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**KEYNOTE ABSTRACTS**

## **Biotechnology: Connecting Trends**

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### **Abstract**

The paper dwells on the biotechnology landscape, specifically as a global market driver and supporting the Sustainable Development Goals (SDGs). An overview of the biotechnology ecosystem in Malaysia will be shared, the success, and challenges of the first National Biotechnology Policy (2005 – 2020) (DBN 1.0) and the way forward with the NBP 2.0 recently launched by the Prime Minister of Malaysia. How will Malaysia embrace the new trends and challenges in supporting the 10-10 MySTIE and Biotechnology policies? Finally, the role of National Institutes of Biotechnology (NIBM) in elevating the biotechnology agenda of Malaysia will be shared.



## New Knowledge on the Ecosystem Role of Bacteria to Make Intensive Aquaculture More Sustainable

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### Abstract

It is still too little realized that bacteria grow alongside the animals in aquaculture systems. The stability and composition of these microbial populations play a crucial role in production success. The management of the microorganisms that grow in the system is as important as the biosecurity measures to keep pathogens out. Whereas disinfectants manage bacteria by unselectively killing them all, the essence of true microbial management is to promote bacteria with a positive effect on production, minimize the development of microorganisms that may harm the animals (typically opportunistic pathogens), and avoid microbial imbalance. This is conceptualized by the ecological r/K selection strategy, i.e., the competition specialized bacteria (called K strategists) are promoted at the cost of fast growing – often opportunistic – bacteria (called r strategists). The result is an improved microbial community dominated by neutral and beneficial microbes, which lead to beneficial fish/microbe interactions and more stable production. When successfully achieved in intensive aquaculture systems, these measures not necessarily ensure higher survival or growth but result in more stable and predictable productions. There also good indications that integration of intensive monoculture systems (marine fish or shrimp) with extractive polyculture (mollusks, tilapia and/or seaweed) result in more microbial stability and thus contribute to the FAO recommendation for a more sustainable intensification of aquaculture systems.

*Keywords:* sustainable aquaculture, microbial management, r/K strategists, integrated aquaculture

## Climate Change and Coastal Marine Resources Management Policy

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### **Abstract**

The Post-2020 global biodiversity framework applies a “theory of change” approach is a strategic planning framework which recognizes that urgent policy action, globally, regionally and nationally is required to transform economic, social and financial models so that the trends that have exacerbated biodiversity loss will stabilize in the next 10 years (by 2030) and allow for recovery of natural ecosystems in the following 20 years, with net improvements by 2050 to achieve the Convention’s vision of “living in harmony with nature by 2050”. As such, governments and societies need to determine priorities and allocate financial resources, internalize the value of nature, and recognize the cost of inaction and its impacts to the economic activities and livelihoods of the stakeholders, namely the coastal and island communities. Climate Change impacts due to ocean warming and acidification as well as sea level rise and coastal erosion aggravated by human intervention and development have shown significant coastal changes over the years. Coastal Management and policies for adaptation measures due to Climate Change impacts are necessary to prepare for the coastal resilience. Comparative studies will be highlighted to address issues, challenges, Integrated Coastal Zone Management, eco-system approach solutions and recommendation for Multi-Functional Zone Planning or Marine Spatial Planning (MSP) for informed and effective decision-making process.

*Keywords:* Biodiversity loss, Coastal Resources Management Policy, Marine Spatial Planning

## The Role of Viruses in Marine Polar Environments and Their Response to Change

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### Abstract

**Background:** Viruses are the most abundant life form on earth with an estimated total abundance in the oceans of  $\sim 10^{30}$ . They are responsible for 10-30% of bacterial mortality, known to control harmful algal blooms and can reduce photosynthesis by up to 78%.

In Prydz Bay, eastern Antarctica, their abundance at all depths is closely correlated with both chlorophylla and bacterial abundance. Metagenomic analyses of surface seawater from the Scotia Ridge and Prydz Bay, identified bacteriophages of the *Caudovirales*, especially the Podoviridae, as the most abundant. Microalgal viruses belonging to the Phycodnaviridae family, which contains most microalgal viruses, especially *Phaeocystis* viruses, were also identified.

Sea ice algae communities comprises a globally significant photosynthetic biofilm. While their microalgal and bacterial constituents are well characterized, there is very little information on their associated viral communities or on the virus-bacteria and virus-algae interactions within them. While high levels of interaction might be expected because of the high density of cells, infection rates, particularly of microalgae, have been found to be low. It remains unclear whether this is a result of environment characteristics, developed resistance or because of the small number of studies. However, research to date has focussed exclusively on DNA viruses. Recent research has shown that key eukaryote group, including diatoms, are mostly infected with RNA viruses. Our most recent research is showing that RNA viruses are abundant and diverse in sea ice

We are investigating how projected global change, including ocean acidification temperature and nutrient availability, will change infection rates in ice edge blooms and sea ice ecosystems.

**Conclusions:** Viruses play a key role in determining the biomass, biodiversity and productivity of polar ecosystems. Projected global change, i.e. ocean acidification, temperature increases and changes to nutrient availability, is likely to enhance viral infection rates, impacting entire ecosystems.

*Keywords:* viruses, sea ice, polar, climate change

## Mechanistic Insights into the Metabolism of Special One Carbon Compounds (DMSP) by Marine Bacteria

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### Abstract

The microbial cleavage of dimethylsulfoniopropionate (DMSP) generates volatile dimethylsulfide (DMS) through the action of DMSP lyases and is important in the global sulfur and carbon cycles. When released into the atmosphere from the oceans, DMS is oxidized, forming cloud condensation nuclei that may influence weather and climate. Our study provides mechanistic insights into the metabolism of special one carbon compounds (DMSP) by marine bacteria, which should lead to a better understanding of these globally-important biogeochemical reaction.

## R&D Challenges for High Value Natural Products: A Case Study for Medical Cannabis

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### Abstract

Medical cannabis is now being considered for treatment based on the framework to be introduced by the end of 2022. To pursue the objectives for the availability of medical cannabis products to patients, the Ministry of Health has formed an expert committee under the Health Technology Assessment Division (HTA) to recommend the introduction of medical cannabis to treat patients under the MOH hospitals for specific indications, including among others: nausea and vomiting due to chemotherapy treatment, cancer associated pain, inflammatory bowel disease (IBD), neuropathic pain, fibromyalgia, cachexia in AIDS (acquired immunodeficiency syndrome) patients, multiple sclerosis (MS), Parkinson's disease (PD), Tourette syndrome, epilepsy, autism, and post-traumatic stress disorder as well as to improve the quality of life to patients. To stimulate the R&D for medical cannabis products especially to come up with generic versions for dried flowers as in the case for Bedrocan<sup>®</sup> there is a need for a relook at the legal provisions under the Dangerous Drug Act, 1952. Two issues will be highlighted in the presentation namely the need to include public university lecturers into section 6(B)2 and the control of R&D facilities to initiate the cultivation of medical cannabis and the introduction of patients medication card to patients undergoing treatment with medical cannabis according to Section 31 of the Dangerous Drug Act 1952.

## When Mechanisms are Necessary to Improve Predictions of Ecological Responses Under Multiple Stressors

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### Abstract

**Background:** The environmental change due to anthropogenic impact of multiple action of global (e.g. climate change as expressed by increasing temperature, heat waves etc.) and local drivers (e.g. pollution, organic enrichment and habitat fragmentation), modifies the habitat quality for many species and causes biodiversity loss affecting the ecosystem functioning, worldwide. This impairs the species persistence and then to investigate the adaptation capability to new conditions is crucial to predict species' distribution and their presence in the local biodiversity. In this context, to increase our mechanistic understanding on how to predict the effects of environmental change due to multiple stressors is crucial to design anticipatory frameworks. The integration of functional traits into vulnerability assessments is a promising approach to quantitatively capture differences in species sensitivity and adaptive capacity to climate change.

**Method:** Integrating manipulative and modelling experiments, here I will present 1) how to measure and use intraspecific trait variability (ITV) in marine bivalves to feed mechanistic functional trait models based on the Dynamic Energy Budget (DEB, Kooijman, 2010) theory and 2) how to test with DEB the effect of disturbance characteristics (i.e. intensity and duration) on life history traits of model species.

**Results:** Results presented here are variegated. While ITV changes across body size and this affects the Darwinian fitness components, the prediction of increasing metabolic costs from an “una-tantum” pulsing event (e.g. marine heat wave) to chronic pressure (e.g. local continuous pollution) shows that body size and fecundity reduce more when and where food is less (oligotrophic conditions) regardless of whether the individual could be located in the core or at the edge of its known distributional area.

**Conclusion:** Mechanistic modelling is crucial to increase our predictive capability with respect to where and when species will become more vulnerable to collapse, serving as a sensitive spatially explicit early-warning system.

**Keywords:** mechanistic approach, predictive modelling, functional trait, climate change, DEB, bivalves

## Conservation of Aquatic Ecosystem by Combination of Environmental Engineering and Technological Advancements with Minimal Environmental Impact

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### Abstract

The re-establishment of a modern aquatic-recycling society may be realized by combination of some key technologies, nutrient recovery technologies through aerobic and anaerobic fermentation, and bioproduction technologies to produce value-added substances such as microalgal mass culture and hydroponic cultivation. Two recent and ongoing international projects to recover nutrients from unused organic waste and produce microalgae and other value-added substances are conducted. The first project, titled, "Continuous Operation System for Microalgae Production Optimized for Sustainable Tropical Aquaculture (SATREPS-COSMOS)" is an international collaboration between Japan and Malaysia supported by JICA, JST and MOE Malaysia. The COSMOS project aims to enhance aquaculture production through new combination of established technologies and innovation of new technologies. The project is establishing a novel microalgae mass culture system using recycled nutrients from aquaculture sludge. The second project, titled, "Eco-engineering for Agricultural Revitalization Towards improvement of Human nutrition (SATREPS-EARTH): Water Hyacinth to Energy and Agricultural Crops" is conducted in Federal Democratic Republic of Ethiopia. The overgrowth of water hyacinth in Lake Tana, Ethiopia, has been causing various problems. This project aims to build a modern "African *Satoumi* recycling society" that achieves both environmental conservation and economic development by producing valuable products such as nutritious superfood "*Spirulina*" and biochar, utilizing those aquatic plants. The goals include development of appropriate environmental technology with abundant natural sunlight for the respective countries, and innovation of new industrial infrastructures through the commercialization of native phytoplankton species. These projects also encourage young engineers and entrepreneurs through environmental, business, and economic education.

*Keywords:* Recycling society, Microalgae, *Satoumi*, SATREPS

## Drug Discovery and Development from Marine Organism

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### Abstract

Investigation of marine organism to be developed as sources for drug discovery and development have been intensively conducted worldwide. Huge numbers of isolated compounds from marine organism been reported in many journals. It is estimated around 28,000 compounds have been isolated in 2016, and increase annually around 1500 compounds. Most of these compounds are isolated from marine fungi, then followed by bacteria, seagrass, cnidarians and mangrove respectively. Contrast with terrestrial organism, however, only limited compounds have been successfully proven their pharmacological activities both pre-clinical and clinical studies so far from marine organism. Low content; isolation techniques; pharmacological activities test and ecological issues if the natural resources are explored and developed in large scale; are the most factors to become bottle neck in development of drug from marine organism. Nowadays, sophisticated development of sciences and technology have become challenges to overcome these problems. Biotechnological techniques could be potential to enhance the content level of compound especially from culturable marine fungi and bacteria. Fast development of instrumentation for isolation and structure elucidation could be used for purification and determination of compounds even with very complex chemical structures. Most important aspects for drug discovery and development is biological activities. These could be performed using in silico study to predict the efficacy of drug candidates, then continued with preclinical such as in vitro and in vivo study to prove activity and safety before conducted the clinical studies with the human subjects. Finally clinical trial consists three phases have to be conducted to prove the efficacy of drug. When the drug candidate passed these serial studies, they could be registered to be used for treatment of disease. All aspects on drug discovery of drugs from marine organism including prospective, challenges and future development will briefly presented.

*Keywords:* Marine organism, marine natural products, drugs, efficacy, safety, preclinical, clinical study



**SCOPE : IMMUNE AND MOLECULAR THERAPEUTICS**

## Improving Culture Efficiency through Nursery Practice

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### Abstract

**Background:** In commercial shrimp production, disease remains as one of the largest challenges. Losses due to EHP, IHHNV, WSSV and YHV are common, but among the most significant are those associated with outbreaks of AHPND which runs into multi-billion losses each year. AHPND is a problem in the grow-out (G/O) phase of production, typically within the first 30-40 days of stocking, where losses can result in the total loss of stock. To highlight this, the Thai production of whiteleg shrimp in 2010 was 630 K MT but following episodes of AHPND, production fell to 220 K by 2014. To address this, Thai farmers focused on the quality of the nursery shrimp they produced.

**Method:** The nursery approach was in the culture of stage PL10-12 shrimp in a smaller culture area with good biosecurity, fed high quality and good nutrient feed equal in quality to that used in hatchery feeds, and to provide health boosters, together with good microbial management practices to promote the healthy and strong growth of the shrimp. The objectives set out to improve the condition of the hepatopancreas so that it could overcome the challenges from pathogens in the G/O pond environment.

**Results:** This talk will look at the husbandry practices used during the nursery phase of production to improve the condition hepatopancreas, including comments on how this is assessed. This will include comments on the culture environment, stocking density, water quality, diets, and the use of probiotics and health care products.

**Conclusions:** Improving the condition of the hepatopancreas in post-larvae during the nursery phase of production has helped produce stronger animals able to deal with pathogen challenges in grow-out reducing the number of crop failures and disease associated losses.

**Keywords:** Nursery, Microbial control, Health booster.

## Cancer Drug Discovery: Apoptosis Inducers of Marine origin as Potential Drug Therapeutics

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### Abstract

**Background:** Cell development in cancer is guided by dysregulated and abrupt cell division; the disease progresses through multiple stages and is influenced by a wide variety of factors. The primary obstacle to eliminating cancer cells is their ability to avoid apoptosis, a cell death process that is primarily regulated in two ways: intrinsically and externally.

**Method:** Potentially more potent chemicals for cancer therapies could be found through the drug discovery process via screening of various extracts and their fractions of plants and marine origins. Several anti-cancer compounds originated from marine organisms are used in clinical trials due to the fact that they induce apoptosis in cancer cells.

**Results:** The induction of apoptosis (Programmed cell death) is a remarkable natural mode of cell death that could be effectively triggered by newly discovered anti-cancer compounds. Successful induction of apoptosis, cell-cycle regulation, apoptosis, and anti-proliferative activity has been observed using a number of extracts and fractions.

**Conclusions:** The drug discovery platform is an excellent tool in the field of cancer drug therapeutics due to ample screening and detailed molecular understanding of drug-protein interactions in the living system. In drug discovery, the understanding of cancer progression holds huge importance. Therefore, more drug screening studies need to be done along with clinical trials to verify the therapeutic efficacy of those potential compounds in cancer chemotherapy.

**Keywords:** apoptosis inducers, cancer, cancer therapeutics, drug discovery, marine compounds

## Comparative Transcriptome Analysis Reveals Differential Gene Regulation on the Cutaneous Growth and Pigment Formation of Two Domesticated Discus

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### Abstract

**Background:** The red melon (RM) and red cover (RC) are two domesticated red discus that descended from the same wild parental lineages through repetitive selective interbreeding. The red pigmentation of the two red discus emerged at differential developmental stages through unknown molecular mechanisms that had never been studied.

**Method:** To understand the underlying mechanisms of the cutaneous growth and pigmentation of the red discus at different developmental stages, transcriptomic analysis and microscopy technique were conducted on the skin samples collected from 3 juveniles aged 60-day post fertilisation and 3 adults aged one year post fertilization of RM and RC.

**Results:** Results revealed that the extracellular matrix (ECM) activities were highly elevated in the juveniles of both discus, in conjunction with the upregulation of numerous genes associated with the establishment of ECM network, such as collagen metabolic genes (*col9a2*, *coll1a2*, *col22a1* and *col5a*, *adamts2*, *lox* and *fdrn3* genes). The findings showed that the erythrophores and xanthophores in red melon juveniles (RMJ) were closely regulated by the pteridine synthetic gene (*gch1*), one-carbon metabolism (*aldh1l2* and *zgc153031*) and lipid metabolism (*apoda* and *klf1*). Further investigation uncovered the transient suppression of melanophore differentiation and development in RMJ, probably due to the upregulated expressions of two Wnt-signalling inhibitor genes, *notum2* and *sost*. Besides that, the melanosome transport in RC mostly depends on microtubules or spectrin-associated cytoskeleton, whereas the genes that are associated with tyrosinase late N-glycan processing (*man1b*) and transport from Golgi apparatus into melanosome (*st8sia5* and *stxbp6l*) that are crucial for melanin synthesis are differentially expressed between RCA and RCJ.

**Conclusion:** This study is the first report describing the differential growth of cutaneous pigments and its associated molecular mechanisms in red discus. The findings indicated that the cutaneous growth and pigmentation of Red Cover and Red Melon varieties were distinctly regulated.

**Keywords:** erythrophore, chromatophore, xanthophore, ornamental, melanophore

## Life Performance of *Artemia Franciscana* Under Laboratory Conditions

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### Abstract

**Selection of small cysts-** A factorial experiment was set up with four factors consisted of selection pressure, food quantity (50 and 100% feeding level), temperature (28 and 32°C), salinity (80 and 120‰) to evaluate their effect on *Artemia* cyst biometrics, selection response and heritability of cyst diameter over successive generations.

Data recorded to display the cyst diameter in parental generation (P), 227±10.7 µm reduced their sizes after two selection generations i.e. 215-219 µm and 214-218 µm for F1, F2, respectively; Beside that, the percentage of small cysts (≤210 µm) was found increasing in F1 (9.6-33.3%); F2 (14.6-40%) compared to P (8.14%). Similarity, the nauplii length of F1 (409 to 427 µm) and F2 (398 to 424 µm) were indicated smaller than P (432±22.7 µm). Results implied that the main factor probably were selection and feeding level (p<0.001). Meanwhile temperature and salinity had little effects on the cyst size.

**Effect of salinity and temperature-** Two factorial analysis indicated that there were significant interaction effects between temperature and salinity on survival, and between temperature and strain on life span and other reproductive parameters (fecundity, brood interval and total offspring), in which temperature could be the main factor affecting to most the parameters tested. It was found that survival of VC and SFB1 strains at day 7 and 14 were significantly higher than in the original SFB strain (VC>SFB1>SFB) in all experimental conditions (p<0.05). Generally, under culture conditions maintained, the life span, reproductive period and reproductive characteristics were significantly higher in the VC strain than in the SFB and SFB1 strain. The SFB cysts (i.e. cyst diameter and embryo size) were larger than SFB1 and VC train. Interestingly, SFB1 strain which was produced by SFB after a first year in Vinh Chau salt field displayed their better adaptation to high temperature and salinity (32°C and 120‰) than the original SFB (parental) strain but less well adapted to these conditions compared to the VC strain.

**Indoor biomass production-** A two-factor experiment was set up in different combinations of 3 salinities (15; 30 và 45‰) and 3 stocking densities (500; 1.000 and 1.500 Ind./L); there were significant interaction effects (p<0,05) between salinity and stocking density on survival, growth and *Artemia* biomass production. At low salinity (15‰) combined with high stocking density (1.000 and 1.500 Ind./L) were unfavorable condition for *Artemia* culture leading to the lowest biomass production (0.24 kg/m<sup>3</sup> và 0.20 kg/m<sup>3</sup>, respectively). In contrast, at high salinity (30‰ and 45‰) and low density (500 Ind./L) biomass production of *Artemia* were superior (i.e. 1,31 kg/m<sup>3</sup> và 1,28 kg/m<sup>3</sup>, respectively) compared to other treatments (p<0,05); beside other set-up to maximize the biomass production and the protocol of harvesting at 30% of culture volume a day to be an appropriate strategy as highest biomass productivity (i.e. 2,20±0,30kg/m<sup>3</sup>) obtained after 5 weeks of culture.

## The Effects of Co-Treatment of Statin and Aaptamine on the Expression of PCSK9 and LDL-R, and, the Uptake of LDL-C by Liver Cells

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### Abstract

**Background:** Atherosclerosis is characterised by the build-up of fatty deposits and plaque due to a constant elevated level of blood LDL-cholesterol (LDL-C). The growth of plaque protrudes into the arterial lumen and decreases its diameter, thus limiting or occluding the blood flow to heart and brain causing myocardial infarction and stroke, the main cause of global mortality. LDL-receptors (LDL-R), responsible for LDL-C uptake from circulation to maintain lipid homeostasis, are subjected to lysosomal degradation when bound by PCSK9. Therefore, PCSK9 forms a viable target to identify inhibitors that increase the expression of LDL-R and LDL-C uptake. However, statin drugs used to reduce hypercholesterolemia via inhibition of cholesterol synthesis, also increase PCSK9 which explains ineffective statin treatment in some patients. Our previous studies demonstrated that aaptamine reduced PCSK9 expression and increased LDL-C uptake. Therefore, the aim of this study was to determine the effect of aaptamine in attenuating the action of statin on PCSK9 and LDL-R expression, as well as, LDL-C uptake.

**Method:** Human liver HepG2 cell line was used as the model system and subjected to the treatment either with simvastatin or aaptamine or a combination of both mediators for 24 hours. Real-time PCR and western blot analysis were utilized to determine the levels of PCSK9 mRNA and protein, respectively. Immunohistochemistry was utilized to determine the level of LDL-R and LDL-C uptake by liver cells.

**Results:** A single treatment of cells with simvastatin significantly increased the gene expression of PCSK9. Interestingly, when simvastatin-treated cells were co-treated with aaptamine, the gene expression of PCSK9 was significantly reduced. Correspondingly, LDL-R protein expression and LDL-C uptake were decreased in cells incubated with simvastatin, and the protein level of LDL-receptor and the rate of uptake were increased when the cells were co-treated with simvastatin and aaptamine.

**Conclusion:** Aaptamine may play a role in attenuating the effect of simvastatin on PCSK9 and LDL-R expression, which in turn, induces the uptake of LDL-C by liver cells.

*Keywords:* atherosclerosis, PCSK9, statin, aaptamine, lipid uptake.

## Targeting Heat Shock Proteins by Botanical Ingredients to Fight Infection Stress in Aquaculture Animals

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### Abstract

Farmed aquatic animal sector has made effective use of antibiotics in maintaining animal's health and production for half a century. However now, their usage is under severe scientific and public scrutiny due to human health concern. Therefore, there is an international pressure on banning antibiotics usage in farmed aquatic animals. However, there are also good indications that this ban could result in a higher frequency of pathogenic bacteria (such as *Salmonella*, *Vibrio*), which in turn could lead to a higher frequency of infections in both animals and consumers. Consequently, alternative anti-infective strategies must be developed to address these concerns without compromising the efficiency of animal production. Data in the literature points to the role of heat shock proteins (Hsps) - a suite of evolutionary conserved cellular proteins produced in response to cellular stress - as an attractive strategy for controlling disease in both farmed animals. Despite the success of Hsps in experimental therapies of human and animal diseases to date, there are still many impediments remaining toward developing an Hsp-induction strategy within the host. Here, based on the published data from our and other research groups, we discuss the potentials of plant-derived ingredients in targeting Hsps for controlling diseases in aquaculture animals, and their putative modes of actions.

## Cytotoxicity Effect of Lysate from *Acanthamoeba* Spp. Against MCF-7 Cell Lines

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### Abstract

**Background:** Breast cancer is the most cancer which had spread in woman worldwide. The current treatment like chemotherapy showed the resistance of cancer to anti-cancer drug. *Acanthamoeba* are free-living protozoa and can be found everywhere such as fresh water, in brackish water, sea water and even in contact lens. *Acanthamoeba* is known as pathogenic organism toward human and caused several diseases in human such as eye keratitis and fatal granulomatous encephalitis. Previous studies showed the pathogenic free-living *Acanthamoeba* can kill and rapidly lysed variety of tumor cells in vitro. Thus, this study was conducted to explore the potential of *Acanthamoeba* as anti-cancer drug source.

**Method:** In this study, anti-cancer activities of *Acanthamoeba* were determined by extraction of 3 strains of *Acanthamoeba* lysate (*Acanthamoeba* sp (SW isolate), *Acanthamoeba castellanii* (IMR isolate) and *Acanthamoeba* sp (HKL isolate)) by conducting cytotoxicity assays. MTT (dimethylthiazol-diphenyltetrazolium bromide) colorimetric assay was used to determine the cytotoxicity of *Acanthamoeba* lysate toward MCF 7 cells. The modes of cells death were conducted through DNA fragmentation and AOPI assays.

**Results:** The exposure of MCF 7 cells with *Acanthamoeba* lysate showed cytotoxic effect on the viability of MCF 7 cells. *Acanthamoeba* sp (SW isolate) was found to be most cytotoxic against MCF 7 cells compared to others, with IC<sub>50</sub> for *Acanthamoeba* sp. (SW isolate) was 15.09±1.12b µg/ml. The other two strains of *Acanthamoeba* did not show significant different, with IC<sub>50</sub> for *Acanthamoeba castellanii* (IMR isolate) was 31.46±1.10a µg/ml and *Acanthamoeba* sp (HKL isolate) was 28.41±1.13a µg/ml. MCF 7 cells treated with *Acanthamoeba* lysate showed a nucleosomal fragment due to endonucleolytic cleavage of DNA into multiple fragment. MCF 7 cells showed the morphological characteristics of apoptosis such as cell membrane blebbing, which resulted in yellowish-green in the cell, and red spots within the cells could be clearly seen through AOPI assays.

**Conclusion:** In conclusion, through this study our funding indicates *Acanthamoeba* lysate to have potential cytotoxic effect on breast cancer.

**Keywords:** *Acanthamoeba*, MCF-7 cells, anti-cancer.



## Propagation, Phenotypic Characteristics and Varieties of the Discus Fish (*Symphysodon* spp.)

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### Abstract

**Background:** This paper discusses the origin and species of the wild discus fish *Symphysodon* spp., as well as discus propagation and domestication.

**Method:** The phenotypic traits were provided, with a focus on main and secondary colours, stress bars, stripes and spots, eye features, 'D' area, markings and rims, body and fin shape, forehead types, and gill plates. Domesticated discus variants were then classified based on their major base colour and distinguishing phenotypic traits.

**Result:** Discus have been grouped into 69 varieties, all of which have distinct characteristics and are currently categorised on the basis of their unique primary colour characteristics. These varieties have been produced through decades of selective and cross-breeding processes, resulting in the fixation of colour genes and distinct features of the original brood. During natural selection and/or mutation, many distinct features of the discus occur. Common trade names of each variety have been presented herein, together with a description of their main characteristics which separate them from others. It should be noted that different trade names may be used for the same discus varieties and are often used interchangeably by hobbyists and/or exporters around the world. It is anticipated that the names presented herein will be used as a standard reference for the classification of discus varieties in the future.

**Conclusion:** These data are critical in assisting aquaculturists, discus enthusiasts, and researchers in understanding general background features as well as identifying common and novel discus types based on their phenotypic appearance.

**Keywords:** Discus, *Symphysodon*, colour, varieties, aquarium fish

**SCOPE : CLIMATE CHANGE AND MARINE ORGANISM ADAPTATION**

## Human and Environmental Adaptations to Climate Change: a Scientometric Analysis

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### Abstract

**Background:** As the world grapples with climate change, human and natural environments both need to adjust in response to climatic effects. There is already a wealth of research that attempts to understand how both environments adapt to climate change adaptation. Synthesizing this body of research is important as it provides a critical perspective on current results, and insights on gaps for future research.

**Method:** We searched for relevant English research articles in the Web of Science Core Collection (WOSCC) database using two different keywords (i.e. climate adaptation and climate change adaptation). In total we obtained 9,098 articles from WOSCC published between 1972 and 31-12-2021. We generated visual knowledge maps using CiteSpace software to analyze the current body of research and to assess development trends of climate change adaptation research.

**Results:** Almost 53% of publications were published by the USA, Australia, and England. Over the last five decades, there were a total of 6,664 participating institutes, 26,814 different authors, and 1,680 different journals involved in climate change adaptation research and development. We identified 14 clusters from CiteSpace software, including spatial planning, nature-based solutions, and smallholder farmers. The top keywords used were “vulnerability”, “management”, “evolution”, “geographic variation”, “body size” and “temperature”.

**Conclusions:** That main research clusters focused on restructuring current human environments through spatial planning or nature based solutions is an indication that science is strongly looking for solutions to adapt to climate change. Much of this research focused on the field of “Ecology, Earth & Marine” as well as “Economics, Economic & Political”. Our

results suggest that policy makers should focus on local adaptation and empowering decision making in order to properly adapt to climate change. Our study benefits scientific communities, philanthropic funders, related governments, and NGOs towards sustainably managing ongoing climate change impacts.

*Keywords:* Scientometric; evolution; body size; geographic variation; temperature

## Effect of Ocean Warming on Feeding Physiology of Mud Crab *Scylla olivacea*

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### Abstract

**Background:** Ocean warming resulting from climate change and atmospheric warming is potentially creating thermal stress environment for aquatic organism, including brachyuran crabs. However, little is known about the effect of increasing water temperature on the physiology and feeding of mud crabs.

**Method:** Therefore, to determine the effect of temperature increase on physiology and feeding of adult male *Scylla olivacea*, crabs were cultured in two temperature treatments (29°C and 33°C) for 14 days. Physiology and feeding parameters measured included oxygen consumption, thermal coefficient, energy consumption, hepatosomatic index, feed conversion ratio, feed conversion efficiency, specific growth rate, and weight gain of adult male orange mud crabs, *S. olivacea* (Herbst, 1796).

**Results:** Thermal coefficient of mud crabs in both temperature treatments (29°C and 33°C) was at 3.5232 on day seven and decreased to 1.2534 on day fifteen. Oxygen consumption, weight gain, and specific growth rate differed significantly between the two treatments during the treatment period (14 days). However, glucose content in hemolymph, hepatosomatic index, feed conversion ratio, and feed conversion efficiency were not significantly different during the temperature treatment.

**Conclusion:** This study is essential as baseline for future studies of intertidal brachyuran crabs and ocean warming.

**Keywords:** ocean warming, mud crab, feeding physiology, oxygen consumption

## Ocean Warming and Acidification Affect the Essential Fatty Acids of the Commercially- Harvested Turbinid Snail *Turbo militaris*

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### Abstract

**Background:** Dissolution of anthropogenic carbon dioxide into the oceans results in ocean warming and acidification, which could alter the nutritional quality of marine organisms for human consumption. Here, we examine the impacts of ambient and near-future elevated temperatures, as well as pCO<sub>2</sub>, on the proximate composition (moisture, ash, protein, and lipids), fatty acids and trace elements within the foot tissue of *Turbo militaris*, a commercially harvested marine snail from south-eastern Australia.

**Methods:** Under controlled flow-through laboratory conditions, snails were exposed to ambient seawater conditions (22 ± 0.2 °C, pH 8.13 ± 0.01–450 µatm pCO<sub>2</sub>), ocean warming conditions (25 ± 0.05 °C), pCO<sub>2</sub> ocean acidification (pH 7.85 ± 0.02, ~880 µatm pCO<sub>2</sub>) or a combination of both for 38 days. Moisture, ash and lipid contents were analysed individually (n=9 replicates for each treatment). Biuret Assay was used to estimate the total protein. Fatty acid methyl esters (FAMES) of the lipid extracts were analysed using GCMS analysis whereas trace elements were determined on a wet basis analysed on an inductively-coupled plasma (ICP) spectrometer (NexION 300 D series) with ESI SC-FAST Auto Sampler.

**Results:** Proximate composition of the foot tissue was unaffected by ocean warming or acidification. However, ocean warming and acidification caused a reduction in healthful polyunsaturated fatty acids (PUFA), demonstrated as a reduction in n–3 fatty acids. The decrease in n–3 PUFAs, particularly eicopentanoic acid, is a major negative outcome from ocean warming. Apart of PUFAs, ocean warming also increased levels of zinc in the tissues; other toxic elements did not appear to be affected by conditions of ocean climate change.

**Conclusion:** Overall, the major impact of ocean climate change on seafood quality is likely to be a decrease in healthy polyunsaturated fatty acids at higher temperature

*Keywords:* Ocean warming, commercial species, fatty acid composition

## Mechanistic Insight into the Action of *Vibrio* Extracellular Metalloproteases on Collagen

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### Abstract

**Background:** Collagen is the major protein of the extracellular matrix (ECM) of animals, providing structural frameworks for skin, bones, tendons, and other connective tissues. The collagenases of *Vibrio* species, many of which are pathogens, have been regarded as an important virulence factor. However, there is little information on the structure and collagenolytic mechanism of *Vibrio* collagenase.

**Method:** Here, a Class III collagenase, VhaC, from pathogenic *Vibrio harveyi* VHJR7 is purified and characterized.

**Results:** We report the crystal structure of the collagenase module (CM) of *Vibrio* collagenase VhaC and the conformation of VhaC in solution. Structural and biochemical analyses and molecular dynamics studies reveal that the triple-helical collagen is initially recognized by the activator domain, followed by subsequent cleavage by the peptidase domain along with the closing movement of CM, which is different from the peptidolytic mode or the proposed collagenolysis of *Clostridium* collagenase.

**Conclusion:** A model for the integrated collagenolytic mechanism of VhaC, integrating the functions of VhaC accessory domains and its collagen degradation pattern is proposed. This study provides insight into the mechanism of *Vibrio* collagenolysis and helps in structure-based drug design targeting of the *Vibrio* collagenase.

*Keywords:* *Vibrio*, collagen, collagenase, collagen degradation

## The Effect of Climate Change on the Phylogeography of Asian Arowana *Scleropages formosus* (Osteoglossidae) in Malaysia

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### Abstract

**Background:** Habitat destruction, deforestation and overexploitation of natural resources are among the factors causing climate change and affecting many wild species. Some have already gone extinct due to this. Currently, one of the most endangered freshwater fishes is the Asian arowana (*Scleropages formosus* and *S. inscriptus*). Once common and widely distributed in Southeast Asia, small remnant populations of Asian arowana are now scattered in Malaysia, Indonesia, south Myanmar, and south Cambodia. In Malaysia, the Asian arowana is presumed to be still present in about ten drainages with some body-colour variation among drainages. Using the specimens collected from drainages in Malaysia, two hypotheses were tested: 1) each colour-variety form a monophyletic group, and 2) Pleistocene paleodrainages shaped the distribution and differentiation among populations.

**Method:** Mitochondrial COI and Cytb genes, and nuclear RAG1 gene were used to amplify the extracted DNA of Asian arowana collected from drainages in Peninsular Malaysia, southwest Sarawak and central Sarawak. The sequences amplified using these three genes were combined with the published dataset to broaden the comparative analysis of the sequences. Maximum-likelihood (ML) and time-calibrated Bayesian trees were constructed for each gene and the lineages formed were compared.

**Results:** The sequences were assigned to three lineages based on the ML and time-calibrated phylogenetic trees, with low genetic variability among the colour varieties. The monophyletic group hypothesis is rejected due to the paraphyletic group formation of the “gold” and “green-nami” varieties from the Malaysian populations which were nested within the green variety. Instead, the assignment of the lineages is based on the Pleistocene paleodrainages system which better explain the distribution of Malaysian populations than the body-colour variations.

**Conclusion:** With the results shown, Asian arowana in Malaysia is in need of urgent conservation actions because all its main genetic lineages occur in this country. This indirectly highlights the importance of habitat conservation in Malaysia to preserve this diversity.

Keywords: Asian arowana, Pleistocene, colour variation, phylogeography, conservation



## Aquaculture Supplements and Specialty Ingredients for Maximum Performance and Efficiency in Marine Aquaculture

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### Abstract

Backed by over 40 years of research and real customer success, Alltech's specialty feed ingredients offer superior nutritional solutions for fish and shrimp. Disease is a significant threat to successful aquaculture. Pathogenic parasites, bacteria and viruses can cause health problems and death, slowing production significantly. Tackling these issues with vaccines, antibiotics or other chemicals may not be desirable or possible. Aquaculture specialty feed ingredients are essential tools to help fish combat pathogens. They promote well-balanced microflora in the digestive system, keeping fish healthy so you can maximize your production yield and increase your profitability. Alltech Prebiotic -Mannan Rich Fraction (MRF) derived from patented *Saccharomyces cerevisiae* has shown to improve aquaculture gut health by reducing gut inflammation, improving the gut structure and overall better health.

Alltech's chelated trace mineral solutions have demonstrated higher bioavailability than their inorganic counterparts. This warrants the use of lower dietary inclusion rates via reduced wastage of unassimilated minerals. Supplemented either separately or in combination with other feed solutions, these technologies have demonstrated many immune and growth benefits in various aquatic species. One example is supplementation with zinc and selenium. Zinc plays a crucial role in modulating immune function, resulting in shrimp and fish with increased immune capabilities. Zinc also helps maintain skin integrity and speeds up wound healing. In addition to zinc, selenium supplementation has been proven to promote shrimp and fish growth and immune health.

The aquafeed industry has made significant progress in developing sustainable fish feeds with the inclusion of plant-based protein alternatives. This increases the mycotoxin risk in aquaculture diets. Detection, prevention and mitigation are critical for an effective mycotoxin strategy to avoid unpredictable losses and maintain a high-producing culture. Alltech 37+® is a mycotoxin analysis test that allows producers to assess feed samples for over 50 different mycotoxins and receive a custom report to evaluate their current risk level. Recent Alltech's survey on mycotoxins analysis in Asia aquafeed samples showed that the aquatic animal could be exposed to high mycotoxin risk. Utilizing this service from Alltech can provide producers with a realistic picture of feed contaminants, which can speed up diagnosis, determine effective remediation and help form a more impactful mycotoxin control plan. In addition, because it is so difficult to protect animals from mycotoxins, the producer can use the mycotoxin binder, especially the modified yeast cell wall which has a broader binding capacity to mycotoxins to negate the effects of mycotoxins in the digestive tract and prevent mycotoxins from being absorbed by the body.

Presented by Henry Wong (Commercial Development Director – Asia Aquaculture Business)

## Thermal Stress: A Bibliometrics Analysis

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### Abstract

**Background:** This study aims to identify the publication trends and development of thermal stress in aquaculture. We would like to determine the trend of thermal stress research in aquaculture in terms of the year, journal, co-cited journal, countries, institution, keywords, and references.

**Method:** We conducted a bibliometric study based on the keyword “thermal stress” and “aquaculture” analysis from the year 1992 until 2022.

**Results:** A total of 298 samples of scientific articles were from thermal stress research in aquaculture. There was a total of 4,571 citations from the keywords of thermal stress and aquaculture, a total of 34.98% citations were from Marine Freshwater Biology followed by Fisheries, 28.57%.

**Conclusion:** There was an increasing trend of articles published from 2014 until 2021 with most of the published articles from the *People's Republic of China*, with Xiamen University having the highest number of publications.

Keywords: thermal stress, aquaculture, bibliometric

## Territorial Behaviour : A Bibliometrics Studies

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### Abstract

**Background:** Territorial behaviour is closely correlated with population fluctuations. However, to the best of our knowledge, there are no bibliometric studies on territorial behaviour-related topics. The purpose of this study is to investigate the trends and development of territorial behaviour of aquatic animals in the world, through visualisation generated from the literature databases. Specifically, we identify the trends of published literature in terms of the year, journal, co-cited journals, authors, countries, institutions, keywords and references.

**Method:** We conducted a bibliometric analysis on territorial behaviour research from the January 1975 to July 2022, by using the Web of Science database. Microsoft Excel software was used to analyzed the qualitative dataset.

**Results:** There was a total of 1,857 publications with 52,780 total citations from the global territorial behaviour research. We found an increase in the number of publications over time, with the USA having the highest publications, followed by Canada and England. A total of 4,448 authors are involved in territorial behaviour research.

**Conclusions:** This study revealed the increasing number of publications focused on areas such as Zoology and Ecology. These findings show the emergence of territorial behaviour research and could attract various stakeholders, especially the early career researchers in various fields to collaborate in territorial behaviour research in the future.

**Keywords:** Bibliometrics, Territorial, Scientometrics, Web of Science

**SCOPE: NATURAL PRODUCTS FROM MARINE BIORESOURCES**

## Local Knowledge of Aquatic Life as a Source of Medicine

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### Abstract

**Background:** Most of the aquatic life is utilized by the locals for various applications such as fish, mollusk, crab, eel, and so on. Most fish can be consumed as a food source. Other species are used to prepare ingredients for traditional medicine. The use of aquatic organisms as a therapeutic ingredient is employed by practitioners who have the expertise to make medicine, and it is a highly precious treasure of knowledge that should be given careful consideration. This local knowledge may be the key to treating illnesses for which there is still no specific cure.

**Method:** This research investigates the biological characteristics and habitat of various therapeutic aquatic life that can be used as references, such as eel, linang fish, kedak fish, snakehead fish, catfish, buffalo leech, mudskipper, and apple snail. The procedures for preparing organisms as a material for making medication are outlined based on information shared by traditional medicine practitioners through interview with the selected respondents in Terengganu and Pahang.

**Results:** From the review, we found that this knowledge are very valuable and through this paper, researchers can refer and study more deeply so that it can be applied in the preparation of modern medicines and can be commercialized more widely.

**Conclusions:** Continuous efforts should be made to increase the potential ability of traditional products based on aquatic life to treat various health problems. Scientific research for aquatic life should also be implemented more widely such as seeding, breeding and pharmaceuticals.

**Keywords:** Aquatic Life, Traditional Medicine, Local Knowledge, Therapeutic

## Metabolomics Study on the Effect of Dietary Fatty Acid Supplementation on Hybrid Grouper (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*) Challenged with *Vibrio vulnificus*

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### Abstract

**Background:** Groupers are one of the most valuable marine fin fish in Asia. However, with the intense farming practices, aquaculture faces challenges in maintaining good cultural conditions and feed suitability in supporting the growth of fish production. Therefore, improvements in diet formulation are necessary to achieve desirable growth efficiency and health performance in fish.

**Method:** A seven-week study was conducted to investigate the effects of oleic acid supplementation on the survival, immune response, and metabolomic changes in hybrid grouper (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*) challenged with *Vibrio vulnificus*. After six weeks of feeding trials and one week of the post-bacterial challenge, the survived-infected grouper immune organs (spleen and liver) were sampled for GC-MS analysis. The metabolite distribution patterns between the experimental groups were then analysed to reveal the possible metabolic effects of dietary oleic acid on the grouper immune response. The survival and immune response activities were also investigated.

**Results:** Following the *Vibrio* challenge, groupers supplemented with dietary oleic acid exhibited a significantly ( $p < 0.05$ ) enhanced immune response compared to the control diet, i.e., lysozyme, respiratory burst, and phagocytic activities. The survival rate was higher in groupers supplemented with dietary oleic acid, at 63.3% compared to only 43% in the control diet. A total of 61 and 46 metabolites were putatively identified from the liver and spleen, respectively. Metabolic changes were characterized by differences in amino acid, carbohydrate, fatty acid, and organic compound contents. L-valine (13.28) and L-leucine (4.95%) were shown to be highly abundant in the liver. Meanwhile, in the spleen, L-threonine (5.27%) and L-serine (4.57%) were highly abundant.

**Conclusion:** The results obtained from this study provide the basis for understanding the fish metabolome and identifying the metabolic responses to dietary oleic acid supplementation in grouper challenged with *Vibrio*. Dietary oleic acid also shows promising results in enhancing immune responses to increase disease resistance in hybrid grouper fingerlings challenged with *V. vulnificus*.

**Keywords:** metabolomics, oleic acid, feeding supplement, hybrid grouper

## The Review on Anti-diabetic Properties of Traditional Herbal Concoction Containing *Eleutherine palmifolia*, *Syzygium polyanthum* and *Momordica charantia*

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### Abstract

**Background:** A traditional herbal concoction consisting of *Eleutherine palmifolia*, *Syzygium polyanthum*, and *Momordica charantia* is used to treat diabetes. To validate its therapeutic properties required extensive scientific investigations. In this review, a thorough analysis of the therapeutic properties of each ingredient was carried out.

**Method:** The methods used to find the therapeutic properties and its mechanism of action are also reviewed. Based on the literature reviewed, all three ingredients are commonly investigated through *in vitro* using *Mus musculus* cell line (3T3-L1) and *in vivo* using Streptozotocin (STZ) induce rat model indicating their potential to treat diabetes.

**Results:** *Eleutherine palmifolia* showed starch degradation capacity through  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitors, reduce blood glucose, increase insulin secretion, and reduced muscle cell degradation. *Syzygium polyanthum* showed to inhibit glucose absorption from the intestine, increased glucose uptake in muscle tissue, starch degradation capacity through  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitors and decrease blood glucose and advanced glycation end products (AGEs) level. *Momordica charantia* also has  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory effects, renewal of  $\beta$ -cells in STZ-diabetic rats, reduced blood glucose and insulin secretion increase. Interestingly the expressions of phosphorylated protein kinase B, glucose transporter4 (GLUT4), and phosphorylated AMP-activated protein kinase (pAMPK) were also significantly increased in skeletal muscle and adipose tissues of *M. charantia* treated rats. Based on the anti-diabetic properties of the three herbs, it could be hypothesized that the traditional concoction could also have similar properties.

**Conclusion:** It is suggested that further investigation to be carried out to prove the anti-diabetic properties of the concoction through *in vitro* model using 3T3-L1 and *in vivo* using STZ induced rat model. The main therapeutic properties to be elucidated are but not limited to blood glucose,  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitor activities, GLUT4 and pAMPK.

Keywords: *Eleutherine palmifolia*, *Syzygium polyanthum*, *Momordica charantia*, diabetes mellitus, anti-diabetic

## PCSK9 Inhibitor from Marine Resources: A Potential Drug in Reducing the Cholesterol Levels?

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### **Abstract**

Atherosclerosis is the main cause of cardiovascular diseases which in turn, lead to the highest number of global mortalities. This pathophysiological condition is developed due to a constant elevated level of plasma cholesterols. Statin is currently the widely used treatment in reducing the level of cholesterols, however, it may cause adverse side effects. Therefore, there is an urgent need to search for new alternative treatment. PCSK9 is an enzyme responsible in directing LDL-receptor (LDL-R)/LDL-cholesterols (LDL-C) complex to lysosomal degradation, preventing the receptor from recycling back to the surface of liver cells. Therefore, PCSK9 offers a potential target to search for small molecule inhibitors which inhibit the function of this enzyme. A marine natural product repository has been established at Institute of Marine Biotechnology (IMB) which to date contains approximately 1,500 collection of marine natural products. By utilising molecular-based high throughput screening platform, two potential compounds were identified in reducing the gene expression of PCSK9 as well as increasing the LDL-R and the uptake of LDL-C by human liver cells. Correspondingly, the compound also decreased the plasma cholesterol and LDL-C levels in rats. Thus, marine resources may act as promising therapeutic agents in decreasing the levels of LDL-C by inhibiting the gene expression of PCSK9, and therefore, may reduce the progression of atherosclerosis.

*Keywords:* Atherosclerosis, PCSK9, Lipid, Marine Resources



## The Effect of *Clinacanthus nutans* and its Bioactive Compounds Against High-Fat Diet-Induced Type 2 Diabetes in Sprague-Dawley Rats

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### Abstract

**Background:** Diabetes mellitus is one of the risk factors for atherosclerosis development and cardiovascular diseases. *Clinacanthus nutans*, widely used as a traditional medicine for diabetes is reported to have hypoglycemic, hypolipidemic, antioxidant, and anti-inflammatory properties. This plant can be grown in most of the habitats, comprising dense or open forests, bushes, damp fields and valleys, seashores and marine regions, swamps, and mangrove areas. This study aimed to determine the effect of *C. nutans* leaves extract (CNE) and its bioactive compounds against the high-fat diet-induced type-2 diabetic rat model.

**Method:** Twenty-eight male Sprague-Dawley rats were divided into four groups: control non-diabetic group (CR), control diabetic group (CD), diabetic group + metformin (D+Met; 300 mg/kg, daily, orally), diabetic group + CNE (D+CNE; 500 mg/kg, daily, orally). Treatment with oral CNE and metformin was administered for 28 days. Blood glucose concentration, body weight change, serum lipid profile, vascular tissue oxidative stress (SOD, MDA), and inflammation (TNF- $\alpha$ ) markers were measured.

**Results:** The CNE-treated diabetic rats have improved body weight, reduced serum cholesterol (total cholesterol, triglycerides, low-density lipoprotein), oxidative stress, and inflammation markers levels ( $P < 0.05$ ). These CNE effects were comparable to those in the metformin-treated diabetic rats.

**Conclusion:** *Clinacanthus nutans* possess anti-hypercholesterolemic properties, which may be due to a reduction in serum cholesterol levels, vascular oxidative stress, and inflammation. Antioxidant and hypoglycemic properties from bioactive compounds present in *C. nutans* may reduce oxidative stress and potentiate to improve dyslipidemia.

**Keywords:** *Clinacanthus nutans*, oxidative stress, type 2 diabetes, bioactive compounds, cardiovascular disease

## Chitosan Nanoparticles Production of Isolated Chitosan from Horseshoe Crab, *Tachypleus gigas*.

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### Abstract

**Introduction:** Chitosan nanoparticles (CSNPs) have been contributed in various works due to its material properties. Chitosan has been isolated from many marine resources involving three methods; demineralization, deproteinization and deacetylation. Characteristic of chitosan has been done by many studies such as its biodegradability, biocompatibility, non-toxic and act as antibacterial agent and also polycationic disposition. However, chitosan has a larger size that limits its application and does not pass through several application criterion. Thus, the technology of nanoparticles shows a promising leading light in many fields. The **objectives** of this study are to isolate and to evaluate chitosan from *T. gigas* and producing chitosan nanoparticles.

**Methods:** Chitosan (CS) has been produced from horseshoe crab, *Tachypleus gigas* carapace through chemically extraction process. Afterwards, the chitosan was transformed to nanoparticles (NPs) size through ionic gelation with tripolyphosphate (TPP) anions as gelating agent. CS-TPP was prepared with various concentration of chitosan (0.25%, 0.5% and 0.75%) to evaluate the particle size. The physicochemical characteristics have been measured and antibacterial activities against; *Bacillus cereus* and *Streptococcus uberis* (Gram-positive), *Salmonella* sp. and *Escherichia coli* (Gram-negative) were analyzed to determine their potential as antibacterial agent. The functional group of CSNPs were examined through Fourier transform infrared spectroscopy (FTIR).

**Results:** The result showed that yield percentage in this study ranged from 10.7% to 19.3% for carapace and 8.7% to 14.3% for book gills, moisture content carapace was 3.80±0.83% which significantly different (p<0.05) from book gills which was 5.04±0.76%, ash content with a significant different (p<0.05) between both samples (1.04% and 3.31% respectively). While the degree of deacetylation for both was 73.66% for carapace and slightly lower for book gills which 66.54% and solubility between 71.87% to 74.51%. Great antibacterial activities exhibit most effective at 0.25% against *B. cereus* and *E. coli*. The structure of chitosan was examined using FTIR showed patterns the bands corresponding to vibration and stretching of O-H, C-H, N-H and CO bonds.

**Conclusion:** CS-TPP can be formed using TPP via ionic gelation method and has anti-bacterial activity.

**Keywords:** Chitosan; Horseshoe crab; Carapace; Sodium tripolyphosphate; Nanoparticles; anti-bacterial activity.

## In-Vitro Biological Activities of Three Marine Sponges from Genus *Theonella* and *Haliclona* Collected from Bidong Island, Terengganu, Malaysia

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### Abstract

**Background:** Marine sponges are primitive sessile animals that have surprised many scientists and researchers with their rich sources of biologically active compounds. This paper aims to evaluate in vitro biological activities from marine sponges from the genus *Theonella* and *Haliclona* species collected from Bidong Island, Terengganu, Malaysia.

**Method:** The evaluation was done on various concentrations of methanolic crude extracts from the sponges for antibacterial (disc diffusion assay), antioxidant (DPPH), and cytotoxicity activity (against HeLa, MCF-7, HepG2 and Vero cell lines via MTT assay).

**Results:** The results exhibited that *Theonella swinhoei*, and *Theonella cf cupola* were low antibacterial capabilities but were able to exhibit IC<sub>50</sub> of antioxidant activity at 23.2507±1.5714 mg/ml and 18.5248±0.8572 mg/ml, respectively. *Haliclona fascigera* showed high inhibition toward most of the bacteria used and high inhibition of IC<sub>50</sub> at 1.8003±0.0674 mg/ml for the antioxidant activity. Results of the MTT assay showed that both genera of marine sponge samples collected show IC<sub>50</sub> below 25µg/ml in all cancer cells, whilst showing no significant changes for normal cells, Vero at the concentration of 25µg/ml

**Conclusion:** Despite showing low antibacterial and medium antioxidant activity for *Theonella* sp, cytotoxicity assay showed high activity whilst genus *Haliclona* shows excellent results in all biological activities that have been conducted. Marine sponges evaluated in all biological activities have promising bioactive compounds that may be a great candidate for drug discovery in potential antibiotics and human anticancer drug research. Further isolation of compounds from the sponge may be able to provide promising compounds for the treatment of human diseases.

**Keywords:** antioxidant activity, antibacterial activity, biological activities, marine sponges, cytotoxicity MTT

## The Potential of Green Seaweed, *Ulva* sp. as a Source for Antibiofilm Activity

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### Abstract

**Background:** Marine natural products especially seaweeds has gained much attention in combat the biofouling phenomenon that is currently encountered marine environment. Seaweed produces metabolites that are useful in wide biological activities such as probable for antibacterial, antimycotic, antifouling, and antifungal. In this study, green seaweed (*Ulva* sp.) from Kota Tinggi, Tanjung Pengelih Johor was analyzed for its effect towards biofilm formation. Thus, the objective of this study is to determine the antibiofilm and antibacterial activity of extract obtained from *Ulva* sp.

**Methods:** The crude extract was prepared via maceration process using methanol (10% w/v) to yield methanolic crude extract (MCE). Then, the antibacterial assessment was evaluated using the disk-diffusion method against Gram-positive (*Bacillus cereus*, *Streptococcus uberis*) and Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Vibrio* sp., *Salmonella* sp.) on five series of concentrations ranging from 0.03125 g/ml to 0.5 g/ml. The antibiofilm activity was screened using the 96-well microtiter plate crystal violet method against biofilm-forming bacteria *P.aeruginosa* by two-fold serial dilution.

**Results:** MCE of *Ulva* sp. exhibited antibacterial activity on Gram-negative bacteria, which were *Salmonella* sp. and *Vibrio* sp. at concentrations 0.25, 0.125, 0.0625, and 0.03125 g/ml indicating a narrow spectrum with moderate activity. Besides, the growth of biofilm produced by *P.aeruginosa* was highly inhibited by MCE at low concentrations, 0.0156 mg/ml. Thus, this result showed that MCE inhibited the growth of biofilm but at the same time not killed the bacteria owing to no inhibition growth detected during the antibacterial assessment of *P.aeruginosa*.

**Conclusion:** *Ulva* sp. could be one of the promising candidates from the seaweed group as an eco-friendly antibiofilm agent.

**Keywords:** biofilm, methanolic crude extract (MCE), *Ulva* sp., *P.aeruginosa*, eco-friendly

## Epigenetics for Resilient Aquaculture Production in Times of Global Change

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### Abstract

Epigenetics is an emerging area of research in agriculture animals, including fishes and shrimps. In recent years, it has drawn significant attention for its key roles in the improvement of nutrition, health and welfare, and the control of infection stress and diseases in aquaculture animals. Epigenetics deals with the mechanisms of heredity, which do not involve modifications of DNA sequence and are reversible. In a series of *in vivo* studies conducted using aquaculture animals, our and other research groups have shown that environmental cues could potential leave permanent marks on the individuals that influence the development of key aspects of their phenotypes (e.g. growth, development capacity, health and robustness) that cannot be explained by genetic codes. Most of these marks are elicited through epigenetic mechanisms, and interestingly some of these developed phenotypes can be inherited by subsequent generations. In this review, we will provide a basic knowledge on the molecular actors of epigenetics, highlighting the developing knowledge, based on the findings of our and research groups, in aquaculture animals, which can be useful for a resilient aquaculture production especially in the light of climate change.

## Novel SNEDDS Formulations of *Pandanus tectorius* Fruit to Improve Anti Atherosclerosis Activity

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### Abstract

**Background:** *Pandanus tectorius* is a coastal plant that may be used as a source of herbal medicine. It has been scientifically proven to be useful for health as an alternative natural HMGCR inhibitor. However, oral administration is hindered due to the semi-polar properties of the active extracts. The solubility of the extract can be potentially improved through the synthesis of a self-nanoemulsifying drug delivery system (SNEDDS).

**Method:** The formulation was determined using a solubility test and preliminary screening for surfactant and co-surfactant. The optimized formulation was characterized to know the self-emulsification time, thermodynamic stability, optical clarity, particle size, and zeta potential. Antiatherosclerosis activity was investigated by measuring the HMGCR inhibition activity of the crude extract and optimized SNEDDS formulation via *in vitro* study.

**Results:** The optimized SNEDDS formulations of *Pandanus tectorius* extract (PTF-SNEDDS) consisted of caprylic triglycerides, kolliphor RH40, and propylene glycol as oil, surfactant, and co-surfactant. It was found that the self-emulsification time of PTF-SNEDDS in water, simulated gastric, and simulated intestinal fluid was 38.17, 49.44, and 45.29 sec respectively, indicating grade A formulation. PTF-SNEDDS showed no sign of thermodynamic instability. The clarity level of PTF-SNEDDS, particle size, and zeta potential were at 99.3%,  $41.5 \pm 1.1$  nm, and  $-25.9 \pm 1.2$  mV, respectively. The antiatherosclerosis activity of PTF-SNEDDS was 1.23 times higher than the crude extracts and 6.38 times higher than Pravastatin.

**Conclusion:** The optimized formulation is robust to dilution in various media and exhibits no sign of separation. It can maintain its nanoemulsion form and be stable in the gastrointestinal tract. *In vitro* antiatherosclerosis activity of PTF-SNEDDS showed that SNEDDS formulation can increase PTF extracts' HMGCR inhibition activity as the solubility of the extracts' in an aqueous solution was improved.

**Keywords:** *Pandanus tectorius*, antiatherosclerosis, oral bioavailability

## Antifouling Studies of Paint Incorporated with *Sonneratia lanceolata* Crude Extract

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### Abstract

**Background:** Marine resources such as mangrove plants favor the production of secondary metabolites that exhibit antifouling properties and can be used to combat the biofouling problem. These natural-derived compounds are considered environmental friendly compared to commercial compounds. This study was conducted to determine the antibacterial and antifouling activities of *Sonneratia lanceolata* (SL) crude extract and their incorporated paints in addition to the separation of secondary metabolites existing in this crude extract.

**Methods:** Antibacterial properties of this crude extract were tested against selected bacterial test strains while *Pseudomonas aeruginosa* was used for the crystal violet biofilm assay that acts as biofilm-forming bacteria. Vacuum liquid chromatography (VLC) was applied to the separation of secondary metabolites present in the leaves of *S. lanceolata*. The incorporated paints with 5% (SL5%) and 10% (SL10%) weight per volume of the crude extract were tested in an aquarium in the laboratory and submerged in the seawater at Kemaman and Pulau Redang for field testing.

**Results:** The highest antibacterial activity of this crude extract was found against *P. aeruginosa* strain with a diameter of 10mm, while there is no inhibition zone found on another five bacterial test strains indicating that there is no antibacterial property. However, crystal violet assay results showed that the crude extract and fractions obtained from the VLC technique reduced the biofilm of the bacteria at different concentrations. A total of 23 fractions were collected and combined based on the identical spots observed on thin layer chromatography (TLC). Bacteria growths were inhibited the most by SL5% followed by SL10% in aquarium testing compared to the blank panel. Based on the photographic observation, all of the paints successfully reduced the settlement of fouling organisms as compared to the blank paint with the best performance of SL5%.

**Conclusion:** The crude extract of *S. lanceolata* might have the potential to be developed as an antifouling agent.

**Keywords:** biofouling, antifouling, mangrove leaves, secondary metabolites, incorporated paints

## Effects of Inoculum Size of *Afifella marina* in the Bioprocessing of *Sargassum* spp. Based Seaweed

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### Abstract

**Background:** Brown seaweed, *Sargassum* spp. is used as one of the alternative feed ingredients for aquaculture in the form of seaweed meal. Nutritional values of seaweed meal are improved by incubation with different inoculum sizes of microbes such as *Lactobacillus*, *Bacillus* or *Streptococcus* using bioprocess technology. Purple non-sulphur bacteria, *Afifella marina* could also be one of the alternative and potential candidates. This study aims to determine the optimum inoculum level and incubation period for the improvement of nutritional values in *Sargassum* spp. seaweed meal inoculated with *A marina*.

**Method:** *Sargassum* spp. was collected from the Sepanggar Bay. Dried seaweed was grinded into fine particles (<400µm). Inoculum sizes of 10% (v/v), 20% (v/v) and 30% (v/v) of *A.marina* were used with 20g *Sargassum* spp powder in one liter bottle with autoclaved synthetic media. Bottles were incubated at 2500 lux light intensity at 30±2°C for 8 days. Every two days destructive samples were taken for the determination of nutritional values of seaweed meal. The measured analytical parameters were crude protein (%), crude ash (%) and crude fiber (%).

**Results:** Nutritional values of processed seaweed meal powder incubated with *A.marina* have improved with the increase of inoculum size. The crude protein percentage of 14.36±0.36% was significantly high ( $p<0.05$ ) with 30% (v/v) inoculum size and obtained on the 6<sup>th</sup> day of the bioprocessing product. Consistent decrease in crude fiber values were observed with the increase of inoculum sizes, where 19.34% of crude fiber decrement was determined with 30% (v/v) inoculum level on 6<sup>th</sup> day. No significant differences ( $p>0.05$ ) were observed among the values of crude protein, ash and fiber within the 6<sup>th</sup> and 8<sup>th</sup> days.

**Conclusion:** *A.mariana* with 30% (v/v) inoculum size on day 6 has the capability in improving the nutritional values of *Sargassum* spp seaweed meal during bioprocessing.

**Keywords:** *Afifella mariana*, brown seaweed, nutritional values, inoculum sizes and incubation period



## Scoping Opportunities of Natural Bioactive Compounds of Medicinal Plants as Potential Agents for Pneumonia Correlated to COVID-19

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### Abstract

**Background:** SARS-CoV-2 can infect the severe respiratory tract and trigger inflammation, so it causes pneumonia. COVID-19 takes a higher risk for the elderly, commodities, and patients with co-infection of bacteria. Natural products have been used for decades and across the countries, there are fewer side effects to the patient and higher acceptance by civilians as an alternative for health care.

**Method:** This study concentrated to explore the plants and its natural bioactive compounds that may have the potency to prevent pneumonia correlated to Covid-19. The data in this review is taken from electronic databases such as Google Scholar, PubMed, Elsevier, etc.

**Results:** The utilization of plants as bioactive compounds to prevent pneumonia correlated to COVID-19 has been discussed in many studies. Several medicinal plants such as *Curcuma manga*, *Moringa oleifera*, *Nelumbo nucifera*, *Etingera elatior*, *Citrus aurantifolia*, *Cullen corylifolium*, *Glycyrrhiza glabra*, *Allium sativum*, *Nigella sativa*, and *Stephania cepharantha* are potentially having an antibacterial and antiviral activity to prevent pneumonia correlated to COVID-19 also they have properties such as anti-inflammatory, antioxidants, immunomodulatory, etc.

**Conclusion:** Several studies of pneumonia correlated to the coronavirus reported the effectiveness of some medicinal plants in preventing seasonal epidemics caused by viruses and bacteria. However, more rigorous scientific research is needed to be explored to validate the bioactive compounds in vitro and in vivo also for antiviral and antibacterial candidates.

*Keywords:* COVID-19, pneumonia, antibacterial, antiviral, medicinal plants

## Anti-biofilm Activity from Methanolic Crude Extract of *Melaleuca cajuputi*

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### Abstract

**Background:** Due to the issue regarding biofouling that become hype in maritime industry and had contribute to economic loss, antifouling paint was introduced and used as control to prevent the problem. *Melaleuca cajuputi* or pokok gelam can easily be found in the East Coast of Peninsular Malaysia such as Terengganu and Pahang. In this study, crude extract and essential oil extracted from *M. cajuputi* were studied to determine their action in antifouling activities.

**Method:** The biota was soaked with n-Hexane (1:5) before continued soaking three times with methanol (1:10) and fractionated by Vacuum liquid Chromatography (VLC) using n-hexane and acetone. Essential oil was using the commercially available in the market. The antibacterial assay of methanolic crude extract and essential of *M. cajuputi* was conducted using disc diffusion method against six bacterial test strains. Crystal violet assay was conducted to determine the sample that highly help in prevent the detachment of biofilm produced by marine bacteria, *Pseudomonas aeruginosa*.

**Results:** The crude was collected in eight different fractions. Antibacterial assay conducted using methanolic crude extract of *M. cajuputi* give positive inhibition against most Gram-negative bacterial strain than Gram-positive bacterial strain with the maximum inhibition range at 19 mm while essential oil showed positive effect against both Gram-positive and Gram-negative bacterial strain with the ranging between 7-17 mm at the concentration of 0.0625-0.25 g/ml. This indicated that crude extract has narrow spectrum while essential oil has wide spectrum. From crystal violet assay, both samples inhibited the detachment of biofilm produced by the marine bacteria but methanolic crude extract has higher anti-biofilm activity at concentration of 0.016 mg/ml compared to essential oil at 0.0064 mg/ml. Thus, crude extract was most likely to have the anti-biofilm properties.

**Conclusion:** *M. cajuputi* crude extract possess both antibacterial and anti-biofilm properties.

**Keywords:** *Melaleuca cajuputi*, pokok gelam, essential oil, biofouling, anti-biofilm

## Discrimination of Marine Polychaeta Species Based on $^1\text{H}$ NMR Metabolomics

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### Abstract

**Background:** Polychaete worm have gained prominence in study of its bioactivity and structurally intriguing compounds. Previously, polychaeta showed antibacterial activity and acute wound healing properties. However, studies on the chemical constituents of polychaeta from different species are still very lacking.

**Method:** The purpose of this study was to conduct the chemical profile and discrimination of marine polychaeta species based on proton magnetic resonance ( $^1\text{H}$  NMR) metabolomics. In this study, some marine worm species including *Diopatra claparedii*, *Marphysa moribidii*, *Sasekumaria selangora*, *Namalycastis* sp. were collected and analyzed.

**Results:** A total of 40 metabolites were identified including organic acids, amino acids, phenolics, vitamin and halogenated aromatics based on their  $^1\text{H}$  NMR characteristic signals. Multivariate data analysis via principal component analysis (PCA) showed an accumulated variance of 67%, while PC1 explained 45.9%, followed by PC2 with 15.0%. The PCA scores plot revealed the discrimination of 4 different clusters of the polychaeta that were formed based on their different species. Samples were discernible from each other based on different metabolite constitutions. In addition, two major groupings were formed and observed in hierarchical cluster analysis (HCA) dendrogram revealed the chemotaxomy relationship among the different species. *Namalycastis* sp. was clearly separate with other polychaeta species.

**Conclusion:** These results provide the basis information on the metabolite variations in polychaeta from different species that might be useful for future studies. Furthermore, approach used in this study might be deciphering the potential of marine polychaeta in particular as new sources of natural therapeutic agents.

**Keywords:** marine polychaeta,  $^1\text{H}$  NMR, metabolite profile, metabolomics

## Evaluation of Antifouling Potential Derived from Marine Bacterium, *Pseudomonas* sp.

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### Abstract

**Background:** Biofouling is defined as the colonization processes of epibiotic organisms ranging from macrofoulers to microfoulers in excessive growth on any surface in contact with water. However, this fouling can cause major problems in marine-based sectors if not prevented from the start. The promising antifouling compounds to solve this problem are by the production of secondary metabolites from marine bacteria. Marine bacteria are chosen as it can be cultured in a large scale easily, subsequently provide the potential sources of environmental-friendly antifoulant. This study was conducted to characterize and identify the bacteria culture which can inhibit the formation of bacteria biofilm.

**Methods:** The bioactive metabolites were extracted using ethyl acetate and concentrated from the bacteria culture prior the bioassay method. The isolate was screened for antibacterial activities against *Bacillus cereus*, *Streptococcus uberis*, *Pseudomonas* sp. and *Vibrio parahaemolyticus* by using disc diffusion technique before further testing for antifouling activities. Then, the isolate was investigated in order to verify its bioactivity in prevention of biofilm formation following bioassay guided method; crystal violet assay. The bacteria was identified through phenotypic and genotypic tests to validate the species of the marine bacteria.

**Results:** It was found that tested isolate, designated as RLimb shows activity to inhibit the biofilm which leading to prevent the formation of microfouling. This active marine bacteria were characterized as a Gram-negative, aerobic and long-rod-shaped bacteria. Based on the 16S rDNA gene sequencing analysis, RLimb revealed as *Pseudomonas* sp. with the closest described as *Pseudomonas neuropathica* with similarity of 90.78%.

**Conclusion:** Results indicated that bioactive metabolites from *Pseudomonas* sp. can be used potentially as a substitute to the toxic antifoulants in preventing the formation of microfoulers. Further studies need to be done before the application as an alternative natural antifoulants to eradicate biofoulers without harming other marine organisms.

**Keywords:** antifouling, biofilm, marine bacteria, *Pseudomonas* sp.

**SCOPE: SYSTEMS BIOLOGY**

## Sequencing the Seas

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### **Abstract**

Many ocean policies mandate integrated, ecosystem-based approaches to marine monitoring, driving a global need for efficient, low-cost bioindicators of marine ecological quality. Most traditional methods to assess biological quality rely on specialized expertise to provide visual identification of a limited set of specific taxonomic groups, a time-consuming process that can provide a narrow view of ecological status. In addition, microbial assemblages drive food webs but are not amenable to visual inspection and thus are largely excluded from detailed inventory.

Environmental DNA (eDNA) sequencing offer advantages over traditional methods and are increasingly being used for studying biodiversity and monitoring ecosystem changes. As organisms shed DNA into their surrounding environment, eDNA analysis picks up these genetic 'breadcrumbs' and provides clues about all the species present in a sample – from microbes (bacteria) to megafauna (e.g. sharks). This emerging technology is rapidly expanding capabilities for cutting edge genomic research, with applications that can help meet marine conservation challenges of food security, biodiversity loss, and climate change. In summary, eDNA will become a key source of data that enables us to make informed conservation decisions. Given the challenges we face in our oceans, rivers and land, it is time when making good decisions really matters.

## *In silico* Selection and Characterization of Anti-Quorum Sensing DNA Aptamers Against *Vibrio parahaemolyticus*

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### Abstract

**Background:** Aptamer has the privilege in clinical applications due to its high binding affinity and specificity. Numerous therapeutic aptamers that were in preclinical and clinical development have been reported. In this study, we aimed to characterize anti-quorum sensing aptamer as the prophylactic/therapeutic agent against *Vibrio parahaemolyticus*.

**Method:** In-silico modeling was approached to design ssDNA aptamers against bacterial quorum sensing receptor, LuxP. Short fragments of nucleotides with highest binding affinity were selected from molecular docking analysis. The protein-aptamer complexes were further analyzed using molecular dynamic (MD) simulation. Subsequently, binding of the selected aptamers was verified using isothermal titration calorimetry (ITC). Lastly, in-vitro treatment of bacterial culture with the aptamer candidate was conducted to evaluate its anti-quorum sensing properties.

**Results:** MD simulation revealed aptamer candidates that possess significant binding affinity where the calculated binding energy was lower than -250 kJ/mol. ITC identified two short hairpin structured aptamers with significant K<sub>d</sub> values. Transcriptome analysis revealed suppression of quorum sensing-related genes expression upon treatment of the selected aptamer.

**Conclusion:** This study demonstrated an in-silico approach to design ssDNA aptamer that interacts with LuxP, which suppressed quorum sensing-related gene expression.

**Keywords:** single-stranded DNA aptamer, anti-quorum sensing, in-silico modelling

## Integrative Taxonomy Reveals Phenotypic Plasticity in Glass-perchlet *Parambassis siamensis* Fowler 1937

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### Abstract

**Background:** Genetic adaptation and phenotypic plasticity is important in the evolutionary ecology of fish. The glass-perchlet *Parambassis siamensis* Fowler 1937 inhabits freshwater ecosystems across Southeast Asia, including Peninsular Malaysia, Thailand, Cambodia, Laos and Vietnam. The species shows body shape and pigmentation variation in morphology, which led to synonymy of this species with *Chanda punctulata* Fraser-Brunner 1955.

**Method:** In this study, the phenotypic variation of *P. siamensis* was assessed using an integrative taxonomic approach. We compare several populations of the glass-perchlet *P. siamensis* sampled from lotic and lentic habitats throughout Peninsular Malaysia.

**Results:** Our morphological analyses support the presence of only one species, with a range of morphometric and meristic characters overlapping among populations. Our genetic analysis using partial sequences of the mitochondrial gene coding for the protein cytochrome c oxidase I (COI) reveals the existence of two clades that diverge from each other by a minimum uncorrected p-distance of 2.2%. The presence of specimens with numerous melanophores on body sides and differences in body shape indicates the phenotypic plasticity in this species.

**Conclusion:** This finding supports the conclusion that *Chanda punctulata* Fraser-Brunner 1955 is a junior synonym of *P. siamensis*. The population of *P. siamensis* in Peninsular Malaysia (along those from Chao Phraya and lower Mekong basins) are conspecific, comprising two genetically distinct, although close lineages.

**Keywords:** *Parambassis siamensis*, *Chanda punctulata*, phenotypic plasticity, integrative taxonomy



## Comparative Transcriptome Analysis Reveals the Mechanisms that Led to Slow-growth of Orange-Spotted Grouper (*Epinephelus coioides*) Larvae

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### Abstract

**Background:** The orange-spotted grouper is one of the most popular fish in aquaculture due to its high economic value as high quality human dietary protein source. However, random slow-growing fishes were often observed in the batch culture due unknown cause and led to profit loss.

**Method:** The total RNA was extracted from slow growing and fast growing groupers (n=3) on 32 and 60 day post hatching (dph). Then the RNA libraries were subjected to RNA-sequencing by the Illumina sequencing platform. Short reads generated from 12 libraries were assembled *de novo* and comparative analysis was conducted between slow-growing groups and fast-growing groups on 32 dph (SG32 versus FG32) and 60 dph (SG60 versus FG60). The differentially expressed genes (DEGs) were subjected to enrichment analysis to uncover the overrepresented terms of Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways within the DEGs.

**Results:** Exploration of DEGs discovered that the expression of GH/IGF axis related genes such as *IGF-1*, *IGF2*, *IGFBP1*, *IGFBP2* in slow-growing groups were significantly down-regulated, in relative to fast-growing groups. Enrichment analysis of these DEGs revealed that enriched GO terms were mostly associated with cellular process and biological regulation, whereas enriched pathways were related to glycolysis/gluconeogenesis, linoleic acid metabolism, protein digestion and absorption, and cholesterol metabolism. The results of DEGs and enrichment analysis were similar on 32 dph and 60 dph.

**Conclusion:** The low expression of several DEGs such as *IGFALS*, *SST* and genes involved in GH/IGF axis and its downstream signalling pathways for metabolism and digestive-system related pathways in slow-growing orange-spotted groupers might be the main reason of the poor growth. These findings provided valuable insights and the candidate genes for the fish farmers and scientists to tackle the current issue and improve the general health of orange-spotted groupers via specific dietary change and feeding.

**Keywords:** orange-spotted grouper, transcriptome, development, gene expression

## Identification of Candidate Effector Proteins of *Vibrio parahaemolyticus* and Their Targeted Proteins in *Penaeus vannamei* Using Host-Pathogen Protein-Protein Interaction Network Analysis

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### Abstract

**Background:** The white leg shrimp, *Penaeus vannamei*, is one of the most important species in shrimp aquaculture and contributes to economic growth worldwide. However, the production of *P. vannamei* is often hindered by diseases caused by pathogens such as *Vibrio parahaemolyticus*, which resulted in a huge loss of profit in shrimp aquaculture. Despite the losses, the pathobiology of *V. parahaemolyticus* infection in *P. vannamei* is unclear. Hence, this study aims to identify the candidate effector proteins of *V. parahaemolyticus* and their target proteins of *P. vannamei* using the host-pathogen protein-protein interaction (HP-PPI) network.

**Method:** The HP-PPI network between *P. vannamei* and *V. parahaemolyticus* was constructed using an interolog-based method. The topological analysis of the network was analyzed using cytoHubba to identify the top 1000 nodes ranked by the Maximal Clique Centrality (MCC). The pathway enrichment of the top 1000 nodes was conducted using hypergeometric analysis followed by the Benjamini-Hochberg test to determine the significant pathway that might involve in the bacterial infection in shrimp.

**Results:** The HP-PPI network consisted of 157431 interactions between 2234 and 11764 of *P. vannamei* and *V. parahaemolyticus* proteins, respectively. The topological analysis identified T3SS2 effector VopL nucleation of actin polymerization and flagellar biosynthesis as candidate effector proteins, and heat shock protein 70, binding immunoglobulin and skeletal muscle actin 6 as the potential targeted proteins. Pathways, such as the adherens junction, adipocytokine signaling pathway, apoptosis, arginine and proline metabolism, and autophagy, presented as significant pathways that might involve in *V. parahaemolyticus* infection in *P. vannamei*.

**Conclusion:** This study has identified the potential proteins and related pathways that are probably involved in *V. parahaemolyticus* infection in *P. vannamei*. Such knowledge might contribute to improving the production of the shrimp aquaculture industry.

**Keywords:** host-pathogen protein-protein interaction, hub proteins, cytoHubba, *Vibrio parahaemolyticus*, *Penaeus vannamei*

## Biomonitoring of Estuarine Fish Community Utilising Environmental DNA Metabarcoding

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### Abstract

**Background:** Environmental DNA (eDNA) metabarcoding, the combination of highthroughput sequencing and DNA-based species identification from environmental samples is emerging as a non-invasive method for the detection and identification of common, rare, and elusive species in a wide range of ecosystems, including aquatic environments such as marine, freshwater, and coastal ecosystems. In order to assess the ecological status of these ecosystems, for example in estuaries, fish communities monitoring is one of the most widespread and established approach, but the conventional method of fish surveys are invasive, timeconsuming and financially expensive.

**Method:** In the present study, we investigate the potential of using eDNA metabarcoding to assess fish diversity, based on the analyses of water samples collected from Merbok Estuary using both 12S and COI markers

**Results:** Our study demonstrates the utility of eDNA metabarcoding for recovering fish diversity in the studied site. eDNA metabarcoding captured higher diversity with more species than previous conventional surveys, within just a two-day sampling period

**Conclusions:** Our study offers a powerful tool for future large-scale biomonitoring research and provide important information essential for effective long-term monitoring of aquatic habitats. However, the successful detection of species and biodiversity study depends on the availability of a comprehensive reference database.

*Keywords:* Environmental DNA, metabarcoding, estuaries, fish community, biomonitoring

## Rhomboid Protease Genes from Extremophiles: An *In Silico* Analysis

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### Abstract

**Background:** Extremophiles are getting attention due to their ability to adapt to extreme environments. One of the exciting enzymes possessed by these organisms is the rhomboid protease. Rhomboids are intramembranous serine proteases that play an essential role in many cell functions and human diseases such as growth factor signalling, mitochondrial homeostasis, protein regulation and parasite invasion. In this study, we compared the rhomboid protease genes that were retrieved from microorganisms found in the hot springs and Antarctica to better understand these proteins which could be used to improve related drug targeting in the future.

**Method:** JGI and IMG databases were used to retrieve thermophilic protease sequences, while PATRIC retrieved Antarctic rhomboid protease genes. Sequences were then aligned using MAFFT. InterProScan was used to analyse all protein domains, while ProtParam was used to identify protease amino acid frequencies. We also used I-TASSER to predict the 3D protein structure of the rhomboid protease.

**Results:** The multiple sequence alignment (MSA) analysis revealed that 169 conserved areas were presented in the gene sequences. Results also showed that Valine was the most dominant amino acid found in Antarctica representatives while the predominant amino acid in hot springs was Isoleucine. The fundamental structure and functional locations of rhomboids have remained unchanged and retaining their proteolytic activity.

**Conclusion:** Analysis on rhomboids from hot springs and Antarctica representative genes have provide us insights on the structural and functional of these proteases that could eventually offers possibilities as potential therapeutic targets.

*Keywords:* Rhomboid Protease, Extremophiles, In Silico Analysis, Hot Springs, Antarctica

## DNA Barcode Approach in Marine Gastropods' (Mollusca: Gastropoda) Diversity Study

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### Abstract

**Background:** Most species delimitation in Gastropoda has been based solely on morphology, yet the high intraspecific morphological variability among marine gastropods due to phenotypic plasticity and additive genetic variance, has made the identification of the taxonomic entities challenging. The DNA barcoding approach has shown to be greatly useful in species identification and biodiversity research to complement morphological techniques.

**Methods:** Here, we review the genetic barcoding approach on marine gastropods by using genetic markers such as the mitochondrial DNAs cytochrome c oxidase subunit I (COI), ribosomal DNAs 16S (16S), and 12S, and discuss their advantages in delimiting taxonomy boundary of the marine gastropods

**Results:** The COI gene has been shown to be a more common and widely used marker, while the 16S and 12S are complementary to the COI sequence data. Overall, studies revealed that an efficient approach to assessing the biodiversity of marine gastropod species is by using a combination of morphological and molecular methods, and the inventory is important as a monitoring tool for a sustainable natural resource

**Conclusion:** We also recommend the construction of a reliable local DNA barcoding reference database of marine gastropods in Malaysia to be undertaken. This effort is essential for a novel way of biodiversity monitoring in the future, i.e. metabarcoding method through analysis of the environmental DNA (eDNA).

*Keywords:* DNA barcode, marine gastropod, COI, metabarcoding, biodiversity

## Insights into Dimethylsulfoniopropionate (DMSP) Synthesis and Catabolism by Marine Bacteria

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### Abstract

**Background:** Dimethylsulfoniopropionate (DMSP) is an important marine anti-stress compound, with key roles in global nutrient cycling, chemotaxis and, potentially, climate regulation. Recently, diverse marine Actinobacteria,  $\alpha$ - and  $\gamma$ -proteobacteria were shown to initiate DMSP synthesis via the methionine (Met) *S*-methyltransferase enzyme (MmtN), generating *S*-methyl-Met (SMM). Diverse DMSP lyases in some algae, bacteria, and fungi cleave DMSP to yield gaseous dimethyl sulfide (DMS), an infochemical with important roles in atmospheric chemistry.

**Method:** We proposed the catalytic mechanisms of key enzymes involved in DMSP synthesis and catabolism through genetic manipulations, bioinformatic analysis, and enzymatic and structural assays.

**Results:** We characterize a roseobacterial MmtN, providing structural and mechanistic insights into this DMSP synthesis enzyme. We propose that MmtN uses the proximity and desolvation mechanism for Met *S*-methylation with two adjacent MmtN monomers comprising the Met binding site. We also identify diverse functional MmtN enzymes in potentially symbiotic archaeal *Candidatus* Woesearchaeota and Candidate Phyla Radiation (CPR) bacteria, and the animalcule *Adineta steineri*, not anticipated to produce SMM and/or DMSP.

We identify a novel ATP-dependent DMSP lyase, DddX. DddX belongs to the acyl-CoA synthetase superfamily and is distinct from the eight other known DMSP lyases. DddX catalyses the conversion of DMSP to DMS via a two-step reaction: the ligation of DMSP with CoA to form the intermediate DMSP-CoA, which is then cleaved to DMS and acryloyl-CoA. The novel catalytic mechanism was elucidated by structural and biochemical analyses. DddX is found in several Alphaproteobacteria, Gammaproteobacteria, and Firmicutes, suggesting that this new DMSP lyase may play an overlooked role in DMSP/DMS cycles.

**Conclusion:** This study provides important insights into the catalytic mechanism of SMM and/or DMSP production, and offers important insights into how marine bacteria generate the climatically important gas DMS from DMSP, leading to a better understanding of the global sulfur cycle.

**Keywords:** DMSP synthesis, DMSP catabolism, marine bacteria, catalytic mechanism, marine sulfur cycling

**SCOPE: MICROBE ENVIRONMENT AND BIOTECHNOLOGY**

## Marine Resources: from Seafloor to Laboratory Cabinet

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### Abstract

**Background:** Marine resources including marine invertebrates, marine and mangrove animals and plants. Institute of Marine Biotechnology, Universiti Malaysia Terengganu treasure all of the above mention and we do have a collection of BIOTA for various usage. Our program, Marine microbes and environment use marine microbes associations as well as the host of the microbes.

**Methods:** Our current research are focusing on the anti-biofilm and anti-fouling to solve the problem occurred on rigs of oil and gas industries. Our studies show some useful compounds isolated from marine bacteria especially for their anti-microbes, anti-biofilms and anti-fouling bioactivities. We have tested all of our extracts using crystal violet assay, Disc Diffusion test as well as tested steel panel materials (materials supplied by oil and gas industries) in the laboratory using an aquarium (mimicking the real environment on site) for anti-biofilm formation. We tested our extracts on steel panel for the above mention bioactivities for a year with one month interval on site of Redang Island and Kemaman Sea.

**Results:** We have extracted many marine resources for screening of anti-bacterial and anti-fouling activities including the anti-biofilm. 70% of our extracts show moderate to high activities for anti-bacterial, anti-biofilms as well as the anti-fouling. We have characterized some of the marine bacteria isolates, mainly focusing on the *Pseudomonas* sp. We also screened mangrove plants especially from the leaves, also show their activities towards our designated guided-assay. It also found that the painted materials after a year of experiments have given a good result in term of anti-fouling activities when compared with the reference standards.

**Conclusion:** Extracts obtained from this study indicating of a potential to develop as anti-fouling agent especially for industrial usage.

*Keywords:* marine resources, BIOTA, Bioactivities, anti-biofilm, anti-fouling



## A Preliminary Report on Metagenomic Analysis of Photosynthetic Purple Non-Sulphur Bacteria Grown on Homebrew Recipe; Insight on Functions and Risks.

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### Abstract

**Background:** Purple non-sulphur photosynthetic bacteria (PNSB) are well known for their benefit to aquaculture and agriculture. Various reports in aquaculture mentioned that PNSB can reduce waste in water, suppress diseases and increase the growth rate of aquatic animals. While in agriculture it was reported that PNSB can accumulate polyphosphate, producing plant growth-promoting substances and directly provide nitrogen, phosphate, and potassium from their dead cells. The demand for PNSB in the market increasing, and many of them were homebrewed based on formulations available on the internet. The composition in terms of the existing microbe and the safety of the brew is not validated. Therefore, we reported the analyses of the microbial communities of a homebrew PNSB.

**Methods:** The metagenome analysis was done on the pellet obtained from homebrew PNSB. The 16sRNA V4 region was amplified using Illumina Iseq100 and the sequences were trimmed and analysed. The metadata was further analysed on the MicrobiomeAnalystCA webserver.

**Results:** Nine most abundant bacteria in the sample were from the genus *Petrotoga*, *Rhodopseudomonas*, *Cloacibacillus*, *Burkholderiaceae* (family), *Ectothiorhodospira*, *Aminobacterium*, *Dysgonomonas*, *Petrimonas* and *Proteiniphilum*. *Petrotoga* sp. was the most abundant found with 25% of relative abundance (RA) in the samples. It is Gram-negative, strictly anaerobic, thermophilic, halotolerant, and associated with oil contamination. The second abundance was *Rhodopseudomonas* sp., with 16% of RA. This PNSB bacteria can exist in many types of environments and is one of the important PNSB studied. The third abundance was from the family *Burkholderiaceae* with 15% RA, this family includes some important pathogenic species such as the *Burkholderia mallei* and *Burkholderia pseudomallei*. The fourth abundance was *Ectothiorhodospira magna* (4%), a PNSB which mostly reported from salt marshes or soda lakes. The rest of the bacteria species detected were from 3 to 2% RA.

**Conclusion:** Two PNSB species were detected, both were second and fourth abundance species in the sample. While a potential family with pathogenic members was also detected (*Burkholderiaceae*). Further studies need to be done to clarify the species of this family before a definite conclusion on risk can be made.

Keywords: PNSB, homebrew, pathogenicity, risks

## A Bibliometric Analysis of Vibriocin: Trends, Challenges and Future Prospects

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### Abstract

**Background:** The aquaculture industry is seriously threatened by vibriosis, while antibiotics are becoming ineffective against *Vibrio* spp. Bacteriocins are well-known, narrow-spectrum antimicrobial agents. Bacteriocins extracted from *Vibrio* spp. are called “vibriocins”.

**Method:** The aim of this review was to review publications on the detection and extraction of vibriocin to determine the feasibility of vibriocin as a vibriosis control measure. A systematic literature review up to 31st December 2021 was carried out using 5 keywords (Vibriocin, bacteriocin AND vibrio, bacteriocin-like inhibitory substances AND vibrio, BLIS AND vibrio) and 2 databases (Web of Science and SCOPUS). After applying the inclusion and exclusion criteria, a total of 42 articles were included in this review.

**Results:** The first paper was published in 1962, however, the number of publications has fluctuated. Canadian researchers initiated the field, while other major contributors are US, India, UK and China. Progress in the field was slow probably due to a lack of collaboration between leading researchers. When collaboration increased, crucial advancements were observed such as isolation, identification and characterization of vibriocins. Besides “bacteriocin”, vibriocins were also called vibriocine, vibriocidin, vibriobactin, harveyicin, and vulnificin. Five major detection/extraction methods were described in the literature ranging from plate-based methods to broth-based cultures. Vibriocins were detected in a total of 12 *Vibrio* spp. while *V. cholerae*, *V. harveyi* and *V. parahaemolyticus* were the most studied. There was a total of 9 influential articles that shaped vibriocin research. These key research articles were pioneer articles and innovations in the detection, extraction and characterization of vibriocins.

**Conclusion:** It can be concluded that vibriocin detection and isolation are still challenging and there is need for more innovations. However, vibriocins have shown the narrow spectrum activity needed against *Vibrio* spp. Vibriocin could be an effective vibriosis control measure in aquaculture as a recombinant or purified protein.

**Keywords:** aquaculture, vibriosis, bacteriocin, vibriocin, bibliometric

## Attached Microalgae Growth System Using Palm Oil Mill Effluent (POME) for Efficient Biomass Feedstock Production and Nutrient Removal

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### Abstract

**Background:** Untreated palm oil mill effluent (POME) can severely affect the environment due to its high nutrient content. Utilisation of its nutrients for microalgae attached system will beneficially improve the biomass productivity and reducing water pollutant. However, the versatility of the microalgae growth in POME under an attached cultivation system is not very well defined. Hence, this study focused on the cultivation of *Nannochloropsis oculata* with POME for wastewater treatment, algal biomass and lipid production using polyurethane foam (PUF) as an attached media.

**Method:** PUF was tested in culturing *Nannochloropsis oculata* by hanging in a column under different organic loading rates (1 & 4 kgCOD/m<sup>3</sup>.day) in a continuous operational mode at 5,000 Lux and light/dark cycle of 12/12 hours. The removal performance of chemical oxygen demand (COD), nitrogen and phosphate removal were measured using DR900 spectrometers. The microalgae in the PUF's were press and the average dry weight was measured to determine the growth. The lipid production were then determined using Bligh and Dyer method.

**Results:** It was found that the *Nannochloropsis oculata* does reduced the pollutant from the wastewater of COD, nitrogen, and phosphate at around 56%, 97 % and 73 % respectively. Moreover, the microalgae used has shown growth rate ranges from 0.14 to 1.65 g/m<sup>3</sup>.day with the lipid production of approximate 36 %.

**Conclusion:** The results indicates that PUF can be used for the cultivation of microalgae for efficient biomass feedstock production and nutrient removal. This study offers crucial information for creating an approach that integrates wastewater treatment with the generation of biofuels from renewable resources in order to be both effective and ecologically benign.

**Keywords:** POME, *Nannochloropsis oculata*, pollutants removal, microalgal cell growth, lipid production

## Characterization of Microbial Community and Predicted Functions Associated with Four Marine Sponges in Karah Island

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### Abstract

**Background:** Marine sponges are important members of marine ecosystems due to their roles in filtering great volume of seawater, stabilizing of substrates, and providing habitats and food for other organisms. Marine sponges harbour a complex microbial consortium that plays important role in the production of chemical compounds and host metabolisms. Symbiotic microbial compositions of marine sponges have been studied in recent decades especially in the Caribbean and the Mediterranean Sea, whereas there is still a paucity of research in Malaysia. This study investigated microbial composition and their putative functions associated with four different marine sponge species namely *Aaptos suberitoides*, *Neopetrosia exigua*, *Theonella swinhoei*, and *Xestospongia testudinaria* and ambient seawater collected from Karah Island, Terengganu, Malaysia.

**Method:** Bacterial genomic DNA of collected sponges and filtered seawater was extracted and 16S rRNA gene amplicon sequencing was conducted targeting V3-V4 variable region using an Illumina MiSeq platform. Paired-end sequences were analysed using QIIME2 and the potential microbial functions related to marine sponges and seawater microbial community were annotated using Functional Annotation of Prokaryotic Taxa (FAPROTAX).

**Results:** The composition of microbial community was significantly distinctive between marine sponges and seawater. The PCoA plots showed that microbial community structures were clearly different between sponges and seawater. Taxonomic analysis clarified that Chloroflexi was the predominant phylum in all sponge species. Proteobacteria and Actinobacteriota were composed of core microbial community in *N. exigua*, *T. swinhoei*, and *X. testudinaria*, whilst Cyanobacteria and Proteobacteria were dominant in *A. suberitoides*. The function of predatory or exoparasitic was found from all sponge samples, and the abundance of aerobic ammonia oxidation and nitrification highly detected from marine sponge samples. FAPROTAX database and our taxonomic result suggested that genus *Bdellovibrio* and *Nitrosopumilus* were involved in the function of predatory or exoparasitic and aerobic ammonia oxidation and nitrification, respectively.

**Conclusion:** This work revealed the composition of core microbiome and the predicted functions of four marine sponges in Karah Island. Further study should incorporate different season or location to reveal the impact of environmental factors.

**Keywords:** marine sponge, microbiome analysis, next generation sequencing (NGS), functional annotation of prokaryotic taxa (FAPROTAX)

## Spatiotemporal Dynamics of Coastal Viral Community Structure and Potential Biogeochemical Roles Affected by an *Ulva prolifera* Green Tide

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### Abstract

**Background:** The world's largest macroalgal green tide, caused by *Ulva prolifera*, has resulted in serious consequences for coastal waters of the Yellow Sea, China. Although viruses are considered to be the main manipulators of microalgal blooms, our knowledge about the relationships between viruses (including DNA and RNA viruses) and the macroalgal green tide is still poor.

**Method:** We uncovered the diversity and spatiotemporal dynamics of DNA and RNA viruses through metagenomic and metatranscriptomic analysis near the coast of Qingdao.

**Results:** Here, a total of 450,523 DNA and 6,080 RNA viral contigs were identified and clustered into 220,616 DNA and 2,351 RNA viral operational taxonomic units (vOTUs), respectively. About 78% of DNA vOTUs and 97% of RNA vOTUs could not be classified and separated from vOTUs in other ecosystems. Both of the DNA and RNA viral community structure was significantly affected by the bloom of the macroalgae *U. prolifera*, such as the DNA viruses of *Pelagibacter* phages, *Synechococcus* phages and *Roseobacter* phages, and the RNA viruses of Picornavirales and Tolivirales. These findings showed a tight coupling between viral assemblages and prokaryotic abundances and activities influenced by the bloom. Abundant DNA viruses were mainly linked to bacterial Gammaproteobacteria, Alphaproteobacteria, Flavobacteriia and Bacilli, and eukaryotic algae Dinophyceae and Bacillariophyceae, while RNA viruses were mainly linked to bacterial Flavobacteriales and Rhodobacterales, and archaeal Maine Group II and Nitrosopumilales. In addition, most of the viral auxiliary metabolic genes (vAMGs) were associated with carbon, sulfur and nitrogen metabolism and showed spatiotemporal dynamics relating to the degradation of the large amount of organic matter released by the green tide.

**Conclusion:** This study for the first time investigated the responses of DNA and RNA viruses to the world's largest macroalgal green tide, and revealed the spatiotemporal dynamics of the unique viral assemblages and vAMGs following the variation and degradation of *U. prolifera*.

**Keywords:** DNA virus; RNA virus; *Ulva prolifera* green tide; spatiotemporal dynamics; meta-omics

## A Review on Potential Marine Macroalgae for Hypertension

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### Abstract

**Background:** Hypertension is the most alarming diseases that cause cardiovascular risk for human health in the Worldwide. Thus, the author has explored marine natural from marine macroalgae that possess antihypertension activity to lower the blood pressure. Marine macroalgae or seaweeds are marine organism from marine biodiversity that distributed widely alongside in the subtropic and tropic oceans. Marine macroalgae have significant role in preventing the hypertension risk with unique biochemical compounds that does not contain in terrestrial plants.

**Method:** This study to identify the continuous important research in reviewing on potential marine macroalgae for hypertension and to find out the analysis gaps of scientific knowledge for the upcoming research or challenges. Angiotensin-I-converting enzyme (ACE) plays pivotal role in blood pressure regulations. Therefore, the investigate of the ACE activity inhibitory sources from marine macroalgae is main target to prevent high blood pressure disease.

**Results:** This study is based on the review of 26 papers regarding antihypertensive activity in marine macroalgae derived from around the word including Japan, South Korea, China, Scandinavia, Canada, Argentine, Ireland, Portugal, and Mexico countries. The author summarized the various macroalgae that have already been screened their biochemical activity shown almost all macroalgae possess potential of antihypertensive activity. There are 4 green, 30 brown, and 36 red macroalgae. Most of papers only discuss the antihypertensive from subtropical regions. Nevertheless, the tropical regions have highly marine biodiversity that has not yet explored.

**Conclusion:** Future research opportunities to study the tropical marine environment that most diverse marine macroalgae to investigate the hypertensive compounds that have promising prospect from upstream to downstream in marine bioprospecting.

*Keywords:* angiotensin-I-converting enzyme, cardiovascular, hypertension, marine macroalgae

## Understanding the Effects of Salinity and *Vibrio harveyi* on the Gut Microbiota Profiles of *Litopenaeus vannamei*

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### Abstract

**Background:** Increasing evidence have revealed a positive correlation between gut microbiota and shrimp health, in which a healthy shrimp gut consists of a complex and stable microbial community. Given that both abiotic and biotic factors constantly regulate shrimp gut microbiota, any changes affecting the levels of these factors could cause modification to the gut microbiome assemblage. The goal of this study was to explore the effects of salinity levels and pathogenic *Vibrio harveyi* infection on the diversity, structure, composition, interspecies interaction, and functional pathways of *Litopenaeus vannamei* gut microbiota.

**Method:** Juvenile shrimp were cultured at 5 ppt, 20 ppt, and 30 ppt for two months prior to *Vibrio harveyi* infection. After pathogenic *V. harveyi* challenge test, genomic DNA was isolated from the shrimp gut, and subjected to the 16S rRNA metagenomic sequencing analysis.

**Results:** We observed that gut microbiota diversity of shrimp cultured at 5 ppt and 30 ppt were lower than those cultured at 20 ppt after exposure to *V. harveyi* infection, suggesting that shrimp cultured at the two former salinity levels were more susceptible to *V. harveyi* infection. Network analysis also showed that shrimp cultured at 20 ppt exhibit a more stable bacterial network with complex interspecies interaction, even after induced by *V. harveyi*. Moreover, the presence of a high number of beneficial bacteria such as *Pseudoruegeria*, *Rhodovulum*, *Ruegeria*, *Shimia* and *Lactobacillus* in shrimp cultured at 20 ppt might have played a role in inhibiting the growth of *V. harveyi* and other potentially pathogenic bacteria. Besides, bacterial functional pathway prediction has also shown that metabolic pathways such as phenylalanine metabolism, glycine, serine and threonine metabolism, starch and sucrose metabolism, glyoxylate and dicarboxylate metabolism, carbon metabolism and biofilm formation process

were significantly higher in shrimp cultured at 20 ppt.

**Conclusion:** Collectively, our results suggested that 20 ppt is an optimal salinity that suppresses the growth of *V. harveyi* and potential pathogenic bacteria in the shrimp gut, which could possibly minimize the risk of pathogenic infection for sustainable production of healthy shrimp.

*Keywords:* Pacific white shrimp, climate change, 16S rRNA metagenomic sequencing, shrimp immunity, abiotic factor



## Environmental Functions of Actinomycetes from Setiu Mangrove Area

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### Abstract

**Background:** Actinomycetes is a group of Gram-positive bacteria that is involved in many ecological functions such as bioremediation, nitrogen fixation, phosphate solubilization, decomposition and antimicrobial potential bioactivities. We investigate the bioactivities of Actinomycetes isolated from Setiu mangrove area. The location is located near local aquaculture and residential areas that are exposed to potential pollutants.

**Methods:** The isolated bacteria were identified via Bergey's Manual and via molecular method. The ecological functions of the isolates were inferred by antimicrobial screenings (cross streak method) and enzymatic activities that were of amylase, lipase, protease, urease, phosphate solubilization, cellulase, and gelatinase.

**Results:** There were eighteen Actinomycetes isolated from the sampling site. Observation of the isolates showed majority with white aerial mycelium only differ by the colour of the substrate mycelium; isolates ASC-5, ASC-1 and ASC-8 are pale yellow; ASCX-10 and AGVX-11 brown in colour, whereas ASC-6 black colour. For Actinomycetes ASCX-9 with transparent colonies with brown colour substrate mycelium. While isolating ASC-7 and ASC-2 orange colour substrate mycelium. Spore structure varies by open loop, branched flexuous, rod and flexuous. These bacteria were identified as *Streptomyces* spp. and *Micromonospora* sp. by Bergey's Manual and 16s rRNA molecular identification. Fifteen percent of the isolates exhibited the highest antimicrobial inhibitions against *Streptococcus uberis* and only 5% killed *Candida albicans*. As for enzymatic screening, 28% showed strong amylase activity and 44% on lipase. Whereas for positive observation protease, urease, phosphate solubilization, cellulase and gelatinase 61%, 78%, 51%, 55% and 50% respectively.

**Conclusion:** Actinomycetes from mangrove is versatile in recycling complex organic and inorganic elements in the location. They are also involved in the phosphate cycle, based on their ability to solubilize this element.

*Keywords:* Actinomycetes, Setiu, mangrove, environment, enzyme.

