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Extraction of Extracellular Polysaccharides (EPS) from Freshwater Microalgae

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Abstract

Microalgae are unicellular and multicellular microorganism existed in freshwater, marine systems and brackish life. Recently, they are gaining wide attention as potential natural resources that highly contributes in human life such as medical and biotechnology aspects. A production and optimization about algal Extracellular Polysaccharide (EPS) are important for several important properties such antithrombotic, immunomodulatory ability, antitumor and cancer preventive, antilipidaemic and hypoglycaemic, antibiotics and anti-inflammatory and antioxidant, making them promising bioactive products and biomaterials with a wide range of applications. Besides, studies on production and optimization of EPS in different species of microalgae may produce different kinds of EPS with different biotechnology properties. Recently, we found another potential of EPS as adjuvant for mucosal vaccine for small ruminants. This study propose a simple method to extract the production of EPS by using the centrifugation process and ethanol as the chemical treatment. This alcoholic precipitation process lead to gain the EPS powder after freeze-drying. The results of EPS extraction can be used as a base line for the future research.

Keywords--- **Microalgae, Freshwater, Extraction, Biotechnology**

Can Metabolomics Help to Elucidate the Metabolome Contributing to Antioxidant and Anti-Inflammatory Defense Properties of *Chaetoceros calcitrans* Extract?

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Abstract

Microalgae are known to contain various bioactives including lipids, fatty acids and natural pigments implicated with natural antioxidants. However, the exact metabolome and the antioxidative as well as anti-inflammatory ability of the indigenous microalga, *Chaetoceros calcitrans*, are still less understood. In this work, two *in vitro* antioxidant test, evaluating the DPPH free radical scavenging and the nitric oxide (NO) inhibitory activity were carried out on different solvent extracts. To identify sensitive biomarkers specific to each association, we have exploited multi-platform metabolomics approaches. NMR and UHPLCMS were employed for generation of metabolic profiles, followed by chemometric analysis. Acetone (Ac) and chloroform (Cl) extracts of *C. calcitrans* exhibited radical scavenging activity, with 43.01 % and 35.03 % percentage inhibition. In addition, the Cl extract inhibited the release of NO production from the LPS-activated RAW 264 cells, with IC_{50} 3.46 µg/ mL after the treatment. We were able to tentatively identify 28 metabolites via NMR analyses from Cl extract including 6 fatty acids, cholesterol, 10 amino acids, 2 sugars and 1 sugar-alcohol, 6 carotenoids and 2 chlorophylls; ion structures were confirmed using tandem mass spectrometry MS/MS. Main secondary metabolites were carotenoids including fucoxanthin, lutein, astaxanthin, canthaxanthin, zeaxanthin and violaxanthin. Discrimination of different solvent extracts revealed clear differences in the metabolite profiles and our quest for antioxidant biomarkers led to discovery of carotenoids with prominent association significance to the tested bioactivities by PLS bi-plot model. The results suggested Cl extract of *C. calcitrans* showed the abundance of high-value metabolites as markers for antioxidant and anti-inflammatory.

Keywords --- ***Chaetoceros calcitrans*, metabolomics, NMR; UHPLCMS, antioxidant**

In Vitro Evaluation: The Anticancer Activities of Skin Mucus from Asian Swamp Eel (*Monopterus albus)* and Identification the Anticancer Related Compounds using LC-QTOF-MS

Ayah Hilles

Abstract

Natural products consider as the main source for alleviate diseases for a long time ago. A lot of people around the world rely on plants as traditional medicines for their primary health care. Marine natural products have attracted the attention of pharmaceutical researches all over the world for developing different pharmaceutical products. Asian swamp eel is a freshwater like fish continuously produce mucus in their skin; there are some evidences that this mucus has anticancer properties. Therefore, this study aimed to investigate the anticancer potential activities in *Monopterus albus* skin mucus aqueous and methanol extracts *in vitro* using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay against human non-small lung carcinoma (A549) and normal mouse embryonic fibroblast (3T3 L1) cell lines. Further, the compounds with anticancer properties were confirmed using liquid chromatography-electrospray-quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS). The results showed that methanol extract has higher anticancer activity compared to the aqueous extract as the viability percentage for methanol extract at 1000 µg/mL and 200 µg/mL is 46.58% and 87.61% respectively while the viability percentage for aqueous extract at 1000 µg/mL and 200 µg/mL is 38.19% and 76.29% respectively, this finding strongly agreed with the LC-QTOF-MS identification results which showed six compounds with anticancer properties (Octylphenol, Peonidin, benzyl benzoate, 3',4',5',5,7,8-hexame-thoxy flavone, progenin II and salvianolic acid G) in the methanol extract while there was only one compound with anticancer properties in the aqueous extract (progenin II), this is confirm the cause of the variation in anticancer activities in methanol and aqueous extract. In conclusion, the present study revealed the anticancer potentials of this species of Asian swamp eel which might be a promising indicator in the development of new anticancer agent.

Keywords--- **Cytotoxic activities, *Monopterus albus*, skin mucus, LC-QTOF-MS**

Administration of Live-Attenuated Vaccine of *V. harveyi* to Improve Survival of Gnotobiotic Brine Shrimp (*Artemia salina*) Model against Multiple *Vibrio* Infection.

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Abstract

Vibriosis is a marine aquatic animal disease that caused by few major *Vibrio* species including *V. harveyi*, *V. alginolyticus* and *V. parahaemolyticus. Artemia salina* larvae is a good model organism that were commonly used for toxicity study and virulence of marine pathogenic bacteria. In the current study, we used the gnotobiotic *A. salina* to study the virulence of multiple *Vibrio* spp., and evaluated the safety and efficacy of newly developed live-attenuated vaccine of genetically modified (stated serine protease) *V. harveyi* in marine environment. During the bacterial safety assay, the wildtype *V. harveyi* and live-attenuated *V. harveyi* were tested for 48 hours. The result showed that the high concentration of live-attenuated mutant *V. harveyi* (MVh-vhs) at concentration of 10^9 CFU/mL is harmless and had improved the *A. salina* larvae survival compared to the untreated control. On the other hand, pathogenic wildtype *V. harveyi* caused lethal effect on *A. salina* larvae by decreasing their survival. In separate experiment, post 6 hours and 24 hours MVh-vhs- incubated *A. salina* larvae were used to test the efficacy of the different concentration 10^5 CFU/mL, 10^7 CFU/mL and 10^9 CFU/mL live-attenuated *V. harveyi* (MVh-vhs) to against 10^9 CFU/mL virulent *V. harveyi*, *V. alginolyticus* and *V. parahaemolyticus* during the challenge test. The surprising result showed that 10^7 CFU/mL MVh-vhs with 6 hours incubated *A. salina* larvae contributed higher survival while 10^9 CFU/mL MVh-vhs with 24 hours incubated *A. salina* larvae contributed higher survival compare with other concentration after multiple *Vibrio* challenge. We postulated that the incubation time had affect bacterial concentration uptaked by *A. salina* larvae on the MVh-vhs strain and affect the effectiveness of Artemia bioencapsulation on MVh-vhs strain for targeted hosts also as vaccine candidate in the future.

Keywords--- ***Vibriosis, Artemia salina,* live-attenuated vaccine*, Vibrio, serine protease***

Phenolic Contents and Antioxidant Capacities of *Morinda citrifolia* Leaf Water Extracts from Different Extraction Times and Identification of Catechins by UPLC-TWIMS-QTOF

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Abstract

Morinda citrifolia is a small evergreen tree that is ubiquitous in tropical countries. Previous studies had found that *morinda citrifolia* leaf (MCL) extract contains catechins. Catechins or known as flavan-3-ols is a subgroup of flavonoids. Catechins is a group of potent antioxidants which able to prevent free-radical related diseases including cancer, cardiovascular diseases, etc. The present study has the aim of investigating the effect of 3 hot water extraction times (15min, 30min, and 45 min) at 80°C on phenolic contents and antioxidant capacities of MCL extracts. In addition, this study also has the objective in identifying the type of catechin present in the MCL extract using ultraperformance liquid chromatography coupled to traveling wave ion mobility/ quadrupole time of flight mass (UPLC-TWIMS-QTOF) spectrometry. Hot water extraction was conducted as to mimic the herbal steeping process. Total phenolic content (TPC) of the extracts was determined with Folin-Ciocalteu method and total flavonoid content (TFC) was determined with aluminium chloride colorimetric assay while antioxidant capacities were investigated with ferric reducing antioxidant power (FRAP) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. MCL extract with overall higher antioxidant profile was selected for catechin identification by UPLC coupled to hybrid mass spectrometer Vion™ IMS-QTOF from Waters. Result of antioxidant assays showed that 30 min extraction time gave better antioxidant profile with 42.66±8.870 mg GAE/g extract for TPC, 6.00±0.408 mg CE/g extract for TFC, 151.50±16.208 μM TEAC/mg extract or FRAP and IC_{50} of 0.57±0.095 mg/ml for DPPH. Result of UPLC-TWIMS-QTOF found that MCL extract contains epigallocatechin-3-O-gallate (EGCG) with m/z 459.0917 $[M+H]^+$, epigallocatechin (EGC) with m/z 307.0818 $[M+H]^+$, catechin-3-O-gallate (CG) with m/z 443.0976 $[M+H]^+$, epigallocatechin(4β,8)-gallocatechin with m/z 649.0947 $[M+K]^+$ and gallocatechin(4α→8)-epicatechin with m/z 633.1000 $[M+K]^+$. MCL extract has shown to have good antioxidant profile and contains several types of catechins.

Keywords---***Morinda citrifolia* leaf, antioxidant, catechin, UPLC-TWIMS-QTOF**

Singlet Oxygen Antioxidant Capacity, Oxygen Radical Absorbance Capacity and Total Phenolic Content of Microalgae

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Abstract

Microalgae is famous for its antioxidant compounds including carotenoids and phenolic contents. Both carotenoids and phenolic compounds are associated with various health benefits. There are many antioxidant assays created to measure the antioxidant capacity of microalgae but most of them are limited to the mechanisms of hydrogen atom transfer and electron transfer reaction mechanisms. So far, no measurement caters for singlet oxygen quenching that can be done by microalgae carotenoids. This study evaluated the antioxidant capacity of microalgae through all 3 mechanisms mentioned above using both lipophilic and hydrophilic Oxygen Radical Absorbance Capacity (ORAC), Folin-Ciocalteu (FCR) method and Singlet Oxygen Antioxidant Capacity (SOAC) assay. Six microalgal biomass were screened for the antioxidant capacity. Microalgae were extracted using organic solvents categorized as Hydrophilic (H), Lipophilic (L) and lipophilic-intermediate-polar (LIP) solvents from methanol and water, hexane and dichloromethane, and chloroform and ethanol respectively. Among the tested microalgae, *Tisochrysis lutea* showed the highest total ORAC value in both LIP and L and H extraction at 1046 μmol TE/gDW and 406 μmol TE/gDW algal biomass, respectively. The FCR assay showed that *Tisochrysis lutea* had the highest phenolic content at 46.0 μmol GAE/gDW in LIP extract and 37.6 μmol GAE/gDW in L and H extract. SOAC analysis revealed that the highest singlet oxygen quenching capacity in LIP extract was *Tetraselmis* sp. with 689.8 α-tocopherol/gDW, but in L and H extract was *Tisochrysis lutea* with 654.4 α-tocopherol/gDW. The results indicate that solvent for extracting antioxidant compounds vary from each species depending on the targeted compounds. It was concluded that singlet oxygen quenching capacity of carotenoids should not be neglected because it contributes a significant amount of antioxidant activity in microalgae.

Keywords--- **Antioxidant capacity, phenolic content, oxygen radical absorbance capacity, singlet oxygen antioxidant capacity, carotenoids**

Isolation and Identification of Tolerant Bacteria from Roots of *Scirpus grossus* Exposed to Mixed Synthetic Dye

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Abstract

In phytoremediation, beside the plants, rhizobacteria in the rhizosphere have important role symbiotically works with plants to render the toxicity and remove pollutants from contaminated soils and water. In this study, an effort was taken to screen and identify effective rhizobacteria that can assist plants to remove dye in synthetic dye effluent. In the present study, during 14 days of a preliminary test, *Scirpus grossus* was exposed to different concentrations (50, 75, 100 mg/L) of mixed synthetic dye of methylene blue (MB) and methyl orange (MO) in sub-surface flow (SSF) reed bed. Twenty seven colonies of rhizobacteria were isolated from the roots and the sand surrounding the roots of *S. grossus* with the highest dye concentration 100 mg/L. All isolates were further analyzed for their colony and microscopic morphology to detect any similar bacteria. The test indicated that there were no similarities between them. Then, these bacteria were exposed to 100 mg/L mixture dye concentration to screen for rhizobacteria that are capable to tolerate with dye. Six bacteria had survived in this concentration of dye based on the CFU results (A5, B1, B2, B3, C6 and E3). Subsequently, the ability of these six rhizobactera to degrade dye was tested with 100 mg/L dye concentration in five days. Only three rhizobacteria (B3, C6, and E3) that could degrade dye based on UV-vis results with removal of 92, 58 and 63% respectively. The three rhizobacteria were identified using polymerase chain reaction (PCR) as the same bacteria as *Alcaligenes aquatilis_ strain_C_11*.

Keywords--- **Methylene blue, Methyl orange, *Scirpus grossus*, (PCR), *Alcaligenes aquatilis_ strain_C_11*.**

Maximum Biological Removal of Ammonium and Manganese in Slow Sand Biofilter (SSB) using Response Surface Methodology (RSM)

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Abstract

Biological removal of ammonium and manganese were conducted in a slow sand biofilter (SSB) operated batchwise using naturally inoculated biomass from lake water. Response Surface Methodology (RSM) with central composite design (CCD) was used to design the experiment with four factors and two responses. A set of 30 experiments were conducted to predict ammonium (NH_4-N_{PR}) and manganese removal (Mn_{PR}), with four input variables of feed concentration for ammonium (NH_4-N_{inlet}) and manganese (Mn_{inlet}), retention time (RT) and aeration rate (AR). The optimum conditions suggested by the RSM were 2.01 mg NH_4-N/L and 3 mg Mn/L feed concentrations at 6 L/min AR and 9.45 hr RT. The treatment resulted in 89% and 98% reduction of ammonium and manganese, respectively. The validation experiment were carried out at the suggested condition with 8% and 2% error for ammonium and manganese respectively indicated that the predicted value is in agreement with the obtained experimental value.

Keywords--- **Ammonium removal, manganese removal, response surface methodology (RSM), slow sand biofilter (SSB)**

Metabolite Profiling of *Chlorella vulgaris* Microalgae by GC-MS and LC-MS Analysis

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Abstract

The commercial cultivation of microalgae has begun in 1960s and Chlorella was the first targeted organism, which has been considered as potential source of renewable energy and an alternative for phytoremediation. Biodiesel production is mainly based on grease raw materials; however, the prices of commodities are regulated, suggesting that new greases sources like microalgae are needed. Microalgae can be used as a growth and immune stimulant because of their contents of essential amino acids, carotenoids, minerals, vitamins, chlorophyll, and some substances that have antioxidant effects, hence can be used in the development of farming aquatic animals. Drugs are currently used to treat most of the cultured fish diseases, but their application is not entirely safe and is expensive in addition to initiation of drug resistance with an accumulation of hazardous residues in the fish body. In this regard more concern should be focused on algal research to understand their tentative components, the mechanism of phytoremediation and its development. This research work aimed at holistic profiling of a general Metabolome of *Chlorella vulgaris* compounds using GC-MS and LC-MS techniques. Microalgae sample of *Chlorella vulgaris* was prepared by raising 200 litres of cultured media from 40 milliliters of starter-culture using Bold Basal's media, BBM. The wet crude product was collected using centrifuge machine and lyophilized using freeze drier. Solid phase extraction, SPE was performed and obtained different polarity extracts for spectroscopic analysis via standard procedures. Fatty acids composition of the crude sample of chlorella vulgaris were first extracted as the whole lipid content using chloroform methanol (1:2 v/v) and then dissolved using Milli Q water. The dissolved fatty acids were then derivatized into FAMEs using acetyl chloride and methanol (5:100 v/v) and analyzed using GC-MS. The results from LC-MS/MS were used to profile the sample's Metabolome in general. So far about 15 fatty acids were documented from GC-MS data and about 20 compounds from LC-MS/MS data and the research is still ongoing to evaluate the vaccination tendencies of *Chlorella vulgaris* in Nile Tilapia *Oreochromis niloticus*.

Keywords--- ***Chlorella vulgaris, Oreochromis niloticus,* phytoremediation, metabolome**

Toxic Effect of Prallethrin and Phenothrin Mixture on Mouse Brain Histopathology using Inhalation Exposures

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Abstract

In the present investigation, there is limited study on histopathological effects of inhalation exposure of mixture compounds, i.e., Prallethrin and Phenothrin. Prallethrin and phenothrin are neurotoxic compounds which are widely used as mosquito repellents. They were administered in male mice (*Mus musculus*) in repeated inhalation exposures of dose A and dose B for 60 days. Dose A is a mixture of 0.000141 mg/L Prallethrin and 0.104 mg/L Phenothrin, while dose B is a mixture of 0.00141 mg/L Prallethrin and 1.04 mg/L Phenothrin. The histochemistry intended for analyzing chemistry of mouse brain tissues and cells. Both of doses produced toxicosis characterized by a change in glial cells and necrosis percentage area in brain compared with control group. Dose B illustrates the larger mean percentage area of necrosis and glial cells proliferase than dose A on 20^{th}, 40^{th} and 60^{th} day. The consecutive results were 33%, 37% and 39% of necrosis whereas for glial cells were 17%, 18% and 18%, respectively. Dose A indicates that there was a decrease in mean percentage in glial cells on day 20, 40 and 60, i.e., 30%, 23%, and 19%, while the mean percentage of necrosis were 9%, 13% and 11%, respectively. The study suggests that repeated inhalation exposures of Prallethrin and Phenothrin indicated considerable harmful effects on *Mus musculus* brain.

Keywords--- **Histopathology, inhalation, *Mus musculus*, prallethrin, phenothrin**

In Vitro Study on Anti Atherosclerosis Potency of *Pandanus tectorius* Fruit via Inhibiting of HMG-COA Reductase Activity

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Abstract

Atherosclerosis is a disease in which plaque builds up inside your arteries and can made up by fat, high cholesterol, and other substances found in the blood, which leading cause of heart attacks. Therapeutic treatment of atherosclerosis or other cholesterol diseases generally utilizes the drug of statin groups. However, the drugs have side effects if it is consumed in prolonged periods. Therefore, the current perspective of study is looking for a 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitor which has been shifted towards a natural agent to decrease the amount of cholesterol through the mechanism of the enzyme in producing cholesterol, thus it could potentially be as an anti-atherosclerosis agent. This study aim was to assess the activity of Pandanus *tectorius* fruit extract which is rich by some bioactive compounds as an inhibitor potential agent for HMG-CoA reductase enzyme. Sample was extracted successively using hexane, ethyl acetate, and methanol to obtain tree kind of fruit extracts, namely hexane (PHK), ethyl acetate (PEK), and methanol (PMK). The samples were prepared using DMSO at concentration of 100, 200, and 400 µg/mL. While, the anti-atherosclerosis activity was done by HMG-CoA reductase kit (Sigma Aldrich) and using pravastatin as a controlled drug. The data was read by ELISA reader at wavelength of 340 nm. The result showed that all samples at all tested concentrations was inhibit the HMG-CoA reductase activity in vitro compared to the pravastatin. The highest activity was obtained by PMK (105.9% higher than pravastatin) at concentration of 100 µg/mL. This study found that *Pandanus tectorius* fruit has very good potency as an anti-atherosclerosis agent via inhibiting of the HMG-CoA reductase activity.

Keywords--- **Atherosclerosis, HMG-CoA reductase, in vitro**

Antioxidant Capacity and Effects on Heat Shock Proteins (HSP70) by Six Malaysian Indigenous Strains of Microalgae

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Abstract

In recent decades, alternatives natural sources of antioxidant had been on surge to replace the synthetic antioxidant due to rise in side effect on human. Six Malaysian indigenous microalgae had been screened for its antioxidant capacity and the study of its effect on the synthesis of heat shock proteins (HSP70) in adult *Artemia franciscana* was carried out. Antioxidant capacities of indigenous strain collected were measured in a concentration of 1-40mg/ml through DPPH assay. At a concentration of 15.0 mg/mL, the DPPH free-radical scavenging activity of microalgae was the highest in the order of: *Chlorella vulgaris* (73.6%), *Ankitrodesmus gracilis* (64.8%), *Auxenochlorella pyrenoidosa* (61.1%) followed by *Desmodesmus sp*. (53.0%), *Chlorella sp1* (52.3%) and lastly *Chlorella sp2* (52.2%). The half-maximal inhibition concentration (IC50) of these six microalgae against radical scavenging activities varies from IC_{50} 0.61 mg/ml (*Desmodesmus sp*.) to IC_{50} 1.35 mg/ml (*Chlorella sp2*). *Auxenochlorella pyrenoidosa* demonstrated the highest value of total phenol content of 48.27 mg GAE/g extract while Chlorella vulgaris possessed the least value of 24.9 mg GAE/g extract. While for Ferric reducing antioxidant power, it ranged from 0.62 Ferrous Equivalents (mM) in Chlorella sp2 to 1.04 Ferrous Equivalents (mM) in *Ankitrodesmus gracilis*. Different microalgae displayed different effect on the synthesis of Hsp 70 in adult artemia but it showed gradual increment in artemia when they were cultured in *Chlorella vulgaris* and *Chlorella sp1* with increased concentration from 0.5×10^6, 1.0×10^6, 2.0×10^6 of microalgae while other microalgae reduce the synthesis of HSP70 in adult artemia. This study showed that different species of microalgae imposed different effect on HSP70 production in adult artemia and *Chlorella vulgaris* and *Chlorella sp1* possess the potential which could be considered for future application to naturally induce the HSP70 production in adult artemia while being used as live feed.

Keywords--- **Antioxidant, heat-shock protein, indigenous microalgae**

Onset and Resolution of Depression-Like Behavior in Rats Fed a Low Zinc Diet

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Abstract

Patients with depression have high blood cortisol levels and low serum zinc levels, suggesting low zinc levels may be associated with onset of depression. In this study, rats were fed a low-zinc diet and evaluated for onset of depression-like behavior, as well as resolution of depression-like behavior after zinc supplementation, to identify the relationship between zinc deficiency and depression-like behavior. Two groups of male Wister rats, 4 weeks of age, were fed normal (52.8 mg Zn/kg) and low-zinc diets (0.37 mg Zn/kg) for 2 weeks. Resting blood samples were collected at the end of Week 2, and serum corticosterone level was measured as a stress marker. Subsequently, rats of both groups completed the 15-minute forced-swim test (FST), which evaluates depression-like behavior, to determine duration of immobility. From Week 2, the low-zinc diet group was fed normal diet. Change in duration of immobility during FST was evaluated in 10 animals each at 0, 1, 4, 7 and 14 days after switching of the diet. In the low-zinc diet group, the negative feedback function of the hypothalamic-pituitary-adrenal (HPA) axis was evaluated by administering dexamethasone. Resting serum corticosterone level at the end of Week 2 was significantly higher in the low-zinc diet group than in the normal diet group (243 ± 50.4 ng/mL vs 7.5 ± 0.7 ng/mL), indicating that the low-zinc diet group experienced stress, compared with the normal diet group. Duration of immobility during FST was significantly longer in the low-zinc diet group than in the normal diet group (49.0 ± 1.7% vs. 41.8 ± 2.3%). High serum corticosterone level and longer duration of immobility during FST in the low-zinc diet group suggested onset of depression-like behavior. After switching from low-zinc diet to normal diet, duration of immobility became similar to that of the normal diet group at 4 days after switching, indicating that animals had recovered from depression-like behavior induced by zinc deficiency. In the low-zinc diet group, dexamethasone administration lowered corticosterone to a similar level as in the normal diet group, which confirmed no impairment in the HPA axis.

Keywords--- **Zinc, depression-like behavior, forced-swim test, corticosterone, HPA axis**

Indirect Antioxidant Activity and Gene Expression of Antioxidant Enzyme Associated with a Phenolic Antioxidant Deriver from the Soft Tissues of the Pacific Oyster, *Crassostrea gigas*

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Abstract

We identified a phenolic antioxidant, 3,5-dihydroxy-4-methoxybenzyl alcohol (DHMBA), in the soft tissues of the Pacific oyster. The concentration at which DHMBA exerts its efficacy was clearly lower than that of other antioxidants, for example vitamin E and vitamin C. These findings suggested that DHMBA has both direct and indirect antioxidant functions. We also speculated that DHMBA as an indirect antioxidant may induce expression of the enzymatic antioxidant gene even in a lower antioxidant concentration. We examined the effects of DHMBA on the Keap1-Nrf2 pathway, which is an important biological defense mechanism against oxidative stress. Human liver cancer cells (C3A) and a reporter gene assay were used to evaluate activation of the Keap1-Nrf2 pathway by DHMBA. A Dual-Glo® Luciferase Assay with FuGENE® HD Transfection Reagent, and two luciferase reporter vectors were used to measure luciferase activity. cDNA was synthesized using the GoScript™ Reverse Transcription System. Real-time polymerase chain reaction was used to measure the expression of Nrf2 target genes in the samples. Cell viability assay against pro-oxidant of 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) and half maximal inhibitory concentration (IC_{50}) by DHMBA were performed using C3A and Cell Counting Kit-8 (CCK-8). The result of reporter gene assay revealed that DHMBA significantly increased luciferase activity in a dose-dependent manner ($P < 0.001$), while chlorogenic acid had no effect on luciferase activity. DHMBA also significantly induced expression of the Nrf2 target genes *HO-1* ($P < 0.001$), *SOD1* ($P < 0.001$), *GPx* ($P < 0.001$) and other genes in a dose-dependent manner. Cell viability assay revealed that DHMBA protected C3A cells against AAPH. The concentration of IC_{50} of DHMBA was determined as 500 μM indicating that DHMBA showed lower toxicity to the C3A cell. These results suggested that DHMBA have indirect antioxidant for hepatoprotection against oxidative stress via the activation of the Keap1-Nrf2 pathway and the induction of phase II enzymes.

Keywords--- **Indirect antioxidant, Keap1, Nrf2, phase II enzymes, 3,5-dihydroxy-4-methoxybenzyl alcohol (DHMBA)**

Improved Method for DNA Extraction from an Individual Copepod

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Abstract

The DNA barcoding is an useful tool for taxonomy, molecular phylogenetics, and population genetics of all kinds of organisms. In our recent study to reveal the genetic diversity and biogeography of copepod *Acartia steueri* Smirnov 1936, the lysis buffer method (Lee and Frost, 2002) was used for DNA extraction from an individual copepod. However, the success rate of whole procedure from DNA extraction through PCR amplification of marker gene was about 50%. This inefficiency resulted unsatisfactory number of genetic data from each population of the copepod, particularly in the samples containing limited number of individuals. In this study, we tried to modify the lysis buffer method with the aim of improving the success rate of DNA extraction and PCR amplification from individual copepod. *Acartia japonica* Mori 1940 which was easy to collect numerous individuals from Japanese coastal areas was used as a model copepod species. The copepod samples were collected in Manazuru Port, Sagami Bay using a 180 μm plankton net and fixed in 5% formalin. Female *A. japonica* was selected by morphological observation under microscope and preserved in 99.5% ethanol. We modified the procedures of removal of ethanol, volume of the lysis buffer used for an individual copepod, and incubation time for lysis. The success rate was evaluated by the result of PCR amplification of mitochondrial cytochrome b gene. As a result of the experiments, DNA extraction procedure from an individual copepod with 90% of success rate was established. This new procedure is also simpler and less time-consuming than the conventional method, and might be useful for DNA barcoding studies of copepod species.

Keywords--- **DNA barcoding, DNA extraction, copepod, *Acartia japonica***

Influence of Dissolved Oxygen on Microalgal Growth and Astaxanthin Accumulation for Mass-Culture Purposes

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Abstract

Microalgae are able to produce astaxanthin pigments which have strong antioxidant activity in oxidative stress conditions, and are widely used in fisheries, medical care, and foods. In closed photobioreactors, oxygen is needed for respiration of algae, but is also known to be a source of stress due to highly reactive intermediates that are generated in the process of reduction, and consequently inhibit the growth of algae when reaching high dissolved oxygen concentrations. Prior studies have shown that the astaxanthin content of a green alga *Chromochloris zofingiensis* increased under oxidative stress conditions (Kobayashi et al., 1997; Ip et al., 2005; Li et.al., 2009). In this study, we modified the dissolved oxygen (DO) concentration from an anoxic state to supersaturated state, and evaluated the effect on algal growth and astaxanthin accumulation. *C. zofingiensis* was cultured for 5 days at 0, 50, 100, 200, and 400% DO concentrations under high-light (450 µmol photons m^{-2} sec^{-1}) and nitrogen-free conditions. The oxygen concentration was regulated by mixed gases of oxygen, nitrogen and 1% carbon dioxide. Growth and astaxanthin accumulation were evaluated by cell number, biomass dry-weight and HPLC analysis, respectively. The biomass dry-weight of *C. zofingiensis* at 200% and 400% DO concentrations were lower than 100% DO, and cell division was inhibited at high 400% DO concentration and low 0% and 50% DO concentrations (<100 % DO). While high DO inhibits the carbon assimilation of algae, low DO may also have inhibited the cell division through limited respiration. The astaxanthin content in <100% DO was significantly lower than ≥100% DO conditions, indicating that a moderate level of DO is necessary for algal astaxanthin accumulation. These results suggest that, while it is important to remove excess oxygen during algal mass-production, careful considerations not to limit oxygen necessary for respiration and astaxanthin accumulation are also important for astaxanthin mass-production.

Keywords--- **Astaxanthin, dissolved oxygen, closed photo-bioreactors, mass-culture**

Isolation and Screening of Marine Diatoms in Coastal Waters of Goto Islands, Japan

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Abstract

We established clonal cultures of some marine diatoms which produced high amount of useful substances such as eicosapentaenoic acid (EPA) and fucoxanthin in order to achieve commercial mass culturing of them. Cultures were obtained from six locations in coastal waters of Goto Islands, western Japan, whose habitats were highly diverse, and therefore a wide range of variability of diatoms species was expected. Cells were isolated by micropipetting under an inverted microscope, and strains were grown in f/2 medium enriched with silicate. Batch cultures were grown at 25°C under an irradiation of 200 µmol m^{-2} s^{-1} with a 12L12D cycle. Growth was monitored by optical density at 680 nm everyday. Subsamples were taken for determination of cellular dry weight, EPA and fucoxanthin content. Nine diatom species were cultured, viz. *Navicula distans*, *Chaetoceros difficilis*, *Chaetoceros pseudocrinitus* strain 1, *Chaetoceros pseudocrinitus* strain 2, *Talassiosira decipiens*, Pennales cf. *Diploneis* sp., *Cylindrotheca closterium*, *Chaetoceros curvisetus*, and *Bacterosira fragilis*. Cellular EPA and fucoxanthin content varied between 2.15 and 12.8 mg g-dw^{-1}, and between 0.34 and 6.32 mg g-dw^{-1}, respectively. Among them, *C. closterium,* Pennales cf. *Diploneis* sp. and *T. decipiens* showed high productivity of EPA in this order, 11.8, 8.80 and 1.91 mg g-$dw^{-1}d^{-1}$, respectively. Regarding fucoxanthin, *T. decipiens* showed the highest productivity of 5.65 mg g-dw^{-1} d^{-1}, followed by Pennales cf. *Diploneis* sp. (4.15) and *C. closterium* (4.04). Thus, we propose *C. closterium,* Pennales cf. *Diploneis* sp. and *T. decipiens* as useful producers of EPA and fucoxanthin.

Keywords--- **Marine diatom, commercial mass culturing, EPA, fucoxanthin**

Metabolite Profiling and Acute Toxicity Study of *Isochrysis galbana*

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Abstract

Malaysia's National Agriculture Policy (NAP 3) has set a target in increasing the aquaculture production by 250% to meet the high demand as fish is one of the essential protein sources for human consumption. In order to meet this target, greater measures at improving aquaculture health have to be taken since disease outbreaks are often encountered in the rapid developing aquaculture industry. Tilapia fish (*Oreochromis niloticus*) is the 2nd highest harvested freshwater species in Malaysia, however it is commonly prone to be affected by *Aeromonas hydrophila* and *Streptococcus spp*. Hence, the use of natural immunostimulants could be an effective method in elevating the immune system of Tilapia in disease resistance. Thus, the present study is designed with aim to profile the metabolites of the indigenous species *Isochrysis galbana* (IG) as a potential fish immunostimulant agent, and its toxic effect on tilapia. The metabolite profile of IG was comprehensively characterized using NMR spectroscopy in complement with LC-mass spectrometry. Among the identified secondary metabolites include carotenoids, polyunsaturated fatty acids, and amino acids. The multivariate data analysis such as Principle Component Analysis (PCA) indicated that the metabolites were extracted better in ethanol. The acute toxicity study was carried out to determine the suitable dosage of microalgae to feed the fish. The IG raw biomass was administered through oral gavage at a dose of 500 mg/kg body weight of tilapia fish. The fishes were observed for any mortality and behavioral pattern in 72 hours. The tilapias were then sacrificed to obtain blood as well as the organs of kidney and liver for toxicity analysis. The analysis has shown that the IG biomass did not cause any mortality or toxic effects as compared with control groups. This finding indicates the possibility of further analyzing *Isochrysis galbana* for natural immunostimulant role in improving aquaculture health.

Keywords--- ***Isochrysis galbana*, tilapia, NMR, LCMS, toxicity**

Enhancement of Microalgae Cultures by Symbiotic Probiont Strains

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Abstract

In this study, four potential probionts with inhibitory effects towards Vibrio pathogens were used to enhance the growth of microalgae. These symbiotic probiont strains were isolated from *Amphora sp*., *Chlorella sp*., and *Spirulina sp*. and identified as *Lysinibacillus fusiformis* strain A-1, *Bacillus sp*. strain A-2, *Lysinibacillus fusiformis* strain Cl-3 and *Bacillus pocheonensis* strain S-2, respectively using 16s rDNA. The inoculation ratio of bacteria and microalgae was fixed to 1:4, and co-culture incubation was monitored for 7 to 8 days. Three out of four probiont strains were able to promote the growth of microalgae while one strain possessed an inhibiting trend when co-cultured with the microalgae. The findings showed that the cell density of *Chlorella sp*. was promoted more than two times due to probiont Cl-3 supplementation (9.9×10^6 cells/ml) compared with the control (4.2×10^6 cells/ml), observed at day 7 of co-culture incubation. *Bacillus sp*. strain A-2 showed a good correlation after co-cultured with *Amphora sp*. evidenced by a steep growth of *Amphora sp*. (8.5×10^5 cells/ml) in comparison with the control (1.4×10^5 cells/ml) at day 8 of co-cultured. On the other hand, it was found that *Lysinibacillus fusiformis* strain A-1 inhibited the growth of *Amphora sp*. on day 5 of co-cultured. The fluorescence of Spirulina sp. co-cultured with Bacillus pocheonensis strain S-2 recorded a slightly higher reading than single *Spirulina sp*. culture. This study proposed three symbiotic bacteria; *Bacillus sp*. strain A-2, *Lysinibacillus fusiformis* strain Cl-3., and *Bacillus pocheonensis* strain S-2 as potential strains in promoting the microalgal biomass.

Keywords--- ***Biomass, co-culture, microalgae, potential probiont.***

Screening of Native Oleaginous Microalgae in Mangrove Area under Nitrate-Replete and Nitrate-Deficient Culture Conditions

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Abstract

Microalgae are diverse microscopic organisms, living either in freshwater or marine environment. They have been recognized for their highly marketable polyunsaturated fatty acids contents such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, indigenous microalgae in Malaysia are untapped resource that led to lack of research on lipid assessment especially on mangrove species. Thus, this study aims to determine the potential of mangrove-isolated microalgae for oil production. A total of 10 microalgae species were cultivated in a small-scale optimized culture system under nitrate-replete and nitrate-deficient BBM mediums. The cells were harvested at stationary growth phase to evaluate the microalgae biomass, total lipid content and fatty acids composition (FAC). The results showed that biomass production was significantly lowered, while the total lipid content was significantly increased in nitrate-deficient medium. On the other hand, the results were reversed in nitrate-replete medium. The analysis of fatty acids compositions revealed that major fatty acids profiles identified in most lipid classes in these isolates were palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic acids (C18:2). Thus, these results demonstrated the potential of mangrove microalgae as feedstock for edible and non-edible oil (biofuel) applications.

Keywords--- **Microalgae, biomass, lipid, fatty acids profiles**

Assessment of Antioxidants Activity in Selected Indigenous Microalgae

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Abstract

Antioxidants are any of various substances that inhibit oxidation or reactions promoted by oxygen and peroxides which help to protect the living body from the deleterious effects of free radicals. Microalgae are photosynthetic organisms that are widely studied due to the variety of bioactive compounds including antioxidants. They show great potential as artificial antioxidant replacements due to their high production of primary and secondary metabolites. Thus, this study takes a look on the antioxidants production of three indigenous marine microalgae species which are *Chlorella vulgaris* (UMT-M1), *Isochrysis galbana* (CB) and *Tetraselmis* sp. (CT) at different algal growth phases (lag, log, linear and stationary phases). Activity of enzymatic antioxidants such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) as well as the non-enzymatic antioxidants such as ascorbic acid, alpha-tocopherol and glutathione were assessed at different algal growth phases. The production of the antioxidants may vary according to the microalgae species and algal growth phases. Potential microalgae with the highest antioxidants activity will be further studied to determine the best culture conditions for enhancement of the antioxidants production. Potential indigenous microalgae can be utilized as natural sources of antioxidants with high nutritional value in pharmaceutical industry. Furthermore, this study is also important toward a realistic commercialization of new native microalgae antioxidants.

Keywords--- **Antioxidants, microalgae, growth phases**

High Biomass Productivity of the Marine Diatom *Chaetoceros gracilis* under High Irradiance

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Abstract

Growth characteristics of the diatom *Chaetoceros gracilis*, which contains valuable compounds such as eicosapentaenoic acid and fucoxanthin, was investigated to establish mass production. A semi-continuous culture using a 1-L column reactor was conducted at three different dilution rates of 0.1, 0.2, and 0.4 d^{-1} under light intensities of 300 and 1000 µmol m^{-2} s^{-1}. The production was evaluated by biomass increase per unit area of illuminated surface of the column per day. Concentrations of nitrogen, phosphorus, and silicon in the medium were 4.0, 0.24, and 0.14 mM, respectively at the dilution rate of 0.1 d^{-1} and 9.9, 0.61, and 0.35 mM at the dilution rate of 0.2 and 0.4 d^{-1}. The culture was replaced with new fresh media once a day. Growth characteristics of *C. gracilis* was examined for 18 days at the dilution rate of 0.1 d^{-1} and for 25 days at the dilution rate of 0.2 and 0.4 d^{-1}. The highest production rate of 20.6 g-dw m^{-2} d^{-1} was obtained at the dilution rate of 0.4 d^{-1} under the light intensity of 1000 µmol m^{-2} s^{-1}. To our knowledge, this rate exceeded the maximum production rate of 1~3 g-dw m^{-2} d^{-1} in previous studies of *C. gracilis*. However, the high productivity was unstable under the high irradiance due to deficiency of silicate, while nitrogen and phosphate concentration remained stably replete. At low dilution rates cells tended to be large in size and cellular dry weight per unit volume increased. This was accompanied by silicate deficiency. These observations suggest that relative nutrient concentrations of nitrogen, phosphorus and silicon is crucial to maintain high biomass productivity and keep physiological and morphological state of *C. gracilis* under high irradiance in semi-continuous culture.

Keywords--- ***Chaetoceros gracilis,*** **eicosapentaenoic acid, fucoxanthin, high irradiance, areal production rate**

Disease (AHPND) *Vibrio* spp. from *Litopenaeus vannamei* (Boone, 1931) Shrimp Ponds.Determination and Characterization of Acute Hepatopancreatic Necrosis

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Abstract

Acute Hepatopancreatic Necrosis Disease (AHPND) emerged as a new disease of shrimp in 2010 and affected the shrimp industry leading to serious global economic losses. The etiological is a bacterial strain *Vibrio parahaemolyticus* (Vp) that carries a plasmid containing toxins (PirA and PirB). However, recent researches show that, Vp is probably not the only species capable of causing AHPND. Thus, the present study focuses on isolation, screening, identification and mitigation of AHPND caused by bacteria from *Litopenaeus vannamei* shrimp ponds. The AHPND causing strains were isolated from shrimps and water samples. The isolated strains were screened for PirA and PirB toxins using AP4, AP3 VpPirA-284F, VpPirA-284R, VpPirB-392F and VpPirB-392R primers. The identification of PirA and PirB positive strains were carried out using Multilocus Sequence Analysis (MLSA) of 16S rRNA, RctB and RpoD genes. The toxicity of AHPND positive strains were evaluated through *in vivo* assay using *Artemia* and *Litopenaeus vannamei* as a model organism. The survival rate and bacterial loads in the *Artemia* and *Litopenaeus vannamei* were determined. A total of 86 strains were isolated from 6 different ponds. Total of 11 strains were recovered from the pond water and 75 strains were isolated from hepatopancreas of shrimp samples. The strains were screened for $PirAB^{vp}$ toxin using AP3 and AP4 primer set (nested PCR). Total of 18 strains were tested positive for $PirAB^{vp}$. The toxicity of AHPND positive strains were evaluated through *in vivo* assay using *Artemia* as a model organism. Twelve AHPND positive strains with $PirAB^{vp}$ genes were selected randomly from the 18 AHPND positive strains demonstrated significant ($p < 0.05$) mortalities of *Artemia* compared to the negative control. Strain BpShHep31 is the most virulent strain compared to other strains and the LD_{50} was attained within day 2. The 12 AHPND positive strains were identified using molecular method (MLSA) of 16S rRNA, RctB and RpoD region amplification. The 12 AHPND positive strains belong to the *Harveyi* clade and closely related to *Vibrio parahaemolyticus* and *Vibrio harveyi*.

Keywords--- **Acute Hepatopancreatic Necrosis Disease (AHPND); *Vibrio parahaemolyticus (Vp)* and Multilocus Sequence Analysis (MLSA)**

Ontogeny of Digestive Tract and Proteolytic Digestive Enzymes during the Early Development of *Hippocampus kuda* (Bleeker, 1852) Juveniles

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Abstract

Low survival remains a significant hurdle in the nursery culture of yellow seahorse *Hippocampus kuda*. To potentially improve the survival and growth of early *H. kuda*, the development of alimentary tract in *H. kuda* juveniles and their proteolytic enzyme activities were evaluated from 0 to 9 days-after-birth (DAB). Juvenile seahorses were fed a combination of live foods consisting of rotifers *Brachionus* sp., *Artemia* sp. and cyclopoid copepod *Oithona simplex*. Seahorse juveniles were fixed for histological analysis and snap-frozen for trypsin and chymotrypsin assays. The wet weight, height, intestine length and relative intestine length (IL:H ratio) were recorded. Premature seahorses were observed to have a round-shaped yolk sac, which was not found in normal newborns. However, histological observations revealed internal yolk globules in normal newborn seahorses. The digestive system of *H. kuda* juveniles consisted of a snout, straight intestinal tube with an opened anus, as well as a gallbladder, liver and pancreas. No stomach was observed. Although the height and length of the intestine increased with seahorse age, IL:H ratios remained around 0.30. Both the specific and total trypsin activities showed a similar trend where the activities were higher on 0–3 DAB, lower on 5 DAB and then slightly increased from 7 to 9 DAB. Meanwhile, the specific and total chymotrypsin activities were low on 0 DAB, increased on 1 and 3 DAB, before decreasing and remaining low from 5 to 9 DAB. The trypsin to chymotrypsin (T:C) ratio was highest on 0 DAB but dropped drastically the following day and remained low until the end of the experiment. The results indicated a transition from partial lecithotrophy to full exogenous feeding in *H. kuda* juveniles when the alimentary system was developing. These findings provided important knowledge to further improve the feeding and nutrient utilization in early *H. kuda* juveniles.

Keywords--- **Yellow seahorse, gut development, trypsin, chymotrypsin, lecithotrophy**

High Oil Accumulation in *Chlorella vulgaris* (UMT-M1) Induced under Lowered Salinity

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Abstract

Salinity can influence oil accumulation in microalgae. Most studies focus on increasing salinity stress in order to achieve high oil production in various microalgae species. Hence this study opts to demonstrate the potential of lowered salinity in inducing high oil accumulation in a mangrove isolated microalga, *C. vulgaris*. *C. vulgaris* was initially cultured in 30 ppt F2 medium. The cells were harvested and transferred to fresh F2 medium of different lowered salinity (20, 15, 10, 5 ppt) and cultured for 12 days. The results revealed that under 15 ppt, *C. vulgaris* accumulated the highest oil content of 63% (per dry weight) as compared to 35% under control conditions (30 ppt). Oil content extracted from other salinity conditions ranged between (30 – 45%). 15 ppt cultures also demonstrated a growth curve which reached stationary phase earlier (Day 7) than other salinity conditions (Day 9). No significant difference was recorded in cell density and chlorophyll content among all cultures, a positive sign of unhindered growth. Fatty acid composition under all treatments will be further identified using GC-FID analysis. The outcome shows that a salinity threshold exists in *C. vulgaris* for high oil accumulation under lowered saline conditions. This characteristic can be further studied towards biodiesel applications.

Keywords--- **Microalgae, salinity, FAME**

The Effects of the Different Proportion of Soil Extracts Added to A Culture Media on The Growth of Targeted Microalgae

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Abstract

Supplementation of soil extracts to culture media can enhance the growth of some microalgae. However, it has not been clearly identified which soil and soil extraction method are suitable for positive growth enhancement of microalgae. It is also unclear of the right optimal proportions of soil extract to culture media used for microalgae. *Nanochloropsis* sp. was incubated in 96-well-microplates in an incubation chamber with temperature of 24°C, light intensity of 7000lux and 12L: 12D cycle using Conway media with soil extract added with different proportions. Two soil samples used in this study were collected from Raja Musa Forest Reserve (Raja Musa) and Ayer Hitam Forest Reserve (Ayer Hitam), Selangor Malaysia. Soil extracts with four different soil extraction conditions were examined. Optical density at 680 nm was measured for every 6 hours up to a total of 90 hours of which the growth rates and division rates were then calculated. Higher growth rate was observed when the highest proportion of soil extract was added to the media whilst no effect of soil extract on the growth was observed at the lowest proportion. Among the different soil extraction conditions, the soil extracts with autoclaved at 105 °C for one hour twice showed the highest growth rates, although the higher concentrations of carbon, nitrogen, and phosphorus in the soil extracts were observed in other soil extraction conditions. Results also showed that the use of soil extracts from Raja Musa showed the higher growth compared to Ayer Hitam. These results showed that soil extracts can enhance microalgal growth but the degree of the growth enhancement is different with soil types as well as soil extraction methods.

Keywords--- **Soil extract, *Nanochloropsis* sp., 96-well-microplates**

Physiological Assessments of *Ankistrodesmus gracilis* under Nitrate Starvation and Induction Culture Conditions

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Abstract

Nitrate is a major nutrient that supports cell growth and development in green microalgae. Cells grown with limitation of nitrate tend to induce stressed responses such as accumulation of oil bodies inside the cell. The current study demonstrates the physiological changes of *Ankistrodesmus gracilis* under nitrate starvation and induction cultures. *A.gracilis* cells (1.0×10^8 cells/ml) were first nitrate starved in F2 medium for a period of 12 days. The cultures were then induced with different concentrations of nitrate (100%, 200% and 300%) for a period of 8 days. Results showed that *A. gracilis* accumulated 62% (per dry weight) of total oil content under starvation period. Upon nitrate induction, significantly higher cell growth was recorded coupled with reduction in total oil content between the ranges of 25 to 35% (per dry weight). Differential physiological responses monitored under nitrate starvation and induction suggests that a dynamic interaction exists towards major nutrient changes. Thus screening for biomarkers and responsible genes can be made under proposed time point so that complete understanding on cell mechanisms can be elucidated. These findings are believed to assist high biomass microalgae culture for feed in aquaculture sectors.

The Effects of Different Soil Extraction Conditions on the Recovery of Soil Extracts Using Soils Collected from Raja Musa Forest Reserve and Ayer Hitam Forest Reserve in Selangor, Malaysia

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Abstract

In natural conditions, soil nutrients are dissolved in water by natural occurrences. However, only a small amount of soil nutrients is extracted by this process. The main objectives of this study were to evaluate the recovery of dissolved organic carbon (DOC), total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) in soil extracts from different extraction methods using soils collected from Raja Musa Forest Reserve (Raja Musa) and Ayer Hitam Forest Reserve (Ayer Hitam) in Selangor, Malaysia. Nine different extraction conditions were tested. Three room temperature extractions (1, 4 and 24 hours) showed that there were very low recoveries obtained with no significant difference between those three conditions. The autoclaved at temperatures 105 0C and 121 0C showed about 100-700% and 200-900% higher concentration of soil extract compared to room temperature extractions respectively. In general, the combination of 24 hr incubation period with autoclaved at temperatures 105 0C and 121 0C does not help to increase extraction yield. The autoclaved at 121 0C generally showed 20-120% higher concentrations of soil extracts compared to the autoclaved at 105 0C, and the autoclaved for one hour twice showed 20-90% higher concentrations of nutrients compared to the autoclaved once. The soil extraction concentrations were nearly 10 times difference between Raja Musa and Ayer Hitam soil. These results showed that autoclave temperatures and duration as well as soil types influence the recovery of soil extracts.

Keywords--- **Soil extraction, total dissolved nitrogen, total dissolved phosphorus, dissolved organic carbon.**