission data were obtained from hospital records of University Malaya Medical Centre. **Results:** Between indoor and outdoor workers, change in mean score of skin dryness and itchiness were significantly different ($p<0.05$) for both cycles. Moderate to strong correlation for skin dryness and strong correlation for skin itchiness was established among indoor workers against $PM_{2.5}$ concentrations. Moderate negative correlation ($r=-0.539$) was established for eczema admission against $PM_{2.5}$ concentration. **Conclusion:** Stronger correlation seen for indoor workers skin symptoms. Findings are suggestive of a potential relationship with $PM_{2.5}$ concentration and could serve as evidence for future studies though a causal link could not be established.

Keywords: Air pollution, skin dryness, skin itchiness, eczema

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Poster

Comparative Proteomics Profiling of Serum of Women with BI-RADS Scores of 1 to 5 Using Gel-Based Approaches

Jaime Jacqueline Jayapalan[a]*, Jumriah Hiji[b], Aik Han Tan[c]

[a]*Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[b]*Translational Core Laboratory, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[c]*Department of Biomedical Science, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia*

*Corresponding author email: jaime_jacklyn@um.edu.my

Abstract

Introduction: Mortality due BrCa can be greatly reduced through an early detection. Mammography, the gold standard technique used for breast cancer screening has few limitations including high false-positive and negative rates thus, leading to overdiagnosis. Currently, there are no protein biomarkers that is capable of detecting BrCa at an early stage. Thus, the present study aims to screen for the potential protein signatures in serum of women with BI-RADS scores of 1 to 5, using gel-based proteomics approach. **Methods:** Silver-stained two-dimensional gel electrophoresis (2-DE) profiles were generated from 34 neat serum samples of women with different BI-RADS scores. The levels of expression of proteins were analysed *via* image analysis. The proteins with significant altered abundance ($p < 0.01$) were identified by MALDI-ToF/ToF and further analysed using appropriate bioinformatics analysis. **Results:** A total of twenty-one proteins (and/or protein species) were found significantly altered in abundance in the silver-stained 2-DE serum profiles of women with different BI-RADS scores ($p<0.01$). Majority of the proteins were acute-phase proteins. **Conclusion:** Serum protein signatures detected in the present study has the potential to complement mammography screening for providing an early and reliable indicator for detection of BrCa. This would in turn facilitates better patient management as well as prognosis. However, the data of the present study needs to be further validated in a larger representative population.

Keywords: Breast cancer, BI-RADS scores, Two-dimensional electrophoresis, Biomarkers, Lectin.

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Poster

Effect of R92L and R92P Mutations in Ornithine transcarbamylase (OTCase) Enzyme

Ernie Zuraida Ali[a]*, Julaina Abdul Jalil[a], Siti Azma Jusoh[b], Zalikha Ibrahim[c]

[a] *Inborn Error of Metabolism and Genetic Unit, Nutrition, Metabolism and Cardiovascular Research Centre, Institute for Medical Research, National Institutes of Health, Ministry of Health Malaysia, Section U13 Setia Alam, 40170 Shah Alam, Selangor, Malaysia*
[b]*Faculty of Pharmacy, Bioinformatics Lab, Universiti Teknologi MARA (UiTM), Puncak Alam Campus, 42300 Bandar Puncak Alam, Selangor, Malaysia*
[c]*Kulliyyah of Pharmacy, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia*

*Corresponding author email: ernie@moh.gov.my

Abstract

Introduction: Ornithine transcarbamylase (OTCase) is an enzyme that is responsible in urea cycle to synthesize urea from ammonia. Mutations found in *OTC* gene usually causes hyperammonemia, which leads to the occurrence of ornithine transcarbamylase deficiency (OTCD). Previous studies found the OTCase binding pocket undergoes an induced-fit conformational change during the binding of the first substrate, carbamoyl phosphate (CP). Several residues located close to the binding pocket have been identified important in this activity which include R92. Therefore, the aim of this study is to elucidate the effect of mutation at this residue using molecular dynamics (MD) simulations. **Methods:** The R92L-PALO and R92P-PALO mutant models were generated using PyMOL software based on reference structure (PDB ID: 1OTH). The validation of the mutant models was done with ProSA, ERRAT and PROCHECK web-servers. MD simulations were carried out and analysed using the GROMACS package. **Results:** The MD simulations analyses of the mutant complexes (R92L-PALO and R92P-PALO) exhibited both mutations caused a local effect to the OTCase structure. The simulations exhibited the substitution of L92 and P92 disrupted the intermolecular interactions between OTCase and PALO. Both mutations also disrupted hydrogen bond interactions with the neighbouring residue (E326) and also residue involved in the catalytic mechanism (C303). **Conclusion:** In conclusion, the findings indicated that mutations of R92 residue affect the catalytic activity of the OTCase, which may influence the occurrence of OTCD.

Keywords: Ornithine transcarbamylase, OTCase, mutation, catalytic

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Poster

Wnt Pathway Gene Expression in Oral Squamous Cell Carcinoma

Nyi Mas Siti Purwaningsih[a], Goot Heah Khor[a,d]*, Nik Mazuan Nik Mohd Rosdy[b], Effat Omar[c]

[a]*Centre of Preclinical Science Studies, Faculty of Dentistry, Universiti Teknologi MARA, Malaysia*
[b]*Centre of Oral and Maxillofacial Diagnostic and Medicine, Faculty of Dentistry, Universiti Teknologi MARA, Malaysia*
[c]*Department of Pathology, Faculty of Medicine, Universiti Teknologi MARA, Malaysia*
[d]*Oral Cancer and Maxillofacial Research Group, Universiti Teknologi MARA, Malaysia*

*Corresponding author email: gootheah@uitm.edu.my

Abstract

Introduction: Oral squamous cell carcinoma (OSCC) is the most common type of oral cancer worldwide. Uncharacteristic regulation of Wnt pathway has been associated with various cancers. Inhibition of Wnt pathway mechanism is efficiently blocked cancer growth in highlighting a great potential of therapeutics treatment. Several studies highlight the contribution of WNT signalling pathway activation in oral neoplastic transformation toward OSCC progression. **Methods:** ORL-48 (OSCC) cell line was derived from Cancer Research Malaysia. Normal gingival fibroblast (hGF) cell line was used for the control group. After cell culture and treatment with 5-aza-2'-deoxyxytidine, cells were preceded for PCR array profiling by Custom RT2 Profiler PCR Array. Data analysis was done by GeneGlobe software (Qiagen). **Results:** In comparison between the untreated control and OSCC, the genes of PPP2R1B, SENP2, Fzd1, PPP3R2, WIF1 and Wnt8a were significantly expressed. After treatment with 5-aza-2'-deoxyxytidine, Wnt8a was not longer extinct. In comparison between treated OSCC and untreated, RAC and SFRP4 genes are downregulated, while Wnt10B, Fzd10, Wnt1 and WIF1 genes show having upregulation role in oral cancer. **Conclusion:** Wnt8a has downregulation role in OSCC, while Wnt10b, Fzd10, Wnt1 and WIF1 have upregulation role in OSCC. Wnt pathway genes can be used as a targeted therapy in OSCC.

Keywords: oscc, pcr array, wnt pathway

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Poster

Mallotus mollissimus and *Solanum erianthum* Carries Potential Phytochemical with Antimicrobial Properties

Lucky Poh Wah Goh[a], Nurul Ain Ismail[a], Ping-Chin Lee[a], Siew-Eng How[a], Jualang Azlan Gansau[a]*

[a]*Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah. Malaysia*

*Corresponding author email: azlanajg@ums.edu.my

Abstract

Introduction: *Mallotus mollissimus* (*M. mollissimus*) and *Solanum erianthum* (*S. erianthum*) plants are underutilized plants with potential medicinal properties and has been used indigenous communities. However, the phytochemical constituents and antimicrobial activities of these plants remain under-reported. This study is aimed to investigate the phytochemicals contents and antimicrobial activities of *M. mollissimus* and *S. erianthum* plant extracts. **Methods:** Plant extracts were obtained and fractionated with column chromatography**.** Antimicrobial activity screening was performed against several Gram-positive and Gram-negative microorganisms by disk diffusion method. Qualitative phytochemicals were performed targeting alkaloid, saponins, steroids and tannins, flavonoids, terpenoid and phlobatannins, anthraquinones and cardiac glycosides. **Results:** *M. mollissimus* and *S. erianthum* plant extracts showed broad spectrum of antimicrobial activities against Gram-positive bacteria. Both plant extracts and their fractions contain alkaloid, steroids and cardiac glycosides. Interestingly, flavonoids, terpenoid, tannins were found in *M. mollissimus* only and correlated to the antimicrobial activity against *Streptococcus pneumoniae*. **Conclusion:** These findings suggested that *M. mollissimus* and *S. erianthum* contains potential antimicrobial agents.

Keywords: *Mallotus mollissimus*, *Solanum erianthum*, antimicrobial, phytochemical

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Poster

In silico Identification of Transcription Factor Families in *Curcuma alismatifolia* (Ornamental Ginger) using Available Transcriptomic Resources

Sima Taheri*

Centre for research in biotechnology for agriculture (CEBAR), University of Malaya

*Corresponding author email: sima_taheri@um.edu.my

Abstract
Curcuma alismatifolia is ornamental ginger from the Zingiberacea family that grows from rhizomes and originated from tropical and sub-tropical areas of northern Thailand and Cambodia. *Curcuma alismatifolia* flowering stems comprise showy inflorescence with several apical bracts on a long and stiff peduncle. The inflorescence comprises several of pink, white, red, and purple bracts in the upper part and green bracts in the lower part. Both types of bracts bear small axillary flower buds. Transcription factors are proteins that regulate the expression of genes involves in the anthocyanin pathway in ornamental plants. However, there is no information on transcription factor identification in *C. alismatifolia*. In this study, by transcriptome-wide mining of inflorescence of two *C. alismatifolia* cvs. 'Chiang Mai Pink' and 'UB Snow 701', 2,740 unigenes encoding 59 classes of transcription factors families were predicted. Based on TF classification, 625 and 130 unigenes encoded MYBs and NACs transcription factors, respectively. These findings provide a valuable resource for gene expression and genetic transformation studies and improvement of flower color in the ornamental *Curcuma*.

Keywords: ulam, luminescent assay, CYP 3A4, inhibition, food-drug interaction

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Poster

Metabolome Analysis of Oil Palm Friable Calli and Its Development

Dalilah Abu Bakar*, Noor Idayu Tahir, Umi Salamah Ramli, Siti Rahmah Abdul Rahman, Meilina Ong Abdullah

Advanced Biotechnology and Breeding Centre (ABBC), Malaysian Palm Oil Board (MPOB), Bandar Baru Bangi, 43000 Kajang, Selangor

*Corresponding author email: dalilah.ab@mpob.gov.my

Abstract

Introduction: Plant development in *in vitro* culture system involves both growth and differentiation processes which depends on multiple factors such as genotype, nutrient availability and culture conditions. Growth, in size or biomass, is a quantitative change that is irreversible. **Methods:** Oil palm liquid culture system was initiated through the selection of friable calli induced from inoculated immature leave explants after 3 months in culture. The friable calli were mass propagated further in suspensions that were subjected to scheduled subculture routines and maintenance then were transferred into maturation stage for further development. The multiplication and transition of oil palm calli to cell aggregates has yet to be delineated from the biochemical perspective. The metabolite profiling and characterization of both calli and cell aggregates were conducted using liquid chromatography-mass spectrometry (LC-MS), while multivariate statistical analyses were employed to facilitate data interpretation. **Results:** At least 21 metabolite peaks were detected in friable calli, while 32 peaks were observed in cell aggregates at 7th subculture. Differential abundance of amino and organic acids were observed and identified from the friable calli tissues and the cell aggregates in parallel with the chemometrics data. **Conclusion:** The distinction of oil palm tissue culture materials by its phytochemical profiles is of major interest to better understand its biosynthesis for future performance and quality improvements. The discovery of phytochemicals in oil palm liquid culture system may provide added prospects in the production of high value compounds for the pharma and nutraceuticals sectors.

Keywords: *Elaeis guineensis*, metabolomics, LC-MS, tissue culture, liquid culture system

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Poster

Classification of Multifunctional and Multisubunit Oil Palm Acetyl-CoA Carboxylase (ACCase) via Domain Analysis

Nadzirah Amiruddin[a], Chan Kuang Lim[a] and Leslie Low Eng Ti[a]*

[a]*Advanced Biotechnology and Breeding Centre, Malaysian Palm Oil Board, No 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia*

*Corresponding author email: lowengti@mpob.gov.my

Abstract

Introduction: Acetyl-CoA carboxylase (ACCase) is considered one of the most important enzymes in the regulation of fatty acid synthesis, as it controls the carbon flux into lipids in plants. The enzyme catalyzes the first step of fatty acid synthesis by converting acetyl CoA to malonyl-CoA. There are four domains (alpha-carboxyl transferase (CT-α), beta carboxyl transferase (CT-β), biotin carboxyl carrier protein (BCCP), biotin carboxylase (BC)) present in ACCase. These domains may be present in a single protein (multifunctional form) and/or as four separate proteins, in multisubunit form, each containing a different subunit. The multifunctional and multisubunit ACCase is found in the cytoplasm and plastid respectively. In oil palm, both the multifunctional and multisubunit forms of ACCase are present. Multisubunit ACCase is involved in oil palm fatty acid production. **Methods**: To identify the oil palm ACCase, the Arabidopsis thalianaACCase gene sequence was downloaded from the KEGG pathway databases and used as a query sequence, resulting in the identification of an ACCase gene from the published oil palm gene models. **Results**: Seven domains were predicted to exist in oil palm ACCase using Interproscan (ACCA, ACC_central, Carboxyl_trans, CPSase_L_D2, CPSase_L_chain, Biotin_carb_C and Biotin_lipoyl). Using the domains identified, a set of identified domain(s)-containing protein sequences were retrieved and annotated using Blastx. **Conclusion**: The oil palm ACCase genes were classified into multifunctional (2 copies) and multisubunit (CT- α [3 copies], BCCP [2 copies], BC [2 copies]) forms.

Keywords: oil palm, acetyl-CoA carboxylase, fatty acid synthesis, multisubunit, multifunctional

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Poster

Variability in Yield Components and Vegetative Traits of 3-Way Crosses Oil Palm Progenies

Nor Azwani Abu Bakar*, Mohd Din Amiruddin and Ahmad Kushairi Din

Malaysian Palm Oil Board (MPOB), 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia.

*Corresponding author email: nor.azwani@mpob.gov.my

Abstract
High yielding and dwarf planting materials has become a goal in oil palm breeding and improvement programmes. A total 30 progenies of MPOB-Nigeria *dura* x Deli *dura* were progeny tested with AVROS and Yangambi were initiated aiming at assessing their yield components and vegetative growth performance. The progenies were laid down in a Randomized Complete Block Design (RCBD) with four replicates in 2011 at the Malaysian Palm Oil Board (MPOB) Research Station, Ulu Paka, Malaysia. Analysis of variance (ANOVA) revealed highly significant results for yield components and vegetative traits, denoting there were substantial genetic variation for breeding and selection for the traits studied. Four progenies had exceeded FFB yield of more than 200 kg palm^{-1} year^{-1} where PKG 136 from MPOB-Nigeria *dura* x Deli *dura* x AVROS was the top scorer for having FFB yield of 207.36 kg palm^{-1} year^{-1}. The plant height varied from 1.91 (PKG 115) to 2.51 m (PKG 126), giving the height increment ranged from 31.8 to 41.8 cm year^{1}. In this study, the correlation analysis showed positive and highly significant between FFB yield with all other yield and vegetative traits except for frond production (FP). Both PKG 136 and PKG 105 from MPOB-Nigeria *dura* x Deli *dura* x AVROS *pisifera* have been identified as the potential parent and will be selected for further evaluation trial.

Keywords: oil palm, germplasm, advance population, progeny test

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Poster

Optimization of Protoplast Isolation From Oil Palm *In vitro*-derived Leaf And Mesocarp

MD Piji Mohd Al Akmarul Fizree[a]*, Noor Azmi Shaharuddin[b], Chai Ling Ho[c], Mohamad Arif Abd Manaf[a], Ghulam Kadir Ahmad Parveez[a] and Mat Yunus Abdul Masani[a]

[a]*Malaysian Palm Oil Board (MPOB), 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia.*
[b]*Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.*
[c]*Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences,Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.*

*Corresponding author email: fizree@mpob.gov.my

Abstract

Introduction: The protoplast represents a truly single-cell system which is useful and beneficial for *in vivo* gene functional study. The different source of protoplasts according to the gene of interest, together with robust isolation and cultivation procedure are beneficial for genetic improvement of the perennial crop, such as oil palm. **Methods:** Previously, a method has been published on the isolation of oil palm protoplast from suspension callus. This study serves as the extension of the previous research to optimize isolation of protoplasts from oil palm mature tissues such as *in vitro*-derived (*iv*) leaf and mesocarp. Isolation of mesophyll protoplasts from *iv* leaf was optimized by identifying the best parameters affecting protoplast yield and viability, such as enzyme combinations and procedure to obtain clean and vital protoplasts. **Results:** By doing this, an efficient protocol for isolation of oil palm mesophyll protoplasts that can produce up to 2.5×10^6 protoplasts/g FW with up to 94.78% viability was developed. Then, optimization for isolation of protoplasts from the mesocarp of the age around 12 WAA was carried out with previously optimized enzyme mixture. After two hours of incubation time, 3.98×10^6 protoplasts/g FW with 85% viability were recovered. **Conclusion:** Overall, we conclude that combination of cellulase R-10, macerozyme R-10, driselase, and pectolyase Y-23 enzymes improved isolation of oil palm protoplast from mature tissues.

Keywords: protoplast isolation, oil palm *in vitro* leaf, oil palm mesocarp

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Poster

Preliminary Analysis of QTLs Associated with Yield Components in Oil Palm (*Elaeis guineensis* Jacq.) through Assocation Mapping

Suzana Mustaffa*, Norhalida Mohamed Serdari, Zulkifli Yaakub , Meilina Ong Abdullah

Advanced Biotechnology and Breeding Centre (ABBC), Malaysian Palm Oil Board (MPOB), No. 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor.

*Corresponding author email: suzana.mustaffa@mpob.gov.my

Abstract

Introduction: Oil palm (*Elaeis guineensis* Jacq.) is the most widely traded vegetable oil in the world, with the current yielding is about 4-6 t/ha. There is a large scope for increasing the oil yield by selecting elite planting material for breeding programs in germplasm evaluation and utilization. Through association mapping (AM), it is now possible to dissect complex quantitative traits and identify a single polymorphism within a gene that is responsible for phenotypic differences. **Methods:** In the present study, a diverse range of 116 Tanzania oil palm germplasm genotypes were characterized using 92K single nucleotide polymorphism (SNP) array through Illumina platform. After SNP filtering using ASSIsT 1.01 and removing markers with minimum allele frequency (MAF) <0.05, a total of 49961 informative SNPs was obtained. These markers were analyzed using FarmCPU package embedded in R software for association mapping analysis. Analysis was combined with three top principal components (PC1-3) analyzed using GAPIT to control the population structure**. Results:** Results from the Manhattan plots revealed that seven quantitative trait loci (QTLs) each were significantly associated with yield components (bunch number and bunch weight). QQ plot showed that the model applied fitted best to the uniform distribution and appropriate for analyzing the association between the oil palm SNPs and trait studied. **Conclusion:** The detection of marker-traits associations will facilitate the implementation of marker assisted selection (MAS) and speed up breeding improvement programs in the future.

Keywords: association mapping, oil palm germplasm, SNP marker

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Poster

Transcriptome Analysis of Virus-Host Interactions between the Lytic Bacteriophage DchS1 and the Soft-Rot Macergen, *Dickeya chrysanthemi*

Ming Wei Lew[a], Sasireigga Jaya Jothi[a,b], Heera Rajandas[b], Sivachandran Parimannan[b], Su Yin Lee[a,b]*

[a]*Faculty of Applied Sciences, AIMST University, 08100, Bedong, Kedah, Malaysia.*
[b]*Centre of Excellence for Omnics-Driven Computational Biodiscovery (COMBio), Faculty of Applied Sciences, AIMST University, 08100, Bedong, Kedah, Malaysia*

*Corresponding author email: su_yin@aimst.edu.my

Abstract

Introduction: Bacteriophages have been used as a biocontrol agent for various crop diseases, including bacterial soft rot that is caused by *Dickeya chrysanthemi*. However, insights into the virus-host interactions are still lacking. Hence, this study aims to elucidate the genome-wide transcriptional response of *D. chrysanthemi* to a lytic phage (DchS1). **Methods:** A lytic phage strain against *D. chrysanthemi* (DchS1) was isolated from environmental sample using enrichment method. The strain was characterized using double agar overlay method, one-step growth curve, nuclease digestion, transmission electron microscopy and stability study. Total RNA was extracted from the culture of *D. chrysanthemi* infected with DchS1 at early, mid and late infection time points. **Results:** DchS1 was a double-stranded DNA phage from the *Myoviridae* family. The double agar overlay result showed clear plaques that indicate the lytic activity of DchS1 against *D. chrysanthemi*. The latent period and burst size were estimated to be 20 min and 209 plaque-forming units/cell, respectively. The phage showed highest survival rate at pH7, 29°C and 37°C, and after exposure to UV light for 5-10 min. Total intact RNA was successfully isolated from both the virus and host. The RNA samples will be sent for sequencing. **Conclusion:** The sequencing of the RNA samples and transcriptomic analysis will reveal the interactions between these organisms and uncover new candidates for antibacterial compounds and phage therapy.

Keywords: *Dickeya chrysanthemi*, bacteriophage, RNA extraction, transcriptome, virus-host

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Poster

Production of Recombinant Iridovirus Coat Protein using a Synthetic Biology Approach

Fernandes Opook[a], Peik Lin Teoh[a], Balu Alagar Venmathi Maran[b], Kenneth Francis Rodrigues[a]*

[a]*Biotechnology Research Institute, Universiti Malaysia Sabah, 88400 Sabah, Malaysia*
[b]*Borneo Marine Research Institute, Universiti Malaysia Sabah, 88400 Sabah, Malaysia*

*Corresponding author email: kennethr@ums.edu.my

Abstract

Introduction: Grouper iridovirus (GI) is a causative agent that is associated with the infection and disfigurement of groupers is aquaculture systems. The Iridovirus coat protein has been reported to be antigen with potential applications for the development of vaccines for the aquaculture industry, however the mass production of this protein is limited by the lack of availability of a suitable gene construct for its large scale production. **Methods:** This study was directed towards engineering a synthetic gene construct of the GI major capsid protein (GI-MCP) followed by insertion into two different bacterial expression vectors, pET-22 and pGS21A. The vectors were transformed into chemically competent E. coli BL21 DE3 and expression was induced at 12°C 25°C and 37°C. **Results:** The synthetic gene containing the synthetically designed GI-MCP was successfully expressed in both plasmids and was validated using protein spectroscopy. **Conclusion:** The gene construct can be applied for the large-scale production and commercial application of the GI-MCP as an antigen for diagnostic purposes as well as a vaccine for aquaculture.

Keywords: Iridovirus, Major Capsid Protein, Grouper Iridovirus, Synthetic Biology, Codon Optimization.

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Poster

In vitro Proliferation of Shoots and Callus of *Talinum paniculatum* (Jacq.) Gaertn.

Shahril Efzueni Rozali[a,b]*, Izzat Danial Muhammad Nasir[a], Jamilah Syafawati Yaacob[b]

[a]*School of Science and Psychology, Faculty of Arts and Science, International University of Malaya-Wales, Block A, City Campus, Jalan Tun Ismail, 50480 Kuala Lumpur, Malaysia*
[b]*Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia*

*Corresponding author email: shahrilefzueni@iumw.edu.my

Abstract

Introduction: *Talinum paniculatum* also known as Javanese ginseng is an important medicinal plant that was used as traditional medicine to treat various illnesses. The present of oleanane-type saponins in this plant demonstrates antifertility, antibacterial, antifungal, antioxidant, and cytotoxic activities. The main goal of this study is to establish a protocol for *in vitro* propagation and callus induction of *T. paniculatum* as an alternative method to ameliorate the pharmaceutical potential of this species. **Methods:** Nodal explants and transverse thin cell layer from the aseptic seedlings of *T. paniculatum* were used in this study. Multiple shoots were induced in MS medium supplemented with BAP (0.5 – 1.5 mg/L) while callus formation was induced in MS medium supplemented with combination of 1.0 mg/L BAP and 2,4-D (0.5 – 2.5 mg/L). **Results:** The highest number of shoots (4.00± 0.62) was recorded in nodal explants cultured on MS medium supplemented with 1.0 mg/L BAP ($p<0.05$). Various concentration of auxins influenced the induction, proliferation, morphology and colour of the callus. Transverse thin cell layer explants cultured on MS medium supplemented with 1.0 mg/L BAP and 2.0 mg/L 2,4-D reached the maximum biomass production in callus induction stage. The highest proliferation of subcultured callus with whitish green colour was observed in MS medium supplemented with 1.0 mg/L BAP and 0.5 mg/L 2,4-D. **Conclusion:** The optimized conditions for shoot multiplication and callus proliferation of this underutilized medicinal plants potentially useful for biotechnological applications in pharmaceutical industry.

Keywords: 6-benzylaminopurine, callus, shoot multiplication, *Talinum paniculatum*, transverse thin cell layer.

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Poster

Identification of Putative Olfactory Proteins from a Key Palm Oil Pollinating Weevil, *Elaeidobius kamerunicus*

Nabeel Ata Abdul Muneim, Muhammad Azwan Zulkifli, Mohd Amin Abd Halim, Siew-Eng Ooi, Meilina Ong-Abdullah*

Advanced Biotechnology and Breeding Centre, Malaysian Palm Oil Board, No. 6 Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia

*****Corresponding author: meilina@mpob.gov.my

Abstract

Olfaction is essential for the survival of many insects. Their sophisticated olfactory systems help them accomplish key tasks, such as seeking food resources, avoiding predators, locating mate partners and selecting egg-laying sites. The olfactory proteins, which are primarily expressed in the olfactory sensilla located in the antennae of insects, play vital roles in the responses triggered by external chemical stimuli. Recent advances in bioinformatics and molecular biological techniques have enabled the identification of numerous olfactory-related genes as well as their functions but information on the molecular chemoreception transduction mechanism is still very limited. A tiny weevil called *Elaeidobius kamerunicus* (*Ek*) of the Coleopteran order of the Curculionidae family that originated from West Africa is the most efficient pollinator of *Elaeis guineensis*. Due to the key role and economic importance of *E. kamerunicus* to the oil palm industry, scarcity of its genetic information should be addressed, particularly pertaining to its genome. Basic genomic features such as K-mer distribution prediction of diploid genome size supported by flowcytometry, heterozygosity ratio, repeats rate percentage and GC content percentage were obtained for both male and female *Ek*. Leveraging the genome survey data, a more targeted in silico characterization of the olfactory proteins such as the odorant-binding proteins (OBP) and odorant receptors (OR) was conducted. De novo functional annotation was performed on the assembled data through OmicsBox platform. A total of 35 to 47 and 24 to 28 scaffolds contained partial putative/candidate OR and OBP genes respectively. These results represent an early effort in providing the *Ek* genome with functional molecular information.

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Poster

Food Operators' Perceptions on Biodegradable Food Container Usage at Subang Jaya: The relationship of Environmental Knowledge, Concern, Attitude and Usage Intention

Nor Hidayah Mohd Kadir[a], Kavita Chirara[b]*

[a]*Department of Food Science, Faculty of Applied Sciences, UCSI University, 56000 Cheras, Wilayah Persekutuan, Malaysia*
[b] *Department of Biotechnology, Faculty of Applied Sciences, UCSI University, 56000 Cheras, Wilayah Persekutuan, Malaysia*

*Corresponding author email: kavita@ucsiuniversity.edu.my

Abstract
Malaysia generated around 1 million tonnes of unmanaged plastic waste, putting the country as the eighth worst country worldwide for plastic waste. The waste management system is not efficient enough to address the pollution in the nation. In recent years, the government has taken steps to educate the citizen on biodegradable packaging, recycling and reduction of plastic usage. The study explores the perceptions on biodegradable food container usage among food operators in terms of sociodemographic characteristics, environmental knowledge, concern, attitude and usage intention. A total of 113 food operators from Subang Jaya, Selangor were surveyed through self-administered questionnaire through convenience sampling method. The findings show that level of education has a significant impact on environmental knowledge and attitude. Besides, the results of Pearson correlation coefficient show medium positive relationship between environmental knowledge with both attitude and usage intention. Medium positive relationship is also observed between environmental attitude and usage intention. Meanwhile, weak positive relationship recorded between environmental knowledge and concern. Environmental concern demonstrates weak positive relationship with both attitude and usage intention. High level of environmental awareness among the food operators reflected that they are more conscious about the environment and the beneficial effects of biodegradable food container usage. However, high price and low product quality may be the reasons the purchase intention is not reflected in the actual purchase of biodegradable products. The collective measure to increase awareness among food operators' on proper waste management is crucial to effectively minimise the amount of waste in general.

Keywords: Environmental, biodegradable, Knowledge, Concern, Attitude

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Poster

Pharmaceuticals and Personal Care Products (PPCPs) Residues in Surface Water Bodies from Selangor (Malaysia): Occurrence and Environmental Risk Assessment

Eugenie Sin Sing Tan[a]*, Yu Bin Ho[b], Chung Keat Tan[a], Farahnaz Amini[a], Normina Bustami[a], Sharmanee Thiagarajah[a], Edmond Siah Chye Ng[a]

[a] *Faculty of Medicine and Health Sciences, UCSI University, No.1, Jalan Menara Gading, UCSI Heights, Cheras, 56000, Kuala Lumpur*
[b] *Department of Environmental and Occupational Health, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.*

*Corresponding author email: eugenietan@ucsiuniversity.edu.my, dr.eugenietan@gmail.com

Abstract

Introduction: Pharmaceuticals and Personal Care Products (PPCPs) are classified as the new emerging class of pollutants by the United States Environmental Protection Agency. Its ubiquitous nature coupled with its high persistency in the environment is alarming. Moreover, some PPCPs are endocrine disrupting chemicals (EDC) responsible for feminization of male fishes via production of vitellogenin. Occurrences of antibiotics in the environment induce high bacterial resistance. PPCPs were widely manufactured and administered in Malaysia but little or no quantification was carried out. Thus, this study aims to investigate environmental occurrences of PPCPs in Langat River Basin and to conduct Environmental Risk Assessment (ERA). **Methods:** River water samples were collected from twelve sampling locations in the Langat Basin. Samples were extracted using Solid Phase Extraction (SPE) Oasis HLB cartridges and analysed using tandem Liquid Chromatography Mass Spectrometry (LC-MS/MS). Environmental Risk Assessment (ERA) was calculated and classified as no risk, low risk, medium risk and high risk. **Results:** All PPCPs were detected at sampling locations except gemfibrozil, levonorgestrel, naproxen, norethindrone, progesterone and trimethoprim whose frequency of detections were 97.2, 91.7, 91.7, 88.9, 83.3 and 63.9% respectively. Two PPCPs were quantified at concentrations more than 1000 ng/L; they were caffeine and naproxen. Thereafter, ERA was used to evaluate possible aquatic toxicities in Langat River. High risks of acute toxicities were found for naproxen and sulfamethoxazole. Several PPCPs exhibited high chronic ecological toxicities namely diclofenac and sulfamethoxazole. Naproxen exhibited medium ecological risk. Metoprolol and DEET exhibited low chronic ecological risk. **Conclusion:** More studies should be encouraged to safeguard the aquatic ecosystem and human health.

Keywords: Pharmaceuticals and Personal Care Products (PPCPs), Endocrine Disrupting Compounds (EDC), Environmental Risk Assessment (ERA)

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Poster

Functionalization of Medium-Chain-Length Polyhydroxyalkanoates with Methyl Acrylate by Free Radical Copolymerization using Azobisisobutyronitrile

Mary Hui-Yee Ling[a], Chong-Boon Ong[a]*, Mohamad S. M. Annuar [b]

[a]*Faculty of Arts and Science, International University of Malaya-Wales, City Campus, Jalan Tun Ismail, 50480 Kuala Lumpur.*
[b]*Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur*

*Corresponding author email: ongchongboon@yahoo.com

Abstract

Introduction: Chemical modifications of PHA and mcl-PHA are important to enhance their performance characteristics and add novel functionalities. **Methods:** Functionalization of mcl-PHA produced by *Pseudomonas putida* Bet001 with methyl acrylate (MA) using 2,2'-azobisisobutyronitrile (AIBN) as a radical initiator was carried out. **Results:** Optimum copolymerization was obtained at 30 mM AIBN, 50°C in 60 minutes. Spectroscopic analysis authenticated the successful grafting of MA to mcl-PHA. **Conclusion:** MA-grafted mcl-PHA exhibits improved flexibility from adjusted crystallinity behaviour.

Keywords: Polyhydroxyalkanoate, *Pseudomonas putida*, graft copolymerization, azobisisobutyronitrile, methyl acrylate

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Poster

Molecular Characterization and Phylogenetic Analysis of an *Enterococcus Faecium* Isolate from a Clinical Sample in Sabah, Malaysia

Nur Nashyiroh Mastor[a,b], Vijay Kumar Subbiah[b], Wan Nazirah Wan Abu Bakar[c], Suzanah Silee[c], Khurshida Begum[d], M. Jahangir Alam[d], Mohammad Zahirul Hoque[a]*

[a]*Department of Pathobiology & Medical Diagnostics, Faculty of Medicine and Health Sciences, University Malaysia Sabah, Sabah, Malaysia.*
[b]*Biotechnology Research Institute, University Malaysia Sabah, Sabah, Malaysia.*
[c]*Department of Pathology, Queen Elizabeth Hospital, Ministry of Health, Kota Kinabalu, Sabah, Malaysia.*
[d]*Department of Pharmacy Practice and Translational Research, University of Houston College of Pharmacy, Houston, Texas, USA.*

*Corresponding author email: drzahir@ums.edu.my

Abstract

Introduction: Enterococcus faecium is a gram-positive bacterium commonly found as a commensal which causes different infections in humans including hospital-acquired infections. We were interested in investigating the molecular characterization of local Enterococcus bacteria isolated from a clinical sample in a tertiary hospital in Kota Kinabalu, Sabah, based on the whole-genome sequencing approach. **Methods:** The collected sample was cultured for bacteria onto blood agar and characterized by using molecular methods. Bacterial DNA was extracted by using the Qiagen DNeasy Tissue Kit (Qiagen, Valencia, CA). The quantification of DNA was done using a Qubit fluorometer (Qubit 3.0, Thermo Fisher Scientific). The sample was then sequenced using the Illumina HiSeq 4000 platform. The quality of the sequence reads was checked using FastQC. This research was approved by the Medical Research Ethics Committee (MREC), Ministry of Health, Malaysia (No. NMRR-19-1770-48622) and University Malaysia Sabah ethical committee (JKEtika 1/19(26). **Results:** There were 221 contigs, an estimated genome length of 2,890,784 bp, and an average G+C content of 37.73%. The N50 length is 38,963 bp and the L50 count is 22. Our phylogenetic tree analysis using comprehensive genome analysis service at PATRIC (a comprehensive bioinformatics resource tool) showed the isolate belongs to Enterococcus faecium. The raw reads were deposited in the NCBI SRA. This whole-genome data was deposited in DDBJ/ENA/GenBank under the accession number JAEFCY000000000. **Conclusion:** The molecular characterization data from our study might be useful in detecting pathogenic microorganism of *E. faecium* infections and would broaden the application of whole genome sequencing in clinical practice.

Keywords: *Enterococcus faecium*, Hospital-acquired infections, Whole genome sequencing, phylogenetic analysis

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Poster

Prevalence and Genetic Diversity of PVL-Positive *Staphylococcus aureus* Nasal Carriers among Patients and Healthcare-Workers in a Tertiary Hospital in Selangor -Malaysia

Syahirah Samsudin[a], Hassanain Al-Talib[b]*, Zaini Mohd Zain[b]

[a]*Institute for Medical Molecular Biotechnology, Faculty of Medicine, Universiti Teknologi MARA (UiTM), Sungai Buloh, Selangor, Malaysia*
[b] *Medical Microbiology and Parasitology Department, Faculty of Medicine, Universiti Teknologi MARA (UiTM), Sungai Buloh, Selangor, Malaysia*

*Corresponding author email: hassanain@uitm.edu.my

Abstract

Introduction: The virulence of *Staphylococcus aureus* (*S. aureus*) is associated with the presence of various toxins including Panton-Valentine leukocidin (PVL) toxin gene. The PVL toxin encodes for extracellular cytotoxin. These toxins interrupt the permeability barrier of leukocytes and erythrocytes by making pores in their cell membranes. The PVL-positive *S. aureus* is a main cause of nosocomial and community-acquired infections. The aim of the present study is to determine the percentage rate, associated risk factors and the phylogenetic relationship of PVL-positive *S. aureus* nasal carriers among patients and nurses. **Methods: c**ollectively, 315 nasal swabs were collected from inpatients and nurses from Hospital Sungai Buloh. Isolation and identification of *S. aureus* was performed by using Staphylase test kit. Multiplex PCR assay was used to confirm *S. aureus* isolates with positive PVL toxin gene. Antimicrobial susceptibility of PVL-positive *S. aureus* was identified by the disk diffusion method. Phylogenetic analysis of PVL-positive *S. aureus* was determined using pulsed-field gel electrophoresis (PFGE). **Results:** One hundred-sixty out of 315 (50.8%) isolates were *S. aureus*. About 7/160 (4.4%) harboured *luk*S gene (six MSSA and one MRSA). The patients with a nasogastric intubation, fever past 2 weeks, runny nose, nose itching and longer hospital stay had a significantly high risk of being PVL-positive *S. aureus* nasal carriers. The phylogenetic of all seven PVL isolates presented five patterns of isolates and they were distantly related. **Conclusion**: The current study provided awareness to the hospital authority to act for prevention and control.

Keywords: *S. aureus*; nasal carriers; PVL; multiplex PCR and PFGE

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Poster

Identification of Lactic Acid Bacteria through Phenotype and Genotype Based Method

Nur ilida Mohamad[a]*, Musaalbakri Abdul Manan[a], Norrakiah Abdullah Sani[b]

[a]*Food Science and Technology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), 43400, Serdang, Selangor D.E., Malaysia*
[b]*Department of Food Science, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600, UKM Bangi, Selangor D.E., Malaysia.*

*Corresponding author email: ilida@mardi.gov.my

Abstract

Introduction: Lactic acid bacteria are a group of Gram-positive, non-sporulating and microaerophilic bacteria that have similar nutritional and growth requirements among their group members. They occur naturally in certain environment including in several type of food products. Due to their many application including for industrial and food processing purposes, isolation and identification work of LAB were continuously done to find a better strain. Identification of LAB was usually done using phenotype and/or genotype identification methods. **Methods:** In this study, preliminary identification of 22 LAB isolates originated from 127 local pickled fruits samples was done based on their morphological characteristics on agar and physiological analysis (Gram staining and catalase reaction) followed by identification based on biochemical reactions (phenotype) using API 50 CHL system and polymerase chain reaction (PCR) amplification of 16S rRNA gene and sequencing (genotype). **Results:** The 22 LAB isolates have been successfully identified as LAB group members based on both methods. Through genotype identification, majority of the isolates were of the *Lactobacillus* genus (19 isolates), while another three belongs to the genus *Leuconostoc* (two isolates) and *Enterococcu*s (one isolate) respectively. The identification using API 50 CHL was not able to identify the *Enterococcus* as it has been identify as *Lactobacillus* sp. Identification results between both methods at species level was not in agreement for certain isolates as seven of the isolates were identified differently by both methods. **Conclusion:** All 22 presumptive LAB isolates based on their morphological characteristics on agar and also physiological analysis have been identified as LAB genus based on both phenotype and genotype identification. However, there were differences in identification result by both methods especially at species level.

Keywords: Lactic acid bacteria, identification, genotype, phenotype, API

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