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FOREWORD BY THE VICE CHANCELLOR OF UNIVERSITI TEKNOLOGI MALAYSIA

السلام عليكم ورحمة الله وبركا ته and Greetings to All,

In accordance with the Universiti Teknologi Malaysia's status as a research university it is important for the university to disseminate new research findings and discoveries to the community, the nation and the world. I am pleased that the Faculty of Biosciences and Medical Engineering has taken the initiative in organizing this International Conference on Biosciences and Medical Engineering 2016 (ICBME2016).

This conference serves as a platform for the discussion and dissemination of research findings, information on research trends, and latest development in the area of Biosciences and Medical Engineering. It is hoped that this gathering of academicians, researchers, and professionals from universities, government agencies, research institutes, corporate bodies, and non-governmental organizations together with undergraduate and postgraduate students can lead to much bigger things in the future.

The gathering would enable attendees to be stimulated to write, research, and discuss with others from all over the world.

Finally, I hope that the conference will be able to meet its goals and objectives and provide a rewarding experience to all participants; both local and international. My appreciation goes to the organising committee for making this conference a success.

Best wishes,

PROF. DATUK. IR. DR. WAHID BIN OMAR
Vice-Chancellor
Universiti Teknologi Malaysia

FOREWORD BY THE FACULTY DEAN

Bismillahhirramanirrahim

It gives me great pleasure in welcoming all guests, invited speakers and participants to ICBME2016 scientific meeting. The theme for this meeting "Enhancing multidisciplinary research areas of Biosciences & Medical Engineering: Transitioning research findings into practical applications" reflectsthe need for us to gather all our specific research expertise and strength to benefit the community in terms of practical applications.R&D activities are vital for progress and nation building. To achieve these goals, R&D projects require constant support with respect to financing, time, and resources. This meeting is important as it provides the avenue for scientists, engineers, industries and researchers, especially in the younger generation to meet and present their research findings in a supportive and receptive environment.In this environmentnew ideas and collaborations are often achieved which ultimately becomesbeneficial for the community.

I take this opportunity to thank the professional organisations and societies, government agencies,university colleagues and companies for their continued and invaluable support to make this meeting a success. I would like also to express a big thanks and congratulations to the organizing committee for their dedication and untiring effort towards making the meeting a success.

I wish you all an enjoyable meeting and fruitful discussion.

PROFESSOR JASMY BIN YUNUS

Dean & Chairman for ICBME2016

FOREWORD BY THE CO-CHAIRMAN - ICBME 2016

I am delighted to welcome all the participants of ICBME2016 for the first time in FBME, UTM Johor Bahru, co-sponsor by various organizations. UTM is popular by its status as a research university in Malaysia and ranked in between 400-500th among the top universities in the world and 70th in Asia by Times Higher Education. UTM places research and education services as its main pillars. The theme for this year "Enhancing multidisciplinary research areas of Biosciences & Medical Engineering: Transitioning research findings into practical applications" and fit into Malaysian Research Assessment Instrument Phase II (MyRAII), that emphasized on Quality Research, Innovations & Networking/linkages.

This meeting is unique, in that it is multidisciplinary in nature with wider participations of people from various field of research in Biosciences and Medical engineering. It will hope to spark collaborations in addressing some of the challenges faced locally and internationally and will hopefully benefit our society.

The international character of this meeting is illustrated by the participants from various Asian, European and African countries. The conference consisted of 3 Keynotes, with 8 invited papers, and the rest are all contributed papers. The main outcome from this meeting are research publications in the form of Scientific Journals and Conference proceedings. However, Book Chapter contributions are highlighted as one of the main conference publications that will be published by a reputable publisher "Penerbit UTM Press". The Book can be used for general references in the form of textbook for university students and general readings for the public.

Finally, I would like to thank to all participants and their respective institutions that have made this meeting possible and I wish you all a pleasant meeting.

PROFESSOR FAHRUL ZAMAN HUYOP
Co-Chairman for ICBME2016

KEYNOTE SPEAKERS

Professor Abdul Latif Ahmad
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Adsorptive Polymeric Membrane for Protein Immobilization

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Abstract

A biomaterial is defined as a material placed in contact with biological fluids and/or living systems and it is expected to function with a desirable response in specific applications without exerting any undesirable effects. The use of synthetic polymeric membranes as selective biomaterials has gained increasing attention owing to their versatility and biocompatibility. In addition, most membrane processes operate at relatively low temperatures and pressures involving no phase changes or chemical additives, thereby minimising the extent of denaturation/degradation of the biological products. There has been an increasing interest in the use of polymeric membranes in biosensor applications such as medical diagnostics and environmental pollution control. A biosensor contains a biological entity such as an enzyme, antibody, or bacteria, which acts as a recognition agent capable of providing quantitative or semi quantitative analysis. For this application, the membrane is referred to as an adsorptive membrane, in which the biological entity is fixed on the membrane surface and acts as a ligand to capture the compound(s) of interest, for example, antibody–antigen diagnostics (immunoassay), species-specific DNA probes, and affinity membrane chromatography. The interaction between the receptor molecule and membrane may arise from simple physical entrapment or covalent bonding between recognition elements.

Keywords: biomaterial; polymeric membrane; adsorptive; biosensor

Professor Christopher Ian Baldwin
Dean of Biomedical Sciences
Newcastle University Medicine Malaysia

Medical Research at Newcastle University
'World class collaboration between basic scientists and clinicians'

Abstract

The Faculty of Medical Sciences at Newcastle University is home to a world leading collaboration of research scientists and clinicians and we excel in tackling challenges in health and healthcare across a wide range of disciplines and specialities. This lecture will focus on a number of areas of current biomedical research at Newcastle University where such collaboration is critical to the success of the studies.

Heart disease is the leading cause of illness in diabetic patients, accounting for more than half of all fatalities. Clinicians and basic scientists in the Institute of Cellular Medicine at Newcastle University have recently shown that Metformin, which is a commonly prescribed drug for Type 2 diabetes could be routinely taken by Type 1 diabetic patients to slow the development or delay heart disease. The researchers have shown that Metformin may be used to lower Type 1 diabetic patients' risk of developing this complication by promoting a patient's ability to repair their own damaged blood vessels by increasing repair mechanisms mediated by vascular stem cells.

Up to nine in 10 children diagnosed with acute lymphoblastic leukaemia, the most common form of childhood cancer, now achieve a long-term cure. The prognosis is much poorer for those children whose disease relapses, however, with fewer than six in 10 children surviving longer than five years. Currently, children with relapsed leukaemia are grouped by whether they are at a standard or high risk of a poor outcome, based on factors such as how long the child had been in remission and if leukaemia cells were present in the bone marrow at the time of relapse. Patients currently deemed to be high risk under these criteria undergo a gruelling stem cell transplant, while standard risk patients are normally treated with further chemotherapy. Clinical researchers and basic scientists in the Northern Institute of Cancer Research at Newcastle University have recently discovered abnormalities in a number of genes which provide both additional information about how and why these children responded poorly to current therapy and can accurately predict how children whose cancer returns after treatment are likely to respond to further treatment .

Obesity has reached epidemic proportions globally, with at least 2.8 million people dying each year as a result of being overweight or obese. Over 40% of the calories in your diet can come from fat (mainly triacylglycerol) and you digest and absorb between 95% and 100% of all the fat that you eat. The enzyme pancreatic lipase accounts for 80% of all fat digestion and if the activity of this enzyme can be reduced we absorb less fat from our diet. *Basic Scientists in the Institute of Cell and Molecular Bioscience at Newcastle University have* shown that alginate (a dietary fibre extracted from brown seaweed) can reduce the activity of pancreatic lipase by up to 80% - thus if you do not digest the fat you can't absorb it.

Basic scientists and clinicians at the Wellcome Trust Centre for Mitochondrial Disease at Newcastle University **have recently completed the first in-depth analysis of human embryos created using the new technique ' early pronuclear transfer' which is designed to reduce the risk of mothers passing on mitochondrial disease,** which is debilitating and often life-limiting, **to their children**. Researchers have shown, in a study involving over 500 eggs from 64 donor women, that **the new procedure does not adversely affect human development and greatly reduces the level of faulty mitochondria in the embryo.** This suggests that **the technique will lead to normal pregnancies whilst also reducing the risk of babies having mitochondrial disease.** The team at Newcastle Fertility Centre, which is part of the Newcastle Hospitals NHS Foundation Trust, will shortly apply for a license to offer pronuclear transfer to women at high risk of transmitting mitochondrial DNA disease to their children and the team is also working to secure the necessary funding to be able to offer clinical treatments on the NHS.

Assoc. Prof. Dr. Sehanat Prasongsuk
Plant Biomass Utilization Research Unit Department of Botany,
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Potential of Plant Biomass and Plant Biomass-Degrading Microbes in Thailand for Bioenergy and Biorefineries

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Abstract

Thailand is rich in biodiversity since it is located in the tropical region. Diversity of plant biomass in Thailand includes various tropical crop plants, weed biomass, agricultural residues and industrial wastes. The major component of plant biomass including cellulose, hemicellulose and lignin can be converted into a variety of bioenergy and biorefineries. In addition, protein from plant can be useful as well. The production of bioenergy (mainly ethanol) and biorefineries (alpha-cellulose, xylan-derived products, lignosulfonate and leaf protein concentrate) from plant biomass found in Thailand will be addressed. Enzymes and other commodities from plant biomass-degrading microbes, such as *Aureobasidium*, *Lysobacter* and some tropical white rot fungi, will also be presented.

INVITED SPEAKERS

Dr. Razauden M.Zulkifli
Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, 254 Phayathai Road Bangkok, 10330, Thailand

Sustainable Source of Resveratrol from Agricultural Waste for Anti-Ageing Cream Formulation

Co-authors: **Nurul Jannah Md Arif[1], Tewin Tencomnao[2] and Farediah Ahmad[3], Razauden M.Zulkifli[1,2]**

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Abstract

Resveratrol is used in cosmetic industry as topical formulation due to its beneficial biological properties. They were found naturally in red wine, pines, blueberries and bilberries, peanuts and pistachios however they are not sustainable for cosmetic industry due to low amounts of raw material. Grape has become a fast growing agricultural sector in Thailand and Malaysia. Along with the increment, this agriculture waste remains to be unutilized. Therefore, the present study was aimed to investigate and utilized the natural resveratrol from *V. vinifera* agricultural waste and develops into an affordable anti-aging product. The dried stems (500 g) were sequentially extracted with n-hexane, ethyl acetate (EtOAc) and methanol (MeOH). The yielded crude were n-hexane (1.84 g, 0.37%), EtOAc (1.16 g, 0.23%) and MeOH (11.13 g, 2.23%) extracts respectively. Analysis by thin layer chromatography (TLC) suggested the presence of resveratrol originated from EtOAc extract. 1 g of EtOAc extract was further fractionated by vacuum liquid chromatography (VLC) to isolate resveratrol (9 mg) as an amorphous yellow powder, Rf = 0.63. The resveratrol was further characterized and confirm by fourier transform infrared (FTIR) and proton nuclear magnetic resonance (1H-NMR) spectroscopy respectively. Finally, anti-aging cream comprising resveratrol with a concentration of 0.05% was formulated and further evaluated on stability and storage characteristics. In conclusion *V.vinifera* agricultural waste can be harness for potential sustainable sources of resveratrol for cosmeceutical and nutraceutical applications.

Key words: resveratrol, *vitis vinifera*, anti-ageing

Prof. Dr. Amir Husni Mohd Shariff
University Malaysian Sabah

Entrepreneurship, Business and Research Association with Institute Of Higher Learning

Abstract

The available funds from government sectors for collaborative research between Institute of Higher Learning and Private Sector is a lucrative sector to be tapped to produce commercially high end valued products, using the brains from the local university researchers to work on the product wants of the commercial sector. This will add value to the university research activates besides the on-going basic research undertaken to strengthen the basic fundamental sciences understanding. This commercial research endeavors will open the doors for our local universities to be financially independent and self-sustained in their future undertakings.

The end objective is to develop spin-off companies using funds from the following appointed government agencies MTDC, MOSTI (Techno Fund), CIDA, TERAJU, TEKUN for commercialization purpose, with funds ranging from a few hundred thousand to an amount of few million Ringgit based on project requirements.

In order to achieve it, a model must be developed by the Institute of Higher Learning to ensure the objective is achieved and the product developed commercialized with a win-win situation for both parties. The issue of profit sharing between researches- University and private sector must be resolved from day one, in order to ensure its success. The issues of Intellectual Property (IP), Royalty, profit sharing, Cash Flow, equity and the available grant to suit the environment and the wants of both parties. The government universities must have a commercial one stop center to cater these needs and manage by professionals with business astute and acumen.

Marian P. De Leon, PhD
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Exploring Philippine Caves for Potential Microbes

Abstract

Microorganisms are ubiquitous and diverse in nature and can grow even in extremes of environment, temperature, osmolarity, pressure and pH. The vast majority of the microbial species is not situated within such energetically favorable environment but rather thrives under extremes of environmental stresses including near-starvation or oligotrophy and may be based on inorganic energy sources rather than sunlight. These diverse oligotrophic communities are usually found in ocean, sediments or subterranean sites such as caves, which is an interesting facet of microbial diversity. This presentation will highlight initial findings on the microbial diversity of Philippines cave environs and its potential biotechnological use.

Professor Ir. Dr.-Ing. Eko Supriyanto
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The Future Role of Biomedical Engineer in South East Asia

Abstract

Biomedical Engineer is the youngest of the engineering professions. This engineering profession has emerged to fulfill the need of health care system due to advancement of technology. Although biomedical technologies have grown very fast in the last decade, the position of biomedical engineer in the job market remains unclear especially in developing countries. This causes the incorrect positioning of many biomedical engineering graduates and lack of health professional understanding on biomedical engineering function. This situation is also caused by mismatch of biomedical engineering education programs in the universities. In this paper, we will present the future demand for biomedical engineers and their roles in South East Asia. The study is based on 20 years demography, epidemiology and economy projection in South East Asia.

Keywords: biomedical engineer, South East Asia, health care system

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Agrobacterium tumefaciens Mediated Transformation of Turkish Upland Rice (*Oryza sativa* L.) with *dehE* gene for Dalapon Tolerance

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[4]Department of Plant Protection, Faculty of Agriculture, Ondokuz Mayis University, Samsun, Turkey.hmaksoy@omu.edu.tr

Abstract

Transgenic rice plants were generated by the introduction of *dehE* gene from *Rhizobium* sp. for herbicide tolerance. A *dehE* gene from *Rhizobium* sp. was sense expressed into Turkish Upland Rice produce transgenic plants tolerant to Dalapon herbicide stress. Mature seed-derived embryogenic calli were transformed *with Agrobacterium tumefaciens* strain EHA105 harboring the binary vector Gateway® TOPO with *dehE* gene. Active proliferating calli selected in hygromycin and cefotaxime were regenerated and transferred to MS medium. Transformation of transgenic rice confirmation realized by PCR and bioassay (dalapon). The putative transgenic rice were transferred to soil and grown further. The putative transgenic plants was sprayed with different concentration of dalapon, they were growing well. But control plants were died when dalapon sprayed on them. As a result, the transgenic plants were highly tolerance to dalapon herbicide as revealed by herbicide bioassay.

Keywords: *Oryza sativa, dehalogenase,* Dalapon, *Agrobacterium tumefaciens*

Professor Muhaimin Rifa'i
Head of Immunology Laboratory in Biology Department,
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Essential Roles of Propolis in the Maintenance of Homeostasis in Diabetic Mouse Model

Co- author: Aris Soewondo[1], Tsuboi Hideo[2], Sasmito Djati[1], Widodo[1]
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[2]Department of Immunology, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan.

Abstract

It is currently known that 4.6% of the total Indonesian population suffering from diabetes mellitus, and this figure is predicted to increase to 7.8% in 2030. During this time, the treatment of various diseases including diabetes depends on a lot of drugs synthesis. On the other hand, Indonesia has natural resources that have the potential to cure various diseases. In general, the severity of diabetes is triggered by inflammatory molecules that continue to be produced by immunocompetent cells. In previous studies, we have obtained evidence that propolis may improve homeostasis in diabetic mice model by increasing $CD4^{+}CD25^{+}$ regulatory T cells. However, the hypothesis that propolis can suppress the expression of Toll-Like Receptor (TLR) could not be confirmed in this study. It looks that propolis rather restores the expression of Toll-Like Receptor becomes normal than suppress the expression. In diabetic mice, TLR expression dropped dramatically, and propolis administration can ameliorate this condition. In this experiment, we showed that propolis at a concentration of 200 mg/mL could increase insulin expression in pancreatic beta cells. Administration of propolis on splenic cell culture can inhibit the rate of apoptosis. Co-cultured of propolis and cisplatin can inhibit apoptosis that usually driven by cisplatin, and the cell remains at synthesis and mitosis phase.

Keywords: Diabetes mellitus, propolis, immunomodulatory, Toll-like receptor.

Associate Professor Dr. Widodo
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Designing Vaccine of Nasopharyngeal carcinoma based on Epitope-conserve region on gp350/220 of Epstein - Barr virus

Co- author: Muhaimin Rifai, Sri widyarti, M. Sasmito Djati, Loly S. Sitompul
Jurusan Biologi, Fakultas MIPA, Universitas Brawijaya, Malang-Indonesia

Abstract

Nasopharyngeal carcinoma (NPC) is a malignant tumor in the nasopharyngeal epithelial cells that caused by many factors, one of which is the viral infection of EBV (Epstein Barr Virus). The standard treatments to cure NPC still have not been encouraging. The prevention through vaccination is an effective way to stop the disease. However, EBV vaccine that being able to cover all variants of the virus is still not available yet. Therefore, we identified the conserved region of glycoprotein 350/220 of EBV which has immunogenic and antigenic properties. The glycoprotein 350/220 is a viral surface protein that responsible for the binding CR2 receptor, mediated EBV to enter the host cell. Further, by blocking CR2 binding domain of gp350/220 using antibody will inhibit EBV's spreading, and provoke an immune system to eliminate the virus in a patient. Glycoprotein 350/220 from all variants of Epstein-Barr virus was retrieved from NCBI. The conserved domain of gp350/220 was identified by aligning the protein sequences and structures. The polymorphic structure was used as a template for docking analysis to determine the resemblance of amino acid from polymorphic variants of gp350/220 that binds CR2. The epitope mapping of gp350/220 was done by Discotope BepiPred method. The result revealed that the conserved region of gp350/220 was predicted to have an epitope, and it does not have any similarities to the human's cell surface protein. We demonstrated the ability of the epitope to stimulate B cells to produce specific antibodies. The epitope has similar structure compare with GP350/220 Intact protein. Further investigation revealed that the epitope has a molecular weight of 2.29 kDa, pI=4:37 and the half-life for 10 hours in *E. coli*. It means the epitope is stable and has a long life in *E. coli*, which is indicated that the *E. coli* is the best host to produce the epitope recombinant. This study illustrates the potency of the epitope as both active and passive immunization agents against nasopharyngeal cancer.

Keywords: EBV; Epitope-based vaccine; NPC; gp350/220

Dr. Ismaila Yada Sudi
Head of Department of Biochemistry, Adamawa State University (ADSU) Mubi, Nigeria.

Response of Coffee Senna (*Senna Occidentalis*) Seeds to Different Fermentation Periods

Co- author: Augustine Clement[1], Ismaila Yada Sudi[2]* and Joseph Uchei Igwebuike[3]

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Abstract

A laboratory analysis was conducted to investigate the effect of varying fermentation period on the proximate composition, amino acid profile and levels of anti-nutritional factors of *Senna occidentalis* seeds. The seeds were fermented for 0, 3, 5, 7 and 9 days, respectively in replicate of three in a completely randomized design. The proximate composition, amino acid profile and level of anti-nutritional factors of the differently fermented *Senna occidentalis* seeds were determined using standard laboratory procedure. Data obtained were subjected to analysis of variance of the completely randomized design (CRD) using Statistix 9.0. The results indicated that the proximate composition of *Senna occidentalis* seeds significantly ($P<0.05$) increases as the fermentation period progresses except for the ether extract and nitrogen-free extract which reduced as the fermentation period increased. The amino acid content similarly showed significant ($P<0.05$) increase as the fermentation period advanced. The level of the anti-nutritional factors were observed to significantly ($P<0.05$) decline as the fermentation period progresses. It can be concluded that *Senna occidentalis* seeds can be fermented up to 9 days without adverse effect on the nutritive value of the seeds. However, fermentation for 5 is recommended due to its minimal reduction of ether extract and nitrogen-free extract when compared to 7 and 9 days fermentation. The fermented seed meals should be used in a feeding trial in order to evaluate their feeding value to animal.

Keywords: Response, fermentation periods, *Senna occidetalis*, lesser-known.

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Imaging Memory through Spike Directivity and Its Solitonic Properties

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Abstract

Memory is stored due to the formation of specific proteins that ensure the specificity of biological responses to appropriate stimuli. The hypothesis proposed by Nancy Woolf [*A structural basis for memory storage in mammals*, Prog. Neurobiol.**55**,59-77(1998)] suggests that memory is stored in the distalmost dendrites of pyramidal neurons of different cortical areas because new branches are the epicenter of novel information storage in-between neurons. However, this hypothesis does not convey the dynamical properties of memory in the distalmost dendrites. The memory process of consolidation leads to selective storage, involving dynamic interaction yet self-preserving. Action potentials dissipate during collision and so long-term memory cannot be in the spike-rate. We have theoretically obtained neuronal solitons that occur within sub millisecond precision for coincidence detection during acquisition of memory and remain stable so they are appropriate for storage of long-term memories. Furthermore, neuronal solitons are sensitive to their initial position with non-stereotypical amplitudes that could carry with it semantic information. Such solitonic properties of membrane voltage changes in distalmost dendrites of neurons contain proteinaceous structures which introduce electrical signalling that is well suited for long-term storage of semantic memory. The measure of non-stereotypy includes spike-directivity. We show in the existence of an intrinsic spatial code within neuronal spikes that images memory of the animal during behavioral tasks. As rats learnt a T-maze procedural task, simultaneous changes in temporal occurrence of spikes and spike directivity are evidenced in "expert" neurons from the dorsolateral striatum. While the number of spikes between the tone delivery and the beginning of turn phase reduced with learning, the generated spikes between these two events acquired behavioral meaning that is of highest value for action selection. Spike directivity is thus a hidden feature that reveals the semantics of each spike and in the current experiment, predicts the correct turn that the rat would subsequently make to obtain reward. Semantic memories can then be revealed as modulations in spike directivity during the time. This predictability of stored information based on changes that occur in the spike (how it propagates, spike directivity) and not just the time of the spike represents an important step forward in our understanding on the dynamical properties of stored information.

Keywords: Spike directivity; non-stereotypical spikes; semantic memory; neuronal solitons; distalmost dendrites; proteinaceous structures; expert neurons; striatum.

Prof. Dr. M. Saleem Haider
Director IAGS, University of the Punjab, Lahore, Pakistan

Advances in Geminivirus research in Pakistan

Abstract

Geminiviruses are plant viruses that contain a circular, single-stranded DNA genome of about 2.8-3.0 to 5.2 Kb in size, encapsidated in a twinned, icosahedral or 'geminate' particle. They are classified into seven genera on the basis of insect vector transmission, host range, and genome organization. Until the early 1990s, whitefly-transmitted geminiviruses were primarily considered problematic in legumes in South and Central American, and in India, and in most cotton-growing regions of the world. Globally, diseases caused by whitefly-transmitted geminiviruses are important pathogens of crops, including bean, tomato, cassava, tobacco, potato, cotton, pepper, squash, and cabbage. More than fifty cultivated and wild plants have been reported as hosts of geminiviruses. *Begomovirus* is the largest and most devastating genus of family geminiviridae. Members of genus *Begomovirus* are transmitted by the whitefly *Bemisia tabaci* (Gennadius) sibling species group in a circulative, persistent manner. Begomoviruses have either a monopartite or bipartite genome. The monopartite genome type has a single DNA component, whereas, the bipartite type have two components, referred to as the DNA-A and DNA-B component, respectively. Some monopartite begomoviruses harbor one or more types of non-viral molecule, referred to as beta (DNA β) or alpha (DNA α) satellites. Begomoviruses have hampered crop production in Pakistan, infecting cash crops and food crops including cotton, tobacco, tomato, chillies, okra, brinjal and cucurbits. The most devastating disease that has severe impact on Pakistan economy is cotton leaf curl disease (CLCuD). Recently sentinel plots were established to devise management strategy and prediction of emerging viruses and to monitor viral diversity and population structure of viruses associated with Cotton leaf curl disease (CLCuD) in Pakistan. Sentinel plots were established for 3 consecutive years (2011-2013) at Vehari, Multan and Lahore. Aside from four varieties of cotton with differential resistance against CLCuD, other plant species Tomato, Okra, Luffa, Cucumber, Squash, Chilli, Bell pepper, Black Nightshade were included in the sentinel plots. Leaf samples were collected from symptomatic and asymptomatic plants fortnightly. Results revealed that sentinel plots may serve as an indicator of emergent viruses into different hosts, so sentinel plots can serve as management of CLCuD by predicting situation of an outbreak. Use of transgenic techniques including RNAi to develop broad spectrum resistance against CLCuD and whitefly has proved effective in initial trials and it is hoped that it will help to combat the disease by producing resistant plants.

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Cytoplasmic Genome Diversity As Revealed By Submergence Stress Responses at Germination and the Early Seedling Stages in Nucleus-Cytoplasm Hybrids of Common Wheat

Abstract

Plant cells contain three different genomes, i.e, nuclear, mitochondrial and chloroplast genomes. These three genomes are located in their respective cellular compartments and have been known to interact with each other to maintain plant homeostasis during its whole developmental stages. In the *Triticum-Aegilops* comlex, cytoplasmic genome diversity and nucleus-cytoplasm (NC) genome interactions can effectively be studied using a series of NC hybrids in which particular nulcear genomes are combined with various cytoplasmic genomes of *Aegilops* species. Objectives of our study are to evaluate cytoplasmic genome diversity and NC genome interactions that could possibly affect agronomically important traits. We evaluated submergence stress responses of NC hybrids at germionation and the early seedling stages. Bioassays revealed a large variability among 41 NC hybrids. Groups of *Aegilops* cytoplasms either enhanced or reduced the sesitivity to submergence stress of NC hybrids as compared with that of their nulcear donor. Observed variability was ascribed to cytoplasmic genome differences in NC hybrids. Because all NC hybrids possess an indentical nuclear genome, our results suggest a possible involvement of retrograde regulation by that different cytoplasmic genomes could regulate expression of the donor nuclear genome in NC hybrids under submergence stress at these early developmental stages of wheat.

Keywords: Cytoplasmic diversity, Nucleus-Cytoplasm (NC) hybrids, retrograde regulation, Submergence stress responses, *Triticum-Aegilops* complex, Wheat

TENTATIVE PROGRAMME ICBME 2016

10 November 2016 (Thursday)	
7:30-8:15	**REGISTRATION** **(Venue: V01 Main foyer)**
8:15-8:30	**Arrival of VIPs** **(Venue: Main Hall)**
8:30-8:35	**Opening Prayer** **(Venue: Main Hall)**
8:35-8:45	**National Anthem/UTM Song** **(Venue: Main Hall)**
8:45-8:55	**Opening Remarks by Dean & Chairman of ICBME 2016** **(Venue: Main Hall)**
8:55-9:05	**Welcome Message by the Deputy Vice Chancellor (Research & Innovation) UTM** **(Venue: Main Hall)**
9:05-9:35	**Keynote Address I: Prof. Abd. Latif** **(Venue: Main Hall)**
9:35-10:05	**Keynote Address II: Prof. C. Baldwin** **(Venue: Main Hall)**
10:05-10:20	**Refreshment (Venue: Main foyer)**
10:20-10:50	**Keynote Address III: Prof. S. Prasongsuk** **(Venue: Main Hall)**

PARALLEL SESSION 1					
	ROOM 1A **Chairperson: Dr. Syafiqah Saidin/Dr. Maheza Irna Mohamad Salim**	**ROOM 2A** **Chairperson: Dr. Aizreena Azaman/Dr. Fazrena Nadia Md. Akhir**	**ROOM 3A** **Chairperson: Dr. Fazilah Abd. Manan/Dr. Wan Rosmiza Wan Dagang**	**ROOM 4A** **Chairperson: Dr. Praseetha A/P Prabhakaran/Dr Haryati Jamaluddin**	**ROOM 5A** **Chairperson: Dr. Zaidah Rahmat/Dr. Dr. Nur Izzati Mohd Noh**
11.00-11.20	**Invited Speaker** **Entrepreneurship, Business and Research Association with Institute with of Higher Learning** **Professor Dr. Amir Husni Mohd Shariff** **Universiti Malaysia Sabah**				**Invited Speaker** **The Future Role of Biomedical Engineer in South East Asia** **Professor Ir. Dr.-Ing. Eko Supriyanto** **IJN-Universiti Teknologi Malaysia Cardiovascular Engineering Centre**
11.20-11.30	**ID: 101** Nanomedicine: Present and Future Prospects Muhammad Ismail Institute of Biomedical & Genetic Engineering (IBGE),	**ID: 118** Comprehensive Characterization of Malaysia Oil Palm Frond Leaves and their Ash Emmanuel Onoja Universiti Teknologi Malaysia	**ID: 128** The Potency of Consortium Bacteria Isolated from Apple Tree-Rhizosphere from Batu as Biofertilizer Tri Ardyati University of Brawijaya, Malang, Indonesia.	**ID: 138** Antihypertensive effect of hydrolysates of melinjo (Gnetum gnemon) protein seed and their angiotensi-I-converting enzyme inhibitory activity Tri Agus Siswoyo University of Jember	**ID:154** Clinical Laboratory Science: The Hidden Profession that Saves Lives Ryan Jay Cafugauan Dela Cruz RMT

11.30-11.40	**ID: 102** Production of Alkaline Protease from Isolated Bacillus Species from Soil on Affordable Molasses Medium Malaz Khansa Universiti Teknologi Malaysia	**ID:119** Antihyperlipidemic Activity of Flavonoid Rich Extract of Vitus vinifera on High-fat Induced Hyperlipidemic Rabbits Teba Abdul Lateef College for Home Economics, Karachi	**ID:129** A Study on the Bacterial Degradation of Polymer Composite Films Shubhalakshmi Sengupta Jadavpur University	**ID:139** The Correlation Study of Transcutaneous Bilirubin Measurements in Neonates Andra Kurnianto University of Sriwijaya	**ID: 155** Motion Comparative Analysis in Equestrian Jumping between Arabian Horses and Thoroughbred Horses: A case study. Mohd Baihaqi Universiti Teknologi Malaysia
11.40-11.50	**ID: 103** Challenges of Heamodialysis Water Treament System, a Major Setback to Renal Quality Care. (Nigeria Case Study) Abolude Paul Oluwaseun University College Hospital, Ibadan	**ID:120** Antiseptic Alcohol Alters the Antibiotic Susceptibility of Proteus mirabilis Janelle Laura Gacad University of Santo Tomas,	**ID:130** Evaluation Of Plant Host Influence On The Secondary Metabolite Production Of Endophytic Fungi Ana Maria D. Cariño Saint Mary's University	**ID: 146** Antiplatelet Activity of Bromelain Isolated from The Extract of Indonesia's Pineapple Core (Ananascomosus [L] Merr.) Siswati Setiasih Universitas Indonesia	**ID:111** Electromyography in the analysis of sitting and standing passive exercise for isometric ankle exercise rehabilitation Hadafi Fitri Mohd Latip Universiti Teknologi Malaysia
11.50-12.00	**ID:104** Upper limb post stroke rehabilitation performance monitoring tools using optical mouse Abdul Shokor bin Abd Talib 3G University	**ID: 121** Perceptive Assessment of the Effects of Biogas Emission from Waste Dumpsites on Proximate Residential Properties in Nigeria. Kazeem B. Akinbola Polytechnic, Ilaro, Nigeria.	**ID:136** Gama Efficacy of Bacillus thuringiensis D142 (MOSNONTM) against Aedes aegypti larvae in Ngebrak Village, East Java Zulfaidah Penata Brawijaya University,	**ID:141** Application of Surfactant Modified Natural Zeolite Loaded NPK for the Growth of Morus alba Noor Asyraf Noor Azman Universiti Teknologi Malaysia	**ID: 137** The Mechanistic Role Of Catalytic Residues In Non-Stereo Dehalogenase (DehE) Muhammad Hasanuddin International Islamic University Malaysia

			Indonesia		
12.00-12.20	**Invited Speaker** **Essential roles of propolis in the maintenance of homeostasis in diabetic mouse model** **Professor Muhaimin Rifa'I** **Brawijaya University**		**Invited Speaker** **Exploring Philippine Caves for potential microbes** **Dr. Marian Pulido De Leon**		**Invited speaker** **Spike directivity: the neural correlate of semantic memory** **Professor Roman Poznanski** **Stanford University**
12.20-12.30	**ID:105** Application of SRAP markers for Diversity analysis and Development of Variety Specific SCAR markers for Basmati rice DNA fingerprinting Umer Maqsood National Institute for Biotechnology and Genetic	**ID: 123** Aorta blood flow using fixed grid method. Mohamad Shukri Zakaria Universiti Putra Malaysia	**ID:133** Molecular characterization of Penicillium ulaiense causing whisker mold a new postharvest threat to citrus of Pakistan Muhammad Nasir Subhani Institute of Agricultural	**ID:143** Community structure of sulfur oxidizing bacteria at Midorikawa mudflat Irfan Mustafa Brawijaya University,	**ID: 122** Actinomycetes as A Producer of Antibacterial Pathogens Isolated Eucalyptus deglupta Blume. Rhizosphere from Lore Lindu National Park, Central Sulawesi, Indonesia M. Alwi University ofTadulako

	Engineering		Sciences, University of the Punjab, Lahore, Pakistan		
12.30-12.40	**ID:106** Purity and concentration of solubilized inclusion bodies in protein refolding Chun Yi Leong Universiti Malaysia Pahang	**ID:124** Effect of pH Condition on Xylanase Production by Immobilizing E. coli onto Graphene Oxide Nur Atiqah Lyana Nor Ashikin Universiti Tun Hussein Onn Malaysia	**ID:117** Total Phenolics, Antioxidant Activity and Biological Screening of an Epilithic Microalgae Desmodesmus sp. (U-AU2) from Los Baños, Laguna-Philippines Eldrin DLR. Arguelles University of the Philippines Los Banos	**ID: 144** Investigating Small Numbers of Trinucleotide Repeat Expansion: Can PCR do the job? Ziske Maritska Universitas Sriwijaya	**ID:134** Morphological characterization of Penicillium ulaiense causing whisker mold a new postharvest threat to citrus of Pakistan Muhammad Nasir Subhani University of the Punjab, Lahore

12.40-12.50	**ID:107** Isolation and Characterization of Bacteria from Seagrass Ming Quan Lam Universiti Teknologi Malaysia	**ID:125** DR4 626 Single Nucleotide Polymorphism; Its Risk Assessment in Breast Cancer Patients from Pakistan Qaisar Mansoor Institute of Biomedical and Genetic Engineering	**ID:135** The role of nitric oxide in Musa sp. during basal resistance against Fusarium oxysporum f. sp. Cubense Noor Baity Saidi Universiti Putra Malaysia	**ID:145** Pharmacological analysis of an obnoxious water weed: Eichhornia crassipes (Mart.) Solms Fakhra Shamim University of the Punjab	**ID:132** The Detection of 8-Hydroxy-2'-Deoxyguanosine and 1-Hydroxypyrene as Biomarker of CancerRisk in Population Exposed by Benzo[a]Pyrene Budiawan Universitas Indonesia
12.50-13.00					
13.00-14.00	**Lunch Hour**				
	PARALLEL SESSION 2				
	ROOM 1B **Chairperson: Dr. Nik Ahmad Nizam Nik Malek/Dr. Muhammad Amir As'ari**	**ROOM 2B** **Chairperson: Dr. Nida Iqbal/ Dr. Helmi Mohd Sani**	**ROOM 3B** **Chairperson: Dr Goh Kian Mau/Asoc.Prof. Dr. Azli Yahya**	**ROOM 4B** **Chairperson: Dr. Tan Tian Swee/ Professor Zaharah Ibrahim**	**ROOM 5B** **Chairperson: Dr. Muhammad Arshad Javed**

14.00-14.20	**Invited speaker** **Agrobacterium tumefaciens Mediated Transformation of Turkish Upland Rice (Oryza sativa L.) with dehE gene for Dalapon Tolerance** **Dr. Yilmaz Kaya :** **Faculty of Agriculture, Ondokuz Mayis University**	**Invited Speaker** **Designing Vaccine of Nasopharyngeal carcinoma based on Epitope-conserve region on gp350/220 of Epstein-Barr Virus** **Dr. Widodo** **Universitas Brawijaya,**			**Invited speaker** **Advances in Geminivirus research in Pakistan** **Prof. Dr. M. Saleem Haider** **University of the Punjab,**
14.20-14.30	**ID:142** Evaluation of Gold Nano flowers For Signal Enhancement of Lateral Flow Immunoassay Mohammad Lukman Yahaya Universiti Sains Malaysia	**ID:160** The Effectivity of Cinnamon Alcoholic Extract to the Total Cholesterol on Hypercholesterolemia Rat Model Nita Parisa Universitas Sriwijaya	**ID:168** Progesterone Receptor Gene Polymorphism Promoter Region +331G/A Increases Risk of Endometriosis in Indonesian Women Syifa Alkaf University of Sriwijaya	**ID:153** Micropropagation and Assessment of Genetic Fidelity of Stevia Rebaudiana (Bertoni) Using ISSR Markers Zarina Zainuddin International Islamic University Malaysia	**ID:127** Isolation and screening of antimicrobial activities of fungal endophytes from Philippine endemic plant Dillenia philippinensis (Rolfe) Seraphim Marie S. Española De La Salle University

14.30-14.40	**ID:112** Alteration of Saccharomyces cerevisiae genes expression involved in G1 to S transisition progression of the cell cycle mechanism as antiproliferative effect of Noni fruit extract (Morindra citrifolia Linn) Hermansyah Sriwijaya University	**ID:131** Physio-agronomic Response of Spring Maize (Zea mays L.) to Foliar Application of Plant Growth Promoting Substances Muhammad Bilal Chattha University of Punjab	**ID: 169** Effect of Low Intensity Exercise to Postsynaptic Density 95 Level and Spatial Memory Ability on Male Swiss Webster Mice Induced by Immobilization Stress M. Irfannuddin Sumatera Selatan Indonesia	**ID:147** Screening of Endegenous Keratinolytic Bacteria for Biodegrading Chicken Feather Sutoyo University of Jember	**ID:108** Isolation and Molecular Identification of Bacteria Composition in Biofloc from Pacific Whiteleg Shrimp, P. vannamei Grow-out Ponds Nor Azman Kasan Universiti Malaysia Terengganu
14.40-14.50	**ID:113** Electromyography analysis comparison of muscle activation between wobble board manual device and Integrated Multiple Ankle Technology Device Hadafi Fitri Mohd Latip Universiti Teknologi Malaysia	**ID:162** Study on the Cytotoxicity Activity of Hibiscus tiliaceus against Breast Cancer Cells (MCF-7) Yosie Andriani Universiti Malaysia Terengganu	**ID:170** Identification of Cry1c and Cry1fa Binding Proteins in Spodoptera Frugiperda Using Proteomics Munawar S. Ahmad University of Swabi, Pakistan	**ID:148** Role of Quality in Crop Improvement Zahid Akram Agriculture University Rawalpindi,Pakistan	**ID:167** Antibacterial activities of the lichen Ramalina and Usnea collected from Mt. Banoi, Batangas and Dahilayan Bukidnon against selected Multi-drug resistant (MDR) bacteria Lawrence Timbreza Eastern University

14.50-15.00	**ID:114** Use of Multiplex-PCR Method in Identification of Candida species Isolated fromPatients with Clinical Vaginal Candidiasis Susilawati Sriwijaya Univerity	**ID:164** Sonochemical Technique In Situ Application On Cellulose Producing Bacteria (Acetobacter Xylinum) During Static Fermentation Muhamad Elias Alamin Kamaludin Universiti Teknologi Malaysia	**ID:171** Potential Effects of Nigella Sativa and Thymoquinone on the Foetal Development in Mice Following Paternal Exposure to Cyclophoshamide Suzanah Abdul Rahman: International Islamic University Malaysia	**ID:149** Screening for Algicidal Activity of Marine-Derived Fungi Isolated from Macroalgae and the Mussel Perna viridis Aaron Joseph M. Macaspac University of Santo Tomas, Philippines	**ID: 181** High-cell density ethanol fermentation of diluted sugarcane syrup using flocculating and high ethanol-yielding Saccharomyces cerevisiae strain Francisco B. Elegado University of the Philippines Los Banos
15.00-15.10	**ID:115** The homology based structural model of L-2-haloacid dehalogenase (DehL) from Rhizobium sp. RC1 Aliyu Adamu Universiti Teknologi Malaysia	**ID:165** Synthesis of Graphene-Based Magnetite Nanocomposite for Lung Cancer Treatment Xin Jie Lee Universiti Putra Malaysia	**ID:172** Hydrocarbon degradation activities by microbes isolated from low temperature environment Nur Hafizah Azizan International Islamic University Malaysia	**ID:150** Differentiation Potential of Mice Dental Pulp Stem Cells Isolated from Enzyme Digestion and Outgrowth Method Farinawati Yazid Universiti Kebangsaan Malaysia	**ID:176** Potential Ecotourism of Ethnobotanicalstudy Traditionalclothes Bada Ethnic in Biosphere Reserves Lore Lindu Centralsulawesi Eny Yuniati Brawijaya University

15.10-15.20	**ID:116** The Irrigation Water Quality Improvement in Phytoremediation Pond Using Some Species of Local Hydromacrophytes Catur Retnaningdyah Brawijaya University	**ID:126** DNA methylation and expression of DNA cytosine-5-methyl transferase 1 (DNMT1) and methyl CpG-binding domain 2 (MBD2) in patients with Rheumatoid Arthritis Attya Bhatti University of Sciences and Technology (NUST)	**ID:173** Analysis of risk factors for hypospadias Alfian Hasbi University of Sriwijaya	**ID:151** Analysis of The Level of Egg Sterility and The Age of Aedes aegypti Mosquitoes After The Release of The Sterile Insect Technique (SIT) in Ngaliyan District Semarang City Dwi Sutiningsih University, Semarang Indonesia	**ID:166** Elucidation of GDC-0941 binding to PI3Kα isoform via in vitro mutagenesis of non-conserved amino acids of PI3Kα Syazwani I. Amran Universiti Teknologi Malaysia
15.20-15.30		**ID: 180** Anisotropic and Isotropic Substrates that Control the Growth and Function of Liver Cells Che Azurahanim Che Abdullah Universiti Putra Malaysia	**ID:156** Motion analysis comparison speed between 2 types of horses with the same breed: A case study Mohd Baihaqi Universiti Teknologi Malaysia	**ID:152** Identification of Bacterial Species Capable of Degrading 2,2-Dichloropropionic Acid Isolated from Cow Dung Siti Nurul Fasehah Ismail Universiti Teknologi Malaysia	**ID:161** Flow Simulation of Patent Ductus Arteriosus to Evaluate Thrombosis Factors on Closure Device Nurulnatisya Ahmad Universiti Teknologi MARA
15.30-15.40					

15.40-16.00	**Invited Speaker** **Cytoplasmic genome diversity as revealed by submergence stress responses at germination and the early seedling stages in nucleus-cytoplasm hybrids of common wheat Prof. Chiharu Nakamura: Ryukoku University**		**Invited speaker** **Response of Coffee Senna (Senna accidentalis) Seeds to different fermentation periodsDr. Ismaila Yada Sudi Adamawa State University (ADSU) Mubi, Nigeria**		**Invited speakerSustainable source of Resveratrol from agricultural waste for Anti-ageing Cream formulationRazauden M.Zulkifli Chulalongkron University Bangkok Thailand**
16.00-16.35	**CLOSING CEREMONY – Co-Chairman ICBME2016** **Tea Break**				
17.00-17.30	**Evaluation form submission & Certificate Collection**				

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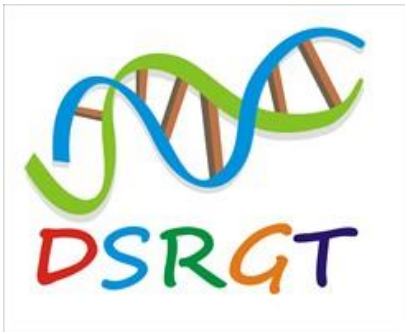
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ABSTRACT

ID 101: Nanomedicine: Present and Future Prospects.

Muhammad Ismail, PhD

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Abstract

Nanotechnology has provoked a new era in the field of biomedical sciences as nano-medicine and nanobiotechnology. The series of basic experimentation and clinical trials has revealed that biocompatible nano-particles alone and in combination with the phytochemicals, synthetic chemical materials and drugs yield promising result in targeting the disease sources. Ag, Zn, Cu, Au, Ni, Graphene nano-particles are being extensively used in nano-medicine research and drug formulation. Substantial fraction of information has shown that there are wide ranging stumbling blocks associated with systemic administration of drugs and how they reach their target site. Bioavailability is a rapidly growing concern that needs in-depth research. Entry of Doxorubicin-loaded anti-EGFR-immunoliposomes and nanoparticle albumin-bound paclitaxel in clinical trials has substantiated valuable role of nanotechnology in drug delivery. Our group has worked on biological applications of Ce doped CuO nanoparticles, Cu and Mg Doped ZnO Nanoparticles. We have reported significant antibacterial activity displayed by nanoparticles. We also provide evidence of targeted killing of Multi-drug Resistant Bacteria by Ni Doped ZnO Nanorods and Ag Doped ZnO Nanorods. Recently, we have experimentally verified that Sn doping induced enhancement in the activity of ZnO nanostructures against antibiotic resistant S. aureus bacteria. Future studies must converge on a better and considerably improved understanding of the toxicological profiling of the nanotechnologically delivered drugs in animal models.

ID 102: A Performance Comparison of DNA Extraction Methods to Optimize Rice (Oryza sativa L.) Leaf Tissue

Samiullah Khan, Muhammad Arshad Javed[1]*, Nusrat Jahan and Cindy Goh Poh Hsin

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Abstract

The traditional liquid nitrogen DNA extraction method is expensive and tedious. Working with segregating population requires smallest possible amounts of tissues. As these populations are grown until maturity, it is not normally recommended to get tissues in bulk. Therefore, there is the need for effective, cheaper and faster methods of DNA extraction for efficient application of marker assisted selection (MAS) for breeding in rice to be able to handle large number of samples at a time. Three DNA extraction methods: Ikeda method, Hosaka method, and an alkaline lysis method were tested on two varieties of rice (Oryza sativa L.) i.e. IR36 and Panderas, based on four different weights (5, 10, 25 and 50 mg). DNA yield, purity and amplifiability from the three methods were compared. The results revealed that the DNA extraction methods and weight of samples affected DNA quality and quantity. The alkaline lysis method provided the highest DNA yield, while the Ikeda and Hosaka methods produced DNA of high purity, integrity and amplifiability. These results suggested that the Hosaka and Ikeda methods were suitable for extraction of rice DNA. The results revealed that the Hosaka method gave the highest DNA quality from 5 mg rice leaves with lower cost and lesser time in DNA extraction analysis. Therefore, the Hosaka method is suitable for the extraction of DNA from rice seedlings. Meanwhile, 10 mg of the samples is chosen as the most suitable amount for DNA extraction for segregating populations.

Keywords: DNA extraction methods; marker assisted selection (MAS); rice DNA; rapid isolation;

ID 103: Production of Alkaline Protease from Isolated *Bacillus Species* from Soil on Affordable Molasses Medium

Malaz Khansa[1,2,*]

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Abstract

Screening was carried out from soil samples collected from garden of FMBE faculty, UTM University, Skudai, Johor Bahru, Malaysia that resulted in isolation of 7 alkaline protease producing alkaliphilic bacterial strains. Strain MK1 showed the highest production (23.95 U/ml) after 48h. This isolate was identified as *Bacillus cereus* by 16S rRNA molecular analysis. This strain was used to study the ability of use the molasses as a cheap nutrient medium for alkaline protease production. Heat treated and H2SO4 treated molasses 10% (v/v) were tested as nutrient media. H2SO4 treated molasses increased the enzyme activity about 5.687 U/ml comparing to heat treated molasses. Addition of yeast extract as nitrogen source at concentration 3g/l gave better activities result but without significant improvement. The main problem was that the specific enzyme activity on molasses media was significantly lower than the specific activity on reference medium. This problem can be overcome by use cheap and efficient downstream processing method. The optimum pH value was 9 and optimum incubation temperature was 30^0C.

Keywords: Alkaline protease, *Bacillus cereus*, molasses, production.

ID 104: Challenges of Heamodialysis Water Treament System, a Major Setback to Renal Quality Care. (Nigeria Case Study)

Abolude Paul Oluwaseun

Dialysis Unit, Department of Biomedical Engineering, University College Hospital, Ibadan.
The Pioneer Teaching Hospital in Nigeria.
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Introduction: Water treatment system for haemodialysis encompasses the entire processes for the production of permeate water used in preparing concentrate and dialysate.The estimated water intake of a healthy individual is 2 litres per day or 14 litters per week. A haemodialysis patients may be exposed to between 350 and 500 litters of permeate water per week, depending upon their treatment time and dialysate flow rate. The production of quality Permeate water is therefore critical to the overall care and survival of all renal patients.

Aims and Objectives: The aim of this paper is to closely examine the challenges involved in the production of permeate water for haemodialysis in Nigeria and the attendant clinical consequences. As a way forward, attempt is made to re-awaken the consciousness of clinicians, nurses and technicians towards the production of quality permeate water to check complications and mortality rate among CKD patients.

Methodology: Our local and international field experiences were used.

Results: We discovered that the correct working of the preparatory process and the final phase of depuration of the system require proper selection, handling and maintenance by a competent and trained staff to guarantee safe and quality dialysate for better care.

Conclusion: All haemodialysis centres require the right water treatment system to provide the needed quality of care and check complications/death in CKD patients.

ID 105: Application of SRAP markers for Diversity analysis and Development of Variety Specific SCAR markers for Basmati rice DNA fingerprinting

Umer Maqsood[1,2] and Muhammad Arif[1,2]

[1] National Institute for Biotechnology and Genetic Engineering
[2] Pakistan Institute of Engineering and Applied Sciences
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Abstract

Basmati rice germplasm is very conserved and have limited diversity. Adulteration of inferior quality non-Basmati grains is another problem for Basmati trade. Currently different SSR and SNP markers are used for diversity analysis and varietal identification. There is need for new markers for precise DNA fingerprinting. In current study, sequence related amplified polymorphism (SRAP) markers were used for diversity analysis and identification of variety specific alleles. In this study 21 rice accessions (8 Basmati and 13 non-Basmati) were subjected to PCR amplification by different combinations of Em and Me primers. PowerMarker 2.5 V software, was used for diversity analysis. Each primer combination generated multiple alleles with average PIC value of 0.221 and major allele frequency ranging from 0.5238 to 1.000. Phylogenetic UPGMA tree was generated. Basmati accessions were differentiated from non-Basmati and placed in same clade. All non-Basmati varieties were further grouped into two clades differentiating *japonica* blood from *Indica* lines. Principle component analysis (PCA) also confirmed these results. Multiple variety specific, Basmati and non-Basmati differentiating alleles were identified and sequenced to develop new SCAR markers. These results proved the potential of SRAP markers for diversity analysis and DNA fingerprinting of Basmati rice.

Keywords: Diversity Analysis of Rice, DNA fingerprinting of Basmati Rice, Varietal Identification, SRAP Markers, *Oryza sativa*

ID 106: Purity and concentration of solubilized inclusion bodies in protein refolding

Few Ne Chew[a], Chun Yi Leong*, Gek Kee Chua, Abu Samah Rozaimi

[1]Faculty of Chemical Engineering & Natural Resources, Universiti Malaysia Pahang, 26300 Pahang

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Abstract

Solubilized inclusion bodies (IBs) refolding process under low protein purity and high protein concentration conditions always re-aggregate targeted functional protein which is not applicable in nano-biotechnology and molecular biology applications. Enhanced green fluorescent protein (EGFP) IBs was used as the model protein in this study for investigating the effects of protein purity and concentration on the protein refolding process. Three different conditions of solubilized EGFP-IBs were self-refolded at 4°C: solubilized EGFP-IBs with cell debris; solubilized EGFP-IBs after detergent washing; and the purified solubilized EGFP-IBs by using preparative native urea polyacrylamide gel electrophoresis (PAGE). High protein concentration and low protein purity in first and second refolding conditions have resulted re-aggregation of solubilized EGFP-IBs. Large molecular structure of self-refolded EGFP were formed and stuck at the top of stacking and resolving gels during the native PAGE protein analysis. The preparative native urea PAGE has successfully clarified and purified the solubilised EGFP-IBs. The result showed that high purity and low concentration of solubilized EGFP-IBs were able to self-refolded correctly. The structure and biological activity of self-refolded EGFP are preserved and has potential to be used in nano-biotechnologies.

Keywords: Protein refolding, inclusion bodies, green fluorescent protein, polyacrylamide gel electrophoresis, protein structure

ID 107: Isolation and Characterization of Bacteria from Seagrass

Ming Quan Lam [1] and Chun Shiong Chong [2,*]

[1] Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia, Johor, Malaysia; mqlam2@gmail.com
[2] Department of Biotechnology and Medical Engineering, Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia, Johor, Malaysia; cschong@utm.my
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Abstract

Three marine bacteria, designated as strain CH1, CH2 and CH4 were isolated from seagrass collected in Desaru, Johor. All strains (CH1, CH2 and CH4) were identified as *Shewanella* sp., *Cobetia* sp. and *Bacillus* sp. respectively based on 16S rRNA gene sequence analysis. All identified strains were catalase-positive, cannot utilized citrate, tryptophan and starch as carbon source. Optimal growth temperature for strain CH1, CH2 and CH4 were observed between 37°C to 40°C while optimal growth of pH for strain CH1, CH2 and CH4 were shown at 5 to 8, 5 to 7 and 5 respectively. Strain CH2 (*Cobetia* sp.) was found to be relatively new (around 100 articles reported in Scopus). Therefore, it can be further characterized. Strain CH1 and CH4 possessed protease(s) that can hydrolyse casein suggesting the strains have potential application value in industries.

Keywords: Marine bacteria; isolation; characterization; phylogeny; *Cobetia* sp., *Shewanella* sp., *Bacillus* sp.

ID 108: The Future Role of Biomedical Engineer in South East Asia

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Abstract: Biomedical Engineer is the youngest of the engineering professions. This engineering profession has emerged to fulfill the need of health care system due to advancement of technology. Although biomedical technologies have grown very fast in the last decade, the position of biomedical engineer in the j ob market remains unclear especially in developing countries. This causes the incorrect positioning of many biomedical engineering graduates and lack of health professional understanding on biomedical engineering function. This situation is also caused by mismatch of biomedical engineering education programs in the universities. In this paper, we will present the future demand for biomedical engineers and their roles in South East Asia. The study is based on 20 years demography, epidemiology and economy proj ection in South East Asia.

Keywords: biomedical engineer, South East Asia, health care system

ID 109: Characterization of a Marine Bacterium, *Roseivirga* sp. strain D-25 with Heavy Metal Tolerance

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Abstract

The issue of heavy metals pollution in marine environment is drawing attention of society and scientists on finding effective methods to prevent and overcome this concern. Bioremediation is one of the promising approaches to clean up the contaminated marine environment. In this study, a marine microorganism identified as *Roseivirga* sp. strain D-25 isolated from seawater of Desaru, Malaysia was characterized for heavy metals tolerance. Five various heavy metals (Zn2+, Al3+, Co2+, Mn2+, and Cu2+) with different concentrations were screened against strain D-25. The heavy metals tolerance analyses were performed at 37ºC by measuring the growth of the strain (OD600nm), and were compared with control with no added heavy metal. The growth profile of strain D-25 indicated that the strain has high tolerance to several metals and the order was Mn > Zn > Al > Co > Cu. The preliminary finding is interesting; since little study has shown that *Roseivirga* sp. strain D-25 to be a potential microorganism in bioremediation of heavy metals in polluted marine environment.

Keywords: *Roseivirga* sp. strain D-25; Heavy metal tolerance; Bioremediation

ID 110: Formation of Polyaniline emeraldine base (PANI-EB) nanoparticles using Langmuir- Blodgett technique

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Abstract

Nanostructures of conducting polymers are of great significant due to their unique properties and application in nano-scale electronic and molecular devices. Polyaniline (PANI) is a conducting polymer which is environmentally stable and easy to prepare. This study intends to study the formation of nanostructures especially nanoparticles of polyaniline (PANI) using Langmuir- Blodgett (LB) technique of molecular deposition. Polyaniline monolayer that forms on water interface prepared by LB technique was deposited on Indium Tin Oxide (ITO) glass and followed by slow drying at room temperature. Decrease in the pH of the sub- phase in LB trough increases level of doping (protonation) of polyaniline emeraldine base to emeraldine salt. Variable presssure scanning electron microscope (VPSEM) reveal that nanoparticles are formed when there is an increase in the subphase pH compare to neutral subphase because of the interaction between PANI and funtionalized acid. This result is supported by XRD which shows that pH 5 have strong diffraction peaks due to the existence of well- defined layer structure. AFM shows that nanoparticles are formed on ITO surface after the deposition of PANI-EB with no significant difference in surface roughness for different pH. As a conclusion, well defined and good molecular arrangement of polyaniline nanoparticles can be achieved by good control of major factor that can influence the deposition process.

Keywords: PANI-EB; nanoparticles; langmuir-blodgett

ID 111: Electromyography in the Analysis of Sitting and Standing Passive Exercise for Isometric Ankle Exercise Rehabilitation

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Abstract

Studies using surface electromyography (sEMG) as clinical and investigational instruments have expended the last few years and the presented results have contributed in different areas of knowledge. The area of rehabilitation may be benefit from this technology, both as an assessment and monitoring of therapeutic treatments. The information in the recorded signal provides significant information including an indication of muscle activity. Analysis of sitting and standing passive exercise needs the respondent to maintain ankle position-that is, to attempt to make all ankle movement using the Integrated Multiple Ankle Technology Device (IMATD), while the proper electrode placement on gastrocnemius and soleus muscles. Therefore the goal of this research is to identify a best position in passive exercise for ankle sprain rehabilitation. This analysis of sitting and standing was using Math lab software to get graph plots of the muscle activation. The result shows that passive exercise in standing ankle rehabilitation more effective in muscle activation for further application especially in ankle exercise rehabilitation.

Keywords: Passive exercise; Surface Electromyography; Integrated Multiple Ankle Technology Device

ID 112: Alteration of *Saccharomyces cerevisiae* genes expression involved in G1 to S transisition progression of the cell cycle mechanism as antiproliferative effect of Noni fruit extract ((*Morindra citrifolia Linn*)

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Abstract

Elucidation of genes involved in G1 to S phase inhibition progression of *S.cerevisiae* cell cycle as effect of noni fruit methanol extract (*Morindra citrifolia Linn*) was carried out. This research aims to elucidate the mechanism of antiproliferative effect caused noni fruit extract which could inhibits growth of *S.cerevisiae*. Transcriptional analysis of genes of cells grown on YPDA containing 1% (w/v) noni fruit extract were performed using quantitaive real time PCR. Ekspression of *CLN3, WHI4, CLN2, CDC7, CDC20, CLN1, YOX1, YHP1, SIC1, MBP1*, and *FAR1* increased while *SWI6, CDC28, CDC6, TAH11, MCM1, FUS3,* and *DBF1* decreased. These results indicated that alteration of these genes expression cause growth inhibition of *S.cerevisiae* cells occurred at G1 phase and this data supports FACS analysis which reveals growth inhibition occurred at G1 phase of cell cycle.

Keywords: *Morindra citrifolia Linn, Saccharomyces cerevisiae,* G1 phase

ID 113: Electromyography Analysis Comparison Of Muscle Activation Between Wobble Board Manual Device And Integrated Multiple Ankle Technology Device

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Abstract

The electromyography is the summation of the motor unit action potentials occurring during contraction measured at a given electrode location. The voltage potential of the surface electromyography signal detected by electrodes strongly depends on several factors, varying between individuals. Changes in the myoelectric characteristics of the muscle during activity can be identified using surface electromyography (EMG), which provides important information on the behaviour of muscles submitted to different types of exercise and device. Therefore in this study Math lab software used for filtered and standardized sEMG data recorded. 20 volunteer choose to do the single leg stance activity on wobble board manual device and Integrated Multiple Ankle Technology Device (IMATD) activity for 20 sec. The results show IMATD single leg stance activity more muscle activation during ankle exercise rehabilitation rather than wobble board manual activity. As a conclusion, it show that, type of an assistive exercise the movement by human and rehabilitation device was also an important part of exercise rehabilitation in term of muscle activation.

Keywords: muscle activation; assistive exercise; Integrated Multiple Ankle Technology Device.

ID 114: Use of Multiplex-PCR Method in Identification of Candida species Isolated from Patients with Clinical Vaginal Candidiasis

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Abstract

Candidiasis vaginalis is fungi inspection caused by various species of the genus Candida yeast which the result may probably be different virulence, thus leading to varied drug of choice for therapy. The objective of this study was to determine the sensitivity and specificity of Multiplex-PCR test in detecting Candida species in women with clinical diagnosis of candidiasis vaginalis who attended Graha Sriwijaya clinic Palembang. There were 79 vaginal swab ssamples which fulfill inclusion criteria. Samples were cultured at ASD medium to detect whether samples infected by Candida. Primers pairs used in Multiplex – PCR was universal primer ITS1 and ITS2, and specific primer CA3 and A4. The diagnostic test of Multiplex-PCR in this study resulted in C. crusei: sensitivity: 100%, spesificity:61,1%, NPP:63,2%, NPN:100%; *C. glabrata*: sensitivity:0%, spesificity:100%, NPP:0%, NPN:76,7%; *C. tropicalis*: sensitivity:100%, spesificity:100%, NPP:100%, NPN:100%; *C. albicans*: sensitivity::33,3%, spesificity:100%, NPP:100%, NPN:93,1% and *C. stellatoideae*: sensitivity:: 100%, spesificity:92,6%, NPP:60%, NPN:100%. Based upon these experiments suggested that Multiplex-PCR test becomes a rapid alternative in identifying species of Candida due to its high sensitivity and high specificity more rapidly.

Keywords: Multiplex-PCR, Fermentation, Sabouraud Agar, Candida sp.

ID 115: The Homology Based Structural Model of L-2-haloacid dehalogenase (DehL) from *Rhizobium* sp. RC1

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Abstract

DehL is an L-2-haloacid dehalogenase (EC 3.8.1.2) that catalyses the conversion of L-2-halocarboxylic acid to D-2-hydroxycarboxylic acid. Although DehL utilises the same substrates as the other L-2-haloacid dehalogenases, its deduced amino acid sequence is substantially different (< 25 %) from those of the rest L-2-haloacid dehalogenases. To date, the 3D structure of DehL is not available. This is severely hindering our understanding of the enzyme's functional mechanisms at molecular level. In this study, we report a homology-based model of DehL from *Rhizobium* sp. RC1, predicted by threading method; and its defined active site. DehL structure constitutes α/β structure that is organised into two distinct structural domains: main and sub-domains. Despite the sequence disparity between the DehL and other L-2-haloacid dehalogenases, its structural model share similar fold as the experimentally solved L-DEX and DehlB structures. The structure of DehL predicted here will play a vital role in deciphering the reaction mechanism and in rational design of the enzyme with enhanced activity and substrate specificity.

Keywords: *Rhizobium* sp RC1, DehL, Dehalogenase, Homology modeling

ID 116: The Irrigation Water Quality Improvement in Phytoremediation Pond Using Some Species of Local Hydromacrophytes

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Abstract

The aims of this research were to determine the effectiveness of phytoremediation models conducted by planting some of local hydromacrophytes in three phytoremediation ponds through a continuous culture system with discharge of 0.3 L / second for improving the irrigation water quality. The quasi-experimental research was conducted in situ in a phytoremediation pond located in Kepanjen District of Malang East Java Indonesia. Pond of phytoremediation is divided into three interconnected sections. Pond 1 and 2 were planted with some of floating leaf and emergent hydromacrophyte, while pond 3 was planted by the combinations of same plants which added with submerged hydromacrophytes. The success of the phytoremediation process is known from some physico chemical parameters of water and trophic diatom index (TDI) that found from artificial substrate been installed in the early treatment. The water quality monitoring performed in each part of the ponds two times after the plants grew steadily. The results showed that planting of hydromacrophytes can significantly increase the physico-chemical quality of water was reflected in the decline in the value of conductivity, turbidity, TSS, nitrates and dissolved phosphates and improve of dissolved oxygen levels in the waters. Based on TDI, there were improvement of the water quality from moderate status (eutrophic) to good status (meso-eutrophic), and from the heavily contaminated with organic pollution to be some evidence of organic pollution. Improvement of water quality is effectively occur after passing through the third pond.

Keywords: Diatom, local hydromacrophytes, phytoremediation pond, water quality

ID 117: Total Phenolics, Antioxidant Activity and Biological Screening of an Epilithic Microalgae *Desmodesmus* sp. (U-AU2) from Los Baños, Laguna (Philippines)

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Abstract

Methanolic extract of the algal strain *Desmodesmus* sp. (U-AU2) were subjected to microtiter plate dilution assay against a wide spectrum of bacteria. U-AU2 exhibited pronounced activity against Gram-positive bacteria, *Staphylococcus aureus* with MIC and MBC of 31.25 and 125.00 μg/ml, respectively. It was moderately active against *Listeria monocytogenes,* Methicillin-Resistant *S. aureus* and *Pseudomonas aeruginosa* (both MIC = 250 μg/ml) as well as *Aeromonas hydrophila* (both MIC = 1000 μg/ml). Minimum bactericidal concentration (MBC) of 1000 μg/ml was observed against *L. monocytogenes,* Methicillin-Resistant *S. aureus, A. hydrophila* and *P. aeruginosa.* Phenolic content of the methanolic extarct was determined using Folin-Ciocalteu reagent and found to have total phenolic content of 652.66 μg GAE/ml. Antioxidant activity was evaluated using DPPH free radical scavenging activity assay and CUPRAC assay. Relative antioxidant efficiency showed that *Desmodesmus sp.* (U-AU2) exerted high potent radical scavenging activity and high ability of reducing copper ions from Cu (II) to Cu (I) in a concentration dependent manner. The results further revealed that the copper ion chelating ability as well as the radical scavenging activity of the extracts were dose-dependent and positively correlated to their phenolic content. The results of this study showed that *Desmodesmus* sp. (U-AU2) could be use as alternative source of bioactive compounds for pharmaceutical industry.

Keywords: Microalgae; methanolic extract; phenolic content; antioxidant activity; microtiter plate dilution assay

ID 118: Comprehensive Characterization of Malaysia Oil Palm Frond Leaves and their Ash

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Abstract

This research work was carried out to unravel the potentials embedded in Malaysian oil palm frond leaves (OPFL). Freshly harvested Malaysian OPFL were subjected to comprehensive physicochemical analyses and the data revealed that the chemical compositions of the untreated OPFL showed presence of carbon, hydrogen, nitrogen and sulfur at mass percentages of 46.98 %, 6.500 %, 1.810 % and 0.150 % respectively. Thermal gravimetric analysis (TGA) and derivative thermal gravimetric (DTG) indicated the existence of moisture, volatile matter and ash with mass percentages as 4.312 %, 67.04 % and 3.239 % respectively. X-ray Florescence (XRF) showed that CaO (39.20%), K_2O (22.10%) and SiO_2 (19.20%) formed the three major inorganic compounds in the untreated sample while the treated sample was majorly made up of SiO_2 (95.20%). X-ray diffraction (XRD) of the ash from the treated sample revealed that the SiO_2 was amorphous and mainly consisted of siloxane and silanol functional as analyzed by Fourier Transform Infrared (FTIR). It can be construed that the OPFL may prove to be an alternative silica source with the potential for applications as adsorbents, support matrix for catalysis, fillers in cement, concrete and plastic industries as well as anti-caking agent in food industries.

Keywords: Characterization; Oil palm frond leaves; silica; treated; untreated; ash

ID 119: Antihyperlipidemic Activity of Flavonoid Rich Extract of *Vitus vinifera* on High-fat Induced Hyperlipidemic Rabbits

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Abstract

Management of hyperlipidemia is considered an important part of medical therapy to prevent cardiovascular disease (CVD). There has been an increase in prevalence of hyperlipidemia due to urbanization which includes sedentary lifestyle and high fat diet. Phytochemicals present in many foods; are found to have antihyperlipidemic activity. In this study, flavonoid rich extract (FREt) of *Vitus vinifera* (raisins) was studied for its antihyperlipidemic effect. The experimental rabbits were divided into three control groups including normal control (distilled water; 1 ml), high-fat induced (HFI) hyperlipidemic control (distilled water; 1 ml) & HFI positive control (simvastatin 20 mg/kg) and a test group (FREt 200 mg/kg) consecutively for 14 days. Lipid profile was determined from the serum (Randox) whereas, LDL-c and antiatherogenic index (AAI) were calculated by formulae. The extract showed significant decrease (p<0.05) in TC, TG and LDL-c levels whereas increase in HDL-c when compared with HFI control group. In addition AAI was improved. Thus concluded that FREt of Raisins possess strong antihyperlipidemic activity in HFI induced hyperlipidemic rabbits.

Keywords: Cardiovascular disease, Flavonoid, Hyperlipidemia, *Vitus vinifera*, Antiatherogenic index.

ID 120: Antiseptic Alcohol Alters the Antibiotic Susceptibility of *Proteus mirabilis*

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Abstract

Proteus infections are becoming a public health problem as their resistance to antiseptics and antibiotics has increased. To investigate the change in the susceptibility of *Proteus mirabilis* to antibiotics after alcohol treatment, the bacterium was isolated from uncooked street-vended chicken intestine ("*isaw*") sold at UST, Manila. The isolate was subjected initially for susceptibility to the antiseptics 70% Isopropyl alcohol and 70% Ethyl alcohol as well as to the antibiotics ciprofloxacin, amoxicillin, and cefalexin. It was then tested likewise for susceptibility to lower concentrations of antiseptics (17.5%, 8.75%, and 4.375%) and again with the different antibiotics. It was observed that *P. mirabilis* is more susceptible to 70% Isopropyl alcohol than to 70% ethyl alcohol and also more susceptible to ciprofloxacin than to the other two antibiotics. Treatments with lower concentrations of the alcohols caused an increase in susceptibility to ciprofloxacin and cefalexin and a decrease in susceptibility to amoxicillin. Statistical analysis showed a significant difference ($p<0.05$) in the susceptibility of the bacterium to antibiotics and alcohols and in the change of its susceptibility after treatments with lower concentrations of alcohols. The results obtained merit investigations to establish the genetic mechanism(s) that caused the change in susceptibility to antibiotics after alcohol treatment.

Keywords: Alcohols, Antibiotics, Isaw, *Proteus mirabilis*

ID 121: Perceptive Assessment of the Effects of Biogas Emission from Waste Dumpsites on Proximate Residential Properties in Nigeria.

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Abstract

Paradoxically, as urbanisation strives to 'kick-start' and advance the cause of the inhabitants of settlements, it inadvertently manifests its ugliest shades, the chief of which is the unprecedented generation of waste of different magnitudes, in terms of size, density, colouration, bio-chemical contents, acidic/basic strengths, contamination impacts, to mention but a few. Sadly enough, insufficient governmental efforts for properly-wholistic management of these wastes, in terms of timely and hygienic collection, transportation, storage, disposal, treatment and recycling for possible re-use, has rendered these once-upon-a-time clean, habitable, healthy and stable settlements to become almost totally unsafe for human and non-human habitation. However, robust measures laced with socio-economic, political, physico-environmental, bio-chemical and even geologic dimensions, are urgently needed to totally exterminate these challenges that urbanisation's phenomenal reckless generation of waste and unwholesome emergence of waste dumpsites have brought upon proximate residential properties and their surroundings' physiognomy, or else, this ugly trend certainly shall continue to deal great blow on the profitability, habitability, longevity, etc. of real estate assets for an unforeseen period of time.

Therefore, in an attempt to validate the occurrence of biogas emission that regularly oozes out from these waste dumpsites and gauge the effects that they have on proximate residential properties, that this study was conducted against the following scorecards: value, health, maintenance, safety/security, economy, psychological trauma, void/emptiness, obsolescence/degradation, land use change, socio-economic denial. A total of 185 structured questionnaires were distributed among proximate residential property owners and tenants, waste collection contractors and consultants, estate surveyors and valuers, town planners, environmental safety officials, as well as local government officials and hospitals. Out of which 173 were retrieved and the integrity checks showed that 9 of these questionnaires were not properly filled, hence 164 were considered for the analyses, using 5 point Likert scale measurement, via both inferential and descriptive statistical tools. The results showed amongst other things, that lack of proper planning and sordid city expansion as well as non-compliance with development-directed instruments were mostly responsible for such unwholesome emergence of these waste dumpsites within residential neighbourhoods. Among the recommendations are purposeful governance to address the sad experiences of property owners' loss of income attendant to void/emptiness in these proximate residential properties as well pragmatic efforts of all stakeholders to properly plan and increase the number of waste collection and treatment plants that commensurate with demographic increase in Nigeria.

Keywords: Perceptive Assessment, Effects, Biogas Emission, Waste Dumpsites, Proximate Residential Properties, Nigeria's Urbanisation.

ID 122: *Actinomycetes* as A Producer of Antibacterial Pathogens Isolated *Eucalyptus deglupta* Blume. Rhizosphere from Lore Lindu National Park, Central Sulawesi, Indonesia

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Abstract

Actinomycete is a cluster of bacteria that have fungus-like morphology due to the soft filament structure called hyphae or mycelia. This research aimed to identify the *Actinomycetes* that associated to the rhizosphere of *Eucalyptus deglupta* Blume. as well as the potential in producing antibacterial pathogens compound toward human. The stages of this research consisted of isolation process and screening by using selective SNA media combined with the method of heating, then continued to the potential test through the antagonist method by using 4 kinds of pathogenic bacteria, such as *Staphylococcus aureus*, *MRSA, EPEC*, and *Vibrio cholerae* strain Inaba. The best isolate for bacterial inhibition was molecularly identified based on 16S rDNA fragment, completed by the observation through scanning electron microscope (SEM). The result of screening process by using 4 bacteria revealed that 15 isolates were potential to produce the antibacterial pathogens compound by the average score of inhibitory 7.48 ± 0.9 mm. From those entire 15 potential isolates of *Actinomycetes*, there was one best isolate by the code L213 which has been proved that it was able to inhibit the 4 kinds of bacteria with the inhibitory score of 7,62 ± 0,4 mm (*V. cholerae* strain Inaba), 15,03 ± 0,7 mm (*S. aureus*), 18,48 ± 0,4 mm (*MRSA*), and 18,52 ± 0,5 mm (*EPEC*). Inhibitory power is classified as a strong inhibitory toward bacteria. Based on the molecular identification, the isolate L213 was allegedly *Streptomyces aurantiacus* strain LMG 19358 by the similarity level of 99%.

Keywords: Potential, *Actinomycetes,* antibacterial pathogens, and rhizosphere *Eucalyptus deglupta.*

ID 123: Aorta blood flow using fixed grid method.

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Abstract

Non-invasive numerical simulation in the biomedical field can provide additional information on the human diseases, for example, in cardiovascular flow. This technique is always hindered by the difficulties in mesh generation due to complex geometry of the patient specific of the aorta. In this paper, numerical simulation using fixed Cartesian grid is presented. The complex geometry of the aorta is derived from CT scan of patient-specific data is used directly without need of conventional grid generation conform to the surface vessel. The simulation is done using opensource computational fluid dynamics (CFD) software Openfoam. It is found that the method could provide a detail flow features downstream of the aorta. This feature is useful, for example, the evaluation of the fluid stress that responsible for hemolysis and thrombosis.
Keywords: computational fluid dynamics (CFD); cardiovascular flow; immersed boundary method, OpenFOAM.

ID 124: Effect of Temperature Condition on Xylanase Production by Immobilizing *E. coli* onto Graphene Oxide

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Abstract

The targeting of recombinant proteins for excretion into culture medium presents significant advantages over cytoplasmic expression. However, during the excretion of recombinant protein, caution must be taken in order to avoid cell lysis due to pressure build-up through overproduction of the expressed recombinant protein in the periplasmic space. The recombinant *E. coli* cells were immobilized on the graphene oxide with temperature 30 degree celcius. The results presented showed that the immobilized cell is a promising method for high enzyme excretion and plasmid stability with less occurrences of cell lysis. The immobilized cell exhibited a 7% increase in the xylanase excretion 39% reduction of cell lysis compared with free cells.

Keywords: Immobilization; Graphene Oxide; xylanase

ID 125: DR4 626 Single Nucleotide Polymorphism; Its Risk Assessment in Breast Cancer Patients from Pakistan

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Purpose: Defective apoptosis is a hallmark for cancer development and progression. The detection of SNPs in the candidate genes is a crucial step before disease association studies. Death receptors DR4 and their ligands are important mediators in extrinsic pathway of apoptosis in cells. In the present study we aimed to determine the association Death receptor 4 (DR4) with breast cancer in Pakistani Population.

Materials: The case control study included 71 breast cancer patients and 51 healthy controls. Using Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) genotypes were analyzed.

Results: The homozygous CC genotype was present in 14.5% patients, 2% in controls. The heterozygous GC genotype in patients was 63.8% and in controls 39.2%.The reduced frequency of GG genotype in patient's accounts for 21.7% as compared to controls 58.8% (p value <0.05) suggests that it may serve as a protection against development and or progression of breast cancer. While the CC genotype being higher in breast cancer patients, p value <0.05 suggests its possible role in regulation of DR4 gene in such way to disturb the normal apoptosis which leads to breast cancer.

Conclusion: Our findings show that DR4 gene polymorphism at 626 G/C may be associated with progression of breast cancer.

ID 126: DNA methylation and expression of DNA cytosine-5-methyl transferase 1 (DNMT1) and methyl CpG-binding domain 2 (MBD2) in patients with Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis (RA) is an autoimmune disease with a characteristic of relentless synovitis, systemic inflammation and auto-antibodies. This patho-physiological condition is developed by genetic, epigenetic and environmental factors. Epigenetic is inherited change in regulation of gene expression without changing in sequence of DNA which includes DNA methylation and histone modification. DNA methylation has role in changing the expression level of gene by blocking the binding of transcription factor with promoter region. The present study aimed to check any expressional changes in DNMT1 and MBD2 gene expression in RA patients in comparison with healthy controls and to determine the role of DNA methylation in these samples. Our data comprise of 45 RA patients and 30 controls in which majority were females. Quantitative analysis of DMNT1 and MBD2 mRNA expression was performed and compared with mRNA levels in control samples. To check DNA methylation status of DNMT1 and MBD2 promoter region, methylation specific PCR was carried out.

The promoter region of DNMT1 was found methylated in RA patients and unmethylated in controls. Moreover, the expression level of mRNA of DNMT1 gene was found less in patient as compared to control. Less than one fold change decrease was found in expression of mRNA in patient with respect to control. Therefore, it is concluded that the methylated promoter may contribute in the low expression of DNMT1 gene in patient and may be involved in the progression of rheumatoid arthritis.

Key words: Rheumatoid arthritis, DNA Methylation, DNMT1, MBD2, Expressing Profiling.

ID 127: Isolation and screening of antimicrobial activities of fungal endophytes from Philippine endemic plant *Dillenia philippinensis* (Rolfe)

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Abstract

Fungal endophytes play an important role as plant symbionts primarily because they synthesize biologically active substances that can be possible sources to modern day drugs. In this study, 33 fungal endophytes were isolated from leaves of the Philippine endemic tree *Dillenia philippinensis.* Morphological identification classified the fungal isolates into eight different genera: *Alternaria* sp., *Aspergillus* sp., *Geotrichum* sp., *Guignardia* sp., *Nigrospora* sp., *Paecilomyces* sp., *Pestalotiopsis* sp., and *Phialophora* sp. Twenty-two representative fungal isolates were mass-produced and subjected to extraction of secondary metabolites using the liquid submerged fermentation set-up. The obtained crude extracts were tested for antimicrobial activities using the disk diffusion assay against Gram-positive bacteria: *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram-negative bacteria: *Escherichia coli* and Multi-drug resistant *Pseudomonas aeruginosa* (MRPA). Results showed that crude extracts associated with *D. philippinensis* are good sources of secondary metabolites of which 73%, 68%, and 55% are respectively effective against *S. aureus*, *E. coli* and MRSA at a concentration of 10 mg/ml.

ID 128: The Potency of Consortium Bacteria Isolated from Apple Tree-Rhizosphere from Batu as Biofertilizer

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Abstract

The usage of chemical fertilizer has effect on decreasing of the soil quality. Biofertilizer contains various microbes involve in improvement of soil nutrition. For example phosphate- solubilizing and nitrogen-fixing bacteria, also Indole Acetic Acid (IAA)-producing bacteria. Recently, plant productivity has been increased by addition of biofertilizer. The objectives of this research were to determine the interaction types among isolates, to observe the effects of consortium on soil organic matter, phosphate, nitrogen content and the abundance of phosphate-solubilizing and nitrogen-fixing bacteria. Interaction among isolates was assayed, isolate with synergistic activity was choosen as consortium. Each isolate was cultivated on Luria Bertani (LB) medium to reached 10^8 cfu/mL. The consortium with dosage 20 mL/5 kg (P1), 60 mL/5 kg (P3) and 100 mL/5 kg (P5) used to watering soil as growth medium of apple tree. Effect of consortium on soil organic matter, phosphate, nitrogen content and total number of bacteria. were detected. All isolates were synergist. Addition of consortium as biofertilizer after 15 d increased soil organic matter to 37.44%. Phosphate content (P5) at 15 d increased in accordance with the abundance of phosphate-solubilizing bacteria. However, nitrogen content (P5) and total number of nitrogen-fixing bacteria reached the highest at 5 d. The optimum dosage of addition consortium as biofertilizer was 100 mL/5kg medium and the highest number of phosphate-solubilizing and nitrogen-fixing bacteria was at 5 d.

Keywords: biofertilizer, consortium, nitrogen fixation, phosphate solubilization

ID 129: A Study on the Bacterial Degradation of Polymer Composite Films

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Abstract

Synthetic plastics like polyolefins, acrylics which are used as consumer products are generally not degraded in the environment and their accumulation results in long term environmental hazards. Therefore, their degradation is an important requirement in today's world. In this present work, polymers (Poly Methyl Methacrylate, PMMA) and its composite (PMMA/Cellulose) were subjected to bacterial degradation by a bacterial strain isolated from a pond water which is regularly subjected to polymer waste disposal. The bacterial strain in pure culture was found to degrade the polymer and its composites as validated by the weight loss studies. The bacteria was found to result in a weight loss of upto 14 % in a time frame of 20 days in the composites. The bacterial growth study in presence of the polymers as the sole source of carbon was also evaluated where in presence of the composite better bacterial growth was reported. Scanning electron microscopy (SEM), field emission scanning electron microscopy (FESEM) and fourier infrared spectroscopy (FTIR) studies of the degraded composites also corroborated the results. Glutaraldehyde fixed SEM images also showed presence of bacterial biomass on the films. Thus, this novel strain of bacterium was capable of polymer composite biodegradation and could be further used for bioaugmentation processes for degrading polymers and their composites.

Keywords: Polymer, Bacteria, Biodegradation, SEM.

ID 130: Evaluation Of Plant Host Influence On The Secondary Metabolite Production Of Endophytic Fungi

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Abstract

Majority of higher plants are colonized by fungi which can live in harmony with their plant host. Endophytic fungi (EF) are asymptomatically and mutually associated fungi with plants in constant balanced antagonisms based on the secondary metabolites the partners produce. Endophytic fungi are known to synthesize metabolites which have biological activities. This study focused on the investigation of the influence of *Cyclorosus interruptus* to metabolite production of its associated Endophytic fungi. EF in *C. interruptus* leaves were isolated, pure cultured, and identified. Selected fungal endophytes were grown and mass produced in Potato Dextrose Broth (PDB) and Potato Dextrose Broth with host plant decoction (PDBD). Secondary metabolites produced by the EF were extracted and identified through Thin Layer Chromatography.

Isolated endophytic fungi belong to different genera. Profiling and identification of metabolites of *Monilia* sp., *Nigrospora* sp., and *Humicola* sp. revealed that these EF synthesized more varied and higher number of secondary metabolites when grown in medium with the host plant phytochemicals. Several metabolites are common to all of the EF. Metabolites produced only in the presence of the host plant phytochemicals belong to the same classification as with the host's metabolites.

Secondary metabolites production of the EF was affected by the presence of its host secondary metabolites. This finding would contribute to the unveiling of host-EF interaction especially in metabolite production which can serve as promising sources of therapeutic products.

ID 131: Physio-agronomic Response of Spring Maize (*Zea mays* L.) to Foliar Application of Plant Growth Promoting Substances

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Abstract

Plant growth promoting substances are being widely used in crop production to increase productivity and to overcome harmful effects caused by environmental stresses. The experiment was planned to evaluate the influence of PGPS on the growth, phenology and yield of spring maize. The growth promoting substances like kinetin (30 ppm), humic acid (2%), *Moringa* leaves extract (2%), salicylic acid (2%), ascorbic acid (2%), and water spray were applied twice as foliar sprays at 6 leave and 8 leave stage. Results showed that foliar application of kinetin, humic acid, MLE, ascorbic acid and salicylic acid significantly improved the growth, phenology and yield attributes of maize as compared to control. Results showed that maximum improvement in days to tasseling, leaf area index, crop growth rate and number of leaves per plant, grains per cob, 1000-grain weight, biological yield, and grain yield were recorded with the application of 2% ascorbic acid, however, the use of PGPS had no significant effect on the days to silking. Further, a maximum plant height was recorded with the foliar feeding of 2% humic acid. Moreover the highest value of chlorophyll (a, b) and total phenolic contents were recorded with kinetin (30 ppm). So, all tested PGPS were variable in their effect, however, they improved the plant performance and may be applied to improve growth and yield of maize.

Keywords: maize; growth promoting substances; growth, yield; phenolic content

ID 132: The Detection of 8-Hydroxy-2′-Deoxyguanosine and 1-Hydroxypyrene as Biomarker of CancerRisk in Population Exposed by Benzo[a] Pyrene

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Abstract

This study was conducted to analyze the profile of DNA Adduct 8-Hydroxy-2′-Deoxyguanosine formation as DNA damage indicator, by measuring 8-Hydroxy-2′-Deoxyguanosine level in serum and 1-Hydroxy pyrene as benzo[a]pyrene metabolites from the population in Palangkaraya during the smoke haze period in 2015. In vitro study using 2′-deoxyguanosine-5'-monophosphate and benzo[a]pyrene also conducted. The object of study included 29 respondents from Palangka Raya as the exposed group and 23 respondents from Kota Batu as a control. The questionnaires were used to collect data related to medical history, smoking habit, occupation, life style and activity during the smoke haze period. The results obtained showed significant differences between 8-OHdG level in exposed group (P-value = 0.005) compared to the control, with the average value of the exposed group are 1.4 times higher than the control. The average value of 1-OHP in exposed group (P-value = 0.002) are 3.8 times higher than the control. The increased level of 8-OHdG and 1-OHP in the exposed group has significant correlation with long exposure. While the in vitro study showed that benzo[a]pyrene can cause increasing 8-OHdG formation. This study provides evidence that there is a correlation between forest and peatland fires with oxidative DNA damage related to risk cancer.

Keywords: 8-OHdG; DNA Adduct; Forest and Peatland Fires; Palangka Raya

ID 133: Molecular characterization of *Penicillium ulaiense* causing whisker mold a new postharvest threat to citrus of Pakistan

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Abstract

Whisker mould caused by *Penicillium ulaiense* has been reported to be a new postharvest disease in many citrus growing regions of the world. Genetic diversity of *P. ulaiense* was analyzed by studying ITS regions of rRNA gene complex. The DNA was isolated by using CTAB technique. PCR amplification with ITS1 and ITS4 was done by using specific primers. ITS-5.8S sequence obtained was compared with those deposited in the GenBank Database. The rDNA genes, generally used in the taxonomic and identification studies, were confirmed in the present study. ITS primers 1 and 4 were used in this study to amplify the entire 5.8S rDNA gene, both ITS1 and ITS4. The most similar ITS sequences up to 99% similarity values were determined by BLAST N comparison of other ITS sequences from penicillia in the GenBank database. The isolate *P. ulaiense* (LN871568) was clustered in a group with *P. expansum* JX869559, *P. chyroysogenum* JX270417 and P. sp. JQ775565, which was supported by a bootstrap value of 63%. The sequence from the isolate and reference strains of *Penicillium* were identical. The sequence of the isolate was deposited in the Genebank. In the maximum parsimony analysis, the isolate was identified as *P. ulaiense.* The isolate and reference strains of *Penicillium* were clustered together in a group, which was supported by a bootstrap value of 63%. The sequence of the isolate was 99% identical to the sequence of *P. expansum* and *P. chyroysogenum.* Phylogenetic tree analyses showed that the studied were prominently divided to three clades. One of them was included *P. chrysogenum*, *Penicillium* species and *P. ulaiense.* Furthermore, *P. ulaiense* was distinctly differentiated from other clade members in the phylogenetic tree. The studied isolate of *Penicillium* had shown 99% genetic similarity with *P. expansum* and *P. chyroysogenum* and it was distinguished on the basis of its morphological characteristics from *P. digitatum* as it produced blue-grey spore mass and from *P. italicum* as it was able to form 1 to 7 mm tall coremia with white stalks.

ID 134: Morphological characterization of *Penicillium ulaiense* causing whisker mold a new postharvest threat to citrus of Pakistan

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Abstract

Whisker mould a new postharvest threat to citrus caused by *Penicillium ulaiense* has been recently reported from many citrus growing areas of the world. During April 2015, orange fruits (*Citrus sinensis*) showing the symptoms of rotting (whisker mould) were collected from commercial markets and packinghouses from Lahore (31.5497° N, 74.3436° E) Punjab province, Pakistan. Rotted part of the fruit was covered with white, compact growth of fungus around olive green spores, fruit shrinked and mummified. Fruit portion were cut into small pieces and surface sterilized and inoculated on PDA media. Petri plates were incubated at 22 ˚C for 7 days in the dark. Obtained colony was white to green having 18-20mm diameter. Colony was round in shape with a cottony texture. Colony reverse was pale yellow to camel brown. This fungus was identified as *Penicillium ulaiense* on the basis of its morphological characteristics and was distinguished from *P. italicum* due to its ability to produce 1 to 7 mm tall coremia having white stalks and from *P. digitatum* due its spore mass of blue-grey colour. *P. ulaiense* produced 2 to 10mm long coremia on white cottony mycelial matt having white colored long stalk which later on turned to greenish conidial cluster. Conidia were cylindroidal or obpyriform 4.8 to 9.6 µm, smooth, thin walled, borne irregularly on compact columns. Conidiophore started from the hyphae and remained along the length of coremium stalk. Conidiophore were 2.0-4.0µm wide, smooth walled. Its penicilli were branched in random pattern monoverticillate, divergent and terverticillate. Matulae were present in 3-4 measuring 15-23×2.2-4.0µm and had smooth walls and long cylindrical. Phialids were present 3-4 per matulae 8-15×2.2-4.0 µm. Conidia are rounded to oval in shape 2-5.0µm and found 2 to 3 per phialid.

ID 135: The Role of Nitric Oxide in Musa Sp. During Basal Resistance against Fusarium Oxysporum F. Sp. Cubense

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Abstract

Nitric oxide (NO) is one of the important signalling molecules that play a key role in various physiological processes in plants. S-nitrosylation, a post-translational modification which involves a covalent attachment of NO moiety to the sulfhydryl group of cysteine residue to form S-nitrosothiol (SNO) is one of the major mechanism by which NO regulates plants development and defence against pathogen attack. Previous study has shown that SNO formation and turnover affects multiple modes of plant disease resistance. In this study, we focus on NO regulation in Musa sp. (banana) in response to Fusarium oxysporum f. sp. Cubense (FOC) infection. In-vitro treatment with NO donor Snitrosoglutathione (GSNO) showed that the proteome of healthy banana was S-nitrosylated and treatment with reductant dithiothreitol proves the reversibility of the cysteine-redox modification. In the following in-vivo study, the roots of banana plants were treated with GSNO and NO scavenger, 2(4-carboxyphenyl)-4, 4, 5, 5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) prior to infection with FOC and disease progression was monitored for four weeks. Protein from roots was then extracted using an optimized method and subjected to Saville assay for SNO quantification. The infected seedlings which were treated with GSNO showed a delay in disease progression as compared to non-treated seedlings whereas the disease progression was accelerated in the infected seedlings that were treated with cPTIO. SNO content was found to be higher in infected sample compared to noninfected sample and sample collected at early stage of infection showed a higher level of SNO content. Our data might provide an insight on regulatory function of NO in Musa spp. during pathogen attack specifically by the host pathogen, FOC.

ID 136: Efficacy of Bacillus thuringiensis D142 (MOSNONTM) against Aedes aegypti larvae in Ngebrak Village, East Java

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Abstract

Mosnon™ is a mosquito bio larvicide that contains of Bacillus thuringiensis D142. There is able to kill of Aedes aegypti larvae. The aim of this study was to analyse the efficacy of Bacillus thuringiensis D142 against Aedes aegypti and to identify community perceptions of Mosnon™ on the field (Ngebrak Village). Preliminary study for determining value of C50 of Mosnon™ already carried out toward third instar of Aedes aegypti, then there were used it in the field scale. Study area is divided into three plots, the first plot treated by 0.02 ppm doses; the second plot treated with 2 ppm doses; and and the thir plot as a control. The result showed that 2 ppm of Mosnon™ dosage was more effective than the other dosages for killing Aedes aegypti larvae within 24 hours of Mosnon™ exposure. Mosnon™ dosage of 2 ppm have residual effects during three weeks of treatment, whereas a dosage of 0.02 ppm has a residual effect for two weeks of treatment with no depletion. The questionnaire result showed that 100 % of respondents said Mosnon™ could reduce dengue vector population. Based on these results it can be concludedthat 2 ppm Mosnon™ dose was more effectively for controlling of third stageAedes agypti larvae.

Keywords: Aedes aegypti, Bacillus thuringiensis, Efficacy, Mosnon™

ID 137: The Mechanistic Role of Catalytic Residues in Non-Stereo Dehalogenase (DehE)

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Abstract

Non-stereospecific α-haloacid dehalogenase E (DehE) from *Rhizobium* sp. RC1 catalyze the hydrolytic dehalogenation of D- and L-2-haloacid to produce L- and D-2-hydroxyl acid respectively. This enzyme catalyzed the removal of halides organic haloacid and can be used as bioremediating agent. In this study, the affinity of DehE binding residues towards ligand was determined by using molecular modeling approach. The interactions of Trp34, Phe37 and Ser188 with both D- and L-2 chloropropionic acids (2CP) were analyzed by using molecular docking simulation and molecular dynamics (MD) simulation. Alanine scanning mutagenesis was virtually performed to clarify the important of these binding residues. Apart from scanning, the complex structure of DehE-H_2O was subjected to MD simulation to study the mechanistic role of catalytic residues, Asn114 and Asp189 with catalytic water. Our findings confirmed that Trp34, Phe37 and Ser188 are important binding residues that interact and stabilize the orientation of substrates in the active site of DehE. Binding residues were formed hydrogen bonds with both ligands at distance less than 3 Å. Apart of that, Asn114 hold the catalytic water at distance ~4 Å while Asp189 was appropriately oriented towards catalytic water for catalysis reaction to be occurred. The results attained here will play important role in elucidating the important binding residues and catalytic residue involve in degradation of halogenated compound by non-stereospecific haloacid dehalogenase.

Keyword: DehE, MD simulation, Haloacid dehalogenase, Catalytic mechanism

ID 138: Antihypertensive effect of hydrolysates of melinjo (*Gnetum gnemon*) protein seed and their angiotensi-I-converting enzyme inhibitory activity

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Abstract

Melinjo (*Gnetum gnemon*) seed contain a high concentration of protein by 9-11% of the seed and has potential for production of a protein hydrolysate. Functional ingredients from melinjo seed protein hydrolysate were determinated by ability of the Angiotensin I-converting enzyme (ACE) inhibitory activity and antihypertensive effect. In the present study, melinjo protein was hydrolyzed by Alcalase for 4 h and the resulted hydrolysate was determined for ACE inhibitory activity in vitro. The antihypertensive effect of rice protein hydrolysate was also investigated in spontaneously hypertensive rats (SHR). The Alcalase-generated hydrolysate showed strong in vitro ACE inhibitory activity with the IC_{50} value of 0,016 mg/ml. A significant decrease in systolic blood pressure in spontaneously hypertensive rats was observed following single oral administration of this hydrolysate at a dose of 30 mg/kg of body weight. A potent ACE inhibitory peptide with the amino acid sequence of CMYLASG was isolated and identified from the hydrolysate. These results suggest that in vitro ACE inhibitory activity and in vivo antihypertensive activity could be generated from melinjo protein by enzymatic hydrolysis. The melinjo protein hydrolysate prepared with Alcalase might be utilized to develop physiologically functional food with antihypertensive activity.

Keywords: Antihypertensive, angiotensin I-converting enzyme, melinjo, protein, hydrolysate

ID 139: The Correlation Study of Transcutaneous Bilirubin Measurements in Neonates

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Abstract

The use of TcB screening reduces the number of blood tests for bilirubin determination. Our aim was to evaluate the correlation of TSB and TcB measurement in neonates. This prospective study was done in Mohammad Hoesin Hospital Palembang from February 2016 through July 2016. Transcutaneous bilirubin measurements were performed on the infant's forehead and sternum. The correlation coefficient between TSB and TcB was performed using Pearson Linear Regression Analysis. TSB/TcB measurements were performed in 150 Asian origin infants age ≤ 28 days (3.77±3.46 days). Most of the infants had a birth weight between 2500 and 4000 g (n=94, 62.7%); 55 neonates (36.6%) were less than 2500 g, and only 1 neonates (0.7%) were greater than 4000 g. The correlation coefficients (r) for TSB and TcB measurements over forehead and sternum were 0.897 and 0.891 ($p<0.001$). Transcutaneous bilirubin assessment by JM-105 at forehead and sternum has demonstrated high and significant correlation when compared to total serum bilirubin measured by chemical laboratory method. It can be favorably used as a screening test to identify the need for serum bilirubin measurement, but it cannot substitute for total serum bilirubin estimation.

Keywords: Neonates; Transcutaneous bilirubin; Total bilirubin serum; Forehead; Sternum; Correlation.

ID 141: Application of Surfactant Modified Natural Zeolite Loaded NPK for the Growth of Morus Alba

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Abstract

A sustainable fertilizer is practically demanded in agricultural sector, especially with zeolite amendment. Here, we investigated NPK-Organo-Zeolite performance as a controlled release fertilizer for the growth of Morus alba (White mulberry). The natural zeolite was modified with hexadecyltrimethylammonium (HDTMA) surfactant. This surfactant modified zeolite (SMZ) acted as a micro-carrier for the major plant nutrients; ammonium (N), phosphate (P) and potassium (K). A leaching test was performed to study the release of NH4+, PO43- and K+ from NPK-Organo-Zeolite, in comparison with monoammonium phosphate (MAP) and monopotassium phosphate (MKP) chemical fertilizers. NPK-Organo-Zeolite released the amount of NH4+ (213.33 mg/L), PO43- (566.67 mg/L) and K+ (8.08 mg/L) lower compared to MAP and MKP because zeolite has a capability in retaining the nutrients. Five different treatments were applied on M. alba; NPK-Organo-Zeolite, MAP, MKP, raw zeolite and control sample, which was harvested after 40 days. The average M. alba treated with NPK-Organo-Zeolite showed higher growth in plant height, number of leaves, total fresh and dry weights parameters (30.63±0.84 cm, 20.66±0.33, 14.69±0.60 g, 2.73±0.09 g; $p<0.05$) which were higher than other treatments, except MAP application. Overall, we conclude that NPK-OrganoZeolite can perform as an alternative controlled release fertilizer for M. alba growth.

Keywords: Zeolite; Surfactant Modified Zeolite, Morus alba, NPK Fertilizer, Controlled Release Fertilizer

ID 142: Evaluation of Gold Nanoflowers for Signal Enhancement of Lateral Flow Immunoassay

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Abstract

Background: Lateral flow immunoassay (LFIA) is a paper-based device commonly used for detection of various types of analytes with the advantages of low-cost, rapid test, mobile and variety of applications. Gold nanoparticles (AuNPs) in spherical shape mostly used as label in LFIA device due to its conjugation properties and visibility by naked eyes. However, disadvantage of AuNPs as labelling agent is less sensitivity. This work evaluates the use of multi-branched gold AuNPs known as gold nanoflowes, (AuNFs) synthesized using the seeded-growth (SG) method to enhance the sensitivity of LFIA devices. Methods: The 40 nm spherical AuNPs and AuNF were synthesized using the SG methods. AuNPs and AuNFs were characterized and conjugated with mouse anti-gram negative endotoxin antibody. Nitrocellulose (NC) membrane HF120 was used with anti-mouse IgG antibody as control dot. Then conjugated AuNP and AuNF was flowed onto NC membrane and finally washed with phosphate buffered saline pH 7.2. Control dot intensity was observed. Results: AuNFs have higher wavelength 630 nm compared to AuNP 526 nm. Morphology observed using transmission electron microscope shows that AuNFs have flower-like shape with 10 to 15 projectile. Control dot with AuNFs gave higher intensity compared to AuNPs. Conclusion: AuNF based LFIA was more sensitive compared to spherical AuNPs.

Keywords: lateral flow immunoassay; gold nanoparticles; gold nanoflowers; enhancement; conjugation

ID 143: Community Structure of Sulfur Oxidizing Bacteria at Midorikawa Mudflat

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Abstract

Midorikawa mudflat is located in a conserved estuary where fresh water from Midorikawa river meets Ariake Sea. The mudflat has active sulfur cycle and released H2S are normally oxidized by sulfur bacteria. However, information on community composition of sulfur oxidizing bacteria (SOB) are limited. This study was aimed to detect the composition of SOB using culture-independent approach based on sulfide:quinone reductase (*sqr*) gene. The gene is involved in initial step of H2S oxidation resulting elemental sulfur. Total DNA was extracted from the sediment. Partial sequence of *sqr* was amplified by PCR and a clone library was established. The results showed that the SOB community was dominated by chemotroph. It mainly composed of Gammaproteobacteria and followed by Betaproteobacteria. All the detected photoptrophs were represented by Alphaproteobacteria. Sequence similarities of *sqr* of the detected bacteria to the isolated reference strains from GenBank database were 72-85%. This indicates that the bacteria containing these *sqr*-like gene sequences are previously undiscovered.

Keywords: Midorikawa mudflat; sulfur cycle; sulfur oxidizing bacteria; *sqr* gene

ID 144: Investigating Trinucleotide Repeat Expansion: Can PCR do the job?

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Abstract

Trinucleotide Repeat Expansion (TRE) in human DNA could lead to various diseases. An expanded CAG repeat (>31 or 37 repeats, depends on the ethnicity) in *Androgen Receptor* gene is suggested to be associated with the occurrence of isolated hypospadias. In an effort to identify the exact numbers of repeats, sequencing has been the most favored method to be conducted despite its cost. This study wished to investigate the possibilities of using Polymerase Chain Reaction (PCR) method to screen expanded repeats in isolated hypospadias, as one of the TRE diseases. Numbers of CAG repeat in twelve hypospadias patients was first predicted from the visualization of PCR products in 3% agarose gel electrophoreses with 20 bp ladder marker before it was finally sequenced. Two samples gave the same result, while the rest showed a range of 1-5 bp differences. Statistically, there was a significant difference between the mean of CAG repeats from PCR method (M=26.1667, SD=6.71272) and the mean of CAG repeats from sequencing (M=23.75, SD=5.70685); t(11)= 4.570, p=0.001. Thus it can be concluded that PCR in TRE disease with small numbers of expanded repeats needs to be followed by sequencing in order to obtain the exact numbers of repeats.

Keywords: Trinucleotide Repeat Expansion, Polymerase Chain Reaction, Sequencing, Isolated Hypospadias

ID 145: Pharmacological Analysis of an Obnoxious Water Weed: *Eichhornia Crassipes* (Mart.) Solms

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Abstract

Current study deals with the qualitative screening of phytochemical of any therapeutic importance accompanying antimicrobial and antioxidant activity by employing different polar solvents *viz*. petroleum ether, chloroform, methanol and nonpolar aqueous solvent extracts of root, stem and leaf of *Eichhornia crassipes*. Antimicrobial activity of the water hyacinth extracts was evaluated with antibiotic susceptible and resistant microorganisms i.e. against two fungi (*Penicillium italicum* and *Botrytis cinreae*) and two bacteria *viz*. *Xanthomonas axonopodis* and *Bordetella pertussis*. The extracts of weed showed significant activity against these microorganisms when compared to standard dose. Maximum antifungal and antibacterial potential was recorded in leaf chloroform extract against *B. cinerea* (29 mm) and in leaf methanol extract against *B. pertussis* (17 mm) respectively among other extracts. The statistical analysis of data depict that *E. crassipes* displayed significant antioxidant activity which may due to the presence of significant metabolites. Qualitative analysis of plant parts have revealed the presence of various components of importance *viz*. alkaloids, coumarins, terpenoids, tannins and flavonoides and absence of saponins, anthraxquinones, phlobatanins and cardiac glycosides by standard procedures. The results obtained indicate it is a powerful source of phytochemical needed for maintenance of good health and can also be exploited in the manufacture of drugs.

Keywords: *Eichhornia crassipes*; Water weed; Antibacterial; Antioxidant activity; Phytochemical.

ID 146: Antiplatelet Activity of Bromelain Isolated from The Extract of Indonesia's Pineapple Core (*Ananascomosus* [L] Merr.)

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Abstract

Indonesia is one of the largest pineapple producer in the world with increasing world's demand of pineapple fruit products every years. Bromelain is a major component of pineapple that has been claimed to have many therapeutic benefits such as inhibiting platelet aggregation. The aim of this study is to isolate and purify bromelain from the extract of pineapple core (*Ananascomosus* [L] Merr.) and examine its antiplatelet activity. Fractionation of the crude enzyme using ethanol gave the highest activity at fraction 30-60% ethanol with the specific activity of 4.53 U/mg, whereas the precipitate obtained by re-fractionation using 0-60% ethanol gave the specific activity of 4.65 U/mg. This fraction has 898 times purity level compared to the crude enzyme extract. The bromelain obtained after purification by ion-exchange chromatography using DEAE-Cellulose had the specific activity of 11.84 U/mg with purity level of 2,277 times compared to the crude extract. The antiplatelet aggregation in vitro test performed using ADP as an aggregation inducer and acetosal as positive control. The results proved that all factions of bromelain enzymes have the antiplatelet activity. The isolate bromelain obtained had the highest antiplatelet activity with the aggregation percentage of 64.04% and the inhibition percentage of 18.47%.

Keywords: pineapple core; antiplatelet; bromelain; inhibition percentages; purification; specific activity

ID 147: Screening of Endegenous Keratinolytic Bacteria for Biodegrading Chicken Feather

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Abstract:
Chicken feather waste contains high protein content. Proteins on the chicken feather is keratin which can not be dissolved because its structure is difficult to be degraded. Keratinolytic bacteria can be used as effectively and environmently bioagen for degrading of chicken feathers. The purposes of this study obtain bacterial isolates degrading the waste chicken feathers. Research is carried out by isolation, assay of semi quantitively keratinolitic and proteolytic activity and phenotypic identification for the selected isolates. Screening of the isolates produced four selected isolates with high keratinolytic activity index, that are *Bacillus* sp. GB22.2 (3,38), *Bacillus* sp. GB23.4 (2.63), *Pseudomonas* sp. GB22.5 (2:55), and *Bacillus* sp. GB22.3 (2.10). The isolates with the high activity index of proteolytic are *Bacillus* sp.GB23.4 (3.72), *Pseudomonas* sp.GB22.5 (2.67), *Acinetobacter* sp. GB22.4 (2.45), and *Stenothropomonas* sp. GB18.1 (2,31). Keratinolytic selected isolates can be developed as an agent of chicken feather keratin biodegradation.

Key words: screening, keratinolytic, bacteria, biodegrading, chicken feather

ID 148: Role of Quality in Crop Improvement

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Abstract

With the advancement of science more and more necessities of life are subject to certain set standards. Human needs have changed over period of time and things which were considered to be luxury in past have become a basic necessity in our present day life. This trend has revolutionized the whole society and even a common man in this age is now more conscious about the quality of the product. Quality can be classified into morphological, organoleptic, biological and nutritional traits which determine the usefulness of the produce. Moreover, the importance of seed quality attributes like physiological, genetic and seed health hold the key for development of high yielding and disease free seed. Modern science technologies are supporting the cause of malnutrition in the developing world and future food security lies not only on the provision of high yielding varieties but also in ensuring the quality of the produce.

Key Words: Quality, crop improvement, standards, quality traits

ID 149: Screening for Algicidal Activity of Marine-Derived Fungi Isolated from Macroalgae and the Mussel *Perna viridis*

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Marine-derived fungi (MDF) can act as biological control agents against toxin-producing algae in shellfish-farming areas. In this study, MDF were isolated from the macroalgae *Gracilaria* sp. and *Enteromorpha* sp. collected from Calatagan, Batangas and from the bivalve *Perna viridis* collected from Bacoor, Cavite in Northern Philippines. A total of eight fungal strains were isolated, purified and cultured – three from *Gracilaria* and one from *Enteromorpha* using the culture medium Potato Dextrose Agar supplemented with marine salts (PDAS), and four from the mussel flesh using the culture medium Dextrose Casein Agar supplemented with marine salts (DCAS). Morphological characterization identified the MDF from the macroalgae as belonging to the genera *Geotrichum, Altenaria,* and *Cladobotryum,* and from the mussel as *Aspergillus* and *Penicillium.* Confirmation of the identities of these MDF will be done using molecular methods. For the production of bioactive secondary metabolites, the MDF from macroalgae were grown on PDBS while the MDF isolated from the mussel were grown on PDB with mussel extract and marine salts (PD-MFBS). All fungal cultures were then extracted with ethyl acetate and the crude culture extracts were tested against representative species of Chlorophyta (*Chlorella vulgaris, Scenedesmus quadricauda, Selenastrum minutum,* and *Chloroccocum humicola*), Bacillariophyta (*Nitzschia palea)*, and Cyanobacteria (*Anabaena variabilis, Nostoc piscinale, Oscillatoria chiliensis* and *Chroococcus minor*) for the screening of their algicidal activity. Results of these assays will be correlated with their potential application as biocontrol agents in aquacultures.

Keywords: algae, bioactivity, fungal natural products, marine fungi, seaweeds

ID 150: Differentiation Potential of Mice Dental Pulp Stem Cells Isolated from Enzyme Digestion and Outgrowth Method

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Abstract

Isolation method for dental pulp stem cells (DPSCs) is still a debatable discussion as researcher aim for a conducive environment for DPSCs to culture and proliferate. Enzymatic digestion and outgrowth method are two commonly used method for DPSCs isolation but it is not well characterized in mice DPSCs. This study focused on comparing mice DPSCs isolation method and its differentiation potential into bone cells. Dental pulp were extracted from mice's incisors and subjected to isolation either by Type I collagenase or culture of pulp tissue in complete medium. Both cells isolated were cultured until passage 4 and subjected to *in vitro* proliferation and differentiation analysis. Both cells exhibited fibroblast-like morphology but cells isolated by enzyme digestion proliferate faster compare to outgrowth method. After 21 days of osteoblast differentiation, DPSCs isolated from enzyme digestion showed alkaline phosphatase (ALP) activity slightly different as compared to outgrowth method. During osteoclast differentiation, DPSCs isolated from both methods did not show any significant different in tartrate-resistance acid phosphatase (TRAP) activity. In conclusion, there is no significant difference of the cells isolated from both method but it is preferable to isolate by enzyme digestion as it is more faster and predictable compare to outgrowth method.

Keywords: Mesenchymal stem cells; adherent cells; osteoblast; osteoclast; alkaline phosphatase, tartrate resistance acid phosphatase

ID 151: Analysis of The Level of Egg Sterility and The Age of Aedes aegypti Mosquitoes After The Release of The Sterile Insect Technique (SIT) in Ngaliyan District Semarang City

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Abstract

Semarang city is a city with the highest dengue cases in Central Java with the district Ngaliyan as the highest endemic areas .Until now, dengue control efforts have not provided adequate results , therefore we need other measures, one alternative is using Technique Male Infertility or better known as Sterile Insect Technique (SIT). This study aimed to observe the level of egg sterility and age of Aedes aegypti mosquitoes after using SIT in Ngaliyan district Semarang city. The method of this research is descriptive study with cross sectional design. Sample of the mosquitoes eggs are all the mosquitoes eggs on paper trap in every house of RW2 population in Ngaliyan district. These samples are the result of the fertilization of male and female mosquitoes in the nature. Data analysis using with Wilcoxon sign test. The result showed that the average of egg sterility percentage in mosquitoes after using SIT indoors was 73.65% and the average of egg sterility percentage outdoors was 69.08%. Wilcoxon test showed significant differences in average age of mosquitoes egg (p 0.000 <p 0:05), males pupae (p 0.005 <p 0.05), females pupae (p 0.001 <p 0.05), and adult female mosquitoes (p 0.016 <p 0.05), before and after SIT. It can concluded from this research that the SIT release in Ngaliyan district Semarang city can increase level of sterility mosquitoes eggs and decrease of the age of Ae. aegypti mosquitoes in this area.

Keywords: Sterile insect technique (SIT), egg sterility, mosquitoes age, Aedes aegypti

ID 152: Identification of Bacterial Species Capable of Degrading 2,2-Dichloropropionic Acid Isolated from Cow Dung

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Abstract:

A 2,2-dichloropropionic acid (2,2-DCP) naturally degrading bacterial species, strain S1 was successfully isolated from cow dung capable of utilizing the substance as the sole carbon source and energy. The isolated bacteria were grown in liquid minimal media containing 20 mM 2,2-DCP. The growth of S1 in various concentrations (10 mM, 20 mM, 30 mM and 40 mM) of the substance was evaluated. Then, species identification *via* Biolog GEN III system and 16S rRNA analysis was performed. Strain S1 was preferred over other strains (S2, S3 and S4) following observations on its rapid growth in 20 mM 2,2-DCP liquid minimal media. The study found the bacteria grew particularly well in 20 mM 2,2-DCP with the highest chloride release while exhibiting a remarkably short doubling time. In view of such notable characteristics, species identification *via* Biolog GEN III system and 16S rRNA analysis was performed and established strain S1 as *Bacillus cereus*. Considering the rapid growth of *B. cereus* strain S1 in such medium, its employment as a bioremediation agent to treat 2,2-DCP contaminated soils may prove beneficial. Moreover, this is the first reported case of a *Bacillus* sp. isolated from cow dung capable of utilizing 2,2-DCP.

Keywords: Cow dung; 2,2-dichloropropionic acid; *Bacillus cereus*; bioremediation

ID 153: Micropropagation and assessment of genetic fidelity of *Stevia rebaudiana* (Bertoni) using ISSR markers

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Stevia rebaudiana, is an economically important sweetening medicinal herb species from the family Asteraceae. To overcome low germination rate of Stevia, *in vitro* clonal propagation provide the alternative to ensure regular supply of planting material. However one of the limitations of *in vitro* propagation is somaclonal variation which affects the genetic fidelity and might give different therapeutic effects to the consumers. Hence this study was conducted to mass produce *S. rebaudiana* accession MS007 using *in vitro* clonal propagation technique using shoot tips and nodal explants. The genetic fidelity between mother plant and *in vitro* plants generated was also determined using ISSR-PCR analysis. The highest frequency of multiple shoot regeneration with maximum number of shoots was noticed on MS medium supplemented with 1.0 mg/L BAP. Highest frequency of rooting with highest number of roots and length from shoot tips and nodes, was observed on half-strength MS medium augmented with 0.5 mg/L IBA. When established in the field it was noticed that 86.67% of plantlets survived which is phenotypically similar to the parental mother plant. For genetic fidelity analysis, a total of 38 ISSR primers were tested, out of which 35 primers produced clear and reproducible bands. The banding patterns produced from amplification of genomic DNA of *in vitro* plants were monomorphic and similar to the mother plant.

Keywords: Stevia rebaudiana, micropropagation, genetic fidelity, ISSR marker

ID 154: Clinical Laboratory Science: The Hidden Profession that Saves Lives

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Medical Technology, also called Clinical Laboratory Science, is a paramedical field which focuses in Chemistry, Hematology, Immunohematology, Serology, Histopathology, Microbiology and other related disciplines.

Medical technologists, also known as Clinical laboratory scientists perform and analyze the results of complex scientific tests on blood and body fluids. These highly trained professionals work in hospitals and independent laboratories using refined procedures and equipment. They are responsible in operating computerized intruments, identifying abnormal cells, assuring safe transfusion of blood products, culturing and identifying bacteria and viruses, correlating test results with patient's condition, selecting and evaluating laboratory equipment, selecting, orienting and evaluating employees and monitoring the quality of testing.

The Clinical Laboratory provide physicians and other health care professionals with information to detect diseases or predisposition of disease, confirm or reject diagnosis, establish prognosis, guide patient management and monitor efficacy of therapy.

Nowadays, the Medical Technology profession is facing a lot of challenges. Some of these are automation and manpower shortage.

ID 155: Motion Comparative Analysis in Equestrian Jumping between Arabian Horses and Thoroughbred Horses: A case study

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Abstract

The case study had involved an equestrian athlete in the show jumping category at UTM Equine Park in order to analyze the comparison between two (2) types of the horse breed through ROM athlete upper body, ROM athlete lower body and ROM horse strides. The objective of this study to identify the best horse breed for Malaysian equestrian athlete to delivered optimum performance levels and emphasis a good posture while doing the show jumping. Video analysis of equestrian jumping training was recorded from the athlete trials for five (5) times of jumping training for every different horse breed and the videos has been transferred into motion analysis software to be studied. The result from the analysis shown that thoroughbred horses', killaney did ROM results in high level and the athlete was able to make ROM at optimum level. The thoroughbred horses are able to jump with harmonies and safe. Finally, doing an equestrian jumping have to look over specific breed in order to achieve at higher level of performance and rider's posture in the show jumping competitions. The specific breed may lead the equestrian athlete get highest point in the competition.

Keywords: Show jumping; posture; optimum

ID 156: Motion analysis comparison speed between 2 types of horses with the same breed which is Arabian type and the speed between 30-40m and 5-15m: A case study

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Abstract

This case study has been held to one of the Malaysian athlete in endurance sport at Equine Centre in UTM to view comparison between 2 different types of Arabian horses to grant of the acceleration riding at a distance 5-15m and 30-40m. The objective of this study was to determine the selection of the appropriate type of riding to horse athletes in the Country to produce optimum performance levels and consistent acceleration. Endurance training video recorded the athletes need to do a total of 5 times of trials acceleration to 2 different types of riding with the different breed Arabian horses. Then the video is inserted into the motion analysis software for review the current level of speed acceleration riding a 5-15 m and 30-40m. The result shows that the horse, Triple A Bold Siber produce mean speed fast at distance 5-15m and 30-40m. In addition, the result of heart rate monitor shows that the Triple A Bold Siber horse has a low reading of heart rate which is 30 per minute compared to the Blue Bronco Horse that has reading of 64 per minute before committing to acceleration. After the acceleration, the heart rate reading for Triple A Bold Saber was (133) and Blue Bronco Horse was (121) which have a slightly different of 12 minutes. This shows that the Triple A Bold Siber is more fit where the rate of the heart break that need to pump the blood mainly lower body. Finally, the selection for a better horses is important for helping the athlete achieve the optimum performance during the competition. A good horse helps athlete to achieve a victory and gold medal at once.

Keywords: Endurance; Acceleration; Heart rate

ID 157: Evaluation of Phytochemical Profilling and Antioxidant Potential of *Cinnamomum zeylanicum* and *Citrus hystrix* Extracts

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Cinnamomum zeylanicum and *Citrus hystrix* are medicinal plants that could act as antiseptic, analgesic, antispasmodic, insecticidal and antimicrobial agent. Consequently comprise phytochemical constituents which can scavenge the free radicals and prevent disease like cancer. The objectives of research are to identify phytochemical constituents and investigate antioxidant activity by DPPH scavenging assay of *C. zeylanicum* and *C. hystrix* extracts. Methanol and acetone were used as solvents to extract both plants essential oil. GC-MS analysis was conducted with two conditions (holding time and no holding time). The antioxidant activity of both *C. zeylanicum* and *C. hystrix* were tested with DPPH assay. Among the major phytochemical constituents analyzed with *C. zeylanicum* were cinnamaldehyde, benzanamine, eugenol, caryophyllene, phenol and oleic acid. While, constituents of *C. hystrix* that had been analyzed were citronellol, caryophyllene, oleic acid, D-Limonene, copaene, citronellal, β-pinene, α-terpineol and safranal. Acetone extract of *C. hystrix* and *C. zeylanicum* gave the best scavenging effect by 95.87%±0.12 at 10mg/ml and 95.20%±0.06 at 1mg/ml, respectively. Methanol extract of *C. zeylanicum* gave inhibition about 94.67%±0.25 at 1mg/ml, while methanol extract of *C. hystrix* gave 93.33%±0.18 of inhibition at 10mg/ml. As conclusion, *C. zeylanicum* and *C. hystrix* can be organic sources for durable antioxidant agent effect of their phytochemical constituents.

Keyword: Antioxidant, phytochemical, *Cinnamomum zeylanicum*, *Citrus hystrix*

ID 158: The Determination of Immunosuppressive Cells and Molecule in EMT6 Breast Cancer in Vivo

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Abstract

Immunosuppressive cells and molecules such as regulatory T cells and CD200 serve as immune marker for tumor progression and may be the important key to therapeutic response. This study aims to determine the infiltration of regulatory T cells (Tregs) in breast tumor and the expression of CD200 on breast cancer cells in murine breast cancer model. To do this, tumor tissue was harvested from Balb/c mice at day 14 following tumor inoculation. Tissue lysate was prepared and analyzed by flow cytometry. Results showed that low frequency of cytotoxic $CD8^+$ T cells was infiltrated at the tumor area. The level of Tregs infiltration was higher and this correlated with level of CD200 that was positively overexpressed by breast cancer cells. These findings shows that CD200 and Tregs were elevated at tumor site thus limit the activation of $CD8^+$ T cells, supressing the immune response and therefore lead to tumor growth. Results from this study provide an early insight of level of these cancer markers in tumor tissue in which this data might be useful in asessing the efficacy of future therapy modalities for the treatment of breast cancer.

Keywords: Immunosuppressive; breast cancer; regulatory T cells, CD200

ID 159: Enhancement of Sirna Delivery System System for Gene Therapy in Lung Cancer Cell

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Abstract

Gene therapy brings the new frontier in cancer treatment as catalysed by the discovery of RNA interference (RNAi) pathway back in 2006. This sequence-specific post- transcriptional gene silencing (PTGS) mechanism that occurs intracellu- larly to degrade foreign double stranded RNA (dsRNA) and regulates endogenous RNA levels. Efficient intracellular delivery of siRNA across biological barriers in general and delivery to target cells in particular is not at all straightforward since cells lack an efficient uptake mechanism for nucleic acids.The pulmonary deposition of siRNA administered to the lung depends on the aerodynamic diameter of the particle in which the siRNA is formulated and on the patient's pulmonary function. Therefore, smaller droplet or particle sizes are required for efficient delivery of siRNA to the respiratory zone (bronchioles and alveoles) where the gas exchange occurs. The magnetic properties of super paramagnetic iron oxide (SPIO) nanoparticles enable it to package siRNA into nanoparticles for it to enter the cancerous cell membrane easily.The usage of semiconductor quantum dots as multi-color biological probes helps in monitoring siRNA delivery.As we co-transfect siRNA with QDs, it leverage the photostable fluorescent nanoparticles to track delivery of nucleic acid, sort cell by degree of transfection and purify homogenously silenced subpopulations.The incorporation of SPIO nanoparticles and QD in the siRNA delivery will help to provide efficient, multifunctional and nontoxic siRNA delivery agents for cancer therapy.

Keywords: Gene therapy; siRNA; SPIO; QD

ID 160: The Effectivity of Cinnamon Alcoholic Extract to the Total Cholesterol on Hypercholesterolemia Rat Model

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Abstract

Coronary Heart Disease is a global health problem. Hypercholesterolemia is a risk factor for CHD. Cinnamon is a herb that is believed to have an antihypercholesterolemia effect. This study aimed to determine the effectivity of Cinnamon to the total cholesterol on hypercholesterolemia rat model. It is experimental study design in vivo with pretest-posttest control group design. Samples consist of 30 healthy Wistar rats were divided into 5 groups with simple random sampling method, Group 1 was treated by giving 20 mg/day, group 2 was 40 mg/day and group 3 was 80 mg/day Cinnamon alcoholic extract, group 4 was treated by giving 0,2 mg/day Simvastatin, and group 5 was given aquadest. The average of the highest decrease of total cholesterol was found in group 3. The results of statistical tests indicated that total cholesterol alteration significantly different in all treatment groups. In conclusion, giving Cinnamon was effective in decreasing levels of total cholesterol on hypercholesterolemia rat model.

Keywords: CHD; hypercholesterolemia; cinnamon; simvastatin; in vivo

ID 161: Flow Simulation of Patent Ductus Arteriosus to Evaluate Thrombosis Factors on Closure Device

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Abstract

Patent Ductus Arteriosus (PDA) is one of the most common congenital heart defects that are treated with minimally invasive surgery using occlusion devices. The occlusion device act as a physical barrier to blood flow in the duct which facilitates thrombogenesis and occludes the duct. Over the past 15 years, there have been significant developments in the devices used to close PDA. Various design of occlusion device effects the flow of blood in the duct. To improve efficiency of the thrombogenesis on the surface of occlusion device and estimate the time needed to occludes the duct, it is important to simulate blood flow through different design of occlusion device. Two design were used which is concave and convex shape of occlusion device. Solidworks was used for modelling, while flow simulation used ANSYS 14.0. Blood was simulated as Newtonian with laminar flow. The simulation showed velocity and pressure around the convex shape were much lower compared to concave shape. The percentage of WSS_{low} on the surface of convex shape was 84.3% while only 22.2% on the concave shape. From the preliminary work on PDA occlusion device, it is suggested that to promote thrombosis, convex shape was much better compared to concave shape.

Keywords: Patent Ductus Arteriosus; Flow analysis; CFD; Thrombosis; Closure devices; Haemodynamic

ID 162: Study on the Cytotoxicity Activity of *Hibiscus Tiliaceus* against Breast Cancer Cells (MCF-7)

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Abstract

Natural products are increasingly being used in the cancer treatment because of their potential anticancer agent with fewer or no side effects compare to the current treatment such as chemotherapy. One of the plant that has potency as the anticancer agent is *Hibiscus tiliaceus* since it was reported had hight antioxidant activity. Extraction on part of leaves, flowers, and seeds of *H. tiliaceus* and were done by maceration using methanol was yielded three methanol extracts. The solvent fractions of each methanol extract were obtained by solvent partitioning using hexane, ethyl acetate and water. The antioxidant capacity of the solvent fractions analyzed by the DPPH free radical scavenging assay. All solvent fractions had high radical scavenging capacity with IC_{50} less than 1 mg/ml. All samples were proceed for cytotoxicity against human breast cancer cells (MCF-7) by using MTT assay. The morphology analysis for detection of apoptosis was done by annexin-V propidium iodide (PI) staining. Result revealed that only ethyl acetate fraction from leaves part (HEAL) was had cytotoxicity activity against MCF-7 cells (IC_{50} = 20 µg/mL). Morphological feature indicated that HEAL was induced authophagic cell death. Further investigation on mechanism of autophagic induction on molecular level will be needed to confirm the cytotoxic activity of *H. tiliaceus* as anticancer potential against MCF-7 cells.

Keywords: *Hibiscus tiliaceus*; cytotoxicity; MCF-7

ID 163: Molecular Evaluation of Selected Malaysian Rice Cultivars Using Specific Primer Targeting *xa13* Gene

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Abstract

Bacterial leaf blight (BLB) is caused by the *Xanthomonas oryzae pv. Oryzae*. It is one of the devastating diseases in rice causing yield losses ranging from 74% to 81% in severe conditions. To date, more than 40 BLB resistance genes have been identified in rice. The present study focused on the screening of *xa13* gene in Malaysian rice cultivars. The *xa13* gene is fully recessive and only conferring resistance in a homozygous state. A total of 39 Malaysian rice cultivars which consist of 35 Malaysian landraces rice and four Malaysian commercial rice varieties were screened using a specific primer targeting *xa13* promoter. The PCR analysis on the positive control varieties (IRBB13 and IRBB66) showed allele with the approximate size of 500 base pairs while the negative control variety (MR84) showed allele with the approximate size of 300 base pairs. All the selected Malaysian rice cultivars showed similar allele size as negative control variety which suggested that none of the selected Malaysian rice cultivars showed the presence of *xa13* gene. Sequence analysis of IRBB13 showed the target region displayed 100% similarity to the IRBB13 resistant allele of *xa13* gene in NCBI database (GenBank id: DQ421394.1). This study provides polymorphism information between the resistant and susceptible varieties which would be useful in marker-assisted breeding (MAB) technology.

Keywords: *xa13* gene, Bacterial leaf blight, Malaysian rice cultivars

ID 164: Sonochemical Technique In Situ Application on Cellilose Producing Bacteria (*Acetobacter xylinum*) During Static Fermentation

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Abstract

Cellulose is the most abundant natural resource that can be found on earth. Usually cellulose is produced from plant however the main drawback in using plant cellulose is it has to be extracted from its source. Moreover the plant has to undergo chemical separation process that use alkali or acid treatment to separate the hemicellulose and lignin in order to get pure cellulose. In order to get cellulose from plant, many trees have to be cut down that leads to deforestation hence causes the global pollution issue. Certain bacteria such as *Acetobacter xylinum* produce cellulose in fermentation process. This technique is more sustainable to obtain high purity cellulose. Static fermentation method using synthetic medium usually take an average of 14 days to produce optimum Bacterial Cellulose (BC). The objective of study is to investigate the effect of ultrasonic wave during static fermentation on the *A. xylinum* as well as cellulose production and determine optimum sonication variables for high yield of BC. Sonication effect on the Gram negative bacteria *A. xylinum* which have thin peptidoglycan layer is studied. Sonochemical technique enhances the transport process of nutrient such as glucose in the medium of to the bacterial cell (*Acetobacter xylinum)*. By incorporating ultrasonic wave that applies 40 kHz of frequency for sonication time of 5 to 7 minutes to selected medium culture in static method, the effect on production of BC is also determined. In comparison to non-sonicated medium, the production rate of BC has speed up in sonicated medium. Results in initial study of BC showed 20-50% improvement in the yield. However, prolonged sonication otherwise influences the behaviour of *A. xylinum* cells and eliminate them at certain level. From the established base-line, further investigation on cell behaviour with the in situ inclusion of other additives into the medium during sonication process and assessment of their performances will be implemented.

Keywords: Sonication, bacterial cellulose, in-situ inclusion, static fermentation, *Acetobacter xylinum*.

ID 165: Synthesis of Graphene-Based Magnetite Nanocomposite for Lung Cancer Treatment

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Abstract

A superparamagnetic graphene-based magnetite nanocomposite (rGO-Fe_3O_4) synthesized via a simple *in situ* chemical approach, was used as a vehicle for targeted drug delivery application that was guided by external magnetic fields. The formation of rGO-Fe_3O_4 was confirmed as the fingerprints of rGO and Fe_3O_4 were identified using X-ray diffraction and the ratio of the D to G band of Raman spectroscopy (1.017) further indicated the formation of rGO. The magnetic and functional properties of rGO-Fe_3O_4 were analyzed using vibrating sample magnetometry and Fourier transform infrared spectroscopy. Next, the synthesized rGO-Fe_3O_4 was successfully functionalized using *Ganoderma lucidum* extract (GL) to enhance the stability (zeta potential of -44.9 mV) and dispersibility of nanocomposites (an average particle size of 481.4 nm). Furthermore, rGO-Fe_3O_4/GL was encapsulated with an amphiphilic polymeric material, Pluronic F-127 (PF-127) via hydrogen bonding. Meanwhile, the chemotherapeutic agent, paclitaxel (PTX) was loaded on PF-127 through hydrophobic interaction and the loading capacity was determined. The controlled release of the PTX was studied using the dialysis method and analyzed using high-performance liquid chromatography. Lastly, *in vitro* studies of rGO-Fe_3O_4/GL/PF-127/PTX was conducted on both human lung fibroblast and lung carcinoma cell lines using MTT assay and LDH assay.

Keywords: graphene-magnetite; drug carrier; drug delivery

ID 166: Elucidation of GDC-0941 Binding To PI3Kα Isoform via *In Vitro* Mutagenesis of Non-Conserved Amino Acids of PI3Kα

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Abstract

Phosphatidylinositol 3-kinase (PI3K) is a lipid kinase that catalyzes the biosynthesis of PI(3)P, PI(3,4)P_2 and PI(3,4,5)P_3 - second messengers that trigger a wide range of downstream signalling cascades involved in cell survival, growth, adhesion and proliferation. The heterodimeric class 1 PI3K proteins are composed of a regulatory subunit (p85) complexed with either one of 4 different isoforms of catalytic subunit (p110α, p110β, p110δ and p110γ). The PI3KCA gene encoding the α-isoform has been found to be frequently mutated in cancers such as breast, prostate, colon, liver and brain.

There has been a growing number of Class I PI3 kinase inhibitors described to date with some showing selectivity to different PI3K isoforms. However, the basis of selectivity of these inhibitors is still ambiguous. Previous studies have shown that specific regions within the catalytic subunit contain non-conserved residues which are involved in isoform selectivity. We have investigated the role of some non-conserved residues using site-directed mutagenesis followed by production of PI3Kα mutant enzymes. The potency of a potent and dual-selective PI3K inhibitor, GDC-0941 was tested against PI3Kα mutant enzymes and compared to the wild type enzyme. This approach will help in understanding the structure-activity relationship of PI3K isoform selectivity and may validate the existing structural information of inhibitor:enzyme complexes reported so far.

Keywords: PIP_2 – phosphatidylinositol 4,5-bisphosphate; PIP_3 – phosphatidylinositol 3,4,5-triphosphate

ID 167: Antibacterial activities of the lichen *Ramalina* and *Usnea* collected from Mt. Banoi, Batangas and Dahilayan Bukidnon against selected Multi-drug resistant (MDR) bacteria.

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Abstract

The scientific community calls this generation as the start of the "Post-Antibiotic Era", wherein even the common bacteria have the capability to resist the common antibiotics drastically. Despite the fast mutations of bacterial strains against antibiotics, scientists are looking for novel bioactive compounds that can inhibit the growth of Multi-drug resistant (MDR) strains. The study fixated on the efficacy of the extracts from the lichen *Ramalina* and *Usnea* in inhibiting the growth of selected MDR bacteria of *Bacillus subtilis, Staphylococcus aureus*, Methicillin-resistant *S. aureus*, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*. The lichen crude extracts exhibited promising activities against the selected bacteria which also surpassed the zone of inhibition (ZOI) of the positive control Vancomycin. The extracts of *U. filipendula* had an average zone of 18mm against MRSA while *U. fragilescens* had an average zone of inhibition of 18mm against *S. pneumoniae*; in comparison with the positive control Vancomycin (30mg/ml) with 17mm and 23mm, respectively. Interestingly, usnic acid and related compounds (e.g.,depsides, depsidones, dibenzofurans, etc.) were detected. Therefore, this study represents lichens as very interesting source of bioactive compounds, which provide unlimited opportunities for new antimicrobial agents. As more metabolites will be investigated, the chance of these re-born microorganisms to survive minimizes.

Keywords: *Usnea; Ramalina;* Multidrug-resistant bacteria (MDR)

ID 168: Progesterone Receptor Gene Polymorphism Promoter Region +331G/A Increases Risk of Endometriosis in Indonesian Women

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Abstract

Endometriosis is a benign gynecologic condition characterized by the presence of endometriotic tissue outside the uterine cavity. Change in progesterone receptor ratio increases the risk of endometriosis. This study wishes to identify relationship between progesterone receptor gene polymorphism promoter region +331G/A and the risk of endometriosis in Indonesian women. An observational case-control study was conducted. Population was Indonesian women with endometriosis and/ or adenomyosis who have undergone laparotomy/ laparoscopy at Obstetrics and Gynecology Department Dr. Mohammad Hoesin General Hospital Palembang on January-November 2013. Subjects who fitted inclusion criteria were given informed consent and have their blood sample taken. The polymorphism was identified by using PCR-RFLP method. There were 26 (54.1%) +331G/A genotype in the case group and 14 (26.4%) in control group. +331A/A genotype was not found in both groups. There was significant increase risk of endometriosis in women with genotype +331G/A to those with genotype +331G/G with OR 3.29 (CI 1.43-7.58 ; $p<0.05$). Polymorphism on progesterone receptor gene +331G/A increases risk of endometriosis in Indonesian women. Keywords: polymorphism, progesterone receptor, +331G/A, endometriosis, Indonesian women

ID 169: Effect of Low Intensity Exercise to Postsynaptic Density 95 Level and Spatial Memory Ability on Male Swiss Webster Mice Induced by Immobilization Stress

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Abstract

Physical Exercise has long been proven as a way to increase metabolic status, synaptic plasticity and also to increase protein regulation in order to maintain the cognitive function and brain health. There has not been many study focusing on the protective effect of physical exercise towards the cognitive function during stress.

Methods: This is a quasi experimental study with post test only control group design, conducted in both animal house and molecular biology laboratory of Medical Faculty Sriwijaya University on April-June 2016. As many as 32 white mice age 10 weeks old weighing around 25-35 grams were divided into four groups. The first group was the control group, the second one was treated with immobilization stress 2 hours daily for 21 days, the third group had been conditioned for 30 minute running at 11 m/min speed for 14 days and the fourth one was treated with physical exercise after being exposed to immobilization stress. The PSD 95 level in hippocampus and serum cortisol of the white mice were measured by using ELISA method, while the spatial memory ability was assessed by using Morris Water Maze Test.

Results: The Induction of immobilization stress for 21 days showed a significant elevation of cortisol serum and revealed a significant decrement of PSD 95 level and also spatial memory ability compared to control group. Low intensity physical exercise showed a significant elevation of PSD 95 level and spatial memory ability compared to control group. PSD 95 level and spatial memory ability was not significant different in stressed-exercise group compared to control group.

Conclusion: Low intensity physical exercise can prevent the decrement of PSD 95 and spatial memory ability due to stress.

Keywords: Low intensity exercise, immobilization stress, PSD 95, Morris Water Maze,

ID 170: Identification of Cry1c and Cry1fa Binding Proteins in Spodoptera Frugiperda Using Proteomics

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Abstract

Bacillus thuringiensis (Bt.) is gram positive endospore forming rod bacterium which forms endospores and produces parasporal crystals. These parasporal crystals are solubilzed in the midgut of insects at alkaline pH and converted to active toxins by midgut proteases. This activated toxin then binds with the receptors (mainly known receptors are alkaline phosphatase (ALP), aminpeptidase N. (APN) and cadherin-like in the the brush border membrane vesicles (BBMV) of the midgut microvilli and forms the pores in the membrane of midgut epithelial cells. When pores are formed then water enters the epithelial cells by osmosis and lyse the cells by osmotic pressure, when epithelial cells are lysed then gut is disrupted, larvae stop feeding and die due to septicemia. We have mainly focused on finding some new receptors/binding proteins in BBMV from S. frugiperda with cry1C and cry1F.

Toxin-receptor/binding-protein interaction was analyzed by using two different cry proteins (cry1C and cry1F) and BBMVs from S. frugiperda. Western blotting, ligand blotting and alkaline Phosphatase (ALP) assay was performed to identify binding-proteins/receptors. Multiple bands (30240KDa) were observed in ligand blots and western blots while isolated bands were seen in ALP assays (68-240KDa). From LC-MS/MS data we concluded that the proteins which are possibly important in the mechanism of action of cry proteins are Vha100-2, isoform B [Drosophila melanogaster], vacuolar ATP synthase subunit E [Bombyx mori], juvenile hormone epoxide hydrolase [Helicoverpa armigera], vacuolar proton-translocating ATPase subunit D [Drosophila melanogaster], elongation factor-1 alpha [Coenonympha mahometana], shock protein 90, aminopeptidase N-like protein [Tribolium castaneum], GA14484 [Drosophila pseudoobscura pseudoobscura], 60S ribosomal protein L15 [Spodoptera frugiperda], glycerol-3-phosphate dehydrogenase [Bombyx mori] and HSP 70 [Trichoplusia ni]. We are reporting these proteins for the first time which can be probably the receptors of Cry proteins. Further study is required to confirm their role as receptors.

ID 171: Potential Effects of *Nigella Sativa* and Thymoquinone on the Foetal Development in Mice Following Paternal Exposure to Cyclophoshamide

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Abstract

Cyclophosphamide (CPA) is an alkylating agent that is widely used as a chemotherapy drug among male of reproductive age. The drug causes chromosomal aberration (CA) in sperm which can decrease fertility and contribute to foetal abnormality. Many studies in animal have showed that foetal abnormalities induced by cyclophosphamide can be ameliorated using antioxidants. Traditional medicine such as *Nigella sativa* (NS) and its active compound, Thymoquinone (TQ), are widely used for their antioxidant activities. However, their antioxidative effects on teratogenicity in animal models remain unknown. Therefore, this study aimed to investigate the possible potential effects of NS and TQ on foetal development after paternal exposure to cyclophosphamide. Male ICR mice (n=3) were assigned randomly into 6 groups: (a) Vehicle control, (b) 200 mg/kg CPA, (c) 10 mg/kg NSE, (d) 10 mg/kg TQ, (e) 200 mg/kg CPA & 10 mg/kg NSE and (d) 200 mg/kg CPA & 10 mg/kg TQ. Male mice were then mated with the same strain of untreated female mice after 32 days. The foetuses were scanned using ultrasound modality to obtain their images and measurement of gestational sac size, biparietal diameter (BPD), crown-rump length (CRL) and heart rate activity. The foetuses were also analyzed for their morphological and skeletal malformations. The positive control and combination group of CPA and TQ showed no pregnancies whilst exposure to CPA supplemented with NS increased fertility rate. Between supplement groups alone, TQ improved pregnancy by the record of the highest number foetuses collected and number of impregnated female mice as compared to NS exposure alone. The measurements of CRL and BPD of foetuses in mice treated with TQ alone were 9.10±0.89 mm and 5.48±0.54mm respectively compared to 10.71±1.35 mm and 6.04±0.52 mm observed in control group. The measurements of BPD and CRL were significantly reduced compared to negative control group. This study indicated that NS could be a protective antioxidant against CPA-induced infertility while TQ alone may have the potential to promote and improve pregnancy success.

ID 172: Hydrocarbon degradation activities by microbes isolated from low temperature environment.

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A group of 18 bacterial isolates were successfully isolated from soil samples taken from Cameron Highland, Pahang. 16S rRNA gene sequencing revealed that out of 17 isolates, 7 were identified as Pseudomonas spp., while the other were identified as Stenotrophomonas spp., Acinetobacter spp., Serratia spp., Bacillus cereus spp. and Exiguobacterium spp. A few enzyme tests were performed for lactase, amylase, protease and lipase activities. The test revealed that most of the isolates produced at least 2 types of enzymes suggesting each of these isolates have their own commercial value to be exploited at the industrial level. Gravimetric analysis was adopted for n-alkanes and polycyclic aromatic hydrocarbons (PAHs) utilization tests. Based on the overall n–alkanes and PAHs utilization experiment, n– alkanes was degraded at a slower rate compared to PAHs. Statistical analysis revealed that the degradation of PAHs by these isolates was significant (p-value 0.014) but not for nalkanes where the p-value was 0.45. Stenotrophomonas sp. 412(2010) was found to be the best degrader for n-alkanes with 53.85 % of degradation. Bacillus cereus strain Aj0803191A however was chosen to be the best degrader for PAHs with 61.05 % of degradation.

ID 173: Analysis of risk factors for hypospadias

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Abstract

Hypospadias is a condition where meatus uretra is ectopically located. The urethral meatus can be placed anywhere along the urethral groove, from the penile, glans to the scrotum or perineum. Hypospadias is one of the most commonly found urogenital birth defect in live newborn boys. The incidence of hypospadias is varied in different countries; around 1:125 to 1:300 in live newborn boys. This Analysis Observational using Case Control was done in Mohammad Hoesin Hospital Palembang from February 2016 through August 2016. The samples data used from Medical record patients at Mohammad Hosein Hospital Palembang. The correlation coefficient between Hypospadia and Non Hypospadia patients was performed using Case control. Risk Factor measurements were performed in 48 samples divided two groups each group consisting 24 samples. Fisher's Exact Test was concluded that there is no significant relationship between genetic factors with the incidence of hypospadias in which the values obtained OR 2.091 (CI95% from 0.177 to 24.734) and p value = 1.000 (p> 0.05). From the Chi Square test was concluded that there is no significant relationship between environmental factors with the incidence of hypospadias in which the values obtained OR 2.000 (CI95% from 0.623 to 6.421) and p value = 0.380 (p> 0.05). OR value of genetic factors to the hypospadias is 2.091, which means the presence of genetic factors 2.091 x higher risk of incidence of hypospadias but not significant (p = 1.000)

Keywords: Hypospadias; Non Hypospadias; Risk Factors; Enviromental; Genetic; Correlation

ID 174: Application of conventional and response surface methodology in optimizing the culture conditions for 1, 3-propanediol production by *Klebsiella pneumoniae*

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Abstract

Background: The present study aims to optimize the 1,3-propanediol (1,3-PD) production from crude glycerol using conventional 'one-factor-at-a-time' (OFAT) and response surface methodology (RSM). The OFAT approach was adopted to identify the potential factors, namely the temperature, pH, glycerol concentration and inoculum size, that affecting the production of 1,3-PD substantially. Subsequently, the statistical screening of factors that exhibited significant contribution to the production of 1,3-PD was carried out by employing RSM based on central composite design (CCD). A total of 30 experimental runs consisting of 16 factorial points, 8 axial points and 6 center points were conducted. The experimental data obtained were analyzed by analysis of variance (ANOVA) and fitted to a second-order polynomial equation using multiple regression analysis.
Results: The preliminary findings has revealed that pH, glycerol concentration and temperature had profound effect on 1,3-PD production. Therefore, these three factors were selected for further optimization using RSM. Under RSM optimized condition, the maximum concentrations of 1,3-PD can be achieved at pH 7.6, with 39.9 g/L glycerol, temperature of 33 °C and incubation period for 59.1 h. The 1,3-PD produced (9.85 g/L) was ~2% higher than the predicted value by RSM (9.69 g/L), hence, the experimental design employed in validating the results obtained was significant. The analysis of variance (ANOVA) showed high coefficient of the determination values (R^2) of 0.9444.
Conclusions: The optimization of culture conditions via statistical approach (RSM) may improve 1,3-PD production by 2-fold as compared to unoptimized conditions. This work provided valuable information for further investigation of economical production of 1,3-PD using crude glycerol generated from biodiesel process.

Keywords: 1,3-propanediol; bioprocess; biodiesel by-product; central composite design; optimization

ID 175: Tolerance of Nickel and Lead by Marine and Terrestrial Strains of *Trichoderma*: Potential Bioremediation Agents for Environmental Clean Up

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Abstract

Trichoderma are commonly isolated in soil and decaying plant materials. Their competitive nature made them a successful colonizer of terrestrial and even marine habitats. *Trichoderma* are also good candidates for bioremediation. In this study, 25 strains of *Trichoderma* were isolated from decayed leaf litter from terrestrial habitats and marine substrata. Preliminary identification of these strains was conducted using morphocultural methods. The *Trichoderma* strains were also tested for their tolerance of heavy metals. Fungal isolates were grown on Trichoderma Selective Medium (TSM) with 50, 100, 300, 500, 700, 900, and 1200 ppm of either nickel or lead. Colony radial growth was measured after 2 days of incubation. Results showed that all *Trichoderma* strains grew on TSM with nickel and lead in all concentrations. However, highest radial growth was observed on TSM with nickel and lead at 50ppm for three terrestrial and three marine strains of *Trichoderma*. Interestingly, all the *Trichoderma* strains can tolerate the presence of lead at 1200 ppm in the culture medium. However, more terrestrial strains (13) grew on this medium as compared to marine strains (8). Our study showed the potential of terrestrial and marine strains of *Trichoderma* for the bioremediation of heavy metals.

Keywords: *Trichoderma*; tolerance; lead; nickel; bioremediation

ID 176: Potential Ecotourism of Ethnobotanicalstudy Traditionalclothes Bada Ethnic in Biosphere Reserves Lore Lindu Centralsulawesi

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Abstract

The research potential of ecotourism ethnobotanical study traditional clothing of bark Bada ethnic in Lore Lindu Biosphere Reserve, Central Sulawesi has been conducted. Bada is one that occupies ethnic neighborhood Lore Lindu and biosphere reserves, including indigenous peoples. Badaethnic located in the district of Poso. Bada ethnic has a natural beauty with a variety of interesting attractions, and one of them is the traditional clothes made of bark. The purpose of this study was able to inventory the biodiversity ahead and examine the utilization of biological resources with respect to indigenous plant-based ecotourism. Data used in this study includes qualitative and quantitative data using exploratory methods. SWOT to provide information about the potential and strategic ecotourism, which uses techniques FGD (Focus Group Discussion) based on key informant information. ICS is used to determine the value of the use of plants. The results of this study, there are three types of plants are used as bark cloth which is Nunu *(Ficus drupacea* Thunb.), Bea (*Ficus virens* Aiton), Malo (*Melochia umbelata* (Houtt.) Stapf.) and god leaves (*Dracaena* sp.). The dyes used in making bark cloth six plant species commonly used, namely Anuda (*Aystasia nemorum* Nees.), Bo'lah (Trema orientalis (L.) Blume), Bonati (*Clerodendrum disparifolium* Blume), Pampolo (*Bischofra javanica* Blume), Damar (*Agathis dammara* (Lamb.) Rich.), Noni forest (*Polyscias cumingiana* (C. Persl.) Fern.-Vill.), and god leaves (*Dracaena fragrans* (L.) Ker Gawl.). Tool grinding/ike stone for one and two made of stone and the handle of the rod enau/saguer (*Arenga undulatifolia* Becc.). Stone for three to seven handle made of tohiti rattan (*Calamus inops* Becc.). The based is used in the process of making bark cloth, there are three types of plants commonly used is iron wood (*Pterospermum javanica* Jungh.), ebony (*Diospyros celebica* Back.), and uru wood (*Elmerillia ovalis* (Miq.) Dandy). The based on the value of the interest or index Cultural Significance/ICS, ironwood (60) and ebony (85) has the highest value. This is because of ebony but used as construction, building materials, container, as well as internal medicine and blood pressure. The based on the results of SWOT analysis obtained three priorities, there are providing knowledge to the community through training in natural resource management ecotourism, create agendas and annual monitoring that is coordinated stakeholders and society, and coordination with officials of government agencies and indigenous communities to provide comfort.

Keywords: Ethnobotany, Ecotourism, Tree Bark clothes, Bada ethnic

ID 177: Genetic Diversity of Malaysian Rice Released Varieties Based On SSR Markers

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Abstract

The purpose of this study is to evaluate the genetic diversity of 42 Malaysian rice released varieties using 13 polymorphic simple sequence repeat (SSR) markers. A total of 45 alleles were detected ranged from 2.0 to 8.0 with 3.5 mean alleles per loci. Expected and observed heterozygosity ranges from 0.04 to 0.76 and 0 to 0.20 with mean of 0.40 and 0.04, respectively. Heterozygosity is expected to be low since the study involved inbred breeding lines which were expected to carry homozygote alleles. Polymorphic information content (PIC) values ranged from 0.04 to 0.72 with RM25 showed the highest PIC value. A dendogram generated based on unweighted pair group method algorithm (UPGMA) showed high divergence between MR269 with other varieties. This dendogram also showed high similarity between MR219 and MR220 since these two varieties are sister lines. MR220-CL1 and MR220-CL2 were clustered together with their respective recurrent parent, MR220. Malaysian rice quality (MRQ74, MRQ76 and Q50) were also observed to be clustered together at approximately 0.8 similarity coefficients.

Keywords: SSR marker; heterozygosity; genetic diversity; Malaysian rice released varieties

ID 178: Sequence Analysis of *Xa5* Gene, a Recessive Gene Controlling Resistance against Bacterial Leaf Blight Disease

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Abstract

The *xa5* gene confers a recessive resistance to the bacterial leaf blight (BLB), one of the devastating diseases in rice which significantly result in yield losses. The *xa5* gene was positioned in the subtelomeric region at chromosome 5. A total of nine pairs of primer were designed to cover approximately 6.3 kb of the *xa5* gene from both resistance (IRBB5 and IRBB66) and susceptible varieties (MR84 and MR263). The sequence analysis showed the target region encodes 106 amino acids and overlapped with two genes namely transcription initiation factor IIA subunit 2 (*TFIIAY*) and Putative uncharacterized protein (BGIOSGA018958). The comparative sequence analyses of the target region revealed 60 variants existed between resistance and susceptible varieties. The variants consist of 55 Single Nucleotide Polymorphism (SNPs) and 5 InDel. This variants is a pre-requisite for the development of functional markers targeting *xa5* gene which essentially useful in marker-assisted breeding (MAB) activities.

Keywords: Bacterial leaf blight, *xa5* gene, Marker-assisted breeding

ID 179: Mining Of SSR Markers from Transcriptomics Data of Three Durio SPP.

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Abstract:

"king of fruit" is a well known moniker to durian due to its characteristics of being thorny, having a thick husk, and sometimes emitting pungent odour. Although durian is well known to the South East Asia community, little is known about its genetic and genomic information. This study aims to look into the transcriptomics properties of three Durio spp. namely *D. graveolens*, *D. kutenjensis*, and *D. lowianus*. RNA sequencing was carried out on all three Durio spp. generating ~41 million, ~27 million, and ~28 million of paired-end reads respectively. A *de novo* assembly was carried out using Trinity assembly pipeline and Bowtie2 for individual mapping producing 65,574,540 (*D. graveolens*), 41,478,216 (*D. kutejensis*), and 44,165,462 (*D. lowianus*) of contigs. Simple Sequence Repeats (SSR) markers were later mined using MISA. A total of 26,262 (*D. graveolens*), 21,549 (*D. kutejensis*), 54,261 (*D. lowianus*) SSR markers (dimmers till hexamers) were successfully identified from each respective Durio spp. The information gathered is stored in an in-house database which later can be used such as for fingerprinting or varietal identification purpose.

Keywords: Durian; Durio; Next-generation sequencing; transcriptome; SSR

ID 180: Anisotropic and Isotropic Substrates that Control the Growth and Function of Liver Cells

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Abstract

Cells sense and respond to the substrate they grown on, thus engineering the substrate is one method to achieve control over cell growth, which is required for tissue engineering. Recent work has established that the cellular behaviour, e.g., adhesion of cells to substrate depends on a number of surface characteristics, specifically their nano-surface properties such as rigidity, roughness and surface topography. It should be noted that the receptors on cell surfaces are themselves nano-scale objects and so it is unsurprising that adhesion can be altered by nano-scale topographical changes. Cell-culture substrates with subcellular patterns have been fabricated via different methods and have been extensively used to investigate how cells respond to surface topography. Engineered surfaces, e.g., with nanoscale substrate surface patterning, can regulate cell behaviour, and this is essential for application in tissue engineering. Here we present results for the cell adhesion and cell morphology and function of liver cell on isotropic and anisotropic substrates, both with nanoscale features based on carbon nanotubes. We find that both cell morphology and orientation are controllable by modifying the nanoscale surface topography of the substrates. Our substrates offer control over topography on both the micro and the nanoscale.

ID 181: High-Cell Density Ethanol Fermentation of Diluted Sugarcane Syrup Using Flocculating and High Ethanol-Yielding *Saccharomyces Cerevisiae* Strain

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Abstract

Improving ethanol production efficiency is again of interest, especially with increasing demand in the Philippines because of the Biofuels Act of 2006, mandating local manufacturers to supply bioethanol to replace 10% of gasoline as fuel for vehicles. High ethanol-yielding yeast strains were screened for flocculating ability. Three *Saccharomyces cerevisiae* strains (TB3, TSJ1 and TW3) were selected for their combined flocculating ability and ethanol yield in flask trials. Batch ethanol fermentation using a 5-L fermentor at 30 °C, 200 g/L initial sugar and pH 4.5 showed that strain TB3 gave the highest amount of ethanol for sugarcane juice equivalent to 76.9 g/L after 24h, corresponding to fermentation efficiency of 88%. At increased initial sugar of 230 g/L and temperature (35 °C) in unsterile conditions (to simulate actual industrial process), the obtained ethanol was 92 g/L after 24 h. Furthermore, a high cell density repeated-batch fermentation trial with cell reuse by flocculation was also implemented using unsterile media containing 230-250 g/L total sugar. Stable fermentation runs at 30ºC were carried out over 10 rounds of cultivation and volumetric productivity was further increased. High cell concentrations (10^8-10^9 cells/ml) were achieved per batch, yielding ethanol contents of up to 86.8-118.5 g/L (11.0-15.0 %v/v) at a shorter fermentation time of 12-14 h. Cell recycle via flocculation was fast and convenient, and the strain was very robust and efficient.

ID 182: Analysis of Genetic Diversity of 64 Varieties of Local Durian (*Durio Zibethinus* Murray.) In Central Java Using Molecular Markers Inter Simple Sequence Repeats (ISSR)

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Abstract

Indonesia is the centre of durian diversity in the world. One type that can be consumed is Durio zibethinus Murray. The markers related material part controlling characterize of an individual, namely DNA molecular markers. Inter simple sequence repeat (ISSR) is one of the molecular markers that can reveal the plant diversity. The study aims are to analyze genetic diversity among local varieties of durian and obtained the durian identity. Durian analyzed totally 64 accessions taken from the Hortimart Gardens Collection, Bawen, Semarang, Central Java. DNA was isolated using the modified CTAB method. Amplification using primer PKBT 4-ISSR and the result was run through the agarose gel. Data were then scored and processed using NTSYS-pc program 2:02 version, dendrogram created by the unweighted pair group with Arithmetical averages (UPGMA). DNA amplification produced 65 polymorphic and 2 monomorphic bands with size variation between 250 bp to 1500 bp. The highest similarity coefficient is 0.96. Accessions separated into two large groups at the coefficient 0.69. Dendrogram analysis showed local durian in Central Java had high variation. This is evident because only found three pairs of accession which has a very high similarity. In conclusion, all accessions analyzed are different accession.

Keywords: Genetic diversity; durian; ISSR

ID 183: A Novel Transformer-based Modeling for Wireless Power Transfer System for Biomedical Devices

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Abstract

Biomedical devices are powered either with an external power cord or batteries. However, an external power cord limits the mobility of a patient and batteries have very limited power capacity and these methods may pose a high risk of infection towards patients whose skin is exposed to them. In order to remedy the issue, an efficient wireless power transfer system which incorporates magnetic coupling technique is proposed to substitute conventional charging mechanisms. The aim of this work is to develop a mid-range wireless power transmitter which consists of a transmitter circuit, receiver circuit and a pair of transmitter and receiver coils to charge the biomedical devices. A transformer based impedance modeling which incorporates a step-up and step-down transformer within the transmitter and receiver circuit is also introduced to improve the charging efficiency by reducing power loss. The experimental results indicate that the proposed wireless power transfer system is a reliable wireless charging technique as it records a transfer efficiency of up to 62% at a mid-range transmission.

Keyword: Wireless power transfer, transformer, magnetic resonance coupling, mid-range

ID 184: Incorporating Fuzzy Logic into an Adaptive Negative Pressure Wound Therapy Device

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Abstract

Negative Pressure Wound Therapy (NPWT) is a rehabilitative technique that promotes wound healing by applying a vacuum through a custom sealed dressing to induce a steady negative pressure on the wound surface area. The practice has been widely adopted in the treatment of trauma wound, chronic wound and deep sternal wound infections as it leads to a shorter recovery time. In spite of its significance, NPWT devices are not made readily accessible to patients due to its exorbitant cost. The aim of this work is to design a cost-efficient NPWT device alongside a robust negative pressure system controller design to regulate the induction of the negative pressure using the principles of Fuzzy Logic for an optimum healing process. The proposed NPWT system comprises adhesive film dressing, wound dressing, fluid collecting canister, drainage tubes, vacuum pump and an Arduino micro-controller as the negative pressure system controller. The proposed NPWT device is capable of generating adjustable negative pressure ranging from 0mmHg to 200mmHg and is a viable replacement to existing NPWT devices.

Keywords: Negative Pressure Wound Therapy (NPWT), fuzzy logic, Arduino micro-controller, wound healing device

ID 185: Implementation of Circular Hough Transform Derivative Algorithm on MRI Images for Eye Globe Detection

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Abstract

An accurate and reliable method to measure the size of the eye globe is essential to avoid the occurrence of pseudo-enophthalmos and pseudo-exophthalmos as well as to achieve a satisfactory post-operation aesthetic result. Manual segmentation on the eye globe boundary is a common measurement method but is not favorable as it is time-consuming and prone to errors due to various inter-operator or intra-operator variability studies. In this work, a derivative algorithm which is based on the Circular Hough Transform (CHT) theory is implemented to automate the measurement of the size of the eye globe using only MRI images without external intervention and is able to accurately render the eye globe for both eyes. This method is able to benefit existing hospital practitioners as they do no longer have to perform a manually drawn estimation of the eye globe based on MRI images or by clinical measurement as it may be exposed to inter-observer bias during the segmentation of the eye globe structure.

Keywords: Circular Hough Transform (CHT); Magnetic Resonance Imaging (MRI); MRI images; eye globe detection

ID 186: The Accumulation Pattern of Metal Ions in Diverse *Capsicum Annuum* Varieties

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Abstract:

Capsicum annuum, also known as chili, is an important crop used in worldwide due to its richness in Vitamin A, B-complex, C, and E along with other nutritive elements. Despite their valuable uses, they are responsible in food preparation as spice in the form of powder, sauce or raw form due to their texture and attractive colors. Quite often, cultivation of *capsicum* possesses great challenge as result of freshwater inadequacy for irrigation purposes. To address this growing problem, usage of industrial and sewage water are favored by farmers as they are cheap, easily accessible and enriched with abundance of essential nutrients and microbes. At the same time, it also contain elevated amount of various heavy metals such as lead (Pb) and Chromium (Cr) that become bioavailable to plants and enter food chain upon consumption. Therefore, this study was aimed to assess the bioaccumulation pattern of heavy metals in different plant parts among diverse *Capsicum annuum* varieties. Two diverse varieties were exposed to Pb and Cr at different levels: 0ppm, 200ppm, 400ppm and 800ppm until maturity and tested for their accumulation in root, leaves and fruits. Analysis of results indicated that both varieties follow the trend: root> leaves> fruit in Pb, while leaves>root>fruit in Cr.

Keywords: *Capsicum annuum*; heavy metals; lead; chromium; accumulation

ID 187: Stress In Plants: Regulations, Mechanisms, Effects and Approaches to Promote Survival

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Abstract

In Malaysia, rice is produced as the major food crop. Paddy ranked the third most widely planted crop after oil palm and rubber. Rice production in Malaysia is dwindled often due to salinity stress in which the effects are very much similar to drought. Viability of seed germination under salinity-prone condition is therefore crucial to ensure survivability. The present study was conducted to evaluate performance of three local rice genotypes (MR211, MR219 and Panderas) against two levels of chloride salinity (0, 150mM and 300m mM NaCl) at germination stage. Germination of the local rice genotypes was evaluated based on three attributes, namely Final Germination Percentage (FGP), Speed of Germination (GS) and Germination Index (GI). Salinity stress was observed to have caused reduction in both quantity of germinated seeds and duration of germination process. All three genotypes were negatively affected by salinity treatments as compared to that of Control. Although all genotypes were extremely sensitive towards salinity level of 300mM, however the magnitude of the effects was different for each genotype. The findings are anticipated to add knowledge regarding the potential of local rice genotype in tolerating salinity stress.

Keywords: Chloride salinity; Malaysian rice; germination; tolerance; genotype variability

ID 188: Responses Germination Attributes to Ferrous Toxicity in *Indica* Rice (*Oryza sativa* L.)

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Abstract

Seed germination is the crucial stage for all stages in direct seeding method of rice cultivation. However, ferrous toxicity is one of the important limitations for rice production in direct seeding method. The response of five rice varieties (Pokkali, Pak Bas, Firat 1, MR211 and MR219) and one local landrace, Panderas against four ferrous toxicity level of ferrous chloride (0, 1.5, 3 and 4.5mM) was studied at germination stage. Data for germination attributes which includes Final Germination Percentage (FGP), Speed of Germination (SG), Mean Germination Time (MGT) and Vigor Index (VI) was obtained to identify the effect of ferrous toxicity at germination stage. Germination was adversely affected at the highest level of ferrous stress (4.5mM) compared to the Control (0mM). All germination attributes were negatively affected with the increment of ferrous stress and Panderas was found to perform better than other varieties. These results would be beneficial for future breeding programs to improve germination attributes in rice.

Keywords: rice; ferrous toxicity; direct seeding method; germination attributes.

ID 189: Estimation of Cadmium (Cd) Toxicological Effects on Two *Indica* Rice at Germination Stage

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Abstract:

Cadmium is very toxic heavy metal for human health. In many countries, the main source of Cd^{2+} intake is crop grain specially rice and contamination of soils by Cd^{2+}. Accumulation of Cd^{2+} in rice grains are the serious agricultural issues in recent years. Contaminated rice and its toxicity are existed in population of many countries like Thailand, China and Japan. To estimate the Cd toxicity on two main *indica* rice cultivars, *Pokkali* and *Pak Basmati* was the main objective of this study. *Pokkali* and *Pak Basmati* were exposed to 1mM/L, 2mM/L, 3mM/L, 4mM/L, and 5mM/L treatments of $CdCl_2$ for 10 days under the laboratory conditions. Final germination percentage, germination energy and speed of germination data were recorded. Analysis of variance and reduction percentage were calculated. Our results showed the significant decreased in germination traits, with increasing concentration of Cd^{2+} at 3mM, 4mM and 5 mM/L extremely ($p < 0.05$). Germination found only in 1mM/L and 2mM/L concentrations of Cd^{2+}. Treatments at 1mM/L and 2mM/ L of Cd^{2+} significantly ($p<0.01$) reduced the germination percentage, germination energy and speed of germination in *Pokkali* and *Pak Basmati* compared with control. Toxicological effect produced more significant effect on germination traits of *Pak Basmati* than *Pokkali*.

Keywords: Toxicitiy; Cadmium, *Indica* cultivars

ID 190: Correlation Studies for Germination and Growth Traits in Rice Germplasm

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Abstract:

Salinity is one of the major sources of stress for the crop plants especially rice. Most of the saline soils are alkaline in nature with high pH values creating saline-alkaline stress conditions. Saline-alkaline stress tolerant rice varieties are very important to bring saline-sodic soils under cultivation but also to address the food security issues. A total of 40 rice varieties were studied for saline-alkaline conditions (pH8: 15 $dS.m^{-1}$) for germination and growth parameters at early seedling stage. The experimental treatments were compared using ANOVA. The mean comparison of growth parameters was formulated using least significant differences. According to IRRI standard evaluation system for tolerance, *Pokkali* was given a score of 3 while *Pak Basmati* was given a score of 8. Hence, *Pokkali* was found saline-alkaline tolerant while *Pak Basmati* was found saline-alkaline sensitive. A highly significant correlation was found between germination and growth parameters measured. High pH and saline conditions affect all germination and growth parameters. Presence of free Na^+ competing with K^+ might be the reason for slower germination and growth rate in rice germplasm. The findings suggest that high Na+ content and its effects on germination and growth traits could be used as saline-alkalinity index for saline-alkaline tolerance in rice. Furthermore, K^+ could be the agent to minimize the deleterious effects of free Na^+ in saline-sodic environment.

Keywords: salinity; saline-alkaline stress, rice, germination

ID 191: Strategies to Manage the Bacterial Blight Disease in Rice

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Abstract:

Bacterial blight is recognised as the oldest known diseases and has been reported in several countries. Bacteria blight caused by *Xanthomonas oryzae* is one of the major destructing bacterial diseases of rice which lead to yield loss in irrigated and rain-fed ecosystem. Several approaches have been reported in bacterial blight disease management such as biological and chemical control, cultivation practice and host plant resistance. Among the various approaches, development of host resistant carrying the resistance gene in commercial breeding programs are the most effective and environment friendly method of controlling bacterial blight diseases. Currently, over 38 resistance genes have been recorded in rice and six of them have been cloned for commercial cultivators. In this review, the current knowledge and biotechnologies tool has been discussed to combat the bacterial blight disease

Keywords: Rice, Bacterial Blight Disease, Resistance Genes, SSR Markers

ID 192: Leaf Proteome and Simulation of Transketolase from *Orthosiphon stamineus*

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Abstract

Orthosiphon stamineus locally known as Misai kucing is a famous traditional medicinal plant and has been extensively used as folk medicine in Southeast Asia to treat various ailments. The pharmacological properties of the plant have been proven including diuretic, antidiabetic, antitumor and antihypertensive. Thus, the leaves of the plant are commercialized as tea product and launched in the market to improve human health. The plant is could also be employed in phytoremediation. The focus of this study was to determine the protein electrophoretic pattern between varieties of the plant and its commercialized product, identify proteins from the leaf proteome and simulate the protein of interet. The pattern between white and purple variety was found to be similar with a slight difference in the intensity of bands whereas the tea product was found to has lesser number of bands. More than 100 proteins have been identified from the leaves of the plant through LC-MS/MS. Transketolase (TKT), a multifunctional protein had been identified from the plant with a role in photosynthesis, act as precusor in several biosynthesis and believes to contribute to phytoremediation. Predicted 3D model of TKT was built by using I-TASSER and validated followed by molecular dynamic (MD) simulation via GROMACS to refine the model. The results obtained from MD simulation reveal that the TKT structure is stable with reasonable flexibility and compactness based on its RMSD, RMSF and radius of gyration which indicates that TKT is stably folded.

ID 193: Molecular Dynamics Simulation of Antioxidant Protein in Upland Rice: Insight into Heat Stress

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Abstract

Global warming is one of the threats for rice production as it leads to the occurrence of heat stress to the plant. Upland rice is known as a stress-tolerant plant as it could survive in harsh environmental condition. Antioxidant protein is vital in combating superoxide radicals resulted from the stress condition. Superoxide dismutase (SOD) [Cu-Zn] is considered as the first line of defense to protect the plant against stress. However, there is no study reported on MD simulation of SOD [Cu-Zn] in upland rice under high-temperature condition. Therefore, in an attempt to determine the stability of SOD [Cu-Zn] during scorching heat condition, wet-lab and bioinformatics techniques were employed. Protein was extracted followed by 2D-PAGE and mass spectrometry analysis. Based on the peptide sequence obtained, 3D model of SOD [Cu-Zn] was developed and MD simulation was performed at three temperatures (38°C, 42°C, 46°C). MD simulation analysis was performed to elucidate the stability of the protein at the aforementioned temperatures. The results strongly suggest that SOD [Cu-Zn] could withstand temperature as high as 42°C. In conclusion, this study accommodates new insights on the ability of upland rice to withstand heat stress condition, which can be manipulated to enhance breeding program.

SELECTED PROCEEDINGS

Response of Coffee Senna (*Senna occidentalis*) Seeds to Different Fermentation Periods

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Abstract: A laboratory analysis was conducted to investigate the effect of varying fermentation period on the proximate composition, amino acid profile and levels of anti-nutritional factors of *Senna occidentalis* seeds. The seeds were fermented for 0, 3, 5, 7 and 9 days, respectively in replicate of three in a completely randomized design. The proximate composition, amino acid profile and level of anti-nutritional factors of the differently fermented *Senna occidentalis* seeds were determined using standard laboratory procedure. Data obtained were subjected to analysis of variance of the completely randomized design (CRD) using Statistix 9.0. The results indicated that the proximate composition of *Senna occidentalis* seeds significantly ($P<0.05$) increases as the fermentation period progresses except for the ether extract and nitrogen-free extract which reduced as the fermentation period increased. The amino acid content similarly showed significant ($P<0.05$) increase as the fermentation period advanced. The level of the anti-nutritional factors were observed to significantly ($P<0.05$) decline as the fermentation period progresses. It can be concluded that *Senna occidentalis* seeds can be fermented up to 9 days without adverse effect on the nutritive value of the seeds. However, fermentation for 5 is recommended due to its minimal reduction of ether extract and nitrogen-free extract when compared to 7 and 9 days fermentation. The fermented seed meals should be used in a feeding trial in order to evaluate their feeding value to animal.

Keywords: Response, fermentation periods, *Senna occidetalis*, lesser-known.

1. Introduction

Lesser-known legume sources are reported to be rich in nutrients such as protein with good array of amino acids and nutritionally needed minerals (Aletor and Agbede, 2005). Fermentation has been reported to improve the nutritional quality feed material (Monein, et al. 1994; Elkhalifa, et al., 2004; Igbabul, et al., 2014)). *Senna occidentalis* is one of such wild legume that is under-utilized as protein source in Nigeria. It is a pan-tropical plant species that belongs to the family of *Fabaceae*, subfamily *Caesalpinioide* and genus *Senna*. It is characterized by alternate compound leaves. The pod is about 12.5cm x 0.7cm containing 23-30 seeds. The flower is ovate yellow (Chang and Miles, 2004). The chemical composition as revealed by Augustine et al. (2013) indicated that the seed meal has promising nutritional value but also contains some anti-nutritional factors such as tannins, oxalates, phytates and saponins which will limit its utilization with adverse consequences on animal performance. In view of the above, it has become imperative to detoxify the seeds before feeding to livestock. Fermentation which is a bioprocess is an ideal detoxification method that can improve the utilization of *Senna occidentalis* seeds. Many

researchers have documented the beneficial effects of fermentation in improving the nutritional quality of feed ingredients (Adebowale and Maliki, 2011; Igbabul et al., 2014). Before recommending fermentation as a method of processing *Senna occidentali*s seeds, it is important to thoroughly investigate the best fermentation time that will enhance optimal utilization of *Senna occidentalis* seeds. At the moment, information on the effects of fermentation time on the chemical composition of *Senna occidentalis* seems to be scanty hence the need to bridge such information gap. This study was therefore designed to evaluate the effect of different fermentation period on the proximate composition, amino acid profile and level of anti-nutritional factors of *Senna occidentalis* seeds indigenous to Mubi area of Adamawa State, Nigeria.

2. Materials and Methods

2.1 Identification, collection and processing of Senna *occidentalis* seeds

Senna occidentalis seeds were identified at the Department of Biological Sciences Adamawa State University, Mubi, Nigeria by a Botanist. The dry seeds were collected in uncultivated areas in Mubi. The seeds were naturally fermented for 0, 3, 5,7and 9 days in triplicates each in a complete randomized design. The fermented seeds were milled and sieved through a 1mm sieve.

2.2 Chemical Analysis

The proximate composition of *Senna occidentalis* seeds was determined using the standard procedure of AOAC (2004). The dry matter content was determined using the oven-drying method and crude protein (CP) was determined using the Kjeldahl procedure. Soxhlet extraction method was used for the determination of ether extract (EE) while the crude fibre (CF) content was evaluated using the trichloroacetic method and the ash content determined using the murfle furnace ignition method. Nitrogen-free extract (NFE) was computed indirectly by using the formula:

NFE = 100 – (% moisture + CP + CF +EE +ash)

The level of anti-nutritional factors was determined using the standard methods of AOAC (2004). The amino acid profile was analyzed using isocratic high performance liquid chromatography (HPLC) equipment (model No. BLC 10/11) as described by Pearson (1991).

3. Results

The result of the proximate composition of *Senna occidentalis* seeds subjected to different fermentation periods is presented in Table 1. The result indicated significant (P<0.05) increase in the crude protein content as the fermentation period progresses from day 0 to the ninth day..

Table 1. Proximate composition of *Senna occidentalis* subjected to different fermentation periods.

Nutrients (%)	T(0day)	T2(3 days)	T3(5 days)	T4(7 days)	T5(9 days)	SEM
Dry matter	92.50	91.00	91.00	92.00	91.00	4.31^{NS}
Crude protein	19.62^{c}	20.00^{c}	21.67^{b}	22.05^{b}	22.61^{b}	0.21*
Crude fibre	13.80^{a}	8.90^{b}	5.90^{c}	5.00^{c}	4.10^{d}	2.05*
Ether extract	4.03^{a}	2.94^{b}	2.87^{b}	2.97^{b}	1.53^{c}	0.11*
Ash	5.70^{b}	6.82^{a}	6.79^{a}	6.50^{a}	6.47^{a}	1.36*
NFE	49.80^{a}	47.90^{a}	44.20^{b}	44.09^{b}	39.07^{c}	1.09*

a,b,c = means in the same row with different superscripts are significantly different (P<0.05)

* = Significant at 5% level of probability, NS = Not significant (P>0.05) SEM = Standard error of the means, NFE = Nitrogen free extract.

The ash content was significantly (P<0.05) influenced by the different fermentation periods. The ash content ranged from 5.70% in the unfermented to 6.82% in the fermented seed meal. The clear trend is that of increase in the ash content as the fermentation period increases.
The crude fibre content was observed to significantly (P<0.05) decreased as the fermentation period increases.

The effect of the different fermentation period on the ether extract was observed to linearly reduce as the fermentation period increases. The nitrogen free extract was seen to have reduced as the fermentation period advances.

The amino acid profile of *Senna occidentalis* seed as affected by different fermentation periods is summarized in Table 2. The result revealed that the amino acid content was significantly (P<0.05) affected by the different fermentation periods. There was an increase in the amino acid profile as the fermentation period increases. This result showed that the fermentation period at day 5, 7 and 9 exhibited similar amino acid profile.

The effect of fermentation periods on the level of anti-nutritional factors of *Senna occidentalis* seeds is shown in Figure 1. The level of the anti-nutritional factors where observed to be lower as the fermentation period increases from day 0 to a period of 9 days.

Table 2. Effects of different fermentation periods on some amino acid profile of *Senna occidentalis* seeds (g/100g).

Amino acid	**T1(0day)**	**T2(3 days)**	**T3(5 days)**	**T4(7 days)**	**T5(9 days)**	**SEM**
Lysine	4.20^{b}	4.30^{b}	4.70^{ab}	4.80^{b}	4.76^{b}	1.02*
Methionine	1.35^{c}	1.42^{b}	1.67^{a}	1.79^{a}	1.76^{a}	0.32*
Isoleucine	2.79	2.97	3.24	3.21	3.19	0.62^{NS}
Phenylalaline	3.60^{c}	4.02^{b}	5.80^{a}	5.94^{a}	6.00^{a}	1.08*
Valine	2.10^{c}	3.31^{bc}	4.25^{a}	4.23^{a}	4.16^{a}	0.67*
Histidine	4.00^{b}	4.20^{b}	4.75^{a}	4.84^{a}	4.77^{a}	0.88*
Arginine	2.30^{b}	3.55^{b}	4.66^{a}	4.61^{a}	3.62^{a}	0.17
Serine	2.01^{c}	3.30^{b}	3.80^{ab}	4.78^{a}	3.89^{ab}	2.42*
Cysteine	2.65^{c}	2.97^{bc}	3.45^{a}	3.61^{a}	3.28^{a}	0.11*
Glycine	6.65^{c}	8.22^{ab}	9.95^{a}	9.00^{ab}	$9.45a^{b}$	1.09*

a,b,c,d = means in the same row with different superscripts are significantly different (P<0.05)
* = Significant at 5% level of probability, NS = Not significant (P>0.05) SEM = Standard error of the means.

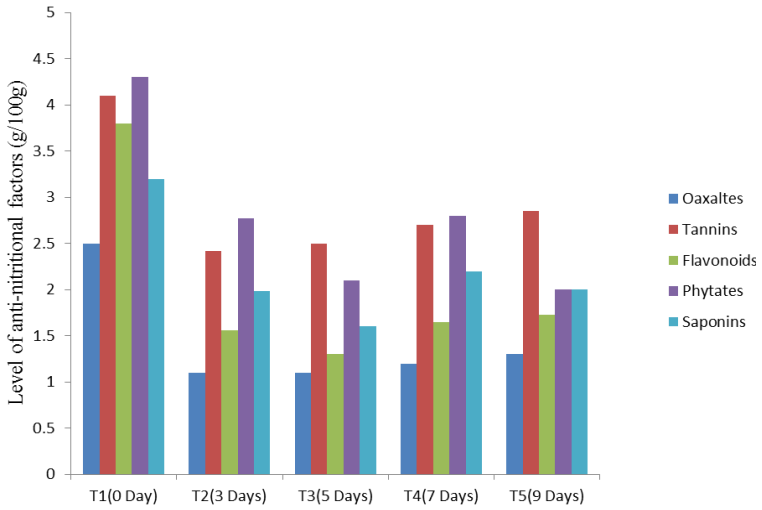

Figure 1. Fermentation periods (days).

□

Patricia *et al*. (2012) further explained that tannin acyl hydrolases have the ability to hydrolyze the ester bond of tannins. The reduction of phytates in fermented feed is attributed to hydrolysis of phytates into lower inositol phosphates (Real, *et al*. 2007; Abdelseed, *et al*., 2011). Similar observations were made by Igbabul *et al*. (2014) and Anthony and Babatunde (2014) for mahogany bean and millet subjected to different fermentation periods

4. Discussion

The proximate composition of *Senna occidentalis* seeds indicated significant (P<0.05) increase in the crude protein content as the fermentation period progresses. Similar effects were reported by Igbabul *et al*. (2014) and Adebowale and Maliki (2011) for *Afzelia Africana* flour and *Cajanus cajan* seed subjected to different fermentation periods. The increase in protein content may be attributed to reasons reported by Anthony and Babatunde (2014) who reported that increase in number of lactic acid bacteria during fermentation can increase protein content of the seeds. This may be linked to increase in microbial protein.

The ash content was significantly (P<0.05) influenced by the different fermentation periods. The ash content ranged from 5.70% in the unfermented to 6.82% in the fermented seed meal. The clear trend is that of increase in the ash content as the fermentation period increases. This finding is in agreement with the report of Uwagbute *et al*. (2000) and Anthony and Babatunde (2014) who similarly reported an increase in the ash content of millet and cowpea as the fermentation period increases. This increase may be linked to the ability of fermentation to lower the dry matter content resulting to an increase concentration of minerals (Adams, 1990)

The crude fibre content was observed to significantly (P<0.05) decreased as the fermentation period increases. Rainbault (2001) observed that such reduction might be due to the enzymatic break down of the fibre during fermentation by lactic acid bacteria which utilized them as carbon source and converted them to microbial biomass thereby reducing the fibre content. Similar finding was reported by Magdi (2011).

The effect of the different fermentation period on the ether extract was observed to linearly reduce as the fermentation period increases. This decrease might be due to the increase in the activities of lipolytic enzymes during fermentation which hydrolysis fat components into fatty

acid and glycerol (Chinma *et al*., 2009). Anthony and Babatunde (2011); Chang and Miles (2004) and Fudiyasa *et al*. (1995) further stressed that break down of fatty acid is responsible for the aroma, taste, odour and texture of fermented feed ingredient.

This reduction effect of nitrogen free extract was similarly reported by Ojewole and Odunta (1992) who attributed such reduction to the utilization of some sugars by the fermenting lactic acid bacteria for their growth and other metabolic activities. This clearly indicated that increase duration of fermentation is likely to reduce the energy value of a feed material (Oyewole and Odunfa, (1992).

The amino acid profile of *Senna occidentalis* seed revealed that the amino acid content was significantly ($P<0.05$) affected by the different fermentation periods. There was an increase in the amino acid profile as the fermentation period increases. This finding was supported by Igbabul *et al*. (2014) who pointed out that increase in microbial mass as fermentation period progresses can cause extensive hydrolysis of protein molecule to amino acids and other simple peptides. However, fermentation period at day 5, 7 and 9 exhibited similar amino acid profile.

The effect of fermentation periods on the level of anti-nutritional factors of *Senna occidentalis* seeds that the level of the anti-nutritional factors lowered as the fermentation period increases. The decrease may be due to the combine effects of cooking resulting to leaching out of these anti-nutritional factors in boiling water and metabolic microbial activity during fermentation. This was buttressed by Ali *et al*. (2009) who reported that tannins act as carbon source for microorganism and as inducer of the endogenous synthesis of the enzymes

5. Conclusions

The outcome of this investigation indicated that progressive increase in fermentation period has beneficial effect on the nutritional value of *Senna occidentalis* seeds. The result indicated that *Senna occidentalis* seed can be fermented up to 9 days. However, fermentation 5 days is recommended due to the minimal reduction of ether extract and nitrogen-free extract when compared to 7 and 9 days. The fermented seeds should be used in a feeding trial to evaluate their actual biological value.

Abbreviations

The following abbreviations are used in this manuscript:

CF: Crude fibre
CP: Crude protein
EE: Ether extract
NFE: Nitrogen free extract

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Effectiveness of commercial antimicrobial agents and commercial antibacterial products towards foodborne pathogens

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Abstract: In food preparation industry, foodborne pathogens contamination are considered as the most critical risk factor that cause foodborne diseases. There are numbers of commercial antimicrobial agents and antibacterial products sold in the market claimed their products able to "sterile" the contaminated kitchen surfaces until 99%, however the effectiveness of the commercial products towards the foodborne pathogens still unclear. Therefore, a study was conducted to evaluate the effectiveness of commercial antimicrobial agents and commercial antibacterial products towards foodborne pathogens, *E. coli*, *Salmonella* spp. and *S. aureus* isolated from surfaces contact with raw chicken sample during chicken preparation. The sensitivity of the three foodborne pathogens isolated from the sampling sites toward four different commercial antimicrobial agents and five commercial antibacterial products were carried out using the disc diffusion method. The foodborne pathogens isolated showed variable susceptibility towards the commercial antimicrobial agents tested. Isolated *E. coli* showed highest susceptibility towards Ampicillin Trihydrate while *Salmonella* spp. showed highest susceptibility towards Tetracycline hydrochloride. In the other hands, *S. aureus* showed the highest sensitivity on Kanamycin Sulfate. The susceptibility testing of the pathogens on the commercial antibacterial products showed that the most effective product in decontaminating the pathogen was the product from Clorox.

Keywords: Foodborne pathogens, commercial antimicrobial agents, commercial antibacterial products

11. Introduction

Kitchen is the main source of cross-contamination of other foods and objects (Flores *et al*., 2012; Humphrey *et al*., 1994; Scott and Bloom, 1993). There are several studies indicate that various pathogenic bacteria like *E. coli*, *Salmonella* spp. and *S. aureus* survive in kitchen utensils, dish cloths, sponges and hands before they can be transferred into foods (De wit, 1979; Gorman *et. al*., 2001; Rusin *et al*., 2002; Scott and Bloomfield, 1990). It is not easily removing the foodborne pathogens on the domestic kitchen surfaces. There are several of commercial antibacterial products in the local market claims their products are effective in reducing the number of foodborne pathogens. Consumers can use commercial antibacterial products sold in the local market to clean their kitchen, but the effectiveness of the commercial antibacterial products in reductions the population is still unclear. This study aimed to evaluate the effectiveness of commercial antimicrobial agents and antibacterial products against *E. coli*, *Salmonella* spp. and *S. aureus* isolated during the chicken preparation. The effectiveness of the products towards the isolated foodborne bacteria was determined using the susceptibility testing.

2. Materials and Methods

2.2 Bacterial strains

Isolates from surface contact with the raw chicken sample during chicken preparation was collected using sterile swab. The isolates were cultured onto a selective enrichment medium followed by plating on specific selective and differential media(Murni A. and Wan Rosmiza W.D., 2015). Table 1 showed the selective enrichment medium, specific selective and different media used to isolate *E. coli*, *Salmonella* spp. and *S. aureus.* Isolates bacteria were undergoing purification before used for susceptibility testing.

Table 1. Selective enrichment medium, specific selective and different media used to isolate *E. coli*, *Salmonella* spp. and *S. aureus* (Murni A. and Wan Rosmiza W.D., 2015)

Foodborne pathogen	Enrichment media	Selective and differential media
E. coli	Lactose Broth	Eosin Methylene Blue (EMB) Agar MacConkey (MAC) Agar
Salmonella spp.	Selenite Cystine (SC) Broth	Hektoen Enteric (HE) Agar Xylose Lysine Deoxycholate (XLD) Agar
*S. aur*eus	Brain-Heart Infusion (BHI) Broth	Baird-Parker Agar (BPA) Mannitol Salt Agar (MSA)

After verifying the purity of the strains, the strains were used for susceptibility testing.

2.3 Susceptibility Testing

The susceptibility testing was performed using the disc diffusion method. Table 2 shows the commercial antibacterial agents and antimicrobial products used for the susceptibility testing.

Table 2. Commercial antimicrobial agents and antibacterial products used for the susceptibility testing

Commercial antimicrobial agents	• Ampicillin Trihydrate • Tetracycline Hydrochloride • Kanamysin sulphate • Chlorine bleach, XY-12
Commercial antibacterial products	• D4 Sanitizer • GERMISEP Disinfectant Tablets (Hovid) • Dettol Disinfectant Multi action Cleaner • KIWI Clean Express Cleaner • Clorox Kitchen Cleaner

2.4 Preparation of the Inoculums

Pure culture of the organism from the nutrient agar was inoculated into a broth medium and incubated at 37°C for 16-18 h. Table 3 shows the bacteria strains and its specific inoculated broth medium.

Table 3. Bacteria strain and its specific broth medium

Bacteria strain	Broth medium
E. coli	Lactose broth
Salmonella spp.	Selenite Cystine broth
S. aureus	Brain-Heart Infusion broth

After proper incubation, the growth stage of the bacteria was determined by measuring the optical density at 600nm (OD_{600nm}). The bacteria should have OD_{600nm} reading below than 1, indicates that the bacteria growth was in lag phase.

2.5 Preparation of the Commercial Antimicrobial Agents and Antibacterial Products

The commercial antimicrobial agents and antibacterial products tested have specific concentration in order to work effectively. Therefore, some of the antimicrobial agents and antibacterial products required specific dilution before it can be use (Table 4). Dilution was performed using sterile distilled water. Prepared antimicrobial agents were stored in icebox and used immediately after prepared. Sterile distilled water was used as a control.

Table 4. Commercial Antimicrobial Agents and antibacterial products with its concentration

Commercial Antimicrobial Agents/ Antibacterial Products	Concentration
Ampicillin Trihydrate	1mg/1mL
Tetracycline Hydrochloride	1mg/1mL
Kanamysin Sulfate	1mg/1mL
Chlorine Bleach, XY-12	0.3 mL/L and 0.6 mL/L
D4	12 L per tablet
GERMISEP	2 L per tablet
Dettol, Kiwi Kleen and Clorox	No dilution required. Used according to the concentration provided by the manufacturer

2.6 Preparation of the Diffusion Discs

The diffusion discs was prepared using sterile filter paper discs (6.0 millimeter (mm) in diameter) immersed into appropriate concentration of the antimicrobial agents. The immersed diffusion discs was allowed to dry for about 5 min. The diffusion disc was immersed into the antimicrobial agents twice to ensure that the solutions completely diffused through the filter paper. The same procedures was repeated using the antibacterial products. Sterile distilled water has used as the experiment control solution.

2.7 Disc Diffusion Method

The Petri plates was divided into four quadrant. 1 mL of prepared inoculums was spread on the Mueller-Hinton agar using L-shaped glass spreader. The plate was allowed to dry for about 5 min before placing the diffusion discs immersed in the antimicrobials. By using sterile forceps, one diffusion disc with appropriate concentration of antimicrobial was placed in each of the four quadrants. The disc was pressed gently on the surface of the agar plates to ensure complete contact with the agar surface. The plates then were inverted and incubated for 24 h at 37 ˚C. After incubation, the diameter of the zone of complete inhibition was measured. The same procedures was repeated using the antibacterial products.

3. Results

The susceptibility of the antimicrobial agents and antibacterial products toward the isolated pathogens were determined based on the size of the inhibition zones.

3.1Commercial Antimicrobial Agents

Figure 1 showed the mean percentage of inhibition of commercial antimicrobial agents testing on the pathogens.

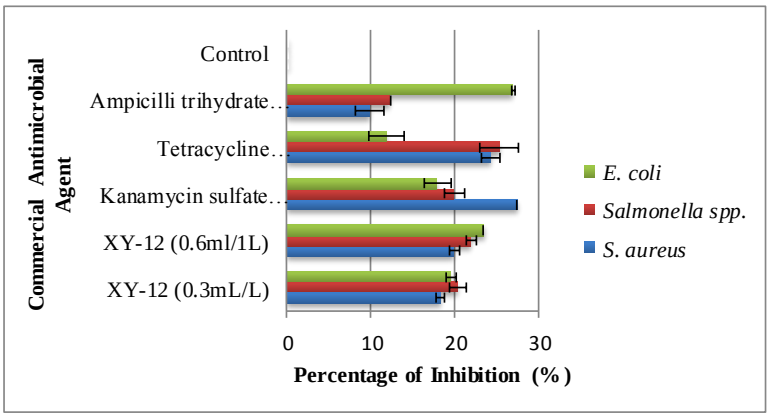

Figure 1. Mean percentage of inhibition of commercial antimicrobial agents testing on the pathogens.

Based on Figure 1, it shows that isolated foodborne pathogens have different susceptibility towards different commercial antimicrobial agents. No inhibition observed on the control (sterile distilled water).

3.2 Commercial Antibacterial Products

Figure 2 showed the mean percentage of inhibition observed when the pathogens tested with the commercial antibacterial products.

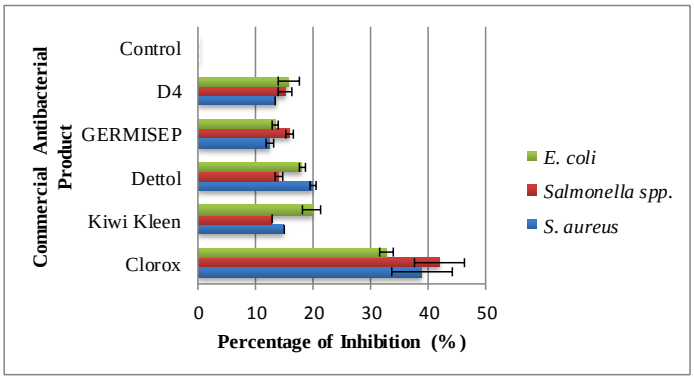

Figure 2 Mean percentage of inhibition zones of commercial antibacterial products testing on the pathogens.

Based on Figure 1, it shows that isolated foodborne pathogens have different susceptibility towards different commercial antibacterial products. No inhibition observed on the control (sterile distilled water).

4. Discussion

4.2 Commercial Antimicrobial Agents

Based on the results in Figure 1, pathogens isolated showed variable susceptibility to the commercial antimicrobial agents tested. *E. coli* showed highest susceptibility towards Ampicillin trihydrate (27%), followed by Chlorine bleach XY-12 (0.6mg/1L) (23.5%), Chlorine bleach XY-12 (0.3mg/1L) (19.6%), Kanamycin sulfate (18%) and lastly Tetracycline hydrochloride (11.9%). Salehi and Bonab (2006) supported this finding. According to the study by Salehi and Bonab on the *E. coli* strains isolated from chicken, *E. coli* has higher susceptibility toward Ampicillin compared to Kanamycin and Tetracyclin.

Meanwhile, *Salmonella* spp. showed highest sensitivity towards Tetracycline hydrochloride (25.3%) and but less sensitivity on Ampicillin trihydrate (12.3%). Tetracycline is a broad-spectrum antimicrobial agent that can inhibit the protein synthesis of the bacteria (Chopra and Robert, 2001). Similar resistance pattern of *Salmonella* spp. on Ampicillin was also observed on *Salmonella* spp. recovered from human, food, water, and animal samples (Elmadiena *et al.*, 2012; Habrun *et al.*, 2010).

The results show that Grams-negative bacteria are more resistant to antimicrobial agents compared to Gram-positive bacteria. This is because Grams-negative bacteria cell wall has high amount of lipopolysaccharide substance (LPS) (Tortora *et al.*, 2012). LPS is made of combination of lipids and carbohydrates, which provide stability to the cells, hence prevent the cell wall from easily breakdown or cell lysis (Mossel *et al.*, 2005). Even though both *E. coli* and *Salmonell*a spp. were Gram-negative bacteria, the susceptibility and the resistance toward the commercial antimicrobial agents was contrast with each other. These results emphasized that resistance of the bacteria towards antimicrobial agents was not only depending on the type of cell wall but also influences by other factors. One of the factors that may affect the resistance of the bacteria towards the antimicrobials is mutation. Different mutation will yield different effect of the

bacteria reaction towards the antimicrobial agents. Some mutation cause bacteria produce potential chemical that can inactivate the antimicrobial agents, where some mutations can allow the bacteria to eliminate the cell target before antimicrobial agents attack by using a special mechanism to export the antimicrobial agents outside, so it will not reach the target cell (Tortora *et al*., 2012).

Meanwhile, compared to *E. coli* and *Salmonella* spp., *S. aureus* showed highest susceptibility on Kanamycin sulfate (27.5%) but resistance on Ampicillin trihydrate (9.9%). Resistance of *S. aureus* towards Ampicilin trihydrateand susceptibility toward Kanamycin sulfate was supported by Brinda and Faur (2010).Based on study by Brinda and Faur (2010), *S. aureus* has highest resistance towards Ampicilin Trihydrate compared to Tetracycline Hydrochloride and Kanamycin Sulfate. The resistance of *S. aureus* towards Ampicilin Trihydrate is caused by the production of Beta-lactamase by the tranducible R-plasmic in the stain (Adeleke and Olaitan, 2003).

4.3 Commercial Antibacterial Product

The only notable difference among the products tested was for product from Clorox. Clorox Kitchen Cleaner showed consistence large inhibition effect, about 32.8-42 % when tested on pathogens. This indicated that most effective commercial antibacterial products to decontaminate *E. coli*, *Salmonella* spp. and *S. aureus* were product from Clorox. Clorox Kitchen Cleaner contains sodium hypochloride. Exposure of bacteria strains to high dose of hydrochloric acid can cause the chlorine dioxide diffused through the bacteria external membrane. The oxidizing effect will inhibit the cellular respiration, resulting lower in the production of adenosine triphosphate (ATP) (Maris, 2005).

Meanwhile, the most less effective antibacterial products in decontaminating the pathogens was GERMISEP. The manufacturer claimed that GERMISEP is effective against broad-spectrum microorganisms but results from the finding were contradicted with the claims. The effectiveness of an antibacterial product depends on several factors. According Tortora *et al.* (2012), the effectiveness of an antibacterial is influenced by the antibacterial concentration, organic content, degree of contact with the microorganisms, exposure time, pH and temperature.

5. Conclusions

E. coli, *Salmonella* spp. and *S. aureus* showed different response towards different commercial antimicrobial agents and antibacterial products. Some of the strains susceptible and some of them resistant towards the commercial antimicrobial agents and antibacterial products. From the results, this prove that there is no single antibacterial agents or antimicrobial products able to remove the foodborne pathogens from surfaces. Commercial antibacterial agents and commercial antimicrobial products can minimize the number of foodborne pathogens on the surfaces but it cannot ensure that the surface is sterile from contamination.

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The Detection of 8-Hydroxy-2′-Deoxyguanosine and 1-Hydroxypyrene as Biomarker of CancerRisk in Population Exposed by Benzo[a]Pyrene

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Abstract: This study was conducted to analyze the profile of DNA Adduct 8-Hydroxy-2′-Deoxyguanosine formation as DNA damage indicator, by measuring 8-Hydroxy-2′-Deoxyguanosine level in serum and 1-Hydroxy pyrene as benzo[a]pyrene metabolites from the population in Palangka Raya during the smoke haze period in 2015. In vitro study using 2′-deoxyguanosine-5'-monophosphate and benzo[a]pyrene also conducted. The object of study included 29 respondents from Palangka Raya as the exposed group and 23 respondents from Kota Batu as a control. The questionnaires were used to collect data related to medical history, smoking habit, occupation, life style and activity during the smoke haze period. The results obtained showed significant differences between 8-OHdG level in exposed group (P-value = 0.005) compared to the control, with the average value of the exposed group are 1.4 times higher than the control. The average value of 1-OHP in exposed group (P-value = 0.002) is 3.8 times higher than the control. The increased level of 8-OHdG and 1-OHP in the exposed group has significant correlation with long exposure. While in vitro study showed that benzo[a]pyrene can cause increasing 8-OHdG formation. This study provides evidence that there is a correlation between forest and peatland fires with oxidative DNA damage related to risk cancer.

Keywords: 8-OHdG; DNA Adduct; Forest and Peatland Fires; Palangka Raya

1. Introduction

Carcinogenic compounds such as Benzo[a]pyrene, which is one of the PAH compound,may contribute to the formation of reactive oxygen species (ROS) in the body. The most important oxygen-free radical,that is hydroxyl radical (HO•), can cause damage to basic biomolecules (proteins, membrane lipids, and DNA) and lead to the generation of a variety of oxidation products. The interaction of HO• with nucleobases of DNA leads to the generation of radical adducts (Valavanidis., 2009).The existence of Poly Aromatic Hydrocarbon (PAH) in nature can come from two sources, namely natural resources and anthropogenic sources. The natural sourcesareforest and peatland fires, oil seepage, volcanoes, plants containing chlorophyll, fungi and bacteria (Ahmad., 2012).In Indonesia, forest and peatland fires happen almost every year and it occurs especially in Sumatera and Kalimantan such as Palangka Raya.Uncontrolled fires could give adverse effects on public health, conservation and significant climate changes (Someshwar., 2011).Therefore, this research was conducted by a scientific approach and correlation studies, to analyze the phenomenon the increased risk of cancer based on adducts level in serum samples collected from respondents in Palangka Raya as an exposed group and Kota Batu as a control group. The 8-OHdG was analyzed from serum samples and 1-Hydroxy pyrene (1-OHP) as biomarker of benzo[a]pyrenemetbolite was analyzed from urin samples. As a comparison, in vitro studies using dGMP and benzo[a]pyrene was also conducted.

2. Materials and Methods

2.1. Chemicals and reagents

8-OHdG 500 µg/mL (Sigma-Aldrich), methanol LC grade (Sigma-Aldrich), Enzyme β-glucuronidase/arylsulfatase (Sigma-Aldrich), Cartridge C18 Waters SEP-PAK (Sigma-Aldrich), 8-OHdG ELISA Kit (Sigma-Aldrich), DMSO, sodium acetate, sodium succinate hexahydrate, calcium chloride, $K_2HPO_4$and KH_2PO_4, benzo[a]pyrene, Na_2HPO_4, NaH_2PO_4, 2'-deoxyguanosine-5'-monophosphate.

2.2. Instrumentation

Incubator shaker (Julobo SW22), Sonicator and Degasser 8510 (Bronson), pH meter, ZORBAX Eclipse Plus C18 (4.6 x150 mm, 5-Micron), Membrane Filter PTFE Polypropylene backed 0.2 µm (Whatman), Eppendorf, vacuum tube 5cc, tourniquet, micro tube, alcoholpads, centrifuge, coolbox, urine tube, Vortex, ELISA reader, and HPLC-Fluorescence Detector, HPLC-UV Detector.

2.3. Sample Collection

Samples were collected from 29 respondents who have agreed to participate in the study and have signed laboratory informed consent to do blood sampling for 8-OHdG analysis. The sample selection was based on questionnaires that have been distributed since February 2015, with an election based on the criteria: no smoking, healthy or do not have a history of chronic diseases (such as asthma, heart disease, diabetes mellitus, dyslipidemia, hypertension, thyroid, liver dysfunction, chronic pulmonary disease , impaired kidney function, tumor/cancer, and other chronic diseases), aged 20-40 years, it does not work or cook using firewood/charcoal, and lived in Palangka Raya over 3 years. As comparison, the selected control group populations (23 respondents) live in area with the minimal contamination, namely Kota Batu.

2.4. Ethics and Study Protocol

The study protocol has been approved by the ethics committee Faculty of Medicine, Universitas Indonesia based on the number 897 / UN2.F1 / ETHICS / 2015. Prior to blood sampling, respondents have signed the informed consent as an evidence that they were approved and without compulsion to be included as a sample.

2.5. Urinary Sampling

Urine samples were used for 1-hydroxypyrene analysis as bioindicator of pyrene compounds exposure. The 24-hour urinary samples were collected and were taken to the laboratory and stored at -20^{o} C. Then 50 mL aliquot urine were separated and stored in -80^{o}C before used for further analysis.

2.6. Analysis of 8-OHdG in Serum

The method used for analysis of 8-OHdG in serum samples was the Enzyme-linked Immunosorbent Assay (ELISA). The kit used is Highly Sensitive 8-OHdG Check Enzyme Linked Immunosorbent Assay (ELISA) kits from Japan Institute for The Control of Aging (JAICA, Fukuroi City, Japan). This method is a competitive antibody that utilizes monoclonal antibody (Clone N45.1) that highly specific for DNA damage. Before analysis, the serum samples undergo a pretreatment using Filter Ultra 10K devices (Amicon, Millipore). Previously, filter incorporated into micro-tubes. Sterilized water 500 mL put in a filter for prerinsing. Then, filter was centrifuged using micro-centrifuge at a speed of 14,000 g for 10 minutes. The residual water was removed from the filters. Furthermore, 500 mL of serum samples was added to the filter and then

was centrifuged at a speed of 14,000 g for 30 minutes. The filtrates were separated for analysis. For about 50mL samples or standards were placed into the plate or well and 50 µL primary antibody solution as a specific monoclonal antibody for 8-OHdGwere added. The plates were shaken gently and sealed using parafilm and then were incubated overnight at 4°C. To eliminate the antibodies binding with 8-OHdG serum, the plates were washed three times with 250 µL wash solution containing phosphate buffered saline (PBS). About 100 µL secondary antibody solution were added to the plate and were shaken gently. The plate was covered by adhesive tape and incubated for 1 hour at room temperature. The secondary antibody contains conjugate Horseradish Peroxidase (HRP) enzyme. The secondary antibody will bind to monoclonal antibody which has been bounded to 8-OHdG in the plate.

To remove the excess of unbounded secondary antibody to monoclonal antibody, the plate was then washed three times with 250 µL cleansing solutions and 100 µL chromogensolution was added to the plate before incubated in the dark for 15 minutes.

As a results, the color of the solution will turn to bright blue, because of HRP enzymes catalyze the oxidation reaction of chromogenic substrates by hydrogen peroxyde generating colored product. The reaction was stopped by added 100 µL formic acid as terminating solutions, which make the color change from bright blue to yellow. The color that produced was equal with amount of antibody bounded to the plate and inversely proportional to 8-OHdG level in serum. The plate was then read using ELISA Reader (Vmaxmicroplate reader, Molecular Device) with software SOFTmax Pro.

2.7. 1-OHP Analysis

Five milliliters of urine were transferred to a conical flask and added 300 µL acetate buffer 2 M (pH 5.0). In additional of 75µL β-glucuronidase/arylsulfatase enzyme, the solutions werethen incubated in a shaker bath at 50ºC for overnight. Furthermore, the hydrolyzed urine samples were loaded into cartridges C-18 (SPE cartridge, SEP-PAK VAC C-18, Waters, Miliford, MA, United States) that had been pre-conditioned with 2 mL of methanol and 2 mL of water. The cartridge was sequentially washed with 2 mL of water. The hydrolyzed urine samples were sequentially eluted by 5 mL of methanol. The samples were then evaporated under nitrogen gas at 60°C and re-dissolved in 1mL of methanol. The solution was filtered using 0.2 µm filter and then stored at −20°C before HPLC analysis using (LC-20AT, Shimadzu) Fluorescence detector (RF-20A), reversed phase C18 column (250 mm x 4.6 mm; 5 µm). The eluentwas a mixture of methanol: water (85 : 15). Analysis were conducted at excitation wavelength of 242 nm and emission wavelength of 388 nm.

2.8. Statistical Analysis

The result of analysis was determined as mean ± deviation standard. The normality data were tested using sharpio-wilk method. The level of 8-OHdG data were normalized by using log10. Analysis of Variance (ANOVA) and t-test were used to identify a significantly difference between risk variable and 8-OHdG level. The significant factor is $P < 0.05$.

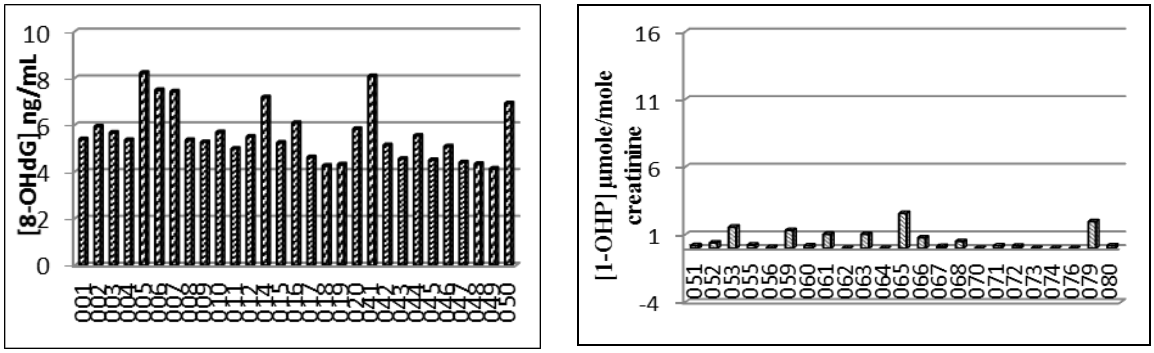
2.9. In Vitro Study

The in vitro studies was conducted by reacting various composition of the reactants. The variations of the compositions were the reaction between500 ppb dGMP pH 7.4 with3000 ppb Fe(II); 500 ppb dGMPpH 7.4 with 3000 ppb Fe(II) and3000 ppbbenzo[a]pyrene; 500ppb dGMP pH 7.4 with 3000 ppb H_2O_2; 500 ppb dGMP pH 7.4 with 3000 ppb H_2O_2 and 3000 ppb benzo[a]pyrene; 500 ppb dGMP pH 7.4 with 3000 ppb Fe(II) and 3000 ppb H_2O_2; 3000 ppb dGMP pH 7.4 with 6000 ppb Fe(II), 6000 ppb H_2O_2, and 3000 ppb benzo[a]pyrene. The solutions were incubated for 5 hours at a temperature of 37°C. The 8-OHdG levels in the samples were then analyzed using HPLC with UV detector.

3. Results

3.1 The level of 8-OHdG in Serum Samples

Analysis of 8-OHdG level showed that the mean and graphic of 8-OHdG level in the exposed group serum samples as much as 5.606 ± 1.162 ng/mL with range of level 4.137–8.228 ng/mL. Meanwhile, the mean of 8-OHdG level in control group was 4.059 ± 0.709 ng/mL with range of 8-OHdG level 2.166 - 4.915 ng/mL (Figure 1 and Figure 2). This results describe that the mean of 8-OHdG level in exposed group was 1.4 times higher than control group. Therefore there was a significant difference in 8-OHdG level between the exposed and control group (P-value = 0.005).



(**a**) (**b**)

Figure 1.a) 8-OHdG analysis of exposed samples b)8-OHdG analysis of control samples

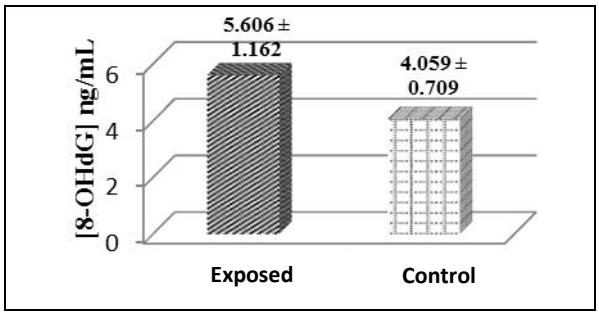
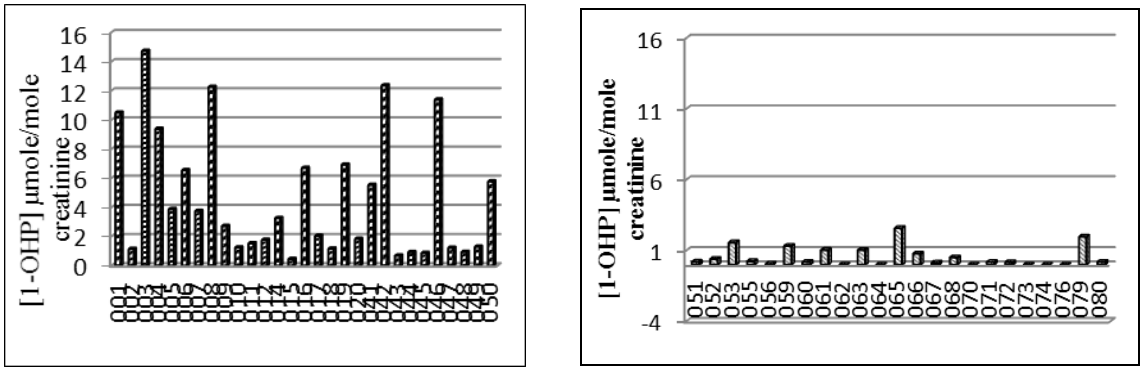


Figure 2. Average of 8-OHdG level of exposed and control samples

3.2. The level of 1-OHP in Urine Samples

Analysis of 1-OHP level showed that the mean in urine samples was 4.57 ± 4.27 µmole/mole creatinine with range of level 0.23 – 14.71 µmole/mole creatinine for exposed group and 0 – 3.735 µmole/mole creatinine for control group (Figure 3 and Figure 4). The results describe that the mean of 1-OHP level in exposed group was 3.8 times higher than control group (0.73 ± 0.75 µmole/mole creatinine).



(a) (b)

Figure 3.a) 1-OHP analysis of exposed samples b)1-OHPanalysis of control samples

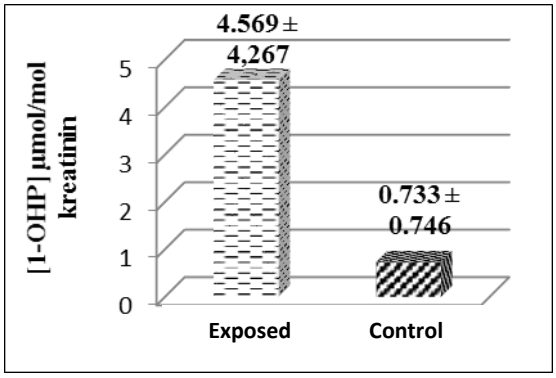


Figure 4. Average of 1-OHP level of exposed and control samples

3.3 In Vitro Study

In vitro studies showed that the addition of benzo[a]pyrene increases the concentration of 8-OHdG. The 8-OHdG levels produced in the reaction between dGMP with Fe(II) were 6.04 ppb; dGMP with H_2O_2 were not detected; and dGMP with Fe (II) and H_2O_2 were 6.38 ppb which are smaller compared to the addition of benzo[a]pyrene that were 12.91ppb, 8.85 ppb, and 12.97 ppb. The effect of benzo[a]pyrene addition in 8-OHdG formation can be seen in Figure 5.

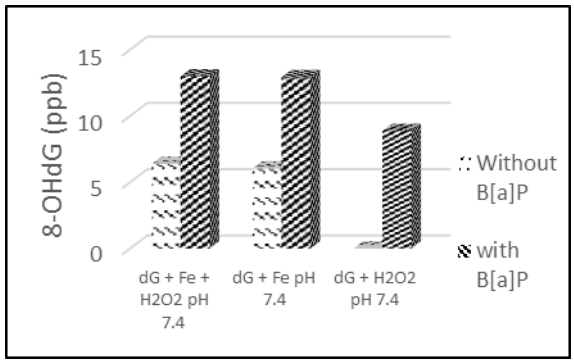


Figure 5. Effect of addition benzo [a] pyrene

4. Discussion

4.1 The level of 8-OHdG in Serum Samples

The increasing of 8-OHdG level in exposed group can indicate that there is an effect of smokes exposure as higher as oxidative stress in exposed group compared with control group. The 8-OHdG is a biomarker of endogenous DNA damage which appear from hydroxyl radical interaction with guanine base in DNA. Hydroxyl radical can be produced in the body either by endogenous or exogenous from xenobiotics exposure such as particulate matter in ambient air (Valavanidis., 2009). The forest and peatland fires in Palangka Raya and some of area in Indonesia have significantly increased the air pollutant in ambient air, such as particulate matter (PM10, PM2.5, PM1). Some studies have reported that there is positive correlation between particulate level in ambient air with the increasing of 8-OHdG level (Benson., 2013).

4.2. The level of 1-OHP in Urine Samples

There was significant difference in 1-OHP level between exposed group and control group (P-value = 0.002). Nevertheless, the average value in each of risk variables shows the significant differences values with the level of 1-OHP. The significant value between 1-OHP level in exposed group (Palangka Raya) and control group (Kota Batu) is P-value = 0.005 ($< \alpha = 0.05$). The detection of 1-OHP in urinary samples of exposed group in Palangka Raya indicates that peatland and forest fire smoke contains carcinogenic compounds such as Benzo[a]pyrene.

4.3. In Vitro Study

Benzo[a]pyrene as a xenobiotic could increase the generation of free radicals. Metabolism of benzo[a]pyrene will generate Reactive Oxygen Species (ROS) such as superoxide, peroxide, and hydroxyl radical (Zhang., 2013). The hydroxyl radical (OH•) could be interacted with DNA and generating DNA Adduct. Furthermore, hydroxyl radical and DNA guanine bases will form 8-OHdG compounds (Gao, 2005).

5. Conclusions

From this research, Benzo[a]pyrene compounds were contained in forest fire smokes proved by the detection of 1-OHP in urine samples of exposed group in Palangka Raya. Benzo[a]pyrene compounds could increase oxidative DNA damage from the detection of 8-OHdG in serum samples of exposed group in Palangka Raya. Statistical data analysis shows the significant increasing of 8-OHdG and 1-OHP in exposed group's serum samples and were affected by the smokes exposure duration. In vitro study shows that the addition of benzo[a]pyrene to the reaction can caused the increasing of 8-OHdG formed. This in vitro study has provide a certainity that benzo[a]pyrene could increase oxidative DNA damage.

Acknowledgments: This research grants from PUPT BOPTN 2015-2016 during the conduct of the study.

Abbreviations

The following abbreviations are used in this manuscript:

8-OHdG : 8-hydroxy-2′-deoxyguanosine
dGMP : 2′-deoxyguanosinemonophosphate
PAH : Poly Aromatic Hydrocarbon

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Evaluation of Gold Nanoflowers For Signal Enhancement of Lateral Flow Immunoassay

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Abstract: Background: Lateral flow immunoassay (LFIA) is a paper-based device commonly used for detection of various types of analytes with the advantages of low-cost, rapid test, mobile and variety of applications. Gold nanoparticles (AuNPs) in spherical shape mostly used as label in LFIA device due to its conjugation properties and visibility by naked eyes. However, disadvantage of AuNPs as labelling agent is less sensitivity. This work evaluates the use of multi-branched gold AuNPs known as gold nanoflowers (AuNFs) synthesized using the seeded-growth (SG) method to enhance the sensitivity of LFIA devices. Methods: The 40 nm spherical AuNPs and AuNF were synthesized using the SG methods. AuNPs and AuNFs were characterized and conjugated with mouse anti-gram negative endotoxin antibody. Nitrocellulose (NC) membrane HF120 was used with anti-mouse IgG antibody as control dot. Then conjugated AuNP and AuNF were flowed onto NC membrane and finally washed with phosphate buffered saline pH 7.2. A control dot intensity was observed. Results: AuNFs have higher wavelength 630 nm compared to AuNP 526 nm. Morphology observed using transmission electron microscope shows that AuNFs have flower-like shape with 10 to 15 projectile. Control dot with AuNFs gave higher intensity compared to AuNPs. Conclusion: AuNF based LFIA was more sensitive compared to spherical AuNPs.

Keywords: gold, nanoflowers, flow

1. Introduction

The lateral flow immunoassay strips (LFIA) is a paper-based biosensor device applying a modern version of immunoassay (IA) with the advantages such as rapid, low limit of detection, high sensitivity, good specificity, low manufacturing cost, less sample operation volume, robustness, user-friendly format and no complicated equipment needed (Quesada-González and Merkoçi, 2015). Furthermore, LFIA strips operated for on field detection which utilizes a dry form of reagents incorporated within this device and read through the naked eye (Fu et al., 2011, Huang et al., 2016). Based on these advantages, it was widely used as a point of care device due to the low-cost material and manufacturing.

Nowadays, LFIA strips used nanomaterials as label which improve their performance for various applications (Quesada-González and Merkoçi, 2015, Huang et al., 2016). Such nanomaterial-based label are colloidal AuNPs, silver, carbon, selenium, quantum dots, up-converting phosphors, dye-doped, magnetic and many more recently which available for qualitative, semi-quantitative as well as quantitative detection with the aid of simple reader

device (Huang et al., 2016). However, AuNPs is the most label used due to easy to synthesize and manipulate, stable in time, size-tunable, biocompatible and have an intense red colour that easy to be detected even by naked eye or usually using colour readers to achieve better detection limits (Quesada-González and Merkoçi, 2015). However, from the previous works the sensitivity of AuNPs based LFIA strips is low (Yonekita et al., 2013, Zhao et al., 2010).

To improve this limitation, signal enhancement strategies have been introduced for AuNPs LFIA strips such as using silver enhancement technology (Shyu et al., 2002, Yang et al., 2011); pre-enrichment treatment of the detection samples by magnetic nanoparticles and AuNPs before using LFIA strips for signal amplification (Nash et al., 2012), post-enrichment such as enzyme signal amplification strategy (Cho et al., 2015a) and surface enhance Raman spectroscopy (Cho et al., 2015b), optimization of antibody conjugated to the surface of AuNPs on the conjugation pad (Zhang et al., 2011), using a dual gold method (Choi et al., 2010, Mei et al., 2013), gold nanocomposites such as core-shell structured Ag/Au nanoparticles (Liao and Li, 2010), magnetic nanogold microspheres (Tang et al., 2009) and shape modification such as AuNFs (Ji et al., 2015).

However, most of introduced methods need complicated instrumentations and multi-steps operation which prevent the wide use for on-field detections in many basal laboratories and un-developed districts. Ji et al., (2015) have shown the simple probe preparation of AuNF LFIA that does not require additional operations for fabrication and relatively has high sensitivity. The multi-branched (>10 tips) AuNFs exhibits higher optical extinction because the tips and core–tip interactions act as an antenna producing electromagnetic field enhancements and exhibit localized surface Plasmon resonances (SPR) resulting in strong optical extinction at visible wavelengths. Besides, the AuNF presents a better colloid-stability and larger total surface area than that of the same size gold nanospheres (AuNS) due to its complex three-dimensional structure. Moreover, the large specific surface area of the AuNFs is in favour of improving antibodies immobilization yield because of the reducing steric hindrance of protein towards the surface of AuNFs.

Despite that, synthesizing AuNFs by Ji et al., (2015) method produces OH^- negative charge covering their surface. According to Makhsin et al., (2012), colour intensity of the AuNPs synthesized with surface OH^- is lower compared to NH_2^- surface charge. To obtain this NH_2^- surface charge, AuNFs need to be synthesized using hydroxylamine as reduction agent as proposed by Zhao et al., (2009). Thus, in this work AuNFs was synthesized using the SG method to produce NH_2^- surface charge. This AuNFs was then conjugated with antibody and tested in LFIA strip. The properties of AuNFs in LFIA was compared accordingly with AuNPs in terms of conjugate colour intensity of control dot.

2. Materials and Methods

2.1 Chemicals and materials

Gold chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), sodium citrate tribasic dehydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$), bovine serum albumin (BSA), potassium carbonate (K_2CO_3), citric acid monohydrate ($C_6H_8O_7 \cdot H_2O$), HCl, HNO_3, Tris-HCl and phosphate buffered saline (PBS) were obtained from Sigma-Aldrich (Milwaukee, USA). Hydroxylamine hydrochloride ($NH_2OH \cdot HCl$), sodium hydroxide (NaOH), sodium chloride (NaCl), absorbance pad and Hi-flow Plus 120 Nitrocellulose (NC) Membrane Cards (HF120) were supplied by Merck Milipore (Darmstadt, Germany). Mouse anti-gram negative endotoxin antibody (302) (GNE) and bovine anti-mouse IgG secondary antibody were purchased from Invitrogen, Thermo Scientific (Massachusetts, USA). Blocking solution was bought from Roche Diagnostics (Risch-Rotkreuz, Switzerland).

The shape and particle sizes of AuNFs were examined using transmission electron microscopy (TEM) (FEI CM12 version 3.L) at 120 kV. The dispersity of the as synthesized AuNPs and AuNFs was characterized using Zetasizer particle size analyser (Malvern Nanoseries N3600).

The UV-Vis-NIR spectrophotometer (Model UV-3600, Shimadzu) was used to characterize absorbance properties of AuNPs and AuNFs before and after conjugation.

Prior to synthesis of AuNPs and AuNFs, all glassware was cleaned in a bath containing aqua regia (HCl: HNO_3, 3:1, v/v). Then this glassware was rinsed with ultrapure water and leaved to dry before used. Ultrapure water was prepared by Milli-Q (18.2 MΩ cm at 25°C), Merck Milipore (Darmstadt, Germany).

2.2 Synthesis of Au seed using the citrate reduction method

The Au seeds of 15 nm and 25 nm sizes were synthesized to make 40 nm AuNPs and AuNFs respectively using a typical citrate reduction method (Grabar et al., 1995, Tang et al., 2011, Makhsin et al., 2012, Zhao et al., 2009). Briefly, $HAuCl_4 \cdot 3H_2O$ (0.01% w/v) was separately prepared in two Erlenmeyer flasks and heated to boiling under continuous stirring. Then, 2.5 and 1 mL of 1 and 5% (w/v) $Na_3C_6H_5O_7 \cdot 2H_2O$ respectively were immediately added into the solutions each to obtain 15 and 25 nm sizes. The solutions were leaved to boiled until the colour changed from light yellow to faint blue, grey-blue and finally to dark red or red wine where spherical particles were formed. After that, the solutions were removed from heat and stirred for a few minutes and cooled to room temperature. Samples from both solutions were taken for characterization using UV-Vis-NIR spectrometer and Zetasizer.

2.3 Synthesis of 40 nm AuNP via the seeding-growth method

The 15 nm Au seeds were grown into 40 nm AuNPs via the seeding-growth method as described previously by Makhsin et al., (2012). In brief, 4 mL of 15 nm Au seeds were added into an Erlenmeyer flask containing 50 mL ultrapure water. Then 1 mL of 0.1 M $NH_2OH \cdot HCl$ was added into the solution and leaved under stirred condition at room temperature for a few minutes. Finally, 0.5 mL of 25.4 mM $HAuCl_4 \cdot 3H_2O$ was added drop wise while stirring. This reduction process completed within 5 minutes. Sample from the solution was taken and characterized using UV-Vis-NIR spectrometer and Zetasizer.

2.4 Synthesis of AuNF via the seeding-growth method

The 25 nm Au seeds were grown into multi-branched gold nanoflowers (AuNFs) via seeding-growth method as described previously by Zhao et al., (2009) except the adjusted pH of Au salt solution was pH 12. In brief, 0.25 mM of $HAuCl_4 \cdot 3H_2O$ was adjusted to pH 12 by adding 1 M NaOH solution. Then, prepared 25 nm Au seeds was mixed with 40 mM $NH_2OH \cdot HCl$ and leaved for a few minutes while mild shaking by vortexer a few times. After that, this solution was added into previously prepared pH 12 of $HAuCl_4 \cdot 3H_2O$ solution at 25°C followed by mild shaking using vortexer. The molar ratio of these mixture $HAuCl_4 \cdot 3H_2O$, $NH_2OH \cdot HCl$ and Au seed was 14:17:1. The colour of the solution turned from light yellow to pale pink and then blue green to form complete reaction. Sample from the solution was taken and characterized using TEM, UV-Vis-NIR spectrometer and Zetasizer.

2.5 Conjugation of mouse anti-gram negative endotoxin (GNE) to AuNPs (AuNPs-MαGNE) and AuNFs (AuNFs-MαGNE)

Firstly, pH of colloidal gold solution was adjusted to slightly higher than the isoelectric point (IEP) of the GNE antibody. For mouse IgG2a, IEP was around 6.4 to 7.6 (Danielsson et al., 1988). Therefore, for AuNPs with lower pH, 0.2 M of K_2CO_3 was added until the pH of the solution became 7. However, for AuNFs, it has higher pH and need to lower down. As a result, 0.2 M of $C_6H_8O_7 \cdot H_2O$ was added until pH lowered to 7. The remaining procedure was similar as

described previously (Makhsin et al., 2012). Briefly, the optimum concentration of MαGNE antibody for stabilizing pH 7 of AuNPs and AuNFs was determined by incubated the serial dilution of MαGNE antibody to the fixed volume of either AuNPs or AuNFs for 15 min at room temperature. Then, the optimum concentration was chosen by observing the minimum concentration of MαGNE that unchanged colour after 10% NaCl was added. After that, 1 ml of pH 7 AuNPs and AuNFs was added with the concentration of previously optimized MαGNE antibody. The solution was then gently mixed and incubated at room temperature in rotary condition for 30 mins. BSA was added into this solution to become 1% at final concentration to stabilize and block unbound sites on the AuNPs and AuNFs surfaces. After vortexing, the solution was centrifuged for 10 min at 10 000 rpm for washing step. The supernatant was removed and pellet was resuspended in 1% BSA. This step was repeated twice. Finally, 1% BSA was added to resuspended the final pellet of conjugation and stored at 4˚C until used. Sample from the conjugation was taken and characterized using UV-Vis-NIR spectrometer.

2.6 Preparation of LFIA and assay procedure

The absorbent pad was attached to the top of HF120 NC membrane backing sheet with 2 mm overlapping area. The strips then were cut into 5 mm width using a strip cutting machine. Then, 1 μL of bovine anti-mouse IgG secondary antibody at concentration of 1 mg/mL was pipetted onto HF120 NC membrane which function as a control dot. No test dot was used in this work as it is just as a proof of concept experiment. The dotted NC membrane was then dried at 37˚C for 2 h in an oven. After that, the NC membrane was soaked in a blocking solution containing 10% casein, Tris-HCl and 0.01 M TBS pH 7.5 to block remaining protein binding sites. After blocked, NC membrane then was dried again overnight at 37˚C in the oven. Figure 1 shows the schematic diagram of LFIA strips. Finally, dried strips were sealed and stored in a desiccator.

The LFIA strips then was tested against AuNPs-MαGNE and AuNFs-MαGNE conjugate. Firstly, AuNPs-MαGNE conjugate was diluted to OD 10 and OD 2 and only OD 2 for AuNFs-MαGNE conjugate using PBS buffer to the final volume of 30 μL per conjugate in a well of microtiter plate. Then, the bottom down of strip was placed in well containing either AuNPs-MαGNE or AuNFs-MαGNE conjugate and at the same time the timer was started. The conjugate then was flowed on the strip until it reached the absorbent pad. After 30 min the strip was washed with solution containing PBS buffer pH 7.2 for about 5 min until it completely reached absorbent pad and removed all background colour on the strip. Colour intensity of the control dot for both conjugate was observed and compared.

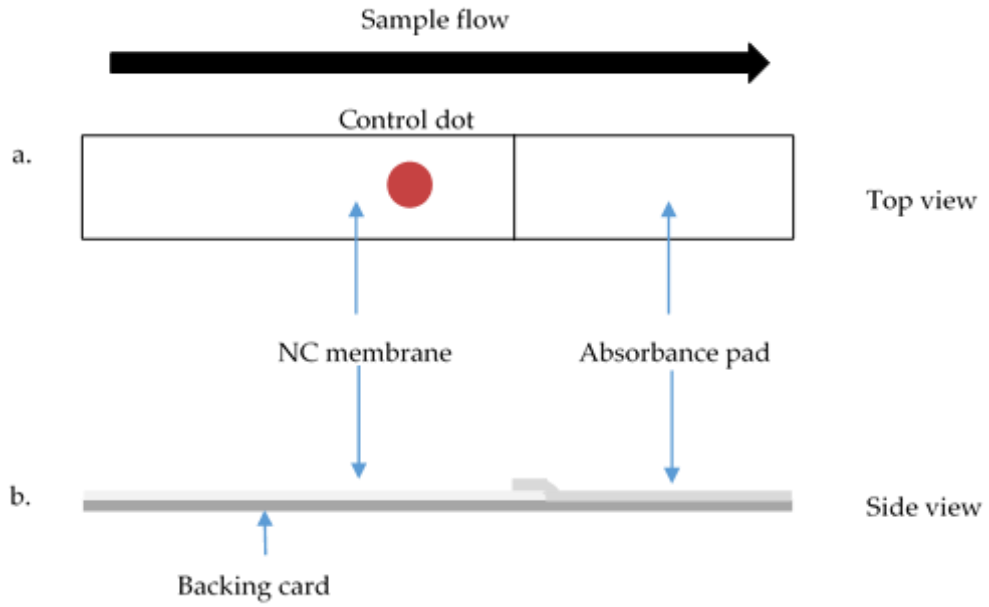


Figure 1.
Schematic diagram of LFIA strips: (a) Top view and arrow of sample flow; (b) Side view of LFIA strips.

3. Results

3.1 Characterization of Au seed, 40 nm AuNPs and AuNFs

The Au seed of 15 nm and 25 nm were successfully synthesized using the citrate reduction method. As shown in Figure 2 (a) and (b), 15 nm and 25 nm Au seeds have a well-dispersed distribution of size with the average particles size (Z average) of 19.96 nm and 29.26 nm, respectively. Characterization of 15 and 25 nm Au seed by UV-Vis-NIR spectrometer as shown in Figure 3 respectively show peak between 519 to 520 nm. These results are consistent with optical colour observed (Figure 4 (a) and (b)) and represent the colloidal gold particles. Moreover, these two Au seeds are almost similar and closely to absorbent OD 1 as shown in Figure 3.

The 40 nm AuNPs and AuNFs were also successfully synthesized using the seeding-growth method. As shown in Figure 2 (c) and (d), 40 nm AuNPs and AuNFs have a well-dispersed distribution of size with the Z size average of 41.46 nm and 49.78 nm, respectively. Figure 3 shows peaks at 526 nm for 40 nm AuNPs whereas AuNFs shows peak at 630 nm. This red-shift from 526 nm to 630 nm are corresponding to optical colour changes from red for AuNPs to blue for AuNFs as shown in Figure 4 (c) and (d), respectively. Besides that, OD for AuNFs is lower than AuNPs which mean low concentration of AuNFs present compared to AuNPs. Result of zeta potentials as shown in Figure 2 (c) and (d) demonstrate the colloidal stability of AuNPs and AuNFs. Zeta potential of AuNFs (-36.8 mV) is higher than AuNPs (-24.0 mV) about 12.8 mV which indicates AuNFs is more stable than AuNPs even though the size is not much different. Similar finding was also observed in previous work (Ji et al., 2015).

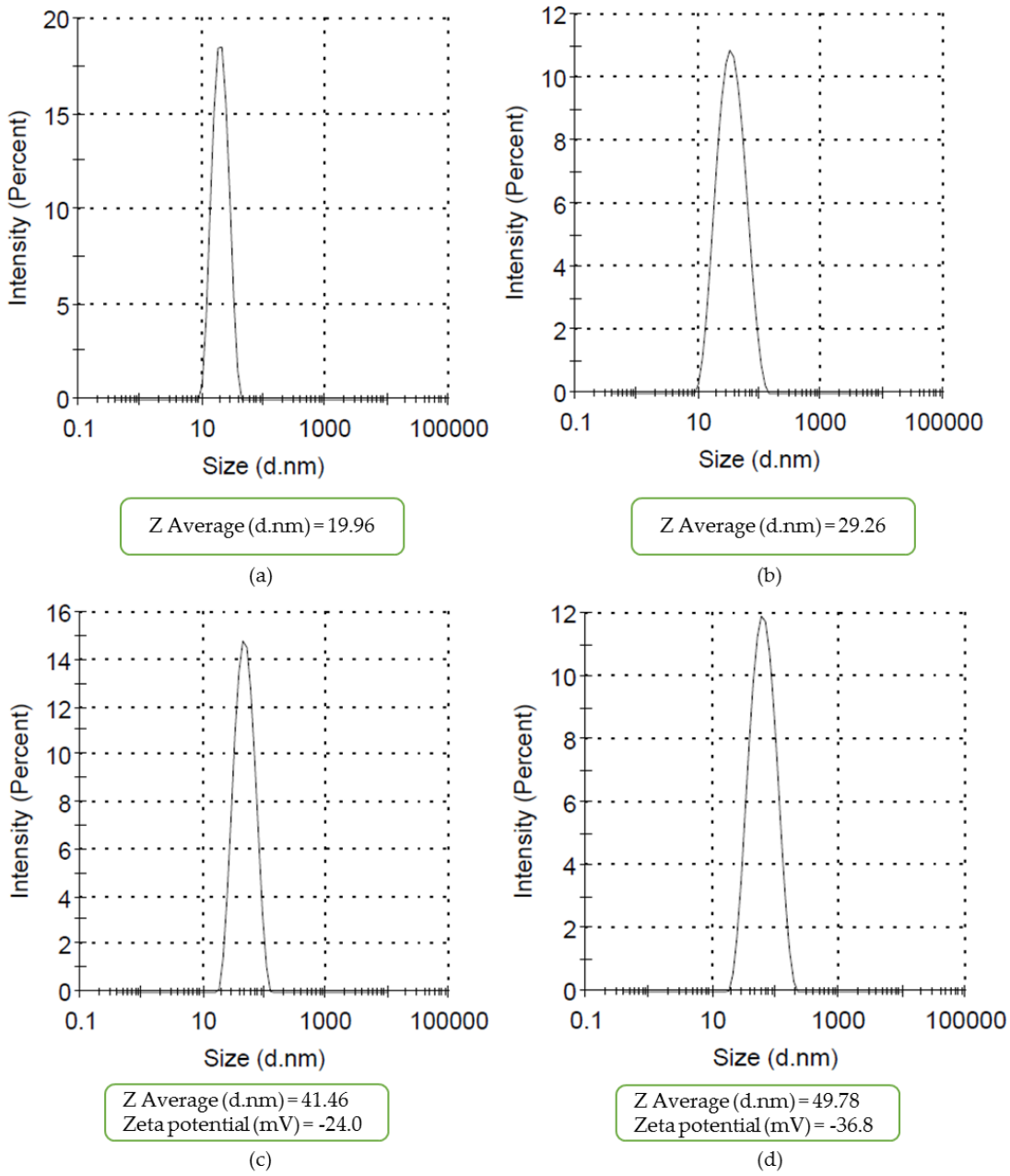


Figure 2. Size distribution by intensity of: (a) 15 nm Au seed; (b) 25 nm Au seed; (c) 40 nm AuNPs with zeta potential result; (d) AuNFs with zeta potential result.

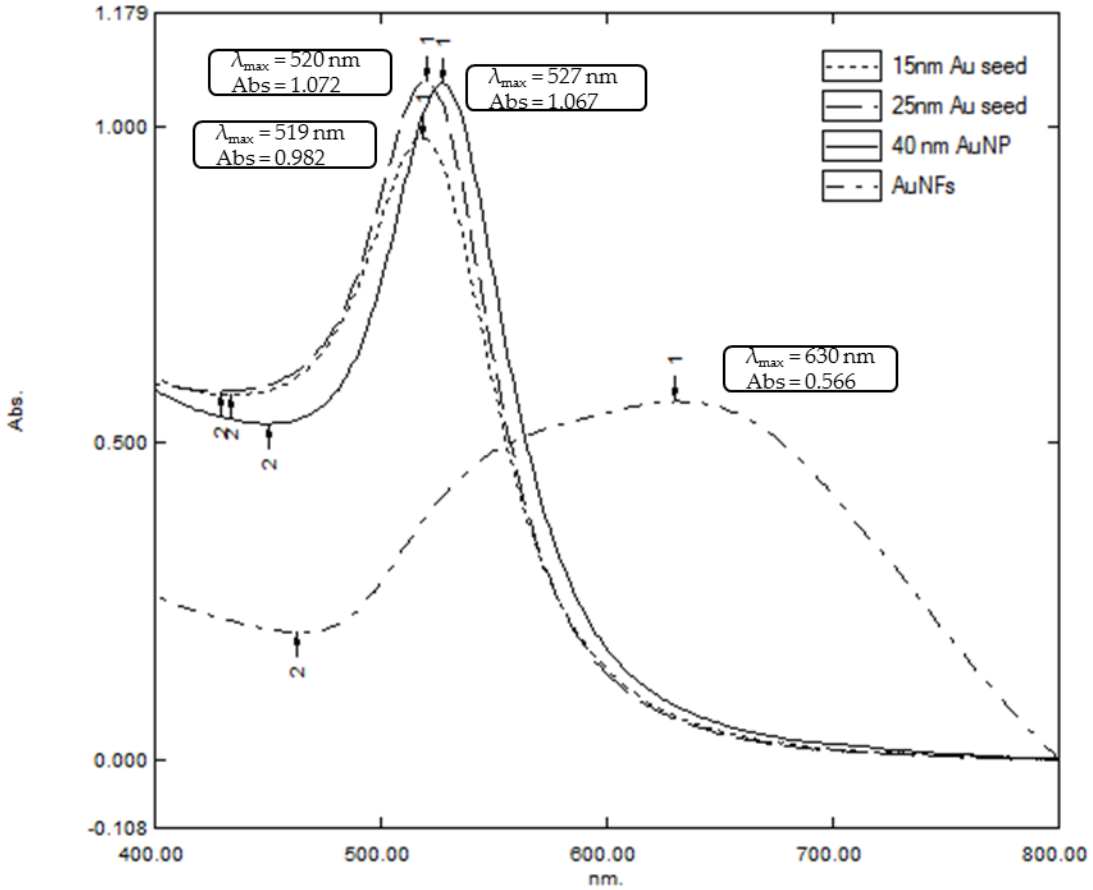


Figure 3. UV-visible spectra of 15 nm Au seed, 25 nm Au seed, 40 nm AuNPs and AuNFs.

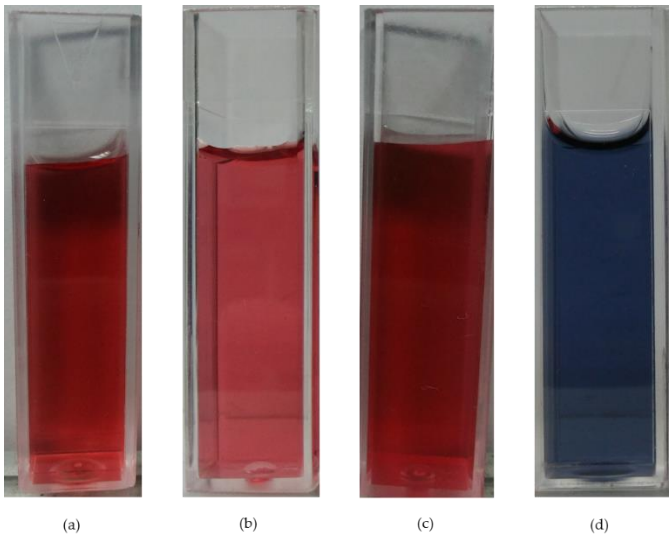


Figure 4. Visual colour of: (a) 15 nm Au seed; (b) 25 nm Au seed; (c) 40 nm AuNPs; (d) AuNFs

To confirm that AuNFs has a multi-branched projectile shape, the sample was observed using TEM. The TEM image for AuNFs (Figure 5) shows a flower-like shape with complex multi-branches with smooth edges of petals attached to solid core in the centres of it. The size distribution of this AuNFs as seen under TEM is between 40 to 50 nm closely to result obtained by Zetasizer analysis.

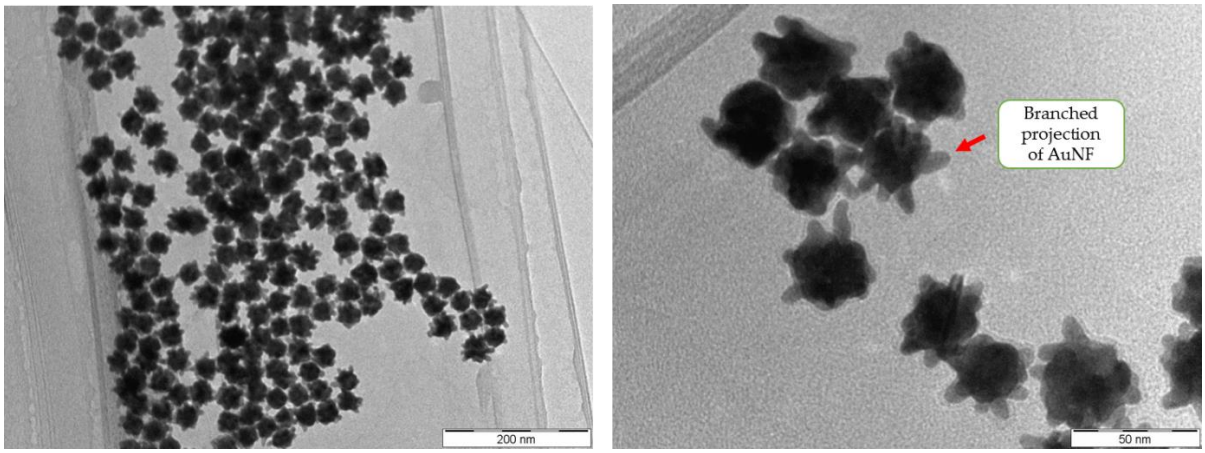
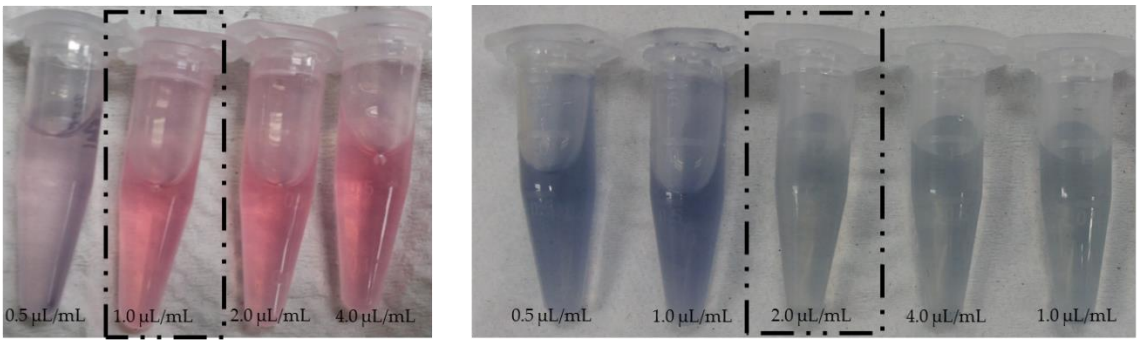


Figure 5. TEM image of AuNFs.

3.2 Characterization of AuNPs and AuNFs conjugates

The minimum amount of M*α*GNE antibody needed to stabilize AuNPs was successfully determined. AuNPs and AuNFs adjusted pH 7 are suitable and contributed to the successfulness of the conjugation. As shown in Figure 6, minimum concentration of M*α*GNE that prevent colour changes to blue was taken as optimum amount for conjugation. In comparison, the AuNFs requires more M*α*GNE antibody (Figure 6 (a)) to be stable compared to AuNPs (Figure 6 (b)).

The successfulness of conjugation can be confirmed by analyse the peak shift using UV-Vis-NIR spectrometer analysis before and after conjugation. The peak shifted for both AuNPs and AuNFs from their initial peak after conjugated with M*α*GNE antibody as shown in Table 1. This analysis proved that AuNPs and AuNFs surface were successfully conjugated with M*α*GNE antibody. At the same time, OD for both conjugates was taken to determine the concentration of the conjugates. Conjugates for AuNPs and AuNFs have concentration of OD 30 and OD 6, respectively. The lower OD for AuNFs conjugate is due to low concentration of AuNFs obtained from the earlier synthesis.



(a) (b)

Figure 6. Red box shown the minimum concentration of MαGNE antibody need to stabilized pH 7 of: (a) 40 nm AuNPs; (b) AuNFs.

Table 1. UV-visible spectra result with peak wavelength and absorbent in OD before and after conjugation of 40 nm AuNPs and AuNFs.

Au shape	Before conjugate		After conjugate	
	λ_{max} (nm)	Abs. (OD)	λ_{max} (nm)	Abs. (OD)
40 nm AuNP	527	1.067	531	0.306
AuNF	630	0.566	579	0.060

3.3 LFIA test

The result for LFIA was obtained by visual inspection (naked eyes) of colour intensity produced at the control dot. The dot colour for AuNPs appeared as red dot whereas for AuNFs, blue dot appeared. When tested with the lowest OD for both conjugate (OD 2), blue dot of AuNFs still appeared whereas red dot for AuNPs could not be seen as shown in Figure 7. The optimum time for both dot to be visible was 15 min. The PBS pH 7.2 was successfully used to remove interference of background colour. This made the dot colour could be clearly observed. The presence of control dots also proved that the LFIA test was valid and the conjugation process was successful.

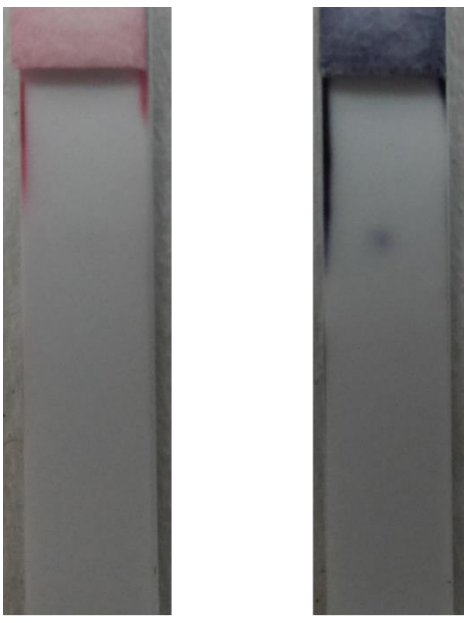


Figure 7. LFIA strips with control dot after flowed with conjugates of OD 2 for: (a) 40 nm AuNPs conjugate; (b) AuNFs conjugate.

4. Discussion

Size and shape of colloidal AuNPs play major role that influence the SPR band, colour, optical absorption intensity and stability. Thus, these properties are critical for LFIA in terms of sensitivity, stability and overall performance (Ji et al., 2015). In order to achieve this, flower-like or multi-branched shape was synthesized using the seeding-growth method. The AuNFs which was synthesized using the seeding-growth method produced the electromagnetic field effect located around the branch of the projectile and useful in surface-enhanced Raman scattering (SERS) (Zhao et al., 2009). In this method, reducing agent was used to promote the growth of AuNFs such as cetyltrimethylammonium bromide (CTAB) (Murphy et al., 2005), Poly(diallyldimethylammonium chloride) (PDDA), ascorbic acid (Zhang et al., 2015), hydroquinone (Ji et al., 2015), hydroxylamine (Zhao et al., 2009) and also used of poly(vinyl pyrrolidone)–sodium dodecyl sulfate aggregations (Ren et al., 2011). Among these, hydroxylamine was chosen due to the same reduction agent used to produce 40 nm AuNPs as compared with AuNFs in this experiment and also due to several advantages as reported by Makhsin et al., (2012).

The synthesized AuNFs was confirmed using TEM as shown in Figure 5. The projectile shape only formed when carried out in basic condition of pH 12 due to the suppressed ripening as reported earlier (Zhao et al., 2009). The size of AuNFs and UV-visible spectra also concordant as reported by Zhao et al., (2009) as seen by Zetasizer graph in Figure 2 and UV-visible graph in Figure 3. Moreover, the synthesized 40 nm AuNPs also has similar properties as reported by Makhsin et al., (2012). All these indicated that the AuNPs and AuNFs were successfully synthesized.

As reported earlier, changing the shape of colloidal AuNPs gave better stability. As a result, synthesized AuNFs has a higher absolute zeta potential compared to AuNPs even though with almost similar sizes. This higher zeta potential above ±30 mV indicate that the formed colloidal particles are stable in suspension due to higher surface charge that prevent particle aggregation within the solution (Sonavane et al., 2008, Ji et al., 2015). Besides, colour of colloidal particles also changed when changing in shape from red for spherical AuNPs to blue for multi-branched AuNFs due to different in SPR band as indicated by higher UV-visible spectra peak. Optical absorption intensity which indicated by OD also changed according to shape where OD for 15, 25 and 40 nm spherical AuNPs are almost similar (OD 1) to AuNFs.

In order to attach M*α*GNE antibody onto the surface of AuNPs and AuNFs, these Au solutions were adjusted to the pH slightly higher than the IEP of the antibody (pH 7 for M*α*GNE antibody). As a result, acidity of AuNPs need to be increased and basic AuNFs need to be lowered to be pH 7. The interaction occurred between AuNPs and AuNFs with M*α*GNE antibody is via electrostatic interaction. The amine group (NH_2^-) on the surface of AuNPs and AuNFs produced using the seeding-growth method provided specific interaction by amine bond to M*α*GNE antibody. Therefore, it required lower concentration of antibody compared to previous study which AuNPs was synthesized by citrate reduction method (Makhsin et al., 2012). Furthermore, AuNFs need more antibody almost half of AuNPs to be stabilized. This because AuNFs has high surface area owned by it branched projection compared to spherical AuNPs. This later improved the sensitivity of the assay (Zhang et al., 2015).

To prove the successful of the conjugation, absorption spectra before and after conjugation of AuNPs and AuNFs with M*α*GNE antibody were analysed. Change and shift in the position of SPR absorption indicate the presence of materials attached on the surface of AuNPs and AuNF due to changes in the dielectric properties and the local refractive index (Kumar et al., 2008). On the other hand, OD after conjugation was measured after diluted with ultrapure water. In this work, conjugate was diluted 100 times. As a result, the actual OD for each conjugate was determined by multiply the obtained OD with 100. The actual OD of AuNPs and AuNFs

conjugate to be 30 and 6, respectively. The OD for AuNFs after conjugation is lesser than AuNPs due to the lower amount of AuNFs particles synthesized before the conjugation process.

The HF 120 NC membrane was used because it has medium sensitivity and flow rate compared to other NC membranes. Control dot contained anti-mouse IgG antibody where it bound and capture conjugated MαGNE antibody. Absorbent pad functioned as sink to absorb the excess conjugate as well as to ensure the continuous flow of the system. When diluted conjugate either AuNPs or AuNFs flowed onto NC membrane, it reached the control dot and reacted. This resulted in accumulation of labelled antibody on the dot which then could be visualized by naked eyes. The intensity of control dot flowed with the lowest concentration of AuNFs conjugate was higher than AuNPs with the same OD (OD 2). This finding supported the theory that multi-branched AuNFs exhibited higher optical extinction due to higher SPR band by its complex 3D structure. The finding also supported by previous study which gave better sensitivity when used AuNFs as LFIA label (Ji et al., 2015, Zhang et al., 2015). The PBS pH 7.2 also was successfully used to remove the excess and background colour for both AuNPs and AuNFs. This buffer has greater solvent properties which could dissolve conjugated antibody and carried it to the absorbent pad. Overall, this assay needed about 15 minutes to complete and comparable to previous study (Ji et al., 2015, Zhang et al., 2015).

5. Conclusions

AuNFs was successfully synthesized and conjugated with antibody at pH 12 slightly higher than IEP of antibody. The AuNFs produced has higher optical extinction 630 nm compared to 40 nm AuNPs at 527 nm that improved the visibility of dot colour intensity. Thus, it improved the performance in term of sensitivity of the LFIA compared to spherical AuNPs at the lowest OD 2. The assay time was 15 minutes and could be considered as rapid test.

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Abbreviations

The following abbreviations are used in this manuscript:

LFIA: Lateral flow immunoassay
AuNPs: Gold nanoparticles
AuNFs: Gold nanoflowers
NC: Nitrocellulose membrane
SG: Seeded-growth method
PBS: Phosphate buffered saline
TEM: Transmission electron microscope
SPR: Surface Plasmon resonance
Ig: Immunoglobulin
IEP: Isoelectric point
GNE: Gram negative endotoxin
OD: Optical density

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Antiplatelet Activity of Bromelain Isolated from The Extract of Indonesia's Pineapple Core (*Ananascomosus* [L] Merr.)

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Abstract: Indonesia is one of the largest pineapple producer in the world with increasing world's demand of pineapple fruit products every years. Bromelain is a major component of pineapple that has been claimed to have many therapeutic benefits such as inhibiting platelet aggregation. The aim of this study is to isolate and purify bromelain from the extract of pineapple core (*Ananascomosus* [L] Merr.) and examine its antiplatelet activity. Fractionation of the crude enzyme using ethanol gave the highest activity at fraction 30-60% ethanol with the specific activity of 4.53 U/mg, whereas the precipitate obtained by re-fractionation using 0-60% ethanol gave the specific activity of 4.65 U/mg. This fraction has 898 times purity level compared to the crude enzyme extract. The bromelain obtained after purification by ion-exchange chromatography using DEAE-Cellulose had the specific activity of 11.84 U/mg with purity level of 2,277 times compared to the crude extract. The antiplatelet aggregation in vitro test performed using ADP as an aggregation inducer and acetosal as positive control. The results proved that all factions of bromelain enzymes have the antiplatelet activity. The isolate bromelain obtained had the highest antiplatelet activity with the aggregation percentage of 64.04% and the inhibition percentage of 18.47%.

Keywords: pineapple core; antiplatelet; bromelain; inhibition percentages; purification; specific activity

1. Introduction

Nowadays, around 171 million people in the world have diabetes, while the death rate is estimated at 3.2 million per year. Indonesia is ranked the 2nd highest diabetes prevalence in the Western Pacific region. WHO estimates the number of people with diabetes in Indonesia would rise dramatically and is predicted to increase to 21.3 million in 2030 (Pusat Data dan Informasi, 2014).

Patients with diabetes mellitus will get a condition that leads to a tendency to activate and form a platelet aggregation. Platelet aggregation plays a major role in acute coronary artery diseases, myocardial infarct and stroke. Cardiovascular disease and stroke are the major cause of death in the world. The death rate from cardiovascular disease as many as 17.3 million people each year. It is estimated that the rate will increase to 23.3 million in 2030. In Indonesia, the prevalence of coronary heart disease in 2013 was 0.5%, and 0.13% for heart failure (Triyono, 2013).

Platelets stick to the damaged vessel wall and attaches to each other to form a platelet mass. In arteries, the release of ADP and Thromboxane A2 can trigger and promote the formation of platelet aggregation or thrombus (Maurer, 2001and Nicholaos, 2011). Aspirin is the commonly used as antiplatelet drug, but continuous usage of this drug may cause side effects such as nausea, headaches, stomach ulcers, stomach cramps and acute bleeding.

Indonesia is the fifth largest pineapple producer in the world with increasing production value every year (Hero, F., 2011). In the last few years pineapple received many attention from scientists because it is thought to have potential as thrombolytic and antiplatelet agents (Metzig, 1999). The thrombolytic and antiplatelet potential are due to their content of proteolytic enzymes, called bromelain. Bromelain can degrade proteins by breaking peptide bonds. Bromelain isolated from the pineapple plant has been used extensively in pharmaceuticals and food (Donald, 1997).

2. Materials and Methods

Pineapples used in this study are obtained from GunungSalak, Bogor, Indonesia. The chemicals used are NaH_2PO_4, Na_2HPO_4, ethanol, Tris (hydroxymethyl)-ammino methane, NaOH, HCl, NaCl, Casein,Tri-Chloroacetic, Bovine Serum Albumin, Na_2CO_3, $CuSO_4.5H2O$, and Follin Ciocalteu obtained from Merck ; Platelet Rich Plasma (Palang Merah Indonesia); 5'diphosphate adenosine (Sigma-Aldrich); and Acetosal(Indofarma). The main equipments used are centrifuge refrigerated, UV-Vis spectrophotometer, sonicator, shaker, vortex, column chromatography, stirrer, micropipette, blender and a cooling box.

2.1 Preparation of Crude Extract Enzymes.

A total of 500 grams of pineapple core were cut and crushed in cold conditions using a blender. Homogenates were filtered using a muslin cloth to remove fiber and were centrifuged at 6000 rpm for 15 min at 4°C. Supernatant obtained is a crude enzyme which will further purified by fractionation methods. Crude enzyme obtained first purified by precipitation method using ethanol at various concentration range, followed by ion-exchange chromatography.

2.2 Ethanol fractionation

Into a crude enzyme (300 mL), pre-cooled acetone was slowly added to get a final required concentration with stirring using magnetic stirrer. Stirring was continued for 15 minutes after the addition of ethanol is completed. The solution was left for 1 hours at a temperature of ± 4°C to achieve the equilibrium between the dissolved and aggregated protein and then centrifuged at 8000 rpm. The precipitate was re-dissolved in potassium phosphate buffer pH 7.0 under cold condition. Whereas, the supernatant was collected and used for further fractionation at a certain concentration range with ethanol.The proteolytic activity of allenzyme fractions were determined using Kunitz method, while the protein content of the fraction of these enzymes were determined using Lowry's method.

2.3 Ion Exchange Chromatography

The enzyme fraction was applied to DEAE cellulose column that has been previously equlibrated with Tris-HCl buffer 0.05M pH 8.0. The column was eluted with a linear gradient of Tris-HCl buffer 0.05M pH 8.0, containing 0.2 M NaCl as the first buffer. Furthermore, the column was eluted by increasing the salt concentration in the Tris buffer (step-wise) containing 0.5 - 1.0 M NaCl.The flow rate was set at 1.5 mL/min and fraction were collected every 5 mL/tube. The fractions were assayed for bromelain activity.

2.4 Protein Determination

The total protein content is determined by Lowry method. The enzyme solution was diluted to 0.5 mL with 0.2 M phosphate buffer pH 7.0. The solution of BSA was prepared as a reference protein. Lowry reagent was added to both solutions and then the solutions were incubated at room temperature. After 10 minutes Follin Ciocalteu was added and the mixture were re-

incubated for 30 minutes. Theabsorbance of the solution was then measured at 595 nm (Plummer, 1982).

2.5 Assay of Bromelain Activity

Proteolytic activity is determined by the Kunitz method using casein as the substrate. As much as 0.1 mL enzyme solution was diluted in Tris-HCl buffer pH 8.0 to 10 mL. Then 1.0 mL of 1% casein was added and incubated at 37°C for 30 minutes. Enzymatic reaction was terminated by the addition of 3 mL of 10% TCA and then centrifuged at 3000 rpm at 4°C for 20 minutes .Control solution used an enzyme solution, which was inactivated by the addition of 10% TCA solution. While the reference solution was made by mixing all the reactants, except the enzyme solution. The mixture is then measured its absorbance at a wavelength of 280 nm (Plummer, 1982).

2.6 Test in vitro antiplatelet aggregation activity

Determination of antiplatelet aggregation activity performed on the enzyme fractions from each purification step which have the highest specific activity. Bromelain fraction of 70 µL was added to the 560 µL of PRP and incubated for 2 minutes at 37 ° C, then stirred gently using a shaker at low speed. The transmittance of the solution is measured both before and after the addition of ADP (70 µL) at 600 nm. In this test, acetosal of 1 mg / mL was used as a positive control, while distilled water is used as a negative control. (Moriyama, et al, 2009).

3. Results

3.1 Crude Enzyme Extraction and Fractional Precipitation by Ethanol

The crude extract bromelain derived from the pineapple core has a proteolytic activity of 20.8889 Units with a protein content of 4033.25 mg of total volume. The crude enzyme has a specific activity of 0.0052 U/mg protein. The results of proteolytic activity and total protein concentration of the crude extract bromelain after precipitated by of using ethanol are presented in Table 1.
Fraction 2 (30-60%) showed the highest proteolytic activity of 135.23 U/mL with a protein content of 29.87 mg/mL compared with other factions. This fraction has a specific activity of 4.5265 U/mg and a purity level 874 times compared to the enzyme extract.

Table 1. Purification of bromelain from pineapple core extract using ethanol

Fraction	Total			Spesific Activity (unit/mg)	Purify Factors
	Volume (mL)	Protein (mg)	Proteolitic Activity (unit)		
Crude Enzyme	325,00	4.033,25	20,8889	0,0052	-
Fraction 1 (0-30%)	7,55	3,46	1,4111	0,4075	79
Fraction 2 (30-60%)	38,00	29,87	135,2333	4,5265	874
Fraction 3 (60-80%)	21,00	35,70	2,9556	0,0027	52
Fraction 4 (0-60%)	6,75	3,37	15,6000	4,6480	898

3.2 Enzyme Fractions of Ion Exchange Chromatography

Results of enzyme purification fractions by using ion exchange chromatography is shown in Figure 1. In the process of purification using DEAE cellulose column chromatography with a linear gradient and step-wise elution gave several protein peaks that were measured at 280 nm.

Chromatograms obtained by making the relationship between the absorbance value of protein and bromelain activity against fractions number. Chromatogram shows 5 peaks (FE1 – FE5) and all are scattered but FE4 has the highest specific activity. The specific activity value of each peaks are shown in Table 2.

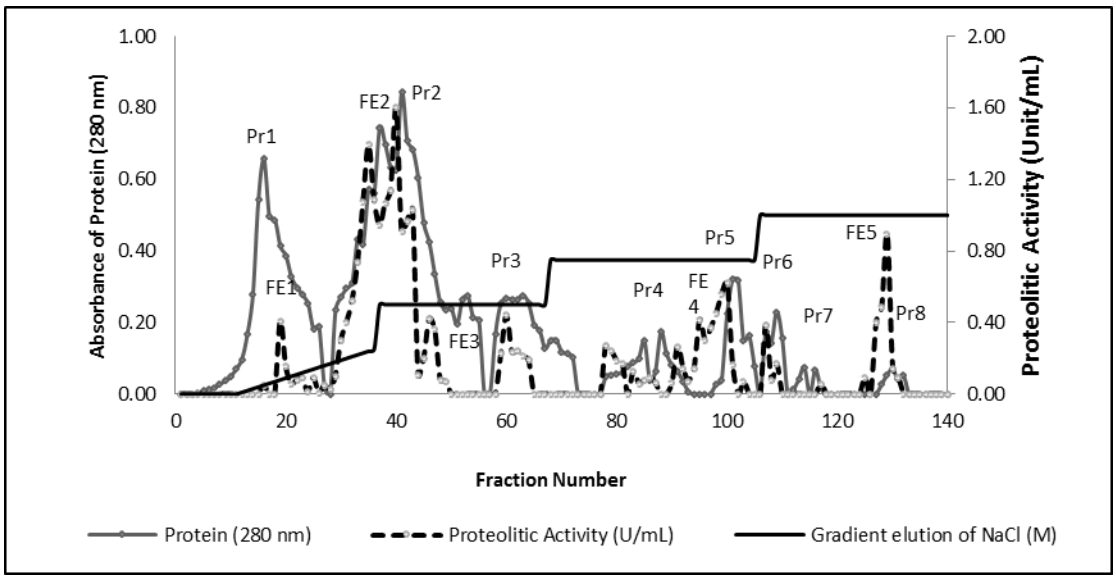

Figure 1. Chromatogram for 0-60% Ethanol Fraction Using DEAE-Cellulose Matrix.
Pr: The Peak of Protein at 280 nm, FE: The Peak of Protein with Proteolytic Activity
Separation conditions: column size 2.3 x 30 cm, matrix 125 mL volume flow rate was set at 1.5 mL/min and fraction were collected every 5 mL. The elution system using a combined method of linear gradient and step wise elution. The initial stage of the column was eluted with Tris-HCl buffer 0.05M pH 8.0; subsequent elution using a Tris-HCl buffer 0.05M pH 8.0 containing 0.25 to 1.0 M NaCl.

Table 2. Purification of bromelain from pineapple core extract using DEAE cellulose chromatografi

Fraction	Total			Spesific Activity (unit/mg)	Purify Factors
	Volume (mL)	Protein (mg)	Proteolitic Activity (unit)		
FE1	70	0,64	0,4000	0,6221	120
FE2	105	0,69	0,1250	0,1806	35
FE3	40	0,15	0,0250	0,1722	33
FE4	45	1,47	1,4750	9,0306	1737
FE5	40	0,13	0,1000	0,7670	148

3.3Purification Steps on Specific Activity of Bromelain from Pineapple Core

Figure 2 shows that bromelain fractions from all purification steps (CE, FE-2, FE-4 and F-chr) have increased the purity level. These are proved by the increasing of enzyme specific activity for each of fractions.

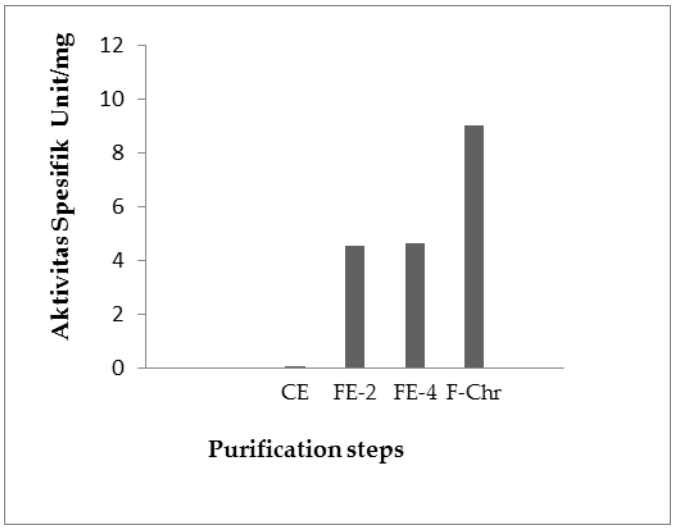

Figure 2. The value of the specific activity of bromelain fractions of purification steps

Information: CE : Crude enzyme, FE-2: Ethanol Fraction (30-60%), FE-4: Ethanol Fraction (10-60%), F-chr :

DEAE cellulose Chromatography Fraction.

3.4 Bromelain Influence toward Platelets Aggregation

Results of antiplatelet analysis through in vitro reaction towards all of bromelain fractions show thatF.P4 (Ion Exchange Chromatography fraction) has the lowest agregation percentage value as much as 64,03% compared to other bromelain fractions (Figure 3). Meanwhile, the percentage of the value of aggregation shown by aspirin as the positive control was obtained by 32.55%.

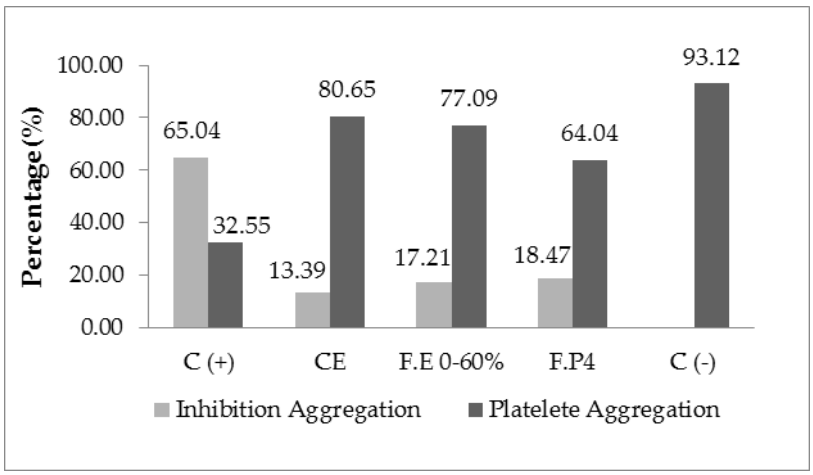

Figure 3. In-vitro antiplatelet activity of all fraction bromelain obtained from pineapple core extracts on every step of purification. C (-): Negative control (aquadest), CE: Crude enzyme, F.E 0-

60%: Fraction Enzyme of Ethanol, F.P4: Fraction Enzyme of Ion Exchange Chromatography, C (+): Control positive (Acetosal).

4. Discussion

4.1 Ethanol Fractionation

Purified bromelain was isolated from the core of pineapple (*Ananascomosus*). Purification of crude enzyme (CE) was carried out in two stages. In the first stage, crude enzyme was purified by precipitation method using cold ethanol (-10^{o}C) followed by ion exchange chromatography using DEAE cellulose column. In ethanol precipitation, the fractionation was carried out in three different ranges: 0-30%, 30-60% and 60-80%. The reason for choosing the concentration of ethanol with three different ranges of concentration aimed to find the interval that can precipitate the maximum proportion of unwanted proteins, while leaving most of the desired protein in solution, or conversely. Each fraction was assayed for its protease activity by Kunitz method using casein as a substrate.

According to Table 1, although at the ethanol fractionation was produced enzyme fractions with a higher specific activity, however other factions also showed specific activity fluctuate. Therefore, it is necessary to re-fractionation of the crude extract bromelain by using ethanol only at a concentration range of 0-60% of the total volume. At this stage, the fraction 4 was produced that showed an increasing specific activity of 4.6480 U/mg with a purity level of 894-fold compared to the crude extract.

4.2 Ion Exchange Chromatograph on DEAE cellulose

In this study, ion exchange column chromatography on DEAE cellulose is one of the advanced stage in the purification of the enzyme. This process is carried out on bromelain from 0-60% ethanol fraction, which has the highest specific activity on the previous fractionation. In Figure 1 and Table 2 are shown seven protein peaks (PrPr 1- 7). Based on the determination of proteolytic activity of all fractions of each protein peak is obtained five peaks of proteins with protease activity (FE1-FE5). Further, all fractions from each peak were collected and determined the specific activity. Apparently, only that 3 protein peaks, namely FE1, FE4 and FE5 that showed protease activity with a relatively high specific activity. The specific activity is highest in the FE4 that is equal to 9.0306 units / mg with a purity level of 1737-fold compared with the crude enzyme. Based on the chromatogram shown in Figure 1, the peaks of protein FE4 and FE5 out respectively from the matrix column, after eluted using the eluent containing negative ions (Cl^{-}) with higher concentrations. Thus, showed that the bromelain in an environment with a pH of 8.0strongly bound to the matrix of positively charged DEAE cellulose.

4.3 Purification Steps on Specific Activity of Bromelain from Pineapple Core

Based on the increase in the value of specific activity and purity levels of bromelain (Figure 2), hence it concluded that the purification bromelain from pineapple core using ethanol fractionation and anion exchange chromatography on DEAE cellulose has worked out quite satisfactorily.

4.4 In-vitro study of the activity antiplatelet of bromelain

Testing platelet aggregation in vitro was conducted using turbidimetric, as proposed by Born's. The principle of this method is to observe the transmission light changes of platelet suspension before and after the addition of the aggregator, which are substances that can lead to aggregation.

Platelet aggregation is the ability of platelets to stick each other to form an aggregate. Meanwhile, the percentage of inhibition describes the ability of a compound to inhibit the aggregation. The greater inhibition percentage of a compound, the more effective of these

compounds as antiplatelet agents. Acetosal is commonly antiplatelet drug that works as an inhibitor at the synthesis pathway of TXA_2 by means of irreversibly inhibiting the enzyme COX-1(Gross, 2009).

All enzyme fractions from each step were tested and showed a decrease in absorbance after adding ADP (Figure 3). This proves that bromelain from the pineapple core extracthas an ability to inhibits platelet aggregation. Bromelain inhibits platelet aggregation by hydrolyzing the enzyme COX-1, thus disrupting the platelet aggregation pathway by decreasing the synthesis ofTXA_2 (Metzig, 1999).Although, bromelain from the pineapple core has an ability of aggregation inhibition relatively smaller (18.47%) compared to aspirin (32,55%), but the antiplatelet activity of this enzyme could still be improved through further purification and a process of concentration by means lyophilization. Hence, it can be concluded that these bromelain has a good prospects as an alternative antiplatelet agent that is natural and safe with no any adverse side effects.

5. Conclusions

These bromelain from pineapple core extract was successfully purified by a series of purification steps. This was proven by an increasing of specific activity value in each fraction of the enzyme which produced. However, it is necessary to perform further purification on the fraction of bromelain from pineapple core by using other methods such as chromatography filtration to enhance its ability as an antiplatelet aggregation agent. Further research is needed to examine the activity of platelet aggregation in vivo to the fraction of bromelain from pineapple core using an animal test.

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Abbreviations

ADP : adensine 5'diphosphate
PRP : platelet rich plasma
TCA : tri-chloroacetic
DEAE cellulose **:** diethylaminoethyl cellulose
BSA : bovine serum albumin
COX-1 : cyclooxygenase
TXA_2 : thromboxaneA_2

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Microbiological Quality of Selected Foods at a Popular Café in UiTM, Shah Alam Campus

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Abstract: A study was carried out to evaluate the incidence of foodborne pathogens; *L. monocytogenes, E. coli* O157:H7 and *Salmonella* spp. and hence determine the microbiological quality of selected samples of foods sold at a popular café in UiTM, Shah Alam campus. Fifteen samples of food from two groups: staple foods and snack foods or locally known as "kuih" were selected. The presence of the three foodborne pathogens in the food samples were preliminary tested with specific biochemical tests and serological tests before the enumeration procedures were conducted. The results of the study showed that *L. monoctogenes* and *Salmonella* spp. were detected in all of the samples tested. However, only nine of the fifteen samples were positive with *E. coli* O157:H7. The microbiological quality and the safety level of the food samples were evaluated by comparing the mean of the colony forming unit per gram sample (CFU/g) with the guideline from the Public Health Laboratory Service (PHLS), U.K. The results of this study indicate that the foods sample were exposed to microbial contaminations and were potentially hazardous for consumption. Analysis of variance of mean CFU of foodborne pathogens per gram of samples tested showed no significant difference at α=0.05 (p>0.05).

Keywords: foodborne pathogen; contamination; microbiological quality

1. Introduction

In Malaysia, the main factor contributing food poisoning is the insanitary food handling procedures which accounted for more than 50 percent (50%) of the poisoning episodes (Ministry of Health. (MOH), 2007). For example, in January 2008 alone, thirty incidents of food poisoning and a food chemical intoxication were reported. Most of the implicated food settings occurred in schools and academic institutions where food preparation premises and inappropriate food handling practices, meals prepared too early and kept at ambient temperature until served and unhygienic practices were the causes of food poisoning cases. This is in agreement with Olsen *et al.* (2000) who demonstrated that foods consumed in institutions and other food services are considered the leading locations for foodborne outbreaks.

For food commodities, more importance is generally placed on the presence and numbers of *Escherichia coli* as indicators of cross-contamination (de Roever, 1999). Coliforms are still considered indicators for assessing general hygienic status of food contact surfaces (Jackson *et al*., 2007). Besides, *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* spp. had also been identified as the major bacterial pathogens that contribute to the foodborne infection. The presences of these pathogens indicate that the foods are contaminated and dangerous for consumption (Forsythe, 2010).

To date, many studies on the biosafety of *L. monocytogenes, E.coli* O157:H7 and *Salmonella* spp. in Malaysia have been reported (Tan *et al*., 2008; Tang *et al*., 2009; Jeyaletchumi *et al*., 2010; Suzita *et al*., 2010; Tunung *et al*., 2011) but there is no published data showing the incidence of these three foodborne pathogens on foods sold in academic institutions of higher learning. Therefore, the aim of this research are to to enumerate the major foodborne pathogens; *L. monocytogenes, E. coli* O157:H7and *Salmonella* spp. in selected samples of foods sold at a popular café in UiTM, Shah Alam campus, hence determine the microbiological quality and the safety level of the selected samples of foods.

2. Materials and Methods

2.2 Selection of food as sample materials

Two groups of commonly consumed foods namely staple foods and snack foods from a selected café in UiTM Shah Alam campus were sampled. Overall, a total of fifteen samples of foods were collected comprising staple foods (8) and snacks or also known as "kuih" (7).

Table 1. Types of food samples for analysis

Group	Food samples
Staple foods	Fried rice, fried noodle, 'roti canai', 'nasi lemak', 'sambal tumis', fried chicken, curry, dhal
Snack foods	'Putu ayu', 'talam', 'ketayap', curry puff, sardine roll, 'pau sambal', 'seri muka'

2.3 Survey and sample collection

All samples were collected between 8.00 am to 10.00 am. Most foods are ready cooked foods, which are locally popular for morning breakfast. Samples were purchased as regular customers using the vendor's own utensils, and arranged into sterile plastic bags for transportation into icebox container to the laboratory. The analyses were performed within 24 hours after the arrival of all samples to the laboratory.

2.4 Identification of foodborne bacteria

There are four steps in the identification of foodborne pathogens the samples; primary enrichment, secondary/selective enrichment, isolation and confirmatory test. Table 2. shows the media used for primary enrichment, secondary/selective enrichment and isolation with the incubation time and temperature for the identification of *L. monocytogenes*, *E. coli* OI57: H7 and *Salmonella* spp.

Table 2. Media for primary enrichment, secondary/selective enrichment and isolation with the incubation time and temperature for the identification of *L. monocytogenes*, *E. coli* OI57: H7 and

Foodborne pathogen	Media			Incubation	
	Primary enrichment	Secondary/ selective enrichment	Isolation	Time (Hour, h)	Temperature (°C)
L. monocytogenes	University of Vermont Modified *Listeria* Enrichment Broth (UVM broth)	Fraser broth	Oxford medium (MOX) agar	24 - 28	35
E. coli O157:H7	Lactose broth	Eosin Methylene Blue (EMB) agar Sorbitol McConkey (sMAC) agar	Blood agar	24	37
Salmonella spp.	Buffered peptone water	Selenite Cystine (SC) broth	Hektoen Enteric (HE) agar	24	37

Salmonella spp.

For pre-enrichment, 25 g of samples were placed in a sterile sandwich bag. 255 mL of appropriate pre-enrichment medium was added to the above sample and mixed for 2 minutes in a blender. Later, the blended homogenate was incubated at specific temperature and time according to the foodborne pathogen strains and media used for enrichment. After proper incubation, the suspensions were transfer into the specific secondary/selective enrichment before plated into the isolation media. Positive growth colonies on the isolated media were undergo two specific biochemical tests to confirm that the colonies isolated are *L. monocytogenes*, *E. coli* OI57: H7 and *Salmonella* spp.

2.6 Confirmatory tests

2.6.1 *L. monocytogenes* confirmatory test

Confirmation of *L. monocytogenes* involved two tests; hemolysis on blood agar and hydrogen sulfide (H_2S) production.

a)Hemolysis on blood agar

A minimum of 20 colonies from the suspected MOX plates were streaked on blood agar plates and incubated at 37°C for 22 hours and then examined under light.

b)Hydrogen sulfide (H_2S) production test

By using aseptic techniques, each of the culture samples was inoculated by means of the stab method into SIM agar. Then the tubes were incubated for 24 hours at 37°C.

2.6.2 *E. coli* OI57: H7 confirmatory test

Confirmation of *E. coli* O157H7 involves two tests; Indole production test and tube agglutination test.

a) Indole production test

The Methyl Red Voges Proskauer (MR-VP) medium was prepared. A loopful of each of the culture samples was inoculated into the MR-VP medium tube. Then the tubes were incubated for 24 hours at 37°C. After 24 hours incubation, five drops of Kovac's reagent were added to the culture.

b) Tube agglutination test

The colonies on the sMAC agar were screened for the O157:H7 strain using *E. coli* O157 antiserum by the tube agglutination technique.

2.6.2 *Salmonella* spp. confirmatory test

Confirmation of *Salmonella* spp. involved two tests; methyl red test and slide agglutination test.

a) Methyl red test

A MR-VP broth tube was inoculated with a loopful of culture and it was incubated at 37°C for 24 hours. After incubation, five drops of methyl red indicator were added to the culture.

b) Slide agglutination test

The *Salmonella* spp. was serologically confirmed using the slide agglutination test of Polyvalent *Salmonella* (A-E and Vi) antisera.

2.7 Bacteria enumeration

The UVM broth emulsion then were used to determine the total aerobic plate count (TAPC), by making serial dilutions using sterile saline and surface plating on MOX agar. Inoculated plates were incubated aerobically at 37°C for 48 hours. Plates containing between 30 and 300 colony forming units (CFU) of *L. monocytogenes* (or the highest number if below 30) were counted. Subsequently, for *E. coli*, the lactose broth emulsion was plated onto EMB agar, while for *Salmonella* spp. the buffer peptone water broth was plated onto HE agar. Both EMB agar and HE agar were incubated for 48 hours at 37°C.

2.8 Statistical Analysis

The means of CFU of the foodborne pathogens per gram of samples tested sample were analysed by using analysis of variance (ANOVA).

2.9 Data analysis

Results and data taken from all the confirmatory tests and the bacteria enumeration were combined and compared to a standard guideline produced by the Public Health Laboratory Service (PHLS), U.K (Robert and Greenwood, 2002) to determine the safety level of all samples.

3. Results

3.1 Identification of foodborne bacteria

Identification of the foodborne pathogens on the isolation media were determined from the growth of positive colonies on the respective media. Different foodborne strains will show different morphology on the isolation media. Figure 1. shows the morphology of the foodborne pathogens on the respective isolation media.

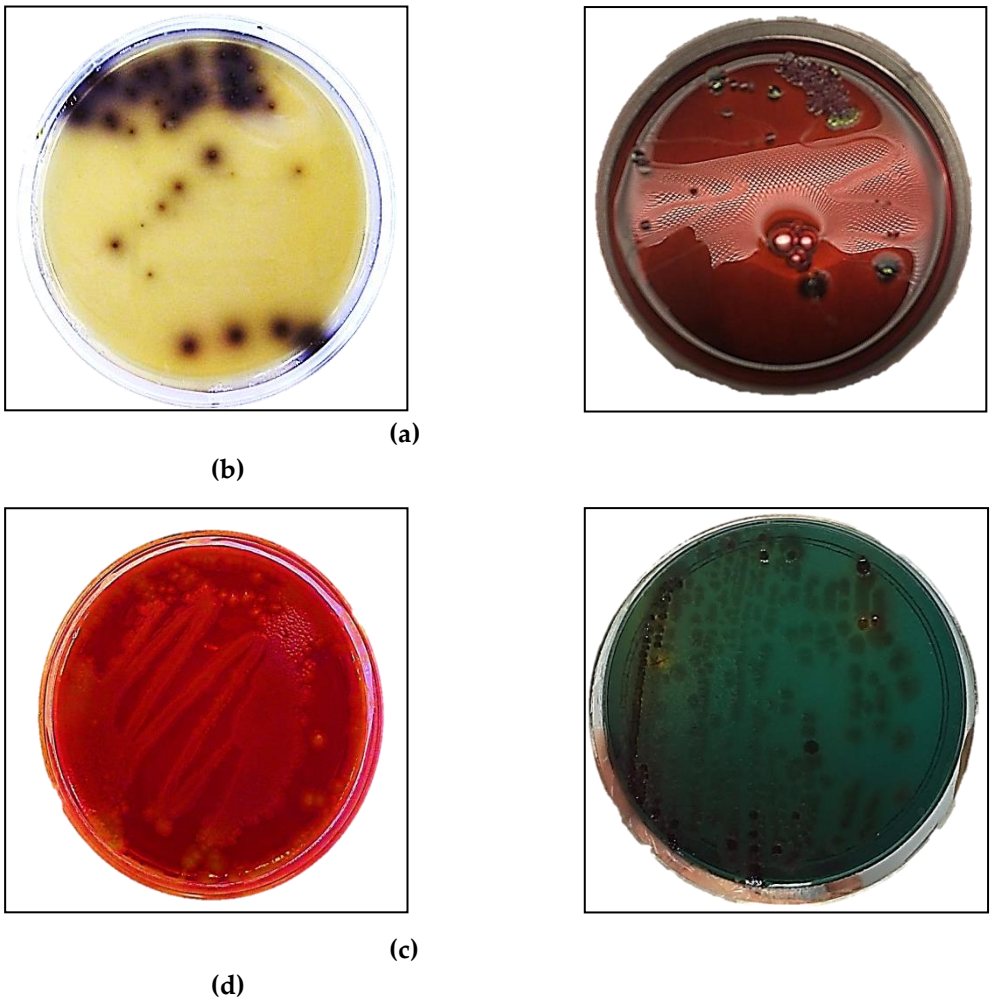
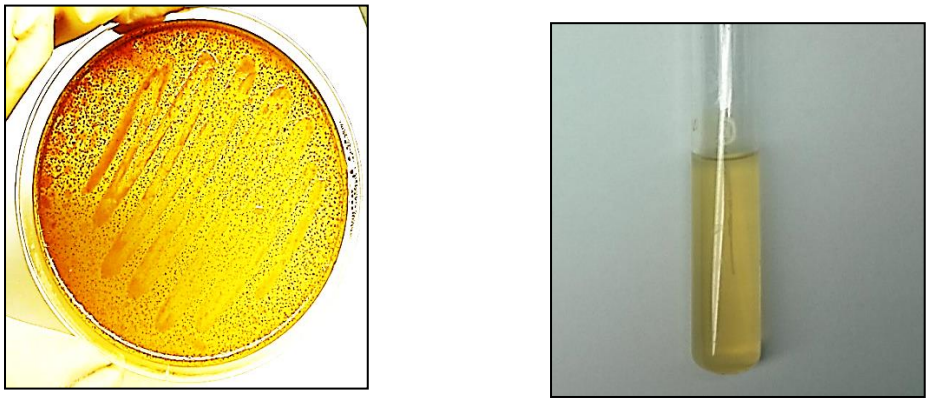


Figure 1. Positive colonies of foodborne pathogens on isolation media: (a) *L. monocytogenes* colonies on MOX agar plate; (b) *E. coli* O157:H7 colonies on EMB agar plate; (c) *E. coli* O157:H7 colonies on sMAC agar plate; (d) *Salmonella* spp. colonies observed on HE agar plate.

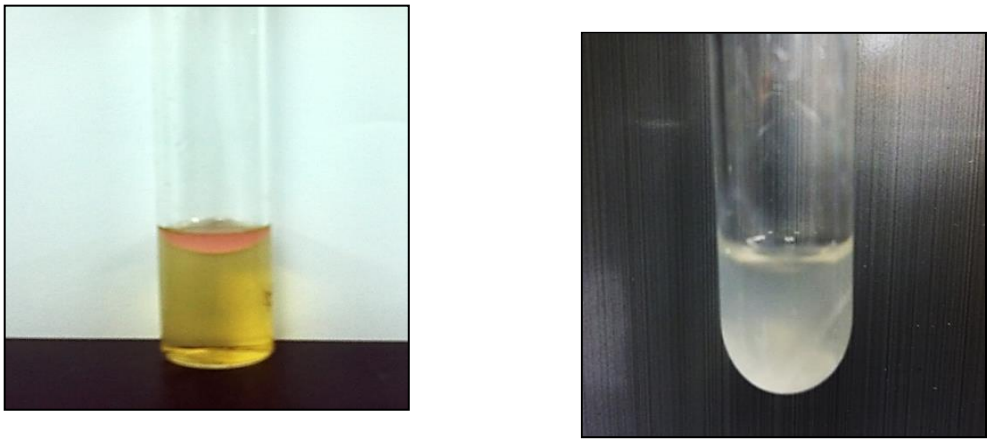
3.2 Confirmatory tests

To confirm the presence of the foodborne pathogens in the samples, two specific biochemical tests were carried out. The biochemical tests were chosen based on the ability of the foodborne to produce certain by-product after utilize a specific source. Figure 2-4 shows the biochemical tests results tested for *L. monocytogenes*, *E. coli* OI57: H7 and *Salmonella* spp.



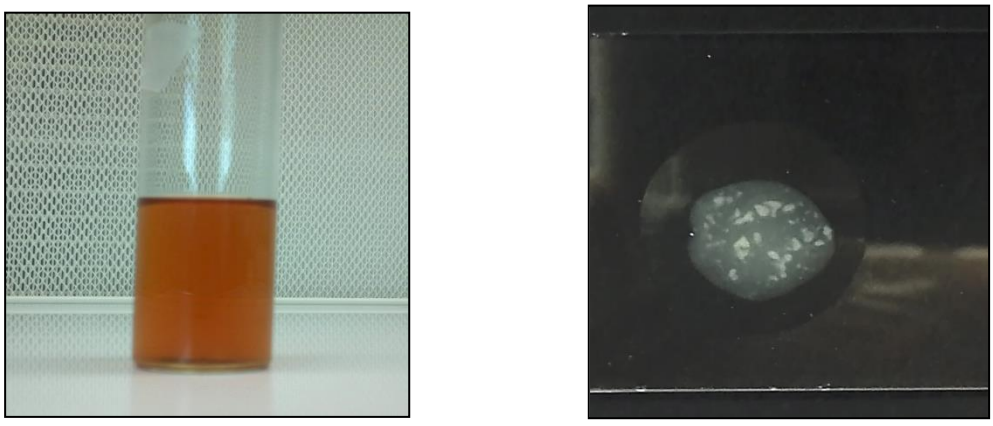
(a) **(b)**

Figure 2. Biochemical tests results for *L. monocytogenes* confirmation: (a) Positive beta hemolysis on blood agar plate; (b) Negative results on hydrogen sulfide production test



(a) **(b)**

Figure 3. Biochemical tests results for *E. coli* OI57: H7 confirmation: (a) Positive results on indole production test; (b) Positive result on tube agglutination test



(a) **(b)**

Figure 4. Biochemical tests results for *Salmonella* spp. confirmation: (a) Positive result on methyl red test; (b) Positive result on slide agglutination test.

Table 3 summarized the biochemical tests results to confirm the presence of foodborne pathogens: *L. monocytogenes*, *E. coli* OI57: H7 and *Salmonella* spp. in the sample tested.

Table 3. Results of the biochemical tests for *L. monocytogenes*, *E. coli* OI57: H7 and *Salmonella* spp. on tested samples.

Sample	Confirmatory test					
	L. monocytogenes		*E. coli* OI57: H7		*Salmonella* spp.	
	Hemolysis on blood agar	**Hydrogen sulfide production**	**Indole production**	**Tube agglutination**	**Methyl red**	**Slide agglutination**
Fried rice	+	-	+	+	+	+
Fried noodles	+	-	+	+	+	+
"Roti canai"	+	-	+	-	+	+
"Sambal tumis"	+	-	+	+	+	+
"Nasi lemak"	+	-	+	+	+	+
Fried chicken	+	-	+	-	+	+
Curry	+	-	+	-	+	+
"Dhal"	+	-	+	-	+	+
"Putu ayu'	+	-	+	-	+	+
"Talam"	+	-	+	+	+	+
"Ketayap"	+	-	+	+	+	+
Curry puff	+	-	+	+	+	+
Sardine roll	+	-	+	+	+	+
"Pau sambal"	+	-	+	+	+	+
"Seri muka"	+	-	+	-	+	+

+, positive; -, negative reaction

3.3 Bacteria enumeration

The enumeration of bacteria colonies was based on serial dilution of 1/10 with 10^{-3}. Table 4. showed the mean count of *L. monocytogenes, E. coli* O157:H7 and *Salmonella* spp. colonies per gram of samples (CFU/g). Table 5. showed the mean count of the colonies and standard deviation, standard error and 95% confident interval of the three different bacteria tested.

Table 4. Mean CFU of *L. monocytogenes, E. coli* O157:H7 and *Salmonella* spp. per gram of samples

Sample	Mean (CFU/g)		
	L. monocytogenes	*E. coli* O157:H7	*Salmonella* spp.
Fried rice	3.5 x 10^3	2.4 x 10^3	3.7 x 10^3
Fried noodles	4.2 x 10^3	2.9 x 10^3	3.2 x 10^3
"Roti canai"	3.2 x 10^3	3.2 x 10^3	4.2 x 10^3
"Sambal tumis"	4.5 x 10^3	3.6 x 10^3	2.8 x 10^3
"Nasi lemak"	3.9 x 10^3	5.1 x 10^3	4.4 x 10^3
Fried chicken	3.0 x 10^3	4.6 x 10^3	6.2 x 10^3
Curry	3.1 x 10^3	2.4 x 10^3	4.4 x 10^3
"Dhal"	2.5 x 10^3	2.7 x 10^3	3.3 x 10^3
"Putu ayu'	3.3 x 10^3	3.1 x 10^3	3.1 x 10^3
"Talam"	5.3 x 10^3	5.4 x 10^3	3.1 x 10^3
"Ketayap"	2.6 x 10^3	4.1 x 10^3	2.9 x 10^3
Curry puff	4.8 x 10^3	2.3 x 10^3	4.8 x 10^3
Sardine roll	4.5 x 10^3	3.0 x 10^3	4.6 x 10^3
"Pau sambal"	3.0 x 10^3	2.7 x 10^3	3.5 x 10^3
"Seri muka"	2.2 x 10^3	3.9 x 10^3	3.1 x 10^3

Note: CFU/g = Colony forming units per gram of samples analyzed

Table 5. Mean total plate count, standard deviation, standard error and 95% confident interval of the three different bacteria tested

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	118.533	2	59.267	.657	.524
Within Groups	3788.267	42	90.197		
Total	3906.800	44			

3.4 Statistical Analysis

Table 6. Analysis of variance of CFU of foodborne pathogens per gram of samples

Pathogen	n	$\bar{x}$ ± SD	Standard Error	(95% CI)
L. monocytogenes	15	35.73 ±9.18	2.37	30.65 -40.82
E. coli O157:H7	15	34.27 ±9.94	2.57	28.76 -39.77
Salmonella spp	15	38.20±9.36	2.42	33.02 -43.38

Table 6. showed the analysis of variance in the CFU of the foodborne pathogens per gram of samples.

4. Discussion

4.1 Identification of foodborne bacteria

The presence of *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella* spp. in the samples tested were determined from the colonies grown on specific media. *L. monocytogenes* were observed as flat, dimpled colonies with black halo (Hwang and Huang, 2010) on MOX agar plate (Figure 1. (a)). The sizes of the colonies were varied in appearance. Meanwhile, colonies of *E. coli* O157:H7 were appeared as blue-black colonies with a green metallic sheen when viewed in reflected light (Hwang and Huang, 2010)(Figure 1(b)). *E. coli* O157:H7 is a non-lactose-fermenting bacteria, hence the growth appeared as colorless colonies in sMAC agar plate (Figure 1. (c)). Figure 1. (d) showed the positive colonies of *Salmonella* spp. observed on HE agar plate. Colonies were observed to be large, glossy and appear as almost completely blue-green or black colonies (Hwang and Huang, 2010).

4.2 Confirmatory tests

The biochemical tests to confirm the presence of foodborne pathogens on the samples were chosen based on the ability or inability of the bacteria to utilize a certain compound and produce specific by-product. Table 3. summarized the results of biochemical tests performed on all samples tested.

Figure 2. showed the results of biochemical tests of hemolysis on blood agar and hydrogen sulfide production to confirm the presence of *L. monocytogenes* in the samples. Figure 2. (a) showed the beta-hemolysis on blood agar. A clear zone, approaching the color and transparency of the base medium which surrounds the colony (Robert and Greenwood, 2002) was observed. Formation of beta-hemolysis on blood agar indicates that the bacteria produce toxic by-products that are capable of destroying the red blood cells. All fifteen foods sampled showed positive results of *L. monocytogenes* on blood agar. Second test performed to confirm the contamination of *L. monocytogenes* in the samples was hydrogen sulfide production. Hydrogen sulfide production test was based on the abilities of some bacteria to liberate hydrogen sulfide and cysteine desulfurase. Cysteine desulfurase can break down the amino acid cysteine to form alanine and hydrogen sulfide (Johnson and Case, 2010). SIM medium contains cysteine and iron ions. If H_2S reacts with the iron, it will form iron sulfide. The positive results of the hydrogen sulfide production were indicated by the blackening of the medium due to the precipitation of the iron sulfide. All samples showed a negative result in this test. No blackening of the medium was observed when the samples were inoculated onto the SIM medium (Figure 2 (b) because *L. monocytogenes* does not have the ability to produce hydrogen sulfide and enzyme cysteine desulfurase (Johnson and Case, 2010).

Two type of biochemical tests, indole test and serological test tube agglutination were performed to identified the presence of *E. coli* O157:H in the samples. The indole tests was performed based on the fact that some bacteria has the ability to convert tryptophan to indole. The production of indole was detected when dimethyl-aminobenzaldehyde (Kovac's reagent) was added. From the test performed, all samples showed a red coloration (Figure 3 (a) after the addition of Kovac's reagent. This indicates that the substrate tryptophan was hydrolyzed to indole (Hwang and Huang, 2010). Serological test for *E. coli* O157:H7 was conducted using the *E. coli* O157 antiserum by the tube agglutination technique. The serological test is based on the

reaction of specific antiserum with its homologous antigen. The samples were only confirmed contaminated with the *E. coli* O157 if it showed positive results for *E. coli* O157:H7antiserum. Tubes that showed a 50% or greater agglutination were considered as a positive result (Figure 3. (b)). Among the fifteen samples tested only nine samples showed positive results. The samples which showed positive results were fried rice, fried noodles, "nasi lemak", "pau sambal", "sambal tumis", "talam", "ketayap", curry puff and sardine roll. Between staple foods and snack foods or locally known as "kuih", snack foods have a higher incidence of *E. coli* O157:H7. The samples that showed negative results for the agglutination tests were *E. coli* but not the enterohemorrhagic*E. coli* (EHEC) or *E. coli* O157:H7 strain.

Methyl red test and tube agglutination were conducted to confirm the presence of the *Salmonella* spp.. Positive results in both tests indicate that the samples were contaminated with *Salmonella* spp. Methyl red test was used to identify enteric bacteria based on their glucose metabolism. All enterics initially produce pyruvate acid to stable acidic end-products. The acids will overcome the buffers of the medium and produce an acidic environment in the medium. When methyl red is added, the methyl red will remained as red, if acidic end products are present (Alexender and Strete, 2000). *Salmonella* spp. is an enteric bacterium; therefore it gives positive results towards the methyl red test (Figure 4 (a)). All samples showed positive result towards the methyl red test. This confirmed the presence of *Salmonella* spp. in all samples. Slide agglutination test of Polyvalent *Salmonella* (A-E and Vi) was used as a serological confirmation for the presence of *Salmonella* spp. in the samples. The test is based on the *Salmonella* spp. antigen reaction with its corresponding antibodies. A granular clumping of somatic antigen in the slide indicated a positive result of the test. A 75% or greater agglutination indicates a positive result of the test (Figure 4 (b)). A partial (less than 3+) or delayed agglutination reaction was considered as a negative result. Fifteen of the samples produced positive results by the slide agglutination test.

4.3 Bacteria enumeration and determination of the microbiological quality and safety level of the samples

Among the three pathogens tested, *Salmonella* spp. had the highest mean of total plate count, followed by *L. monocytogenes* and lowest is the *E. coli* O157:H7 (Table 5.). This showed that the incidence of *Salmonella* spp. in the foods sampled is the highest.

The microbiological quality and safety level of the samples were determined by comparing the counts of the colonies with a standard guideline produced by the Public Health Laboratory Service (PHLS), U.K (Robert and Greenwood, 2002). Table 7. showed the PHLS Guidelines for the microbiological quality of various ready-to-eat foods.

Table 7. PHLS Guidelines for the microbiological quality of various ready-to-eat foods (Robert and Greenwood, 2002).

Foodborne pathogen	Microbiological Quality (CFU/g)			
	Satisfactory	Marginal	Unsatisfactory	Potentially hazardous
L. monocytogens	Not detected in 25g	20 – <100	-	≥ 100
E. coli O157:H7	Not detected in 25g	-	-	Detected
Salmonella spp.	Not detected in 25g	-	-	Detected

Note: CFU/g = Colony forming units per gram of samples analyzed.

Among the fifteen foods sampled, "talam", curry puff, "pau sambal" and "sambal tumis" showed the highest mean number of CFU of *L. monocytogenes* per gram of samples. These four samples are highly suspected to be contaminated with *L. monocytogenes*e especially during storage. This is because *L. monocytogenes* is psychrotropic, therefore it can survive even at refrigeration temperatures (Beumer *et al*., 1996). Another issue of *L. monocytogenes* contamination is that they may get cross-contaminated during handling and may initially contain small numbers of organism (Hwang and Huang, 2010). Post-processing contaminated meat and seafood products may also contribute to the high contamination of *L. monocytogenes*in the samples (Jacquet *et al*., 1995). Since all of the samples CFU/g exceed the PHLS Guidelines for the microbiological quality of various ready-to-eat foods (≥100), the samples can be considered as potentially hazardous for human consumption by the consumers.

From the fifteen foods sampled, "talam", "nasi lemak" and fried chicken had the highest contamination of *E. coli* O157:H7. There are several possibilities that may lead to the contamination. According to Rangel *et al.* (2005), more than half (53%) of the product-associated with outbreaks of *E. coli* O157:H7 did not involve cross-contamination of *E. coli* O157:H7 in the kitchen. The outbreaks were due to produce already contaminated with pathogens before purchases. The items may have become contaminated in the field from manure or contaminated irrigation water; during processing due to contaminated wash water or ice; contaminated equipment, or poor handling practices; during transport or through contaminated storage equipment (Hwang and Huang, 2010). Among the fifteen samples, only fried rice, fried noodles, "nasi lemak", "pau sambal", "sambal tumis", "talam", "ketayap", curry puff and sardine role showed positive results by the serological test of *E. coli* O157:H7. Therefore, only these nine samples are considered as potentially hazardous to be consumed due to *E. coli* O157:H7 contaminations.

Fried chicken, curry puff and sardine roll were the three highest samples highly contaminated with *Salmonella* spp.. Poultry products, egg products, seafood, milk and fresh products are common products associated with *Salmonella* spp. contaminations. According to Cabedo *et al.* (2008), among 1379 animal origin samples tested in Catalonia, Spain, 1.5% *Salmonella* spp. was isolated from frozen chicken. Due to its enteric nature, meat such as chicken may be contaminated from the intestinal contents during evisceration of animals, during washing and during post-harvesting process (Hwang and Huang, 2010). Vegetables and fruits may also carry

the *Salmonella* spp. when contaminated with fecal matter or washed with polluted water. Consumption of the foods sampled in this research can be considered as potentially hazardous because the presence of *Salmonella* spp. was detected in all samples.

5. Conclusions

In conclusion, all the fifteen samples tested showed positive results in both confirmatory tests for *Salmonella* spp.. All samples which undergo biochemical testing for *L. monocytogenes* showed positive results in the hemolysis of blood agar test but showed negative results in hydrogen sulfide production test. Meanwhile for *E. coli* O157:H7, all samples showed positive results in the methyl red tests but only nine out of fifteen samples showed positive results towards the slide agglutination test. The samples that produced negative results in the serological test were probably from another group of *E. coli* and not the enterohemorrhagic *E. coli* (EHEC). Comparing the results of the total plate count in the enumeration of the three foodborne pathogens; *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella* spp. to that of the PHLS Guidelines for the microbiological quality of various ready-to-eat foods, the results indicate that the samples tested were potentially hazardous for human consumptions.

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Molecular Evaluation of Selected Malaysian Rice Cultivars Using Specific Primer Targeting *xa13* Gene

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Abstract: Bacterial leaf blight (BLB) is caused by the *Xanthomonas oryzae pv. Oryzae*. It is one of the devastating diseases in rice causing yield losses ranging from 74% to 81% in severe conditions. To date, more than 40 BLB resistance genes have been identified in rice. The present study focused on the screening of *xa13* gene in Malaysian rice cultivars. The *xa13* gene is fully recessive and only conferring resistance in a homozygous state. A total of 39 Malaysian rice cultivars which consist of 35 Malaysian landraces rice and four Malaysian commercial rice varieties were screened using a specific primer targeting *xa13* promoter. The PCR analysis on the positive control varieties (IRBB13 and IRBB66) showed allele with the approximate size of 500 base pairs while the negative control variety (MR84) showed allele with the approximate size of 300 base pairs. All the selected Malaysian rice cultivars showed similar allele size as negative control variety which suggested that none of the selected Malaysian rice cultivars showed the presence of *xa13* gene. Sequence analysis of IRBB13 showed the target region displayed 100% similarity to the IRBB13 resistant allele of *xa13* gene in NCBI database (GenBank id: DQ421394.1). This study provides polymorphism information between the resistant and susceptible varieties which would be useful in marker-assisted breeding (MAB) technology.

Keywords: *xa13* gene, Bacterial leaf blight, Malaysian rice cultivars

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1. Introduction

Rice is the staple food across the world, especially in Asia region. Rice consumption was expected to increase with the increase of human population. However, biotic and abiotic stress has affected the rice production. Bacterial leaf blight disease which caused by *Xanthomonas oryzae pv. Oryzae* (*Xoo*) is one of the biotic stresses which significantly lead to yield losses across the world. Cultivating the resistant varieties has been proven to be the most efficient, reliable, and cheapest way to control BLB disease. However, most of the Malaysian commercial rice varieties were susceptible against BLB infestation. Hence, there is a needed to develop resistant rice varieties against BLB infestation. Improvement of commercial varieties through introgression of the resistant gene using Marker assisted breeding (MAB) technology represents the best approach in developing resistant rice variety. MAB technology relied on the application of molecular marker during the selection process. MAB increase the efficiency and accuracy in the selection process. Only the plant with the presence of resistance or desired gene will be selected (Collard & Mackill,2008).

To date, about 40 resistant genes for BLB has been identified. The *xa13* gene is a recessive gene and only conferring their resistance only in homozygote recessive status (Hajira et al., 2016). As reported previously, this gene confers resistance effectively against Philippine *Xoo* race 6, which was reported as the most virulent race pathotype and could not overcome by most of the dominant resistance gene (Khush & Angeles, 1999). The *xa13* gene was discovered in the traditional rice variety namely BJ1. The gene was mapped on the rice chromosome 8 (Sanchez et al., 1999). The *xa13* gene has been successfully cloned and sequenced in order to understand their defense mechanism against BLB disease. The previous studies have revealed that some of the defensive genes activated in *xa13* are functioning and operating differently from other dominant R genes (Zhou et al., 2002; Chu et al. 2004).

Chu et al., (2006a) have identified the variants in the promoter region of *Os8N3* gene in *xa13*, which responsible and encodes for sugar transporter. Gene expression analysis of *Os8N3* showed the dominant alleles of *Os8N3* is induced by companionable *Xoo* strains which carrying the transcription activator-like (TAL) effector namely pthXo1. This pthXo1 will bind to the *Os8N3* promoter to induce the expression (Chu et al., 2006b). In the recessive gene, pthXo1 unable to bind to the *Os8N3* promoter owing to the presence of variants thus lead to the inability to induce sugar transporter to establish the infection (Anthony et al., 2010). A study by Hajira et al., (2016) has successfully developed an ideal functional marker targeting variants in the promoter region of *xa13*. The marker will be used in this present student in order to assess the presence of the *xa13* in Malaysia rice cultivars.

2. Materials and Methods

2.1 Plant Material

The seed of 35 landraces rice cultivars, four Malaysian commercial rice varieties, two positive control varieties (IRBB13 and IRBB66) and one negative control variety (MR84) were germinated for 3 weeks prior to DNA extraction purpose. The seeds were obtained from MARDI Breeder seed and MARDI rice Genebank, Seberang Perai. Leaves of three individual from each cultivar were collected and dried using silica gel before proceeding to DNA extraction. Details about the plant materials were summarized in Table 1.

Table 1. List of 43 cultivars used in this study

No	Variety	Type	Origin	Accession No*
1	IRBB13	Positive control	IRRI	-
2	IRBB66	Positive control	IRRI	-
3	MR84	Negative control	Malaysia	-
4	Chempa Padi Huma	Landrace	Malay Peninsula	MRGB05080
5	Siong Pelandok	Landrace	Malay Peninsula	5101
6	Santap Wangi	Landrace	Malay Peninsula	6292
7	Mayang Lega	Landrace	Malay Peninsula	6004
8	Anak Cina	Landrace	Malay Peninsula	5103
9	Janda Muda	Landrace	Sabah (Borneo)	9936
10	Purak	Landrace	Sabah (Borneo)	9953
11	Nangka	Landrace	Sabah (Borneo)	7516
12	Putus Tunang	Landrace	Sabah (Borneo)	7540
13	Semilai	Landrace	Sabah (Borneo)	7543
14	Pagalan	Landrace	Sabah (Borneo)	7560
15	Kungkuling	Landrace	Sabah (Borneo)	7565
16	Kedinga	Landrace	Sabah (Borneo)	7583
17	Turayan	Landrace	Sabah (Borneo)	7571
18	Padi Kolomintuhon	Landrace	Sabah (Borneo)	9866
19	Dihangkang Putih	Landrace	Sabah (Borneo)	9894
20	Tahi Ayam	Landrace	Sabah (Borneo)	9962
21	Padi Solung	Landrace	Sabah (Borneo)	9930
22	Padi Emas	Landrace	Sabah (Borneo)	9963
23	Gebokong	Landrace	Sabah (Borneo)	9959
24	Padi Susiah	Landrace	Sabah (Borneo)	9968
25	Padi Mansud	Landrace	Sabah (Borneo)	9954
26	Padi Beruang	Landrace	Sabah (Borneo)	9956
27	Padi Adong	Landrace	Sabah (Borneo)	10001
28	Padi Tiga Bulan	Landrace	Sabah (Borneo)	9958
29	Padi Tubowan	Landrace	Sabah (Borneo)	10003
30	Padi Pengalaan	Landrace	Sabah (Borneo)	9971
31	Mayang	Landrace	Sabah (Borneo)	9873
32	Liwagu Antap	Landrace	Sabah (Borneo)	9993
33	Mansau	Landrace	Sarawak (Borneo)	3369
34	Biris	Landrace	Sarawak (Borneo)	6891
35	Padi Cigarette	Landrace	Sarawak (Borneo)	11816
36	Chelom	Landrace	Sarawak (Borneo)	7155
37	Alek	Landrace	Sarawak (Borneo)	9037
38	Chelum	Landrace	Sarawak (Borneo)	9467
39	MR219	Advanced Line	MARDI	Breeder Seed
40	MR220-CL2	Advanced Line	MARDI	Breeder Seed
41	MR263	Advanced Line	MARDI	Breeder Seed
42	MR284	Advanced Line	MARDI	Breeder Seed

* MARDI rice Genebank

2.2 Genomic DNA extraction

Total genomic DNA was extracted using protocol developed by Rodica et al. (2011) with some modification in term of lysis buffer ingredients. Approximately 1 gram of each cultivar's leaves was ground using TissueLyser (Qiagen, Netherlands). The ground samples were treated

with 600 µL extraction buffer (2% PVP, 4 mM DIECA, 5mM Ascorbic acid, 1.4 M NaCL, 100 mM Tris-HCL (pH 8.0) and 20 mM EDTA) for1 hour at 65°C. The DNA then was precipitated with an equal volume of cold isopropanol before washed twice with 70% ethanol. The DNA pellet was air-dried and resuspended in 50 µl TE (Tris-EDTA) buffer. The DNA was resolved on 0.8% agarose gel and the DNA concentration was measured using Thermo Labsystems Fluoroskan Ascent ™ (Thermo Scientific, USA).

2.3 Marker Selection and PCR Genotyping

A specific primer namely, xa13-prom (Forward: GGCCATGGCTCAGTGTTTAT and Reverse: GAGCTCCAGCTCTCCAAATG) were used in this study. The primer was designed based on the InDel in the promoter region of *xa13* gene (Hajira et al., 2016). The PCR amplification was performed for each individual in a total reaction volume of 10µl consisting of 1.0 µl of template DNA (approximately 40ng/µl), 1 PCR rxn Buffer, 2.0mM $MgCl_2$, 2 mM dNTPs mixtures, 10 µmol of forward and reverse primer and 1U of *Taq* DNA polymerase (Invitrogen, USA). Amplification was performed using Peltier Thermal Cycler, DNA Engine Tetrad 2 (Biorad, USA). The PCR profile was set with the initial of denaturation at 94 °C for 2 minutes, then followed by 34 cycles of second denaturation at 94 °C for 30 seconds, annealing at 64.4 °C for 45 seconds, and extension at 72 °C for 45 seconds extension, and finally followed by one cycle of post-extension for 5 minutes at 72 °C. Then, the PCR products were resolved using 2% agarose gel and were viewed using UV Transilluminator.

2.4 Sequencing and sequence analysis

For confirmation of target region, PCR assay was performed on IRBB13 sample with a total volume of 40 µl followed the above profile and parameters. The PCR product was purified using Wizard® SV Gel and PCR Clean-Up System (Promega, USA) followed standard company protocol. The purified PCR product was sent to First BASE Laboratories SDN BHD for sequencing purpose. Forward and reverse sequences were viewed, aligned and edited using MEGA7 software before blast at NCBI database to search for sequence similarity.

3. Results

PCR analysis using specific primer targeting *xa13* gene showed none of the Malaysian rice cultivars showed the presence *xa13* gene. The primer was designed based on the variant existed in the promoter region of candidate gene namely, *Os8N3* gene which encodes for sugar transporter. PCR analysis of resistant varieties (IRBB13 and IRBB66) showed the presence of allele with the approximate size of 500 base pair while susceptible or negative control variety (MR84) showed the allele with the size of 300 base pair (Figure 1). All the selected Malaysian rice cultivars showed the same allele size as the MR84 variety which suggested none of them carries resistant allele of *xa13* gene.

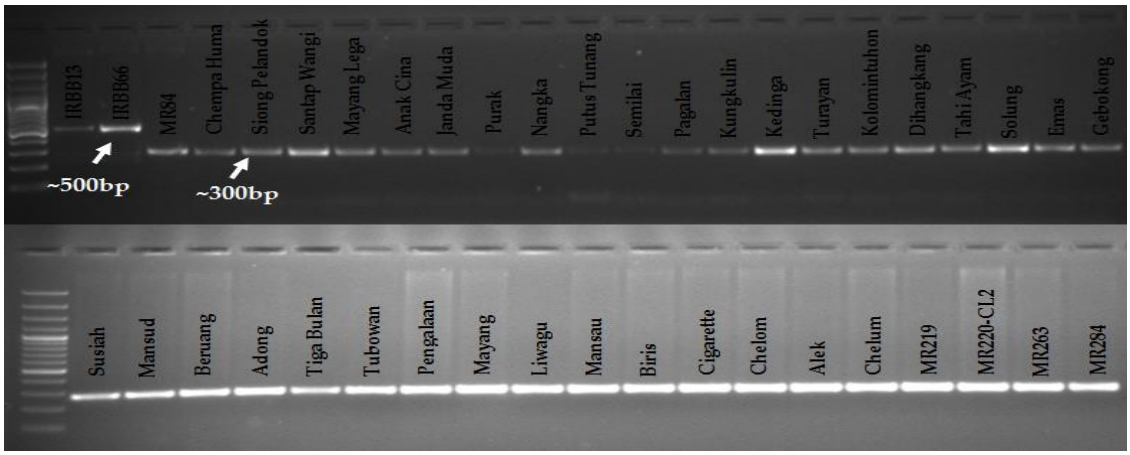
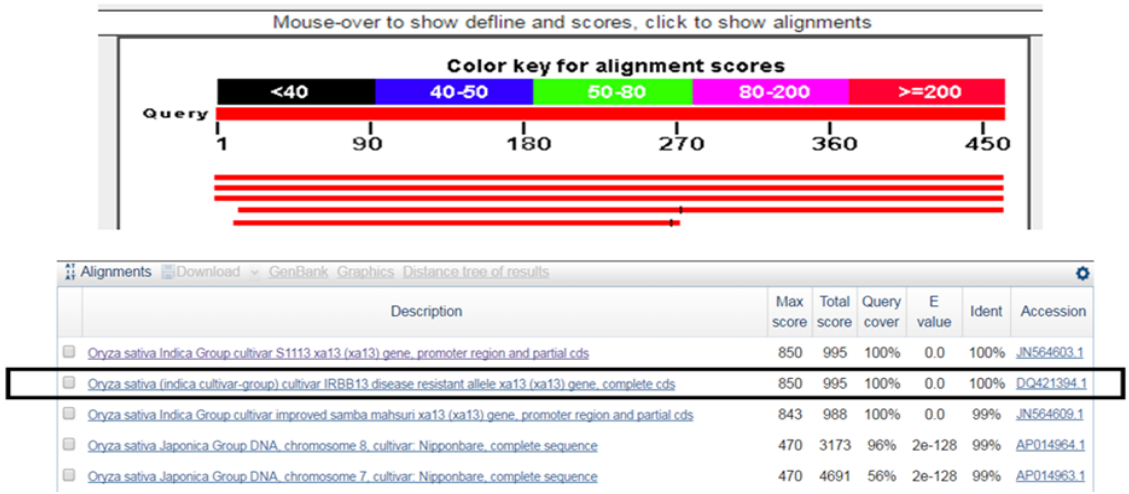


Figure 1. Amplification of the selected Malaysian rice cultivars using *xa13* specific marker on 2% agarose gel

The sequencing analysis of target region for the IRBB13 showed 100% identical to the IRBB13 resistant allele of *xa13* gene in NCBI database with the GenBank accession number of DQ421394.1 (Figure 2). This analysis conforms that the target region is within *xa13* gene.



Description	Max score	Total score	Query cover	E value	Ident	Accession
Oryza sativa Indica Group cultivar S1113 xa13 (xa13) gene, promoter region and partial cds	850	995	100%	0.0	100%	JN564603.1
Oryza sativa (indica cultivar-group) cultivar IRBB13 disease resistant allele xa13 (xa13) gene, complete cds	850	995	100%	0.0	100%	DQ421394.1
Oryza sativa Indica Group cultivar improved samba mahsuri xa13 (xa13) gene, promoter region and partial cds	843	988	100%	0.0	99%	JN564609.1
Oryza sativa Japonica Group DNA, chromosome 8, cultivar: Nipponbare, complete sequence	470	3173	96%	2e-128	99%	AP014964.1
Oryza sativa Japonica Group DNA, chromosome 7, cultivar: Nipponbare, complete sequence	470	4691	56%	2e-128	99%	AP014963.1

Figure 2. Blast result of target region sequence for IRBB13 which the query sequence showed identical to IRBB13 in NCBI database.

4. Discussion

The screening analysis showed none of the selected Malaysian rice cultivars showed the presence of *xa13* gene. A total of 35 landraces rice cultivars which originated from Peninsular Malaysia, Sabah and Sarawak were included in order to cover the diverse genetic background of rice cultivars. Even the landraces rice is a source of genetic resources or genetic reservoir (Gept, 2006), unfortunately, none of the selected Malaysian landraces rice showed the presence of resistant allele for *xa13* gene. Four commercial rice varieties namely MR284, MR263, MR219 and MR220-CL2 were also included in the analysis to represent Malaysian advanced breeding lines. Most of these selected advanced breeding lines are susceptible against BLB disease. Since the marker showed functional polymorphism for xa13 gene, it would be greatly useful to apply this specific marker to introgress xa13 gene into Malaysian advanced breeding lines for durable resistance against BLB disease.

5. Conclusions

Finding of this research showed that none of the selected Malaysian rice cultivars showed the presence of *xa13* gene resistant allele. The use of this specific primer targeting xa13 gene will increase the efficiency and effectiveness in MAB programme since the marker represents functional polymorphism of xa13 gene. The polymorphisms information from this study will be greatly useful for breeders and geneticist to introgress xa13 gene into Malaysian rice cultivars.

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Abbreviations

BLB: Bacterial leaf blight
MAB: Marker Assisted Breeding

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Application of Surfactant Modified Natural Zeolite Loaded NPK for the Growth of *Morus alba*

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Abstract: A sustainable fertilizer is practically demanded in agricultural sector, especially with zeolite amendment. Here, we investigated NPK-Organo-Zeolite performance as a controlled release fertilizer for the growth of *Morus alba* (White mulberry). The natural zeolite was modified with hexadecyltrimethylammonium (HDTMA) surfactant. This surfactant modified zeolite (SMZ) acted as a micro-carrier for the major plant nutrients; ammonium (N), phosphate (P) and potassium (K). A leaching test was performed to study the release of NH_4^+, PO_4^{3-} and K^+ from NPK-Organo-Zeolite, in comparison with Monoammonium Phosphate (MAP) and Monopotassium Phosphate (MKP) chemical fertilizers. NPK-Organo-Zeolite released the amount of NH_4^+ (213.33 mg/L), PO_4^{3-} (566.67 mg/L) and K^+ (8.08 mg/L) lower compared to MAP and MKP because zeolite has a capability in retaining the nutrients. Five different treatments were applied on *M. alba*; NPK-Organo-Zeolite, MAP, MKP, raw zeolite and control sample, which was harvested after 40 days. The average *M. alba* treated with NPK-Organo-Zeolite showed higher growth in plant height, number of leaves, total fresh and dry weights parameters (30.63±0.84 cm, 20.66±0.33, 14.69±0.60 g, 2.73±0.09 g; $p<0.05$) which were higher than other treatments, except MAP application. Overall, we conclude that NPK-Organo-Zeolite can perform as an alternative controlled release fertilizer for *M. alba* growth.

Keywords: Zeolite; Surfactant Modified Zeolite, *Morus alb*a, NPK Fertilizer, Controlled Release Fertilizer

1. Introduction

Modern agriculture depends on the efficiency of the fertilizers applied to boost the plant production and yield, which mainly for crops. There are two classifications of mineral nutrients which are known as macronutrients and micronutrients. The macronutrients that required for plant growth including nitrogen (N), phosphorus (P) and potassium (K) (White and Brown, 2010). These three macronutrients are required in the high amounts for the plant growth. Nitrogen (N) is used for plant growth especially leafy plants such as spinach. Phosphorus (P) is utilized for big fruit and root development and potassium (K) plays a major role in the regulation of water in plants and also largely responsible for many important internal and external fruit quality characteristic including fruit size, rind thickness and colour. However, rapid development and industrialization nowadays have led to the increasing level of pollution, contamination, and erosion of soil. Hence, there are some problems of fertilizers that can cause serious health and

environmental problems (Bhardwaj *et al.*, 2012). The problem that occurs is the accumulation of heavy metals in the soil and leaching of large amounts of nitrogen (N), phosphorus (P), and potassium (K) into groundwater and surface water (Hartemink, 2000). Besides, the problem of leaching caused eutrophication which is the blooming of microalgae and green plants on the surface of lake and groundwater.

In order to minimize the problem of conventional fertilizers, scientist and agronomist have given serious intention to these issues. A slow release fertilizer (SRFs) is one of the ways to overcome those problems. Moreover, a slow release fertilizer which is also known as control release fertilizer (CRFs) have the potential to minimize hazards such as soil toxicity that occurred in the environmental problem from nitrate (N) leaching while maintaining or improving nutrients use efficiency (Shaviv, 2005). There are three types of CRFs which are slightly soluble such as urea, supergranules, and coated fertilizers. Coated fertilizers are physically prepared by coating granules of conventional fertilizers (Tomaszewska and Jarosiewicz, 2003). Previously, Fan and Singh (2012) noted that there was four types mode of slow release fertilizer can be categorized which are diffusion, erosion or chemical reaction, swelling and osmosis. Furthermore, zeolite is one of the useful minerals to produce control release fertilizers. The unique chemical and physical properties of natural zeolite such as high cation-exchange capacities, cation selectivity, and molecular sieving make the zeolite most useful mineral produce control release fertilizers (CRFs) (Ming and Allen, 2001).

Another problem in the agricultural area is that the slow release fertilizers are often expensive and slower nitrate release when the high N is needed (Li, 2003). Clinoptilolite is one of natural zeolite that is typically used as a fertilizer because of the high cation exchange capacity and an inexpensive cation exchanger to load nutrients. This clinoptilolite can be found abundantly in the volcanic rock areas and it was easily obtained and also low cost (Mumpton, 1999). Besides, zeolite increased the nutrients uptake like N, P, and Zn in leaves (Ozbahce *et al.*, 2015). The utilization of original zeolite will cause a problem which is unable to hold phosphate anions into its framework structure. This is because of the repulsion between the anions and negatively charge zeolite surface (Li, 2003). However, the modification of original zeolite with surfactant molecules such as hexadecyltrimethylammonium bromide (HDTMABr) could be used to retain the phosphate (anions) on the zeolites (Ming and Allen, 2001). Surfactant modified zeolite (SMZ) can be defined as the modified form of zeolite in which surface of zeolite have been covered with a cationic surfactant (Ersoy and Celik, 2004). **Figure 1** shows theoretical illustration of the formation of HDTMA bilayer on the surface of zeolite and the adsorption of elements N, P and K (Malek *et al.*, 2014).

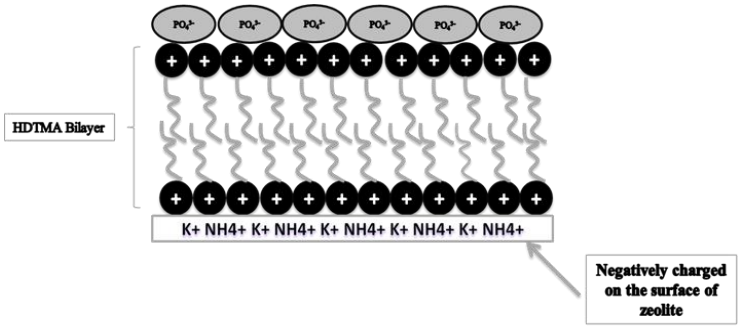

Figure 1. Formation of HDTMA bilayer on the surface of zeolite and the adsorption of N, P and K on the SMZ (Malek *et al.*, 2014)

The modification of the zeolite surface is performed to attract anions on the surfaces of zeolite since SMZ has the ability to attract N, P, and K to bind inside the zeolite framework (Malekian *et al*., 2011). This unique ability of SMZ producing the final product called as NPK-Organo-Zeolite contributed to a vital part of macronutrients component released in plants growth. Although NPK-Organo-Zeolite has been proven to improve the growth of *Clinacanthus nutans* (Abdullah, 2014) and okra (Hamzah *et al*., 2014), there are no studies on the effect of NPK-Organo-Zeolite on a mulberry tree named *Morus alba* (Mulberry).

This *M. alba* plant that belongs to the genus *Morus* of the family *Moraceae* was chosen in this study because it can grow rapidly, deciduous and medium to large size tree from 10-20 m (Singhal *et al*., 2010). Furthermore, mulberry contains high number of bioactive compounds including polysaccharides, anti-diabetes, and anti-oxidation which are beneficial for human (Yuan *et al*., 2015). Since mulberry has an antioxidant agent, this plant has been utilized as a dietary supplement (Yang *et al*., 2010). On the other hand, mulberry can also be utilized as pharmaceutical food (Salinas-Cahavira *et al*., 2011). Another benefits of *M. alba* plant is that all parts of the plant can be used in several purposes such as to treat liver and spleen disorder and also for cardiovascular complications (Lee *et al*., 2011) and extraction from mulberries can be utilized to treat and prevent coughing (Nam *et al*., 2002). Apart from the investigation of NPK-Organo-Zeolite performance as a controlled release fertilizer for the growth of *M. alba*, a leaching test was also conducted for NPK-Organo-Zeolite and in comparison with commercial fertilizers for the released of the major nutrients. Thus, in return, it is expected that the environmental friendly approach of NPK-Organo-Zeolite compared to chemical or conventional fertilizers (Siang, 2015) could reduce environmental problems and improve the crop yield in agriculture.

2. Materials and Methods

2.1 Preparation of NPK-Organo-Zeolite

The natural zeolite used in this research was imported from Indonesia and supplied by Provet Group of Companies Sdn Bhd, Serdang, Selangor. Ammonium acetate ($NH_4C_2H_3O_2$), surfactant hexadecyltrimethylammonium-bromide (HDTMA-Br) and monopotassium hydrogen phosphate $KHPO_4$ were supplied by Qrec (Asia) Sdn Bhd for the modification of the natural zeolite into NPK-Organo-Zeolite. Acetone was supplied by Fisher Scientific, for the chlorophyll test. Nanocolor kits including ortho-phosphate, ammonium and potassium were obtained from Macherey-Nagel, Germany which incorporated with the Nanocolor Vis spectrophotometer to measure the concentrations of ammonium, phosphate and potassium in the solution. Monommonium Phosphate (MAP) and Monopotassium Phosphate (MKP) fertilizers were purchased from Greentrade Sdn. Bhd, Shah Alam, Selangor. The fresh stem of *Morus alba* was obtained as a plant materials from Institute Veterinary, Kluang, Johor.

Firstly, ammonium-clinoptilolite (NH_4-cli) was prepared by mixing 5 g of ammonium acetate, $NH_4C_2H_3O_2$ in 1000 ml with 40 g of natural zeolite. Then, the filtration of the suspension through 185 mm Macherey-Nagel filter paper was conducted in order to separate solid particles with the solution. After that, the solid residual (NH_4-zeolite) on the filter paper was dried in an oven at 80°C overnight. The mortar and pestle were used to ground the dried NH_4-cli and then, the powder was sieved in order to separate the difference size of NH_4-cli. The whole process of the preparation of NH_4-cli was repeated 3 times. Secondly, organo-ammonium-zeolite (Organo-NH_4-zeolite) was prepared by adding 0.7289 g of HDTMA-Br in 500 ml distilled water with 10 g of NH_4-cli. At the final stage, NPK-Organo-Zeolite was prepared by mixing 5 g of monopotassium hydrogen phosphate, $KHPO_4$ with 40 g of Organo-NH_4-Cli in 1000 ml. The final solid residual was dried in an oven at 80°C for 15 hours.

2.2 Characterization of NPK-Organo-Zeolite

Raw zeolite, NH_4-zeolite, organo-NH_4-zeolite, and NPK-Organo-Zeolite were characterized by using Fourier transform infrared (FTIR) (Nicolet iS5-IR, Thermo Fisher). Attenuated Total Reflectance (ATR) technique of FTIR was applied with the OMNIC™ software. Next, X-ray diffraction (XRD) method on a Bruker AXS GmbH, German XRD machine was conducted for the same samples and XRD patterns were recorded with CuKα radiation at λ=1.5406 Å at 40 kV.

2.3 Leaching Test

Five g of NPK-Organo-Zeolite and other fertilizers was applied evenly on top of 200 g sand to ensure the even distribution of NPK-Organo-Zeolite on the sand. Three replicates for each treatment was setup. The experimental setup for controlled release study is shown in **Table 1**. By using retort stand, polystyrene cups with 4×0.5 cm diameter of the hole at the bottom and polystyrene cups were placed under the hole in order to collect the leachate. Then, 200 g of the sand were placed in the polystyrene cups. The polystyrene cups were loosely packed and was watered by 100 ml of distilled water for one day. Columns were allowed to drain for 24 hours before collecting the leachate and all the solutions inside the polystyrene cup were completely permitted to drain (Siang, 2015). After that, the leachate was collected into 50 ml falcon tube after 24 hours in order to identify the concentration of ammonium ions (NANOCOLOR® VIS Ammonium kit (Test: 1-05; REF: 918 05)), phosphate ions (NANOCOLOR® VIS ortho-Phosphate kit (Test: 1-77; REF: 918 77)), and potassium ions (Agilent 700 Series ICP Optical Emission Spectrometers (ICP-OES)) inside the respective leachate.

Table 1. List of samples for leaching test

Sample	Description
P	Aquarium Sand (5g)
C	Aquarium Sand (5g)+ Raw Zeolite (5g)
NPK-Organo-Zeolite (NPK)	Aquarium Sand (5g)+ NPK-Organo-Zeolite (5g)
MonoAmmonium phosphate (MAP)	Aquarium Sand (5g)+ MAP (5g)
MonoPotassium phosphate (MKP)	Aquarium Sand (5g)+ MKP (5g)

2.4 Plant Growth Study of *Morus alba*

Plant growth experiment was carried out at semi-green house, Agriculture Unit, Faculty of Education, Universiti Teknologi Malaysia. This study was performed in a pot about 15 cm x 15 cm in size. The approximately 20 cm long of fresh stems *M. alba* plants were cut and 1 kg of loamy soils was filled in pots for sowing the *M. alba*. The stems cutting of *M. alba* were embedded in the soils for 14 days and watering every days. After 14 days, *M. alba* in the seedling pots were transferred into the pots containing 1.5 kg of mixed soil. The composition of soil ratio was 3:2:1 (loamy soil: slit loam: sand) (Kumar *et al.*, 2011). Then, 5 different treatments (control, raw zeolite, NPK, MAP and MKP) were used as top dressing towards the plants with 5 replicates for each treatment. Next, all the parameters were measured and recorded every 3 days in the morning time. The parameters that were recorded including the height of plants, total number of leaves, width and length of leaves, fresh mass, dry mass and chlorophyll test (Dzkulfli *et al.*, 2014). For the collection of fresh mass, dry mass and chlorophyll tests, they were carried out on the harvest

time that was day 40. After the samples were dried, the fresh mass of all samples were measured on an analytical balance.

2.5 Collection of Data and Statistical Analysis

The plant performance and optimization studies were analyzed by using one way ANOVA and Post-hoc test in SPSS Statistics version 16.0 to determine the effect of treatments on plant height, number of leaf, fresh mass and dry mass of the *M. alba*. The significance value of the analyzed data was set at 0.05.

3. Results

3.1 Characterization of NPK-Organo-Zeolite

3.1.1 Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectra of raw zeolite and modified zeolite were analyzed in order to identify the changes happened in the structure of the zeolite before and after surfactant modification as well as chemical insertion process. **Figure 2** shows the FTIR spectra of raw zeolite and NPK-Organo-zeolite.

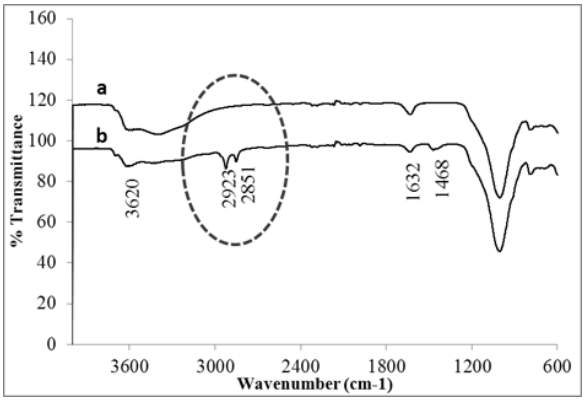

Figure 2. FTIR spectra of (a) raw zeolite and (b) NPK-Organo-Zeolite

For the FTIR spectrum of (a) raw zeolite, there are no bands revealed between 1400 cm^{-1} to 1500 cm^{-1} wavelength. However, it showed that there is strong band shown at the wavelength of 1440 cm^{-1} for (b) NPK-organo-Zeolite. This band referred to the stretching vibration of NH_4^+ (Zazoua *et al.* 2013). Hence, this band corresponds to the presence of ammonium ion on the NPK-Organo-Zeolite structure that penetrates into micro-porous of zeolite after insertion of ammonium (Mohammad *et al.*, 2016). There are two bands on the FTIR spectrum of (b) NPK-Organo-Zeolite at 2924 cm^{-1} and 2865 cm^{-1} but not in (a) raw zeolite. This band can be indicated to the HDTMA molecules that have been adsorbed on the zeolite surface (Bansiwal *et al.*, 2006). These two intense bands are assigned to symmetric and asymmetric C-H stretching vibration of surfactant molecule (HDTMA) (Bhardwaj *et al.*, 2012). This is because the HDTMA molecule has long alkyl tail CH_3 $(CH_2)_{15}N$ (Br) $(CH_3)_3$ constituted with the alkane groups of CH_3 and CH_2 (Bardakçi and Bahçeli, 2010).

3.1.2 X–Ray Diffraction (XRD)

X-Ray diffraction analysis was conducted on unmodified and modified zeolite in order to confirm the crystalline structure and identification of the samples. Most of the researcher

performed XRD analysis to identify and determine the crystalline structure of particularly zeolite at the atomic level (Mishra *et al.,* 2014). In this study, the structural characteristic of raw zeolite and NPK-Organo-Zeolite were characterized by using XRD analysis as shown in **Figure 3**.

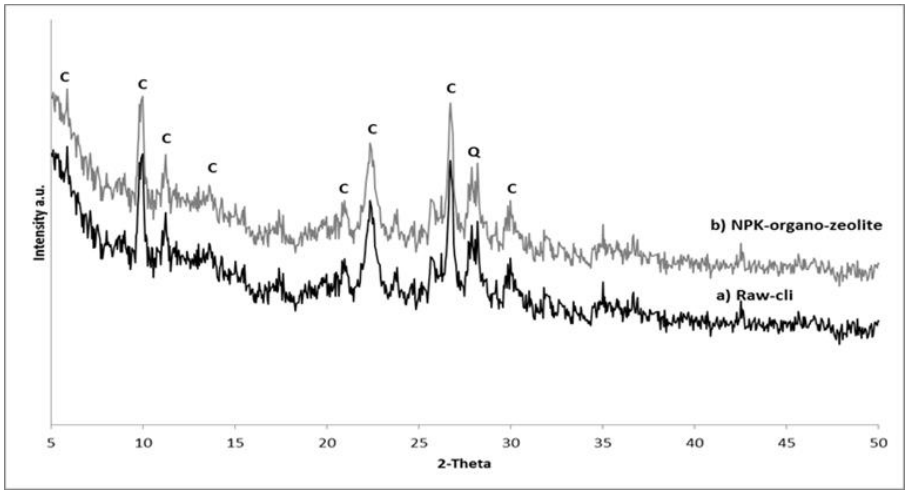

Figure 3. X-ray diffractograms of (a) raw zeolite and (b) NPK-organo-zeolite. (Legend: C-Clinoptilolite, Quartz)

Figure 3 shows that the characteristic bands of unmodified and modified zeolite were found on the X-ray diffractogram. Based on the XRD patterns of raw zeolite and NPK-Organo-Zeolite, it showed that the samples are in the crystalline form as the diffractogram showed important bands from 5° to 50° (Guinier, 1994). Besides that, there were no changes in the XRD patterns of raw zeolite and NPK-organo-zeolite indicates that the structure of zeolite remain unchanged after modification with HDTMA molecules on the zeolite surfaces because the bands showed similar XRD patterns between them (Malek *et al*., 2015).

3.2 Leaching Test

The experiment for the leaching was done in order to analyse nutrients ammonium (NH_4^-), phosphate (PO_4^{3-}), and potassium (K^+) released from different fertilizers samples (Siang, 2015). **Figure 4** shows the concentrations of ammonium (NH_4^-), phosphate (PO_4^{3-}) and potassium (K^+) released from different fertilizers, respectively. Based on **Figure 4(a)**, MAP showed the highest concentration of ammonium ion (NH_4^+) released compare to other fertilizers. However, **Figure 4(c)** shows that MKP fertilizer displayed the highest concentration of K^+ released from the sample. This is because, there is no adsorbent or micro carrier that can hold NH_4^+ and K^+ from leached out easily from the sample. Besides that, this is also due to the MAP and MKP which are water soluble fertilizers and they were easily solubilized after watering (Lindsay *et al.,* 1962).

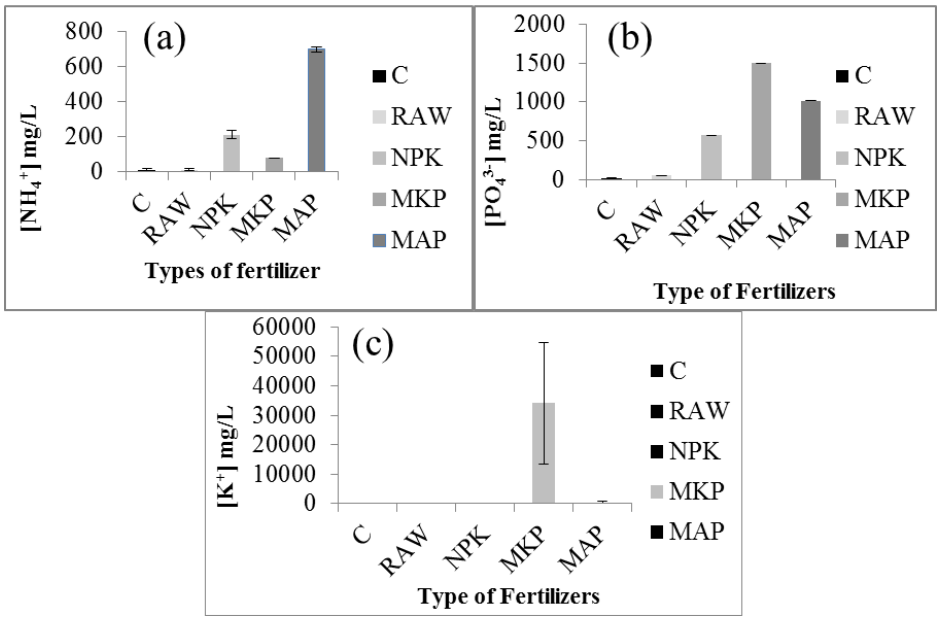

Figure 4. The release profiles of (a) ammonium (NH_4^+), (b) phosphate ion (PO_4^{3-}) and (c) potassium (K^+) from different fertilizers. Legends: C: Control, Raw: Clinoptilolite, MKP: Monopotassium Phosphate, MAP: Monoammonium Phosphate, NPK: NPK-Organo-Zeolite.

3.3 Plant Growth Study

From **Figure 5(a)** to **5(d)**, it can observe that sample from MAP fertilizer provides the highest growth profile including the height of *M. alba*, a number of leaves, the width of leaves, the length of leaves, fresh and dry weight and followed by growth profile of *M. alba* treated with NPK-Organo-Zeolite. On the other hand, *M. alba* that treated with NPK-Organo-Zeolite also showed excellent growth profiles include the height of the plant, the number of leaves, the width of leaves, the length of leaves, fresh and dry weight. The growth profiles that fertilized under MKP fertilizer showed that the overall parameters were lower than plant growth under NPK-Organo-Zeolite. It shows that, there was an excess of nutrients required for the plant growth of *M. alba*. Apart from that, the application of raw zeolite as fertilizer on the growth of *M. alba* displayed the slowest growth profile.

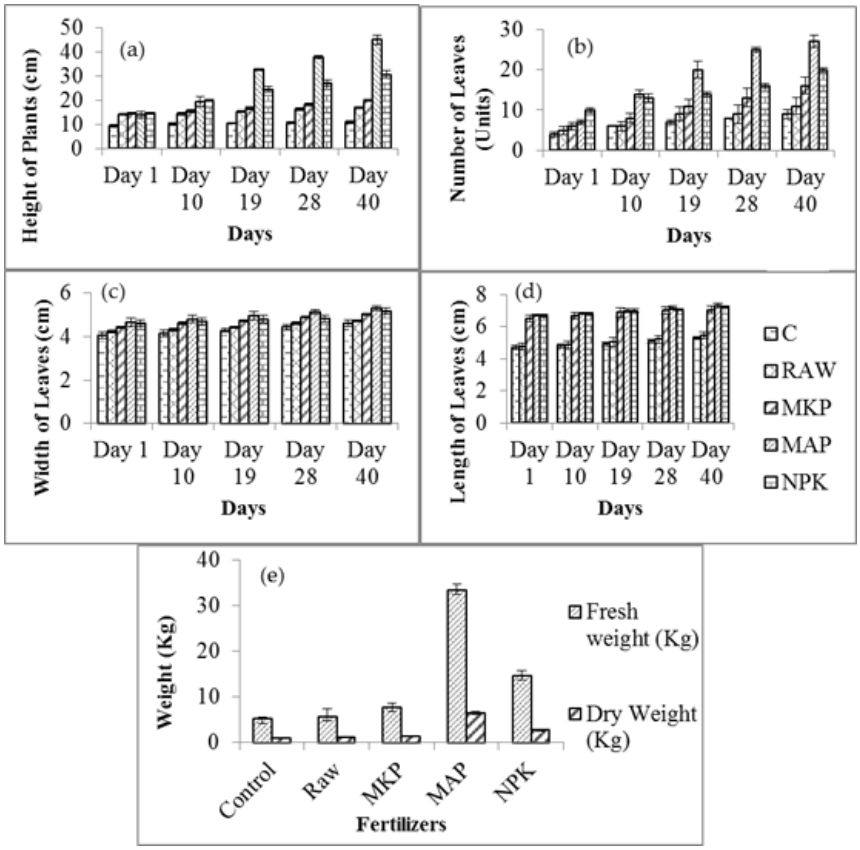

Figure 5. The growth profiles ((a) height, (b) Number of Leaves, (c) Width of Leaves, (d) Length of Leaves and (e) Fresh and dry weight) of *M. alba* using different fertilizers.

Table 2 presents the result for one-way ANOVA statistical analysis for differences of plant height between all five different treatments were significant [F (3, 15)] =363.419, p=0.00]; number of leaves [F (3, 15)] = 53.148, p=0.00]; width of leaves [F (3, 15)] =16.409, p=0.00]; length of leaves [F (3, 15) =115.600, p=0.00]; fresh weight [F (3, 15)] =329.796, p=0.00] and dry weight [F (3, 15)] =171.242, p=0.00]. Hence, the null hypothesis was rejected. Post-Hoc comparison using the Tukey HSD test indicates that the mean values for all parameters (plant height, number of leaves, length of leaves, width of leaves, fresh weight, dry weight) between sample were highly significantly different (p=0.00).

Table 2. Overall parameter for plant growth study of NPK-Organo-Zeolite

Parameter	Treatment					*p*-value
	Control	Raw zeolite	MKP	MAP	NPK	
Plant Height	$10.90^{a}\pm0.28$	$16.93^{b}\pm0.26$	$19.83^{c}\pm0.57$	$44.96^{d}\pm1.13$	$30.63^{e}\pm0.84$	0.00
Width of leaves	$4.63^{a}\pm0.08$	$4.73^{b}\pm0.03$	$5.03^{c}\pm0.06$	$5.30^{d}\pm0.05$	$5.16^{e}\pm0.08$	0.00
Length of Leaves	$5.30^{a}\pm0.05$	$5.46^{b}\pm0.12$	$7.06^{c}\pm0.13$	$7.33^{d}\pm0.08$	$7.26^{e}\pm0.03$	0.00
Total No. Leaves	$9.66^{a}\pm0.66$	$11.33^{b}\pm1.20$	$15.66^{c}\pm1.45$	$27.33^{d}\pm0.88$	$20.66^{e}\pm0.33$	0.00
Total Fresh Mass	$5.19^{a}\pm0.24$	$5.67^{b}\pm0.94$	$7.76^{c}\pm0.52$	$33.45^{d}\pm0.73$	$14.69^{e}\pm0.6$	0.00
Total Dry Mass	$0.97^{a}\pm0.03$	$1.09^{b}\pm0.1$	$1.42^{c}\pm0.09$	$6.53^{d}\pm0.35$	$2.73^{e}\pm0.09$	0.00

Note: Each data print represents the mean ± SEM of 3 replicates

a, b, c, d, e = means in a row with different letters differ ($p<0.05$)

4. Discussion

4.1 Characterization of NPK-Organo-Zeolite

Generally, FTIR spectroscopy is utilized to characterize the nature of bonds and the functional groups in the original zeolite and modified zeolite samples (Malek *et al*., 2014). Besides that, it also used to confirm the structural stability of modified zeolite and raw zeolite. In this study, the main focus of the characterization using FTIR was the possible explanation for the production of NPK-Organo-Zeolite. After modification on zeolite surface with HDTMA molecules, the zeolite surface is accumulated with the HDTMA bilayer. This bilayer formation forms after the HDTMA molecules tend to self-aggregate and formed micelles on the zeolite surface at the concentration of HDTMA above its critical micelle concentration (CMC) value. Hence, the negative charge of natural zeolite is changed to positive charge and the physical properties of natural zeolite are also changed after the modification process (Malek *et al*., 2015). As shown in **Figure 1,** the long alkyl tail of HDTMA molecules CH_3 $(CH_2)_{15}N$ (Br) $(CH_3)_3$ forming bilayer on the zeolite surfaces.

There are bands exist in the FTIR spectra at a wavelength 600 cm^{-1} to 1200 cm^{-1} and these bands remain stable after the modification of zeolite. Hence, it was proven that even the zeolite has been modified by surfactant molecules (HDTMA); it would not much affect and distort their original structure after modification process. The FTIR band at wavelength 1000 cm^{-1} corresponds to the asymmetric stretching vibration modes of internal T-O bonds TO_4 tetrahedral (T= Si and Al) of zeolite while band around 792 cm^{-1} is assigned to the vibration modes of O-T-O groups (Yusof *et al*., 2015). As a conclusion, the production of NPK-organo-zeolite was completely achieved and the chemicals used in the modification process did not distort the original structure framework of zeolite (Li, 2003).

By comparing the XRD patterns in **Figure 3** with the database of XRD powder pattern of zeolite (Treacy and Higgins, 2007), clinoptilolite and quartz exist as impurities in the zeolite material. XRD bands at around 5.85°, 9.8°, 11.2°, 13.25°, 20.80°, 22.35°, 26.70° and 29.85° represent natural clinoptilolite (Malek *et al*., 2015). Besides, traces of quartz also present as minor impurities at band 27.85°. Therefore, after natural zeolite was modified with surfactant molecule (HDTMA) and insertion of NPK elements, the crystalline structure of zeolite remain the same and this can be proven by comparing with the XRD pattern of unmodified zeolite and modified zeolite because there was no significant change of bands between raw zeolite and NPK-Organo-Zeolite. This is because natural zeolite has negatively charged and it can attract ammonium and potassium cations into the micro porous of zeolite. Then, the loading of surfactant molecule HDTMA on the surface of zeolite will form HDTMA bilayer and it will adsorb phosphate anions onto the zeolite without disturbing the aluminosilicate framework structure of the zeolite (Zazoua *et al*., 2013).

4.2 Leaching Test

In a leaching test, a lower concentrations of NH_4^+ and K^+ released was detected from the NPK-Organo-Zeolite sample compared to MAP and MKP fertilizers. This is due to the retention of NH_4^+ and K^+ on the exchange sites of the zeolite which contributed by high cation exchange capacity (CEC) and high affinity of zeolite towards NH_4^+ and K^+ into it micro pore (Malekian *et al*., 2011). This property makes zeolite a potential carrier for NH_4^+ and K^+. Hence, NH_4^+ and K^+ were effectively adsorbed onto the surface of clinoptilolite and releasing it slowly from their framework structure (Rabai *et al*., 2016). Furthermore, based on the physical properties of the zeolite, it can be used as controlled release fertilizer because it has the capability to hold

ammonium cation in and another plant nutrient such as potassium cation in the micro-porous of zeolite structure (Zazoua *et al.*, 2013).

Based on **Figure 4(b)**, it can be clearly seen that the concentration of phosphate ions (PO_4^{3-}) released from MAP and MKP fertilizers was the highest as compared to other treatments. This is because both fertilizers are water soluble and have fast release nutrient property especially phosphate ions. Furthermore, it also due to negatively charge of phosphate ions that have a lower affinity toward particles soil or sand surface (Li, 2003). Besides that, the concentration of phosphate ions from NPK-Organo-Zeolite displayed significantly lower amount of NH_4^+ (213.33 mg/L), PO_4^{3-} (566.67 mg/L) and K^+ (8.08 mg/L) compared to MAP and MKP because zeolite has a capability in retaining the nutrients. In addition, before modification of natural zeolite with surfactant molecule (HDTMA), it cannot adsorb phosphate ions since it is negatively charged and it cause repulsion force between natural zeolite and phosphate ions during nutrient insertion. As a conclusion, these controlled release studies proved that the NPK-Organo-Zeolite has high affinity and high cation exchange capacity (CEC) to retain ammonium and potassium cation in the framework structure of the zeolite.

4.3 Plant Growth Study

MAP fertilizer has a high amount of ammonium ions (NH_4^+) and phosphate ions (PO_4^{3-}) that important for plant growth especially in the shoot and root development (Creger and Peryea, 1994). However, by using MAP fertilizer it can cause several environmental effects such as leaching of nutrients to the groundwater and destroyed soil structure due to the high amount of nutrients release to the soil. This is because MAP is water soluble and fast release fertilizer. Besides that, this fertilizer does not have adsorbent that can retain NH_4^+ and PO_4^{3-} from leaching out easily to the environment when watering, so this fertilizer needs to be applied frequently in order to provide sufficient nutrients for plant growth. On the other hand, NPK-Organo-Zeolite comprised complete nutrients which are ammonium (N), potassium (K) and phosphate (P) inside the zeolite. In addition, N, P, and K are vital for the plant growth especially in leaves, roots, shoots and fruits, respectively (Dogan *et al.*, 2016). Since zeolite has high cation exchange capacity, these properties can help to retain nutrients inside its micro porous of the zeolite and released them slowly or gradually to the plant (Malek *et al.*, 2014). This can be supported by the leaching test where NPK-Organo-Zeolite displayed the lowest concentration of NH_4^+, K^+ and PO_4^{3-} released from the system. Hence, these unique properties of NPK-Organo-Zeolite could be alternative controlled release fertilizer for the various plant growths.

For MKP fertilizer, it shows that, there was an excess of nutrients required for the plant growth of *M. alba*. However, according to Tyburski and Sienkiewicz, (2014) the excess of nutrients from chemical fertilizer such as MKP could turn the soil pH from neutral to acidic condition and this situation is not suitable for plant growth. Furthermore, it could be explained that MKP is water soluble and fast release fertilizer (Lindsay *et al.*, 1962). Application of MKP fertilizer can cause a high amount of nutrient leaching to the soil because MKP fertilizer does not have physical entrapment or micro carrier that can hold nutrients from MKP system from leached out easily during its application on the plant treatment. *M. alba* plant does not grow well after being treated with raw zeolite because the raw zeolite did not have important nutrients to support plant growth especially ammonium, phosphate and potassium in order to assist plant growth (Beqiraj *et al.*, 2008).

5. Conclusions

The results from XRD and FTIR analyses displayed that the original structure of zeolite remains stable and intact after modification of zeolite using surfactant molecule, HDTMA. After

modification on zeolite surface using HDTMA, it allows the attachment of ammonium ions, NH_4^+, phosphate ions, PO_4^{3-}, and potassium ions, K^+ into the framework structure of the modified zeolite. This attachment was proven from the leaching test of the nutrients (NH_4^+, PO_4^{3-}, and K^+) released from NPK-organo-zeolite where it was the lowest as compared to Monoammonium Phosphate (MAP), Monopotassium Phosphate (MKP) and raw zeolite. Furthermore, the application of NPK-organo-zeolite displayed excellent growth of *M. alba* including height, the number of leaves, width and length of leaf, the fresh mass and dry mass, and chlorophyll content except for sample MAP fertilizer. Even though NPK-organo-zeolite has a lesser concentration of nutrients (NH_4^+, K^+, and PO_4^{3-}) compared to MAP fertilizer, NPK-Organo-Zeolite can provide sufficient nutrients to *M. alba* growth and this was proven in the plant growth study. Thus, the environmental problems such as leaching could be reduced or prevented by using NPK-Organo-Zeolite as plant treatment alternative.

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Abbreviations

The following abbreviations are used in this manuscript:

SMZ: Surfactant modified zeolite
HDTMA: Hexadecyltrimethylammonium
SRFs: Slow release fertilizer
CRFs: Control release fertilizer
MAP: Monoammonium phosphate
MKP: Monopotassium phosphate
NPK: NPK-Organo-Zeolite

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The Effectivity of Cinnamon Alcoholic Extract to the Total Cholesterol on Hypercholesterolemia rat Model

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Abstract: Coronary Heart Disease is a global health problem. Hypercholesterolemia is a risk factor for CHD. Cinnamon is a herb that is believed to have an antihypercholesterolemia effect. This study aimed to determine the effectivity of Cinnamon to the total cholesterol on hypercholesterolemia rat model. It is experimental study design in vivo with pretest-posttest control group design. Samples consist of 30 healthy Wistar rats were divided into 5 groups with simple random sampling method, Group 1 was treated by giving 20 mg/day, group 2 was 40 mg/day and group 3 was 80 mg/day Cinnamon alcoholic extract, Control group was treated by giving 0,2 mg/day Simvastatin, and Placebo group was given aquadest. The average of the highest decrease of total cholesterol was found in group 3. The results of statistical tests indicated that total cholesterol alteration significantly different in all treatment groups. In conclusion, giving Cinnamon was effective in decreasing levels of total cholesterol on hypercholesterolemia rat model.

Keywords: CHD; hypercholesterolemia; cinnamon; simvastatin; in vivo

1. Introduction

Atherosclerosis is a condition of thickening in the walls of blood vessels that can lead to coronary heart disease complications (Amelia and Widyaningsih, 2014). These circumstances are closely related with hypercholesterolemia cholesterol levels in the blood exceeds the normal limit (Guyton & Hall, 2008).

Atherosclerosis can be casued by varied risk factors such as hypercholesterolemia, heredity, consumption of high-fat foods, lack of exercise and smoking habits (Setiati, 2009). Hypercholesterolemia can be prevented by maintaining healthy diet, regular exercise, cease smoking and drinking alcohol, along with reducing high cholesterol and saturated fats food consumption. Therefore, a modality of an effective, safe and affordable preventive therapy is needed, like one provided by herbal medicines.

One of the herbal medicines that is widely known is cinnamon. Cinnamon sticks bark contains tannin and approximately 1-3% volatile oil consisting of safrole, eugenol, and sinamaldehid (Mursito 2005). Tannin is an antioxidant compound that is believed to be able to lessen cholesterol levels, which in turn can prevent coronary heart disease (Azima, 2004). Many studies have reported that the tannin content in plants may play a role in preventing and lowering the risk of coronary heart disease.

2. Methods

2.1. Animal and Housing

Adult male albino rats (Rattus norvegicus) weighting 150 – 200 g and 8 – 10 weeks of age were used. Rats were breed in the Animal House of Medical Faculty University of Sriwijaya and placed in ventilated polypropylene cages, with four rats per cage with access to standard diet and tap water ad libitum under standard laboratory conditions (12 h light dark cycle, at 20 – 24 ºC) and acclimated to the laboratory conditions for 1 week before experiment.

The samples were divided randomly into 5 groups and treated as follows: The placebo group, was given distilled water, The control group, given the drug of choice that is Simvastatin 0.2 mg/day, The treatment group I, given Ethanol Cinnamon extract as much as 20 mg/day, The treatment group II, given Ethanol Cinnamon extract as much as 40 mg/day and The treatment group III, given Ethanol Cinnamon extract as much as 80 mg/day

2.2. Preparation of Alcoholic Extract of Cassia Cinnamon

The cinnamon extract was prepared by adding 200 g dried powdered cinnamon into 2 L of ethanol 70% in a bottle, to be mixed manually before then kept in the place at a room temperature for 3 days. The mixture was filtrated with Whatman filter paper and evaporated til dry by using Rotary Evaporator to remove the ethanol.

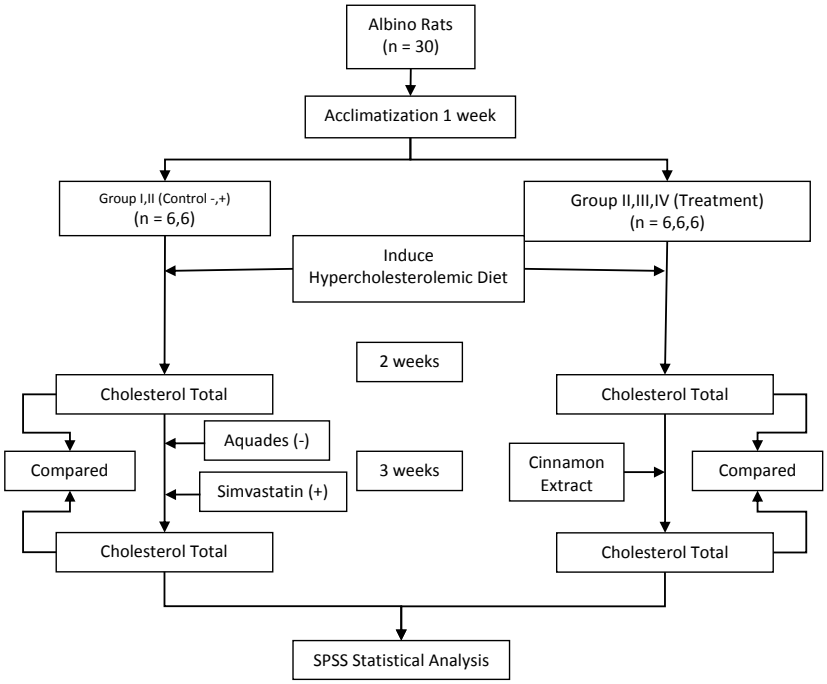
2.3. Induction of Hypercholesterolemia

Wistar male rats were given standard food mixture obtained from the animal house Laboratory of Pharmacology Faculty of Medicine, University of Sriwijaya Palembang and hyperlipidemia diet for 14 days. Hyperlipidemia diet consisted of offal, waste cooking oil and two yellow duck eggs in a blender. The diet then would be given as much as 3-5 ml through a stomach sonde every day.

2.4. Examination Blood cholesterol levels

Examination of blood cholesterol levels was performed before and after treatment. Examination reagent used is "DiaSys-Cholesterol FS" spectrophotometrically.

2.5. Flowchart Research



3. Results

The sample in this study was a white rat (Rattus norvegicus) Wistar male. Prior to treatment, samples were first conditioned of having hypercholesterolemia by feeding them with offal (brain and lard beef) fried in cooking oil, along with two egg yolks, fed directly into the stomach of rats. The induction was conducted in over 2 weeks. After being induced for 2 weeks, the levels of total cholesterol in the sampled were mesdured by using a spectrophotometer brands smartspec plus. Below is the table showing the average total cholesterol level in samples (Table 1) The total cholesterol level in samples were being examined after three weeks treatment,a s shown in table 2.

Table 1. Average total cholesterol Rats Prior Treatment

Group I	Group II	Group III	Group IV	Group V
204	219	215	216	216
215	208	220	205	205
219	221	206	207	210
210	214	209	220	220
218	205	218	218	218
213.2	213.4	213.6	213.2	213.8

Table 2. Mean Total Cholesterol Rats Before and After Treatment

Group	Pre-Test	Post-Test	Difference
I	213.2	220.4	7.2
II	213.4	191.2	22.2
III	213.6	187.2	26.4
IV	213.2	184.6	28.6
V	213.8	182.8	31
Average	213.44	193.24	23.08

To determine the significance of the effect of cinnamon extract on cholesterol-lowering done with SPSS 22 statistical test using the test one way ANOVA followed by post-hoc test. The statistical result showed there is a significant difference between the control and treatment groups $p < \alpha$ (0.000).

4. Discussion

From the results in Table 1, it is showed that cholesterol levels of rats had increased above normal. Cholesterol reagent used is Diasys, where the standard normal cholesterol was 200 mg/dl. After that, begin treatment of hypercholesterolemia in which the feed is continued and each group began treatment for 4 weeks. 1 negative control group was given distilled water 1 ml, Group II, III and IV respectively given cinnamon extract dose of 20 mg/day, 40 mg/day and 80 mg/day, while the V group awarded Simvastatin 0.2 mg/day. According to Lee, et al. (2007), phenolic compounds found in cinnamon can inhibit the activity of HMG-CoA reductase and suppresses lipid peroxidation via increased activity of antioxidant enzymes in the liver that causes a decrease in cholesterol levels. Where statins have a working mechanism to lower cholesterol by inhibiting the activity of HMG-CoA reductase.

From table 2 above can be seen there is a decrease in the average cholesterol level in Group II, III, IV and V. In order to determine the significance of the effect of cinnamon extract on cholesterol-lowering statistical test with SPSS 22 using one way ANOVA test followed by a test post-hoc.

From one way ANOVA test value (sig) $p < \alpha$ (0.000) that there are significant differences between the control and treatment groups. After doing ANOVA test, conducted post hoc test. From the post hoc test showed that cinnamon extract did not have a significant effect on the negative control. In addition, the effect of cinnamon extract have not been able to match the standard drug effect is simvastatin 0.2 mg/day. According to Sonia, et al. (2013), extract of cinnamon for 35 days with a dose of 0.2 g/kg body weight in rats with induced hypercholesterolemia and normal rats that can lower total cholesterol, LDL (low density lipoprotein) and Trigeliserida significantly, while the increase in HDL (high-density lipoprotein) is not very influential. However, from this study, the effect of cinnamon extract 0.2 g/kgBB can't match the effect of the drug hypocholesterolemia Atrovastatin a dose of 0.2 mg/KgBW.

5. Conclusions

Cinnamon (Cinnamomum spp.) is a plant that is common and can be found in Indonesia. The main content of phenolic compounds in cinnamon extract can lower cholesterol levels in the blood, making it useful as a herb plants are hypocholesterolemic. This has been proven in some research conducted in vivo, using animal models, as well as directly to human studies, where

after being given cinnamon extract decreased the total cholesterol significantly. From the results it can be concluded that cinnamon extract has an effect to lower the cholesterol level although the potention is still below the standard drug (Simvastatin).

Abbreviations

LDL : Low Density Lipoprotein
HDL : High Density Lipoprotein

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Elucidation of GDC-0941 binding to PI3Kα isoform via *in vitro* mutagenesis of non-conserved amino acids of PI3Kα

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Abstract: Phosphatidylinositol 3-kinase (PI3K) is a lipid kinase that catalyzes the biosynthesis of PI(3)P, PI(3,4)P_2 and PI(3,4,5)P_3 - second messengers that trigger a wide range of downstream signalling cascades involved in cell survival, growth, adhesion and proliferation. The heterodimeric class 1 PI3K proteins are composed of a regulatory subunit (p85) complexed with either one of 4 different isoforms of catalytic subunit (p110α, p110β, p110δ and p110γ). The PI3KCA gene encoding the α-isoform has been found to be frequently mutated in cancers such as breast, prostate, colon, liver and brain. There has been a growing number of Class I PI3 kinase inhibitors described to date with some showing selectivity to different PI3K isoforms. However, the basis of selectivity of these inhibitors is still ambiguous. Previous studies have shown that specific regions within the catalytic subunit contain non-conserved residues which are involved in isoform selectivity. We have investigated the role of some non-conserved residues using site-directed mutagenesis followed by production of PI3Kα mutant enzymes. The potency of a potent and dual-selective PI3K inhibitor, GDC-0941 was tested against PI3Kα mutant enzymes and compared to the wild type enzyme. This approach will help in understanding the structure-activity relationship of PI3K isoform selectivity and may validate the existing structural information of inhibitor:enzyme complexes reported so far.

Keywords: PIP_2 – phosphatidylinositol 4,5-bisphosphate; PIP_3 – phosphatidylinositol 3,4,5-triphosphate

1. Introduction

Phosphatidylinositol 3-kinase (PI3K) belongs to a lipid kinase family responsible for the phosphorylation of phosphatidylinositols specifically acting on the 3'OH position of the inositol ring producing the second messenger substrates, phosphatidylinositol 3-monophosphate [PI(3)P], phosphatidylinositol 3,4,-bisphosphate [P1(3,4)P_2] and phosphatidylinositol 3,4,5-triphosphate [P1(3,4,5)P_3] which regulates diverse cellular signalling mechanisms including cell growth, proliferation, adhesion, migration, survival, motility, metabolism, gene expression as well as cytoskeleton rearrangement [1-4]. There are eight known mammalian PI3Ks divided into three classes based on their structure, function and substrate specificity [4]. Class 1 PI3Ks, the focus in this study, are a heterodimer proteins consisting of a regulatory subunit complexed with one of the catalytic subunits designated p110α, p110β, p110δ and p110γ. The members of the class I PI3Ks catalyse the same lipid substrate *in vivo* by converting phosphatidylinositol-4,5-bisphosphate (PIP_2) to phosphatidylinositol-3,4,5-triphosphate (PIP_3) which leads to regulation of cell proliferation, cell growth and cell survival [2-5]. This is in accord with findings that drugs targeting class 1 PI3K demonstrated anti-cancer activity. However, individual isoforms in class 1 PI3K have special functions and as a result, PI3Ks are also associated in a wide range of diseases. PI3Kβ isoform has been shown to be involved in platelet aggregation and has become a

promising target for thrombosis while PI3Kγ and PI3Kδ isoforms are thought to be involved in immune cell regulations and motility have been targeted for treatment of immune inflammatory disorders [2]. A lot of attention has been given to the PI3Kα isoform because the gene that encodes for p110α subunit (PIK3CA gene) is frequently mutated in human cancers. High incidence of PIK3CA mutations have been identified in a number of human cancers including breast, colon, pancreas, ovarian, liver and brain [2,6]. Therefore, a small molecule inhibitor which shows selectivity towards p110α isoform could become a promising anti-cancer drug.

The earliest inhibitors discovered were LY294002 and wortmannin which became primary pharmacological tools as both compounds recorded high potency but were non-selective for the isoforms. These compounds compete for ATP binding by forming interactions with conserved residues within the catalytic site [8-9]. Targeting an individual PI3K isoform is challenging but isoform selective inhibitors are necessary to reduce the possible side effects of inhibiting overlapping functions of different PI3K isoforms. To date, there have been a number of PI3K inhibitors developed from different chemical classes that exhibit either broad class I activity or class I isoform selectivity and some are currently in clinical drug trials. For example, GDC-0941 inhibitor has completed phase 1 clinical study and was reported to be well tolerated in patients even though it inhibits other PI3K isoforms as demonstrated in *in vitro* studies [8, 10]. Unfortunately, little is known about the biochemical criteria that define inhibitor selectivity and this project is aimed at understanding the binding mechanism of small molecule PI3K inhibitors which will contribute information for future PI3K isoform selective drug design.

Comparison of the crystal structures of p110γ bound with ATP and the p110γ-LY294002 complex showed that LY294002 induces structural heterogeneity within the ATP binding site region [8]. Similarly, binding of PIK39 inhibitor induced conformational change of the Met804 side chain leaving Lys802 residue exposed for binding to the inhibitor [17]. These structural observations led to an examination of the sequence homology surrounding the amino acids identified above. This showed that there were two distinct of non-conserved amino acids regions (referred as region 1 and region 2 in this article) that potentially confer isoform selectivity and could be targeted in the development of novel isoform-specific PI3K inhibitors [8, 12].

Previous work has shown that site-directed mutagenesis of non-conserved residues in region 1 was able to assess their role in binding to selected inhibitors. Substituting the corresponding non-conserved residues between p110α and p110β isoforms showed that residues Glu859 and His855 can affect the potency of PI3Kα inhibitors [13]. As a continuation of this effort, further PI3Kα mutant enzymes have been generated using site-directed mutagenesis. We screened the role of non-conserved residues using known PI3K inhibitor, GDC-0941 which was aimed at understanding the binding mechanism of this inhibitor within the catalytic pocket.

GDC-0941 has been reported as a potent but non-selective inhibitor with respect to class 1 PI3K isoforms. It has a published IC_{50} of 3nM for p110α/p110δ while it exhibited partial selectivity with an 11 and 25-fold increase relative to the p110α isoform, in the IC_{50} for the p110β and p110γ isoforms respectively [14]. The availability of GDC-0941 liganded p110α, p110β and p110δ crystal structures has facilitated the understanding of the binding mechanism hence GDC-0941 serves as important research tool in elucidating the mechanism of selectivity of the p110α isoform [14-16]. Eventhough GDC-0941 shows little selectivity among class 1 PI3K members, phase 1 clinical studies reported that this inhibitor showed signs of anti tumour activity and it is well tolerated in patients [10].

This report will present our current understanding of the binding mechanism of GDC-0941 based on its inhibition profile facilitated by the availability of crystallography data of GDC-0941 in complex with the p110β, p110γ and p110δ isoforms [14-16].

2. Materials and Methods

2.1 Generation of baculovirus-containing p110α mutant DNA

The procedure involved creating mutant plasmids using site-directed mutagenesis by the polymerase chain reaction (PCR) using pFastBac/p110α plasmid as the template according to the Bac-to-bac Baculovirus Expression Kit (Invitrogen). The expected mutation on the p110α sequence was confirmed by DNA sequencing. This was followed by transposition of the mutant sequence from the pFastBac plasmid into a baculovirus vector called bacmid. Following transfection of the bacmid DNA into Sf21 insect cells, the resulting recombinant virus was amplified to a range of 10^7-10^8 pfu/ml. Western blot was carried out to validate the presence of p110α protein using isoform-specific antibodies.

2.2 Protein Expression and Purification

Sf21 insect cells were co-infected with His-p85α and p110α (wild type or mutants) baculoviruses for 48 hours at 27°C in serum free insect medium (Sf900, Invitrogen). Cells were pelleted and resuspended in 50mM NaH_2PO_4, 300mM NaCl, 100mM imidazole, 1x EDTA-free complete protease inhibitor cocktail (Roche), pH 8, 1% (v/v) Tween-20, sonicated and centrifuged at 14000xg for 15 minutes at 4°C. Ni-agarose beads were added to the protein lysates and mixed for 1 hour at 4°C. Ni-beads were then pelleted by centrifugation at 5000xg for 3 minutes at 4°C. The resultant lysates were filtered through a column trapping the beads with the attached PI3K, washed and then eluted from the nickel beads using 350μM imidazole. Protein fractions were analysed by SDS-PAGE and purified PI3K was dialysed overnight in 50mM Tris-HCl, 300mM NaCl pH 7.5. The purified protein solutions were adjusted to 2mM DTT and 20% glycerol. Protein concentration was estimated before storage at -80°C.

2.3 Inhibition Assays

a) Radioactive kinase assay

Kinase reactions (50 μL) were conducted in a mixture of phosphatidyl inositol substrate, PI3K enzymes (WT or mutants), buffer containing 50mM Hepes, pH 7.5, 3mM MgCl2, and 1mM EGTA and increasing concentration of inhibitor ranging from 0.01nM to 10μM. Reactions were initiated by adding ATP to a final concentration of 100μM (containing 1.25 μCi [γ-^{32}P]ATP) and incubated for 30 minutes. Reactions were stopped using 50 μL of 1M HCl. Lipids were extracted by adding 100 μL of 1:1 chloroform:methanol solution followed by 250μl of 2M KCl and centrifuged briefly to separate the aqueous and organic phases. 40μl of the organic phase was transferred into vials and mixed with 4ml Opti-Fluor O scintillant (Packard Bioscience). The amount of phosphatidylinositol (3)-trisphosphate (PIP_3) product was determined by scintillation counting. Counting was corrected for background counts obtained from preparation of a control tube (without enzyme). All figures were calculated using the PRISM Graphpad program to generate dose response curve and determine IC_{50} values for each PI3K enzyme:inhibitor pair. All assays were performed for each compound in duplicates.

b) Kinase-Glo® Plus Luminescent assay

GDC-0941 inhibitor was dissolved in DMSO to 10mM concentration and serial dilutions were performed using 20% DMSO. Phosphotidyl inositol solution was prepared by adding 85µl of 10mg/ml PI in chloroform solution with 85µl of 10mg/ml phosphoserine, N_2-dried, resuspended in 0.5ml of sonication buffer (25mM Hepes, pH 7) and sonicated for at least 20 minutes. Reaction mixture was prepared using 125µl of kinase buffer (containing 200mM Hepes, pH 7.4), 125µl of PI solution, 125µl of 100µM ATP (prepared using 5mg/ml $MgCl_2$ solution) and 250µl of water.

According to order, 5µl of inhibitor solution was deposited in the 96-well plate at increasing concentration followed by 20µl of diluted PI3Kα enzymes (diluted in 1xkinase buffer) and 25µl of reaction mixture. Reactions were terminated after 1 hour by adding 50µl Kinase-Glo reagent (Promega) followed by 10 minutes incubation to allow for colour development by luciferase. Luminescence signals were measured using Fluostar and counts were corrected for background obtained from preparation of a control well (without enzyme). All figures were calculated using the PRISM Graphpad program to generate dose response curve and determine IC_{50} values for each PI3K enzyme:inhibitor pair. All assays were performed in duplicates.

3. Results and Discussion

3.1 Analysis of the catalytic activity of PI3Kα wild type and mutant enzymes

Our hypothesis is that mutation of a non-conserved residue should not change the conformation of the catalytic pocket and as such, no significant change in the catalytic activity was found in the mutant PI3K enzymes compared to the wild type. Since LY294002 binds at the ATP binding site, all p110α mutant enzymes were tested against LY294002 to examine the conformational change of the ATP binding pocket. A change in LY294002 potency would signify the ATP binding site was altered. Figure 1 showed all p110α mutants exhibited a similar IC_{50} value to the wild type. This demonstrates that the ATP binding pocket remained intact upon mutagenesis of non-conserved p110α amino acids.

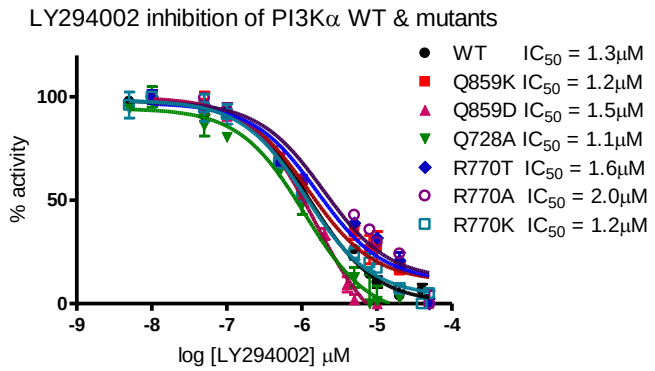


Figure 1. This is a figure, Schemes follow the same formatting. If there are multiple panels, they should be listed as: (**a**) Description of what is contained in the first panel; (**b**) Description of what is contained in the second panel. Figures should be placed in the main text near to the first time they are cited. A caption on a single line should be centered.

In addition, we also determined the kinetic properties of PI3Kα mutant enzymes with respect to ATP (Table 1). The Michealis-Menten constant of the mutant enzymes when compared to the wild type showed no significant different in the K_m values. This result confirmed that the

catalytic pocket of the mutant enzyme did not disrupted by mutagenesis of non-conserved amino acids.

Table 1. Kinetic parameters of purified PI3K (p110α/p85) wild type and mutants with respect to ATP

PI3K	Amount of protein (mg/ml)	K_m (μM) for ATP
p110α, WT ǂ	0.52	82 ± 18
p110α, WT#	1.42	115 ± 30
p110α, R770A ǂ	0.74	74 ± 19
p110α, R770T ǂ	0.85	195 ± 90
p110α, R770K ǂ	0.19	109 ± 26
p110α, Q859K ǂ	0.91	24 ± 10
p110α, Q859D ǂ	1.90	122 ± 38
p110α, Q728A#	1.80	123 ± 28
p110α, K729A#	1.31	83 ± 26

3.2 Analysis of GDC-0941 potency profile

In this section our focus is to investigate the binding interactions of PI3K inhibitor, GDC-0941. We have determined PI3Kα WT inhibition by GDC-0941 which results in IC_{50} values 54.5nM ± 1.2 (n=6) using the radioactive kinase assay while the luminescent kinase assay resulted in consistent IC_{50} value of 36.3nM ± 1.2 (n=6). These values are comparable to the reported IC_{50} considering different assays and conditions used.

A comparison of GDC-0941 liganded to p110β, p110γ and p110δ structures displayed different binding modes (Figure 2). GDC-0941 bound to p110γ showed that the sulphonyl group of the inhibitor participates in a hydrogen bond to non-conserved Lys802 however inspection of GDC-0941/p110β structure display that the equivalent p110β residue, Lys771 did not bind to the inhibitor [14-16]. We had determined that the equivalent residue p110α, Arg770 (region 2) was not involved in binding to GDC-0941 as the GDC-0941 IC_{50} was unaffected by the alanine mutant, p110α,R770A (Figure 3). We also produced isoform mutants by replacing αArg770 with the equivalent amino acid, γLys802 and δThr750 (R770K and R770T respectively) according to methods described in the experimental section. Our data from the luminescent assay showed no change in GDC-0941 potency for R770K (Figure 3) which demonstrates that Lys amino acid has no influence on GDC-0941 selectivity. Similarly, the potency of GDC-0941 was unchanged for R770T mutant in relative to wild type which complies with p110δ/GDC-0941 crystal structure report where the sulphonyl oxygen of the inhibitor does not interact with Thr750 but retain its affinity towards p110δ by interacting with a basic Lys708 residue. This result also demonstrated that the binding mode of GDC-0941 in p110α is different from the one observed in p110γ isoform.

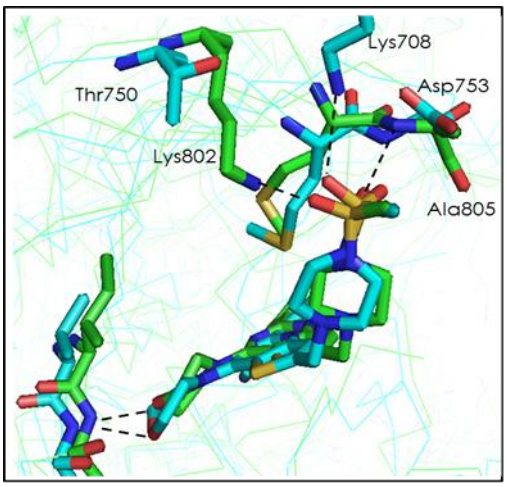


Figure 2. Overlay structures of GDC-0941 bound in p110γ (PDB : 3DBS, green backbone) and p110δ (PDB : 2WXP, cyan backbone) showing the different binding interactions between the inhibitor and the enzyme residues.

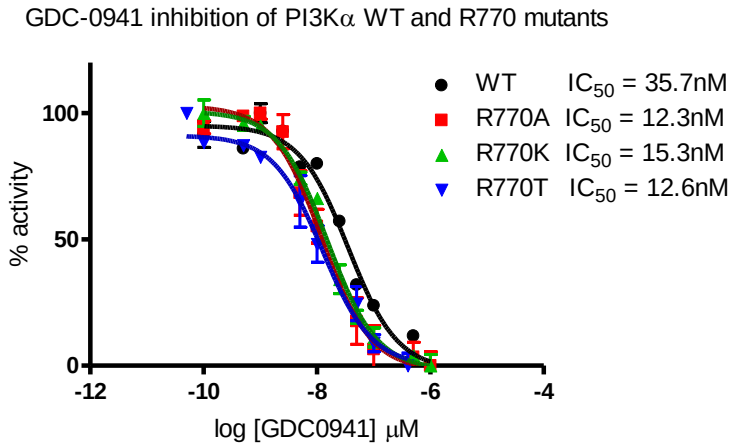
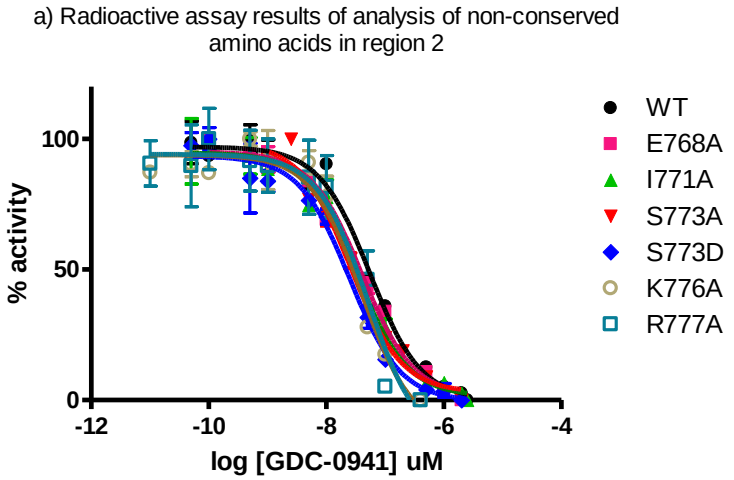


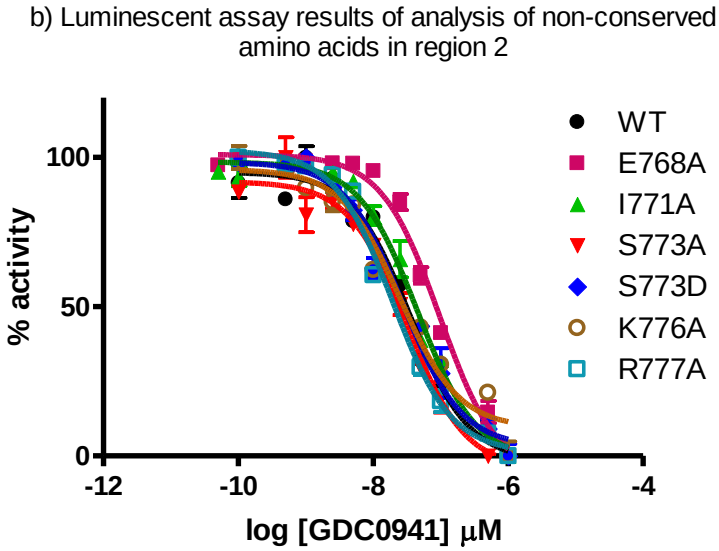
Figure 3. Comparison of GDC-0941 inhibition between PI3Kα WT and R770 mutant enzymes using luminescent kinase assay

Since mutations of p110α R770A and R770K did not show any effect on GDC-0941 binding, this led us to investigate other non-conserved residues within the heterogeneity region 2 of p110α to look for any interacting amino acids. Five alanine mutants of non-conserved amino acids of region 2 were tested in order to identify if any of these amino acids were involved in GDC-0941 binding. Alanine screening test results against the GDC-0941 inhibitor are presented in Figure 4 with similar IC_{50} values recorded using both radioactive kinase assay and luminescent assay with less than 3-fold overall difference between mutants and wild type enzymes. The p110γ/GDC-0941 crystal structure showed that a sulphonyl oxygen binds to Ala805 (γ-numbering) of non-conserved region 2. Two isoform mutants of p110α were produced which are S773A (p110α to p110γ mutation) and S773D (β/δ equivalent mutation). No significant change in GDC-0941 potency was observed with these mutants presumably because the inhibitor makes interactions with the backbone amide of these residues and the side chains of these residues has no effect on

the binding of GDC-0941. The identical inhibition observed between the alanine mutants and WT indicated that this region has no effect on GDC-0941 binding and selectivity.



PI3K	IC_{50} (nM)
p110α, WT	55.4
p110α, E768A	40.5
p110α, I771A	33.2
p110α, S773A	26.0
p110α, S773D	25.5
p110α, K776A	40.8
p110α, R777A	51.5



PI3K	IC_{50} (nM)
p110α, WT	35.7
p110α, E768A	96.7
p110α, I771A	45.4
p110α, S773A	31.6
p110α, S773D	42.5
p110α, K776A	25.2
p110α, R777A	19.5

Figure 4. Dose-response curves and IC_{50} values of GDC-0941 inhibition of non-conserved amino acids of region 2 using a) radioactive kinase assay and b) luminescent assay.

Berndt and colleagues from Cambridge, UK published the p110δ/GDC-0941 crystal structure on 2010 which showed an interaction between the sulphonyl oxygen with Lys708 (p110δ numbering) that protrudes towards GDC-0941 binding pocket. Sequence analysis of the catalytic subunit showed that the amino acids surrounding this residue are not conserved across the different class 1 PI3K isoforms and we have designated this region of heterogeneity as region 3 (Table 2). Alanine mutants were generated for the equivalent residues in the p110α isoform, Lys729 as well as the adjacent amino acid, Gln728 to test the role of these residues in GDC-0941 binding. The K729A mutant showed insignificant change in GDC-0941 potency indicating GDC-0941 does not bind to Lys729 in p110α (Figure 5). This shows that the binding mode of GDC-0941 in p110α is different from the one observed in p110δ. Similarly, no significant different in GDC-0941 IC_{50} against Q728A was observed (2-fold difference) (Figure 5) hence, GDC-0941 binding is not affected by this amino acid either.

Table 2. Amino acid sequence alignment at position 726-736 of p110α representing heterogeneity region 3

726	α	E	T	Q	K	V	Q	M	K	F	L	V	α
732	β	K	L	N	R	A	K	G	K	E	A	M	β
757	γ	Y	D	V	S	S	Q	V	I	S	Q	L	γ
705	δ	K	T	T	K	P	Q	T	K	E	L	M	δ

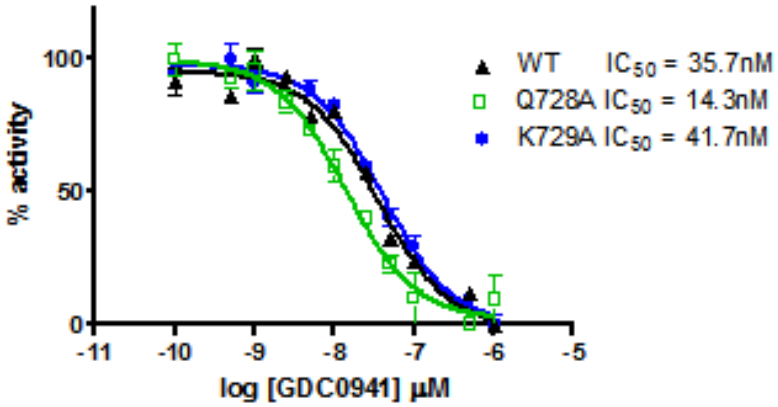


Figure 5. Dose-response curves and IC_{50} values of GDC-0941 inhibition of PI3Kα WT and region 3 mutants using luminescent assay.

Further inhibition studies were undertaken to test the involvement of region 1 non-conserved amino acids in GDC-0941 binding eventhough GDC-0941 was not shown to occupy this region (Figure 6). As seen in Figure 7, the isoform mutants p110α Q859K, Q859D and H855E exhibited up to 1.6-fold difference in the IC_{50}. Overall, isoform mutations of non-conserved amino acids in region 1 had little effect on GDC-0941 inhibition which demonstrated that mutagenesis of these non-conserved residues did not influence GDC-0941 binding and selectivity.

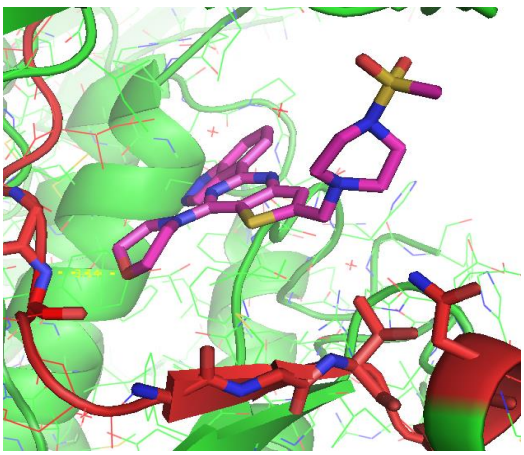


Figure 6. Structure of GDC-0941 bound in p110γ (PDB : 3DBS, green backbone) showing the location of heterogenous region 1 amino acids (in red) within the enzyme binding pocket.

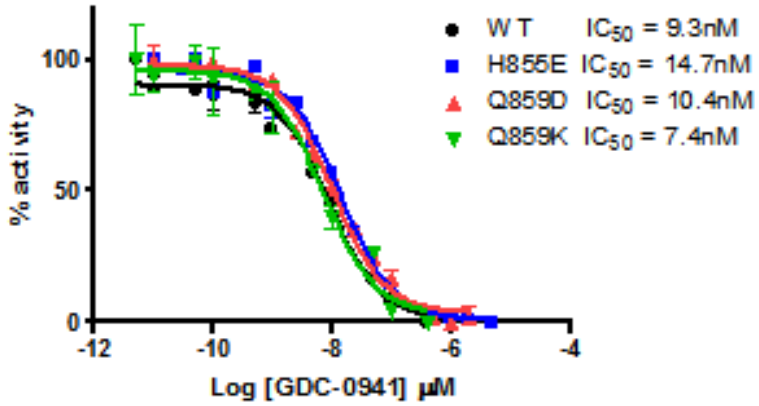


Figure 7. Radioactive results of analysis of non-conserved amino acids in region .

4. Discussion

The comparable inhibition observed between the PI3Kα mutants and WT concluded that GDC-0941 binding and selectivity are not affected by the non-conserved amino acids tested in this study. Examination of the reported crystal structures of GDC-0941 bound to three different PI3K isoforms demonstrated that in each case binding was supported by interactions with different non-conserved residues. This could be of key importance in the pursuit of isoform selective inhibitors as modifications that could restrict the interactions to just one non-conserved residue might lead to isoform selective inhibition.

In our study, no specific interaction was found that governed affinity for a specific isoform and every mutation attempted resulted in only moderate changes in the IC_{50} of GDC-0941. For example, replacement of Arg770 with Ala, Lys (to reflect corresponding Lys802 of p110γ that binds to the inhibitor and Lys777 in p110β) or Thr (matching Thr750 in p110δ) did not influence GDC-0941 binding [1, 3-4]. Nor did replacement of Lys729 by Ala (identifying a residue position that binds to GDC-0941 in p110δ (Lys708)) result in altered affinity. Finally, targeting replacement in region 1, for which the structure of GDC-0941 bound to p110β yielded no impact upon IC_{50} either. The conclusions that can be drawn from this study are mixed. Results may imply that the binding pose of GDC-0941 in p110α is different from that observed in each of the other reported crystal structures, since the positions in p110α were basically unaffected by mutation and so the key residue that contributes to p110α affinity is still to be determined. More likely, our data suggest that GDC-0941 has multiple binding orientations available to it within the binding site of different PI3K isoforms of approximately equivalent strength, such that in the absence of one, another can be accessed without affecting potency.

5. Conclusions

This work has shown that site-directed mutagenesis is able to manipulate the non-conserved residues in the targeted regions within the catalytic pocket. Screening of GDC-0941 against the selected mutant enzymes in region 1, region 2 and region 3 has demonstrated that GDC-0941 was not greatly affected by mutation at the non-conserved amino acid residues tested. We propose that GDC-0941 binds with high flexibility within class 1 PI3K family without affecting its potency utilizing multiple but redundant isoform selective interactions. In future, the use of less flexible inhibitors will be necessary to identify selective:enzyme interactions.

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Abbreviations

The following abbreviations are used in this manuscript:

PIP_2 – phosphatidylinositol 4,5-bisphosphate;
PIP_3 – phosphatidylinositol 3,4,5-triphosphate

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Effect of pH Condition on Xylanase Production by Immobilizing *E. coli* onto Graphene Oxide

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Abstract: The significant utilization of the immobilization technology and knowledge in targeting the recombinant enzyme expression in the culture medium, presents substantial preferences over cytoplasmic excretion. Nonetheless, the main downsides when utilizing *Escherichia coli* as a host is bacterial cell lysis; as a result of pressure build-up through overproduction of the expressed recombinant enzyme in the periplasmic space. Thus, in this research study, the impacts of pH conditions on the cell lysis and protein excretion of immobilized *E. coli* were explored. The immobilized cell culture was performed at 30 ̊c on a rotary shaker at 200 rpm. The culture was harvested after 24 hours and the optimum pH condition was investigated. The xylanase activity was found to be optimum at pH 7 and demonstrated a 29% increase in xylanase excretion with a 22% reduction of cell lysis compared to other pH conditions.

Keywords: Immobilization; Immobilized cell, Graphene Oxide; xylanase

1. Introduction

Recombinant DNA technology has facilitated researchers to bring about an extensive amount of various proteins and enzymes in microbes that were hard to gain in quantity, unattainable before or relatively high-priced. Whereas the foreign genes expression has been recorded in a range of microorganisms as well as cell lines; and the enzymes production includes cloning of the desired gene into the plasmid vector for the recombinant proteins production under the regulation of an inducible promoter (Fakruddin *et al*., 2013; Kuniechick *et al*., 2009). In addition, the expression of recombinant enzyme in which they do not typically take place in the cell is recognized as heterologous protein; and the most frequently utilized expression systems for heterologous gene production of both prokaryotic and eukaryotic origins is microbial expression system (Fakruddin *et al*., 2013).

The usage of plasmid as a vector for production of recombinant enzyme or protein is vital to produce high beneficial enzymes, in unexceptionable supply for research and industrial prospect. However, in commercial production of recombinant microorganisms, plasmid instability; an inclination of the transformed cells loosened their engineered characteristics by alterations to, or loss of, plasmid has become one of the major issues during this process. As a consequence of plasmid instability, it may impact in a critical loss in targeting recombinant protein throughput.

Meanwhile, the most often utilized expression system to produce the heterologous protein is in the bacterial system, *Escherichia coli*. (Fakruddin *et al*., 2013; Kuniechick *et al*., 2009).

E. coli is the most preference host system for proteins and enzymes production by recombinant DNA technology, considered to its promising traits such as, faster and higher cell growth, easier to cultivate, lower cost, suitable and simple for genetically modify and the obtainability of a difference *E. coli* strains and vectors. Consequently, there are lots of molecular procedures and hand-tools for more desirable heterologous proteins expression, for instance; an abundance of strain-engineered, various cultivation approaches and a great number of plasmids for expression purposes. Theoretically, the procedures required to acquire a protein enzyme are quite straightforward and uncomplicated. Nevertheless, in the process, tons of issues can possibly happen for instance; inactivity of the protein enzyme, formation of the inclusion body (IB), unfavorable cell growth rate and even not achieving any enzyme at all are certain complications frequently coming down the pipeline (Rosano & Ceccarelli, 2014; Birkholtz *et al*., 2008).

Throughout the expression of heterologous protein, the composition of insoluble aggregates or lower production might be resulted from the dissimilarities in identical codon usage between natural also expression hosts and the protein overexpression in *E. coli* could bring to insoluble protein synthesis in bacterial inclusion bodies by cause of metabolic burden and it is worthy to discover various expression conditions for instance; provide the enriched culture medium for higher soluble proteins expression as well as reduced the incubation temperatures (Angov *et al*. , 2008; Birkholtz *et al*., 2008). When a foreign DNA is brought into the bacterial strain, the new synthesis recombinant polypeptide will be expressed in the microenvironments of *E. coli*, which might vary from the initial condition relating to osmolarity, cofactors, folding mechanisms, pH as well as redox potential and both spatial and temporally control of gene expression is disoriented. Furthermore, in high-level expression, the polypeptide's hydrophobic stretches are obtainable for interaction with equivalent areas and existing at high concentrations and these aspects altogether bring on aggregation as well as protein instability (Rosano & Ceccarelli, 2014).

In commercial, *Escherichia coli* is generally recognized as safe (GRAS) organism and verified to be a cost-effective host cell line for providing proteinaceous produces. Nevertheless, protein enzymes are barely accumulated for maximum level in *E. coli* and the desired protein production involves bioprocess media optimization for targeting the high cell density culture of *E. coli*. Thus, the defined mineral media have been utilized until now (Basaret *et al*., 2010). Meanwhile, Luria-Bertani broth is the most regularly utilized medium culture for *E. coli* as it contains high nutrients, simple preparation of medium and optimum osmolarity for the early exponential growth phase. All these aspects make it sufficient for enzyme production and recompense for the statement that claiming it is not the best preference for attaining high cell density cultures (Sezonov & Ari, 2007).

The substrate utilized for this research; polysaccharides of xylan are generally correlated with lignin as well as cellulose, which act as an essential structural support to build up the plant cell walls. The degradation of hemicellulose constituent, that predominantly comprises of xylan, necessitates effective xylanolytic enzymatic systems; involving certain biocatalysts such as β-xylosidase, endo-β-1,4-xylanase, α-glucuronidase, α-L-arabinofuranosidase, p-coumaric acid esterase, acetyl xylan esterase and ferulic acid esterase. Recently, there has been a rising diligence in xylanase enzyme fermentations concerning their wide-ranging applications in a number of industries, particularly in the pulp and paper commerce (Rusli *et al*., 2009).

Therefore, xylanases enzymes accountable for the hydrolysis of xylan to xylooligosaccharides (XOS) and have drawn intentness by virtue of their prospective in numerous industrial progresses. Moreover, xylose is the primary constituent of the hemicellulose xylan and the secondary most sufficient plant biomass derivative sugar (Salamanca cardona *et al*., 2014). Xylanase in combining with other enzymes, act proficiently to degenerate xylan to sugar constituents and it can naturally produce by wild type filamentous fungi species for instance; *Trichoderma spp*. as well as *Aspergillus spp.* In addition, xylanase can be applied in wide-ranging utilization for industrial scales such as to enhance the bread quality, improve the digestibility of

ruminant feeds and as a pre-bleaching of kraft pulp (Basaret *et al*., 2010; Rusli *et al*., 2009; Farliahati *et al*., 2010).

On the other hand, with an advance in Nanotech, the Graphene Oxide (GO) has arisen as one of the topmost deliberated nanoparticles presently and has drawn the worldwide interestedness, when it was isolated from graphite in the year 2004; owing to its unique features, for instance; the remarkable mechanical also thermal feature, exceptional electrical conductivity, large surface region and great solvent dispersion which is predominantly caused by the existence of oxygen-containing functional groups (Chook *et al*., 2015; Perreault *et al*., 2015; Wang *et al*, 2015). Moreover, the huge planar dimensional as well as the large surface activity of Graphene Oxide could role as biocomposites with potent nano-interfaces proposing to gas barrier and concurrent promote to mechanical characteristics (Xu *et al*., 2016).

The adaptability and multipurpose of Graphene Oxide (GO) are correlated to the great specific surface region of GO nanosheets, that permits discharge as well as storage of electric charges, biological macromolecules, ionic species, and hydrogen atoms (Xu *et al*., 2016). Moreover, the GO characterization using microscopic has disclosed a structured layer of twist carbon atoms which form tetrahedrons, and that makes grooves also wrinkles on the GO surface layer. Additionally, the π-conjugated planes of GO comprised of the chemical functional groups that act as an anchor to the covalently bonded nanometer and micrometer particles onto GO (Zhong & Yun, 2015). Thus, in this study, the effect of pH conditions on xylanase production by immobilizing *E. coli* onto Graphene Oxide was investigated.

2. Materials and Methods

2.1 The recombinant *Escherichia coli* strain

The recombinant *E. coli* strain that holding a gene of xylanase from *Aspergillus fumigatus* af293, was cloned into expression host, *E.coli* BL21 (DE3) that carrying vector pET21a (+) and was built earlier by Wahab *et al.* The *E.coli* BL21 (DE3) was operated as the heterologous expression host, while *E.coli* JM109 was functioned as storing purposes. While, the vector constitutes a signal peptide M5 that can direct the recombinant enzyme excreted into the extracellular space.

2.2 Determination of optimum pH for xylanase production

The pH effect on the immobilized recombinant *E. coli* was examined by directing the expression at the different pH medium of 5, 6, 7, 8 and 9. The different buffer solution systems for the pH mediums were Tris base-HCL buffer (pH 9), Na_2HPO_4- NaH_2PO_4 buffer (pH 7 and pH 8), citric acid- Na_2HPO_4 buffer (pH 6) and sodium acetate-citric acid buffer (pH 5). The strain was expressed on graphene oxide as a support matrix in Luria Bertani (LB) medium with 0.01 mM IPTG inducer, 30 °C temperature and 200 rpm agitation rate for 24 hours.

2.3 Assay for xylanase activity

Xylanase activity was measured by incubating the dilute enzyme in sodium acetate-acetic acid buffer solution (pH 5.0) at 50°C for 10 minutes using a 1% (w/v) substrate solution, Beechwood Xylan from Merck. The total amount of reducing sugar was assayed by using 500 µL of 2-hydroxy-3, 5 dinitrosalicylic acid (DNS) reagent, 5 minutes boiling, cooling and the absorbance reading was quantified at 540 nm. The definition of one unit of xylanase activity was described as the number of proteins discharging 1 µmol of reduced sugar per minute during the assess conditions.

2.4 β-galactosidase activity (cell lysis)

Cell lysis was calculated by identifying the β-galactosidase value of the extracellular medium with 0-nitrophenyl-β-d-galactopyranoside (ONPG). The total amount of 1 ml buffer substrate consists of 4 mg/ml ONPG soluble in phosphate buffer solution (0.1M) in 7.4 pH condition and supplemental to 0.1 ml analysis sample which was incubated earlier in a 37 ◦C bath water for 10 min. Finally, the cell activity was finished by mixing 0.5 ml of 1M sodium carbonate, and then the absorbance reading was quantified at 420 nm. The definition of one unit enzyme activity was described as the total of enzyme protein that builds 10−8 moles of ONP per minute during the assess conditions.

3. Results

The impact of different pH medium on the stability of xylanase from recombinant *E. coli* was studied as the pH of culture media has a prominent effect on activities of cells throughout expression also growth, enzyme synthesis and excretion as well as its stability (Che *et al*., 2016; Vengadaramana *et al*., 2014). Hence, the *E. coli* was cultivated at varying pH values (5.0, 6.0, 7.0, 8.0 and 9.0) and the activity of xylanase as well as Beta-galactosidase was determined and presented in **Figure 1**. The results demonstrated that when xylanase was cultivated at pH 8.0 and 9.0, the activity was low with 0.048 U/ml and 0.047 U/ml respectively at 30 ℃. Nevertheless, xylanase was slightly stable at pH 7.0 and 6.0 with 0.052 U/ml and 0.049 U/ml respectively. While at pH 5.0 the reaction for xylanase activity was the lowest with 0.037 U/ml in 0.01M sodium acetate buffer.

3.1 Figure

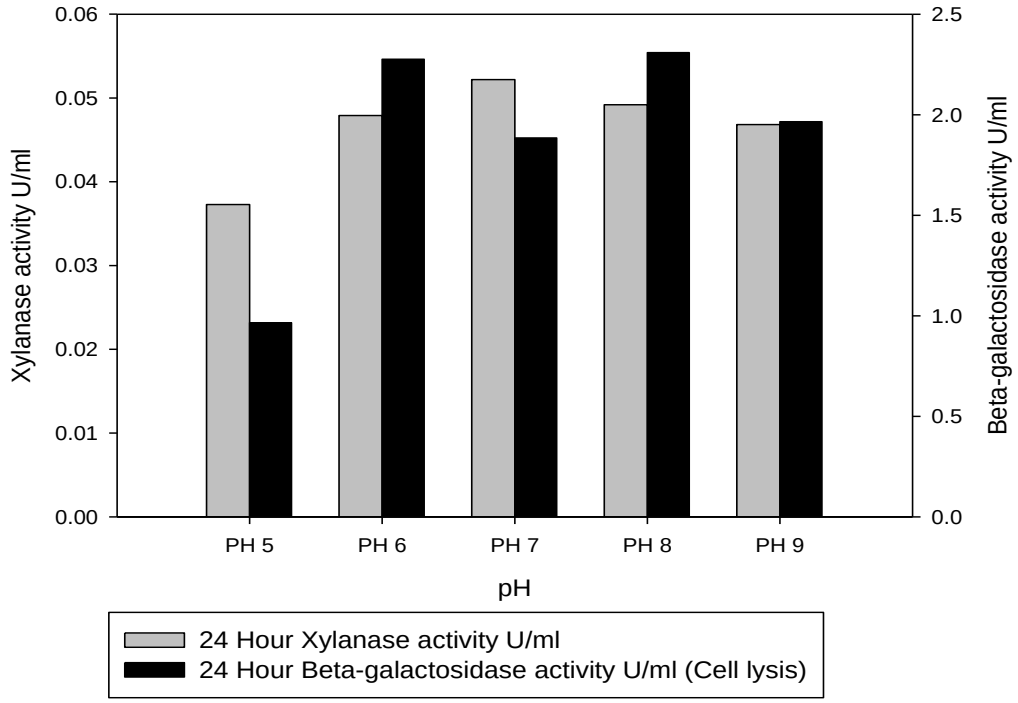

Figure 1. The effect of pH medium on xylanase activity and B-Galactosidase (cell lysis) activity of the immobilized cells.

4. Discussion

The maximum xylanase excretion demonstrated at pH 7.0 (0.052 U/ml) with soluble Xylan as the carbon source and substrate, possibly due to the extracellular enzyme usually stable in surroundings where the pH is retained near neutral conditions and in several experimental conducts with lower pH values, the protein excretion was too low or null (Silva *et al*., 2016). Certain researchers also described that the optimum pH for isolated xylanases from different bacterium are at neutral pH condition and the xylanase isolated from *Aspergillus fumigatus* was active in the pH range from 6.0 to 8.0 (Das *et al*., 2011; Cavalieri *et al*., 2016). Consequently, any alteration in pH can possibly change the structure of an enzyme and a drop in enzymatic activity apart from the optimal pH is because of the protein instability or its inactivation (Battestin & Macedo, 2007).

Moreover, the impact of pH on the enzymatic activity is depending on nature's amino acids active site, that goes through protonation also deprotonation, and by the conformational alteration induced by the amino acid ionization (Sabu *et al*., 2005). pH also influences the hydrogen and ionic bonds that are significant to enzymatic activity, as well as its shape and vary enzymes, has varied inclinations for pH requirement. Accordingly, it is vital to equip the convenient pH surroundings for enzyme excretion (Eed, 2013). Nevertheless, the optimum pH could differ depending on the reaction and system used in the process for instance; enzyme and substrate concentration (Salwanee *et al*., 2013).

Furthermore, Salwanee *et al*. (2013) has described that, in the enzymatic catalysis reactions, usually pH may cause alterations in the catalyst, for instance; the ionic character interruption of the substrates that in turns, can alter the substrate specificity and the enzyme active site or the probable protein structure denaturation of the enzyme (Salwanee *et al*., 2013). Hence, enzymes are easily affected by pH changes and their best performance over an exact optimal pH, with a limited range factor (Sabu *et al*., 2005). It is conceivable that the higher pH condition causes excessive stress on the metabolic performance of bacteria (Zhang *et al*., 2013) and it proved from the results data that the recombinant xylanase from *E. coli* required neutral protein surroundings to be active.

Meanwhile, the lowest Beta-galactosidase activity was demonstrated in pH 5 (0.97 U/mL), followed by pH 7 (1.89 U/mL), pH 9 (1.97 U/mL), pH 6 (2.28 U/mL) and pH 8 (2.31 U/mL). The previous study exhibited indication of increment in bacteria cell autolysis under acidic environment and low salt concentration due to the elastic, flexible and extensible of the cell wall (Ramírez-Nuñez *et al*., 2011). Peptidoglycan is accountable for bacterial cell wall properties and if it is enzymatically damaged by autolysins, it will come to be water-soluble and unable to work as structural mechanical support to produce cellular lysis. Moreover, the alterations in the electrochemical properties of the bacterial cell wall influence the change of autolysins adhesion thus causing the peptidoglicans hydrolysis (Ramírez-Nuñez *et al*., 2011).

Whereas, the most encouraging environment for immobilized cells to excrete enzyme is neutral condition that correlated with lower autolysis compared to acidic pHs; considering of higher H+ ions, thus causing the formation of unstable biofilm and weak attachment due to the cations repulsive forces (Che *et al*., 2016). Thus, varying medium pH condition consequently, can affect autolysins interaction and electrochemical properties in the cell wall, thus causing expansion or contraction of the cell wall as well as decreasing or increasing cellular lysis (Ramírez-Nuñez *et al*., 2011).

5. Conclusions

It is crucially important to inspect the effect of pH medium on xylanase activity as well as B-galactosidase activity throughout this research study. Hence, this study demonstrates that the encouraging condition for immobilized recombinant *E. coli* for production of high levels xylanase excretion with less occurrence of cell lysis was found at neutral pH of 7. Thus, it is dependent on

the system that is desired to be analyzed and it is essential to employ the most appropriate buffer for the cultivation of cell. Furthermore, it is vital to evaluate the effect of buffers on the enzyme sensitivity and the chemical speciation of the examined expression enzyme. Hence, the xylanase enzyme demonstrated an optimal activity at pH 7.0 with a 29% increase in protein excretion and a 22% decrease of cell lysis compared to other pH surroundings.

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The Detection of 8-Hydroxy-2′-Deoxyguanosine and 1-Hydroxypyrene as Biomarker of Cancer Risk in Population Exposed by Benzo[a]Pyrene

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Abstract: This study was conducted to analyze the profile of DNA Adduct 8-Hydroxy-2′-Deoxyguanosine formation as DNA damage indicator, by measuring 8-Hydroxy-2′-Deoxyguanosine level in serum and 1-Hydroxy pyrene as benzo[a]pyrene metabolites from the population in Palangka Raya during the smoke haze period in 2015. In vitro study using 2′-deoxyguanosine-5'-monophosphate and benzo[a]pyrene also conducted. The object of study included 29 respondents from Palangka Raya as the exposed group and 23 respondents from Kota Batu as a control. The questionnaires were used to collect data related to medical history, smoking habit, occupation, life style and activity during the smoke haze period. The results obtained showed significant differences between 8-OHdG level in exposed group (P-value = 0.005) compared to the control, with the average value of the exposed group are 1.4 times higher than the control. The average value of 1-OHP in exposed group (P-value = 0.002) is 3.8 times higher than the control. The increased level of 8-OHdG and 1-OHP in the exposed group has significant correlation with long exposure. While in vitro study showed that benzo[a]pyrene can cause increasing 8-OHdG formation. This study provides evidence that there is a correlation between forest and peatland fires with oxidative DNA damage related to risk cancer.

Keywords: 8-OHdG; DNA Adduct; Forest and Peatland Fires; Palangka Raya

1. Introduction

Carcinogenic compounds such as Benzo[a]pyrene, which is one of the PAH compound, may contribute to the formation of reactive oxygen species (ROS) in the body. The most important oxygen-free radical, that is hydroxyl radical (HO•), can cause damage to basic biomolecules (proteins, membrane lipids, and DNA) and lead to the generation of a variety of oxidation products. The interaction of HO• with nucleobases of DNA leads to the generation of radical adducts (Valavanidis., 2009). The existence of Poly Aromatic Hydrocarbon (PAH) in nature can come from two sources, namely natural resources and anthropogenic sources. The natural sources are forest and peatland fires, oil seepage, volcanoes, plants containing chlorophyll, fungi and bacteria (Ahmad., 2012). In Indonesia, forest and peatland fires happen almost every year and it occurs especially in Sumatera and Kalimantan such as Palangka Raya. Uncontrolled fires could give adverse effects on public health, conservation and significant climate changes (Someshwar., 2011). Therefore, this research was conducted by a scientific approach and correlation studies, to analyze the phenomenon the increased risk of cancer based on adducts level in serum samples collected from respondents in Palangka Raya as an exposed group and Kota Batu as a control group. The 8-OHdG was analyzed from serum samples and 1-Hydroxy pyrene (1-OHP)

as biomarker of benzo[a]pyrene metbolite was analyzed from urin samples. As a comparison, in vitro studies using dGMP and benzo[a]pyrene was also conducted.

2. Materials and Methods

2.1. Chemicals and reagents

8-OHdG 500 µg/mL (Sigma-Aldrich), methanol LC grade (Sigma-Aldrich), Enzyme β-glucuronidase/arylsulfatase (Sigma-Aldrich), Cartridge C18 Waters SEP-PAK (Sigma-Aldrich), 8-OHdG ELISA Kit (Sigma-Aldrich), DMSO, sodium acetate, sodium succinate hexahydrate, calcium chloride, $K_2HPO_4$and KH_2PO_4, benzo[a]pyrene, Na_2HPO_4, NaH_2PO_4, 2'-deoxyguanosine-5'-monophosphate.

2.2. Instrumentation

Incubator shaker (Julobo SW22), Sonicator and Degasser 8510 (Bronson), pH meter, ZORBAX Eclipse Plus C18 (4.6 x150 mm, 5-Micron), Membrane Filter PTFE Polypropylene backed 0.2 µm (Whatman), Eppendorf, vacuum tube 5cc, tourniquet, micro tube, alcoholpads, centrifuge, cool box, urine tube, Vortex, ELISA reader, and HPLC-Fluorescence Detector, HPLC-UV Detector.

2.3. Sample Collection

Samples were collected from 29 respondents who have agreed to participate in the study and have signed laboratory informed consent to do blood sampling for 8-OHdG analysis. The sample selection was based on questionnaires that have been distributed since February 2015, with an election based on the criteria: no smoking, healthy or do not have a history of chronic diseases (such as asthma, heart disease, diabetes mellitus, dyslipidemia, hypertension, thyroid, liver dysfunction, chronic pulmonary disease , impaired kidney function, tumor/cancer, and other chronic diseases), aged 20-40 years, it does not work or cook using firewood/charcoal, and lived in Palangka Raya over 3 years. As comparison, the selected control group populations (23 respondents) live in area with the minimal contamination, namely Kota Batu.

2.4. Ethics and Study Protocol

The study protocol has been approved by the ethics committee Faculty of Medicine, Universitas Indonesia based on the number 897 / UN2.F1 / ETHICS / 2015. Prior to blood sampling, respondents have signed the informed consent as an evidence that they were approved and without compulsion to be included as a sample.

2.5. Urinary Sampling

Urine samples were used for 1-hydroxypyrene analysis as bioindicator of pyrene compounds exposure. The 24-hour urinary samples were collected and were taken to the laboratory and stored at -20 ^{o} C. Then 50 mL aliquot urine were separated and stored in -80^{o}C before used for further analysis.

2.6. Analysis of 8-OHdG in Serum

The method used for analysis of 8-OHdG in serum samples was the Enzyme-linked Immunosorbent Assay (ELISA). The kit used is Highly Sensitive 8-OHdG Check Enzyme Linked Immunosorbent Assay (ELISA) kits from Japan Institute for The Control of Aging (JAICA, Fukuroi City, Japan). This method is a competitive antibody that utilizes monoclonal antibody (Clone N45.1) that highly specific for DNA damage. Before analysis, the serum samples undergo a pretreatment using Filter Ultra 10K devices (Amicon, Millipore). Previously, filter incorporated into micro-tubes. Sterilized water 500 mL put in a filter for prerinsing. Then, filter was

centrifuged using micro-centrifuge at a speed of 14,000 g for 10 minutes. The residual water was removed from the filters. Furthermore, 500 mL of serum samples was added to the filter and then was centrifuged at a speed of 14,000 g for 30 minutes. The filtrates were separated for analysis. For about 50mL samples or standards were placed into the plate or well and 50 µL primary antibody solution as a specific monoclonal antibody for 8-OHdG were added. The plates were shaken gently and sealed using parafilm and then were incubated overnight at 4°C. To eliminate the antibodies binding with 8-OHdG serum, the plates were washed three times with 250 µL wash solution containing phosphate buffered saline (PBS). About 100 µL secondary antibody solution were added to the plate and were shaken gently. The plate was covered by adhesive tape and incubated for 1 hour at room temperature. The secondary antibody contains conjugate Horseradish Peroxidase (HRP) enzyme. The secondary antibody will bind to monoclonal antibody which has been bounded to 8-OHdG in the plate.

To remove the excess of unbounded secondary antibody to monoclonal antibody, the plate was then washed three times with 250 µL cleansing solutions and 100 µL chromogen solution was added to the plate before incubated in the dark for 15 minutes. The plate was then read using ELISA Reader (Vmax microplate reader, Molecular Device) with software SOFTmax Pro.

2.7. 1-OHP Analysis

Five milliliters of urine were transferred to a conical flask and added 300 µL acetate buffer 2 M (pH 5.0). In additional of 75µL β-glucuronidase/arylsulfatase enzyme, the solutions were then incubated in a shaker bath at 50°C for overnight. Furthermore, the hydrolyzed urine samples were loaded into cartridges C-18 (SPE cartridge, SEP-PAK VAC C-18, Waters, Miliford, MA, United States) that had been pre-conditioned with 2 mL of methanol and 2 mL of water. The cartridge was sequentially washed with 2 mL of water. The hydrolyzed urine samples were sequentially eluted by 5 mL of methanol. The samples were then evaporated under nitrogen gas at 60°C and re-dissolved in 1mL of methanol. The solution was filtered using 0.2 µm filter and then stored at −20 °C before HPLC analysis using (LC-20AT, Shimadzu) Fluorescence detector (RF-20A), reversed phase C18 column (250 mm x 4.6 mm; 5 µm). The eluent was a mixture of methanol: water (85 : 15). Analysis were conducted at excitation wavelength of 242 nm and emission wavelength of 388 nm.

2.8. Statistical Analysis

The result of analysis was determined as mean ± deviation standard. The normality data test using sharpio-wilk method. The level of 8-OHdG data were normalized by using log10. Analysis of Variance (ANOVA) and t-test were used to identify a significantly difference between risk variable and 8-OHdG level. The significant factor is $P < 0.05$.

2.9. In Vitro Study

The in vitro studies was conducted by reacting various composition of the reactants. The variations of the compositions were the reaction between 500 ppb dGMP pH 7.4 with 3000 ppb Fe(II); 500 ppb dGMP pH 7.4 with 3000 ppb Fe(II) and 3000 ppb benzo[a]pyrene; 500ppb dGMP pH 7.4 with 3000 ppb H_2O_2; 500 ppb dGMP pH 7.4 with 3000 ppb H_2O_2 and 3000 ppb benzo[a]pyrene; 500 ppb dGMP pH 7.4 with 3000 ppb Fe(II) and 3000 ppb H_2O_2; 3000 ppb dGMP pH 7.4 with 6000 ppb Fe(II), 6000 ppb H_2O_2, and 3000 ppb benzo[a]pyrene. The solutions were incubated for 5 hours at a temperature of 37°C. The 8-OHdG levels in the samples were then analyzed using HPLC with UV detector.

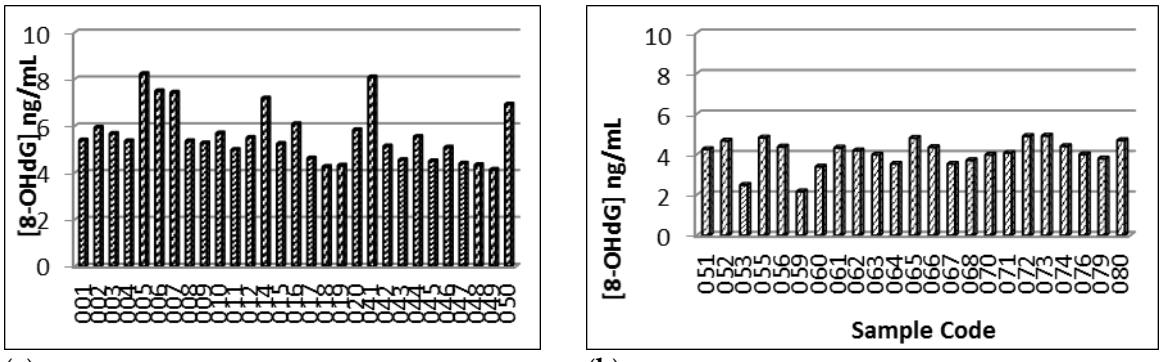
3. Results

3.1 The level of 8-OHdG in Serum Samples

The result of serum analysis, the color of the solution turn to bright blue, because of HRP enzymes catalyze the oxidation reaction of chromogenic substrates by hydrogen peroxyde generating colored product. The reaction was stopped by added 100 µL formic acid as terminating solutions, which make the color change from bright blue to yellow. The color that produced was equal with amount of antibody bounded to the plate and inversely proportional to 8-OHdG level in serum (Figure 1).

Figure 1. Color changing a) after addition of chromogen solution b) after addition of terminating solution
(1st column was the standards, 2nd column was the samples)

Results of analysis of 8-OHdG level shows in Figure 2 and Figure 3 shows the mean and graphic of 8-OHdG level in the exposed group serum samples as much as 5.606 ± 1.162 ng/mL with range of level 4.137–8.228 ng/mL. Meanwhile, the mean of 8-OHdG level in control group was 4.059 ± 0.709 ng/mL with range of 8-OHdG level 2.166 - 4.915 ng/mL. This results describe that the mean of 8-OHdG level in exposed group was 1.4 times higher than control group. Therefore there was a significant difference in 8-OHdG level between the exposed and control group (P-value = 0.005).



(**a**) (**b**)

Figure 2. a) 8-OHdG analysis of exposed samples b)8-OHdG analysis of control samples

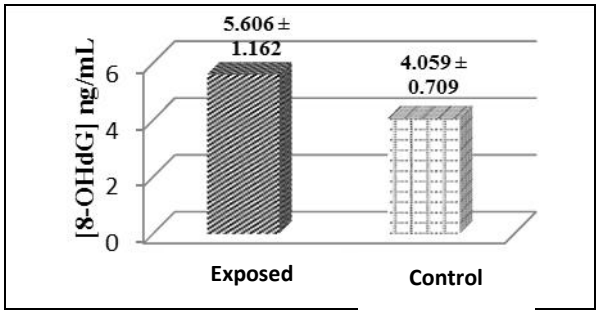
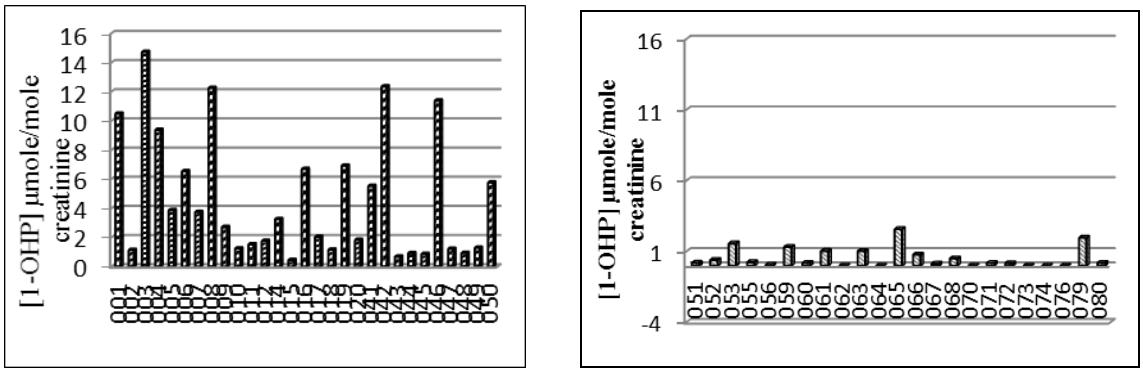


Figure 3. Average of 8-OHdG level of exposed and control samples

3.2. The level of 1-OHP in Urine Samples

Results of analysis of 1-OHP level shows in Figure 4 and Figure 5 shows the mean and graphic of 1-OHP level in the exposed group the mean of 1-OHP in urine samples was 4.57 ± 4.27 µmole/mole creatinine with range of level 0.23 – 14.71 µmole/mole creatinine for exposed



(a) (b)

Figure 4. a) 1-OHP analysis of exposed samples b)1-OHPanalysis of control samples

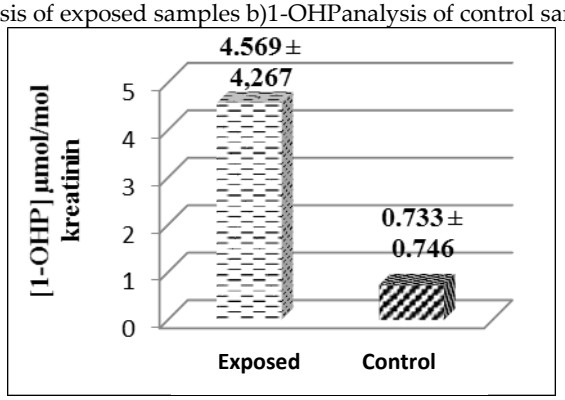


Figure 5. Average of 1-OHP level of exposed and control samples

The results describe that the mean of 1-OHP level in exposed group was 3.8 times higher than control group (0.73 ± 0.75 µmole/mole creatinine).

3.3 In Vitro Study

In vitro studies showed that the addition of benzo[a]pyrene increases the concentration of 8-OHdG were formed. 8-OHdG levels produced in the reaction between dGMP with Fe(II); dGMP with H_2O_2; and dGMP with Fe (II) and H_2O_2 are 6.04 ppb, not detected, and 6.38 ppb which are smaller compared to the addition of benzo[a]pyrene that are 12.91ppb, 8.85 ppb, and 12.97 ppb. The concentration of 8-OHdG in comparison of the results of the addition reaction influence benzo[a]pyrene can be seen in Fig. 5.

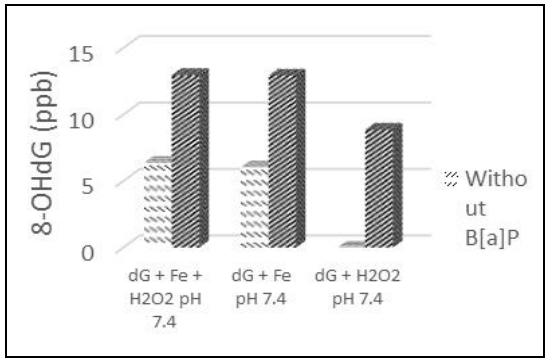


Figure 5. Effect of addition benzo [a] pyrene

4. Discussion

3.1 The level of 8-OHdG in Serum Samples

The increasing of 8-OHdG level in exposed group can indicate that there is an effect of smokes exposure as higher as oxidative stress in exposed group compared with control group. The 8-OHdG is a biomarker of endogenous DNA damage which appear from hydroxyl radical interaction with guanine base in DNA. Hydroxyl radical can be produced in the body by endogenous or exogenous from xenobiotics exposure such as particulate matter in ambient air (Valavanidis., 2009). The forest and peatland fires in Palangka Raya and some of area in Indonesia have significantly increased amount of air pollutant in ambient air, such as particulate matter (PM10, PM2.5, PM1). Some studies have reported that there is positive correlation between particulate level in ambient air with the increasing of 8-OHdG level (Benson., 2013).

3.2. The level of 1-OHP in Urine Samples

There was significant difference in 1-OHP level between exposed group and control group (P-value = 0.002). Nevertheless, the average value in each of risk variables shows the significant differences values with the level of 1-OHP. The significant value between 1-OHP level in exposed group (Palangka Raya) and control group (Kota Batu) is P-value = 0.005 ($< \alpha = 0.05$). The detection of 1-OHP in urinary samples of exposed group in Palangka Raya indicates that peatland and forest fire smoke is contained by carcinogenic compounds such as Benzo[a]pyrene.

3.3. In Vitro Study

Benzo[a]pyrene as a xenobiotic which could increase the generation of free radicals. Benzo[a]pyrene metabolism will generate Reactive Oxygen Species (ROS) such as superoxide, peroxide, and hydroxyl radical (Zhang., 2013). The hydroxyl radical (OH•) could be interacted with DNA and generating DNA Adduct. Furthermore, hydroxyl radical and DNA guanine bases will form 8-OHdG compounds (Gao, 2005).

5. Conclusions

For the results of biomonitoring in Palangka Raya, Benzo[a]pyrene compounds were contained in forest fire smokes proved by the detection of 1-OHP in urine samples of exposed group in Palangka Raya. And Benzo[a]pyrene compounds could increase oxidative DNA damage from the detection of 8-OHdG in serum samples of exposed group in Palangka Raya. Statistical data analysis shows the significant increasing of 8-OHdG and 1-OHP in exposed group's serum samples was affected by the smokes exposure duration. In vitro study shows that the addition of

benzo[a]pyrene to the reaction caused the increasing of 8-OHdG formed. This support the result of biomonitoring research that benzo[a]pyrene could increase oxidative DNA damage.

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Abbreviations

The following abbreviations are used in this manuscript:

8-OHdG: 8-hydroxy-2′-deoxyguanosine
dGMP: 2′-deoxyguanosinemonophosphate

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