



15th Asian Congress on Biotechnology

in conjunction with

7th International Symposium on Biomedical Engineering

October 2nd – 6th 2022

Merusaka Hotel, Nusa Dua, Bali

Book of Abstract

organized by:



RESEARCH CENTER FOR
BIOMEDICAL ENGINEERING
Faculty of Engineering - Universitas Indonesia



Agricultural and Food Biotechnology

Screening of Bangladeshi Chili Germplasm against Anthracnose by Inoculation of *Colletotrichum capsica*

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Abstract. Anthracnose or Fruit rot disease is a major constraint to chili production in many countries of tropical and subtropical regions. It affects the yield and fruit quality of chili. This study aimed to screen sixty germplasm of chili against anthracnose on the basis of *Colletotrichum capsici* inoculation to mature fruits. Ten isolates of *Colletotrichum capsici* causing chili anthracnose were isolated from infected fruits collected from different areas of Bangladesh and pure cultures were developed. Experiments were conducted to identify anthracnose resistant/susceptible germplasm of chili by pathogen inoculation on mature fruits. A set of disease scales, with 0 to 10 scores, were used to assess the degree of anthracnose resistance/susceptibility of the forty one chili germplasm. Fruits of all germplasm were infected with the pathogen except Comilla morich, in which no infection was detected. BD-2035, BD-9737, BD-2064 and BD-2006 had very slight disease symptoms. Highly susceptible germplasm were Dhani morich, BD-2091, Angoor, BD-2048 and Naga. Nine morphological data and per cent of infection area were recorded and multivariate analysis was used to identify the genetic variations among the forty one germplasm by using Euclidean distance following Ward's method. Principal component analysis showed that percentage of infected area and yield per plant were the traits responsible for major variation among the genotypes. In cluster analysis, different chili genotypes produced six distinct groups. The germplasm of the cluster VI (BD2035, Comilla, BD2006, BD2064) with remarkable higher yield and anthracnose resistance have the potential use in future breeding program for the improvement of chili to produce anthracnose resistant chili in Bangladesh.

Fermentation Medium Optimization of *Streptomyces sp.* Antifungals Producer for Oil Palm Pathogen *Ganoderma boninensis*

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Abstract. This research aimed to obtain the best formulation of carbon and nitrogen sources and the optimum concentration as fermentation medium for *Streptomyces sp.* for production of antifungal compound *Ganoderma boninensis*. This research was an experimental research using Completely Randomized Design (CRD) and Response Surface Methodology (RSM) Central Composite Design using the Design Expert 10.0.7. The results showed that the best sources of carbon and nitrogen were liquid glucose and monosodium glutamate (MSG) in the inhibition zone of 16.7 mm and 6.3 mm, respectively. The best concentration levels were 20 g/L (18.67 mm inhibition zone and 44.45% HPLC chromatogram area (%)) and 14.19 g/L (14.17 mm inhibition zone and 38.08% HPLC chromatogram area (%)). The results of optimization I showed that the response of the inhibition zone and area (%) of the optimum HPLC chromatogram was 24.39 mm and 62.68%. Optimization II using the optimization suggestion I obtained the suggestion of 15.2 g/L and 8.3 g/L. The predicted value of inhibition zone was 21.47 mm and area (%) of the HPLC chromatogram was 53.44%. The validation results of the 22.01 mm inhibition zone and area (%) of the HPLC chromatogram were 54.86%. The difference between the predicted value and the 2.45% inhibition zone validation value and 2.58% HPLC chromatogram area (%) is smaller than 5%, so that the validation value is in accordance with the value predicted by Design Expert software 10.0.7.

Keywords: (*Ganoderma boninensis*, Secondary Metabolites, Optimization of Fermentation Media, Response Surface Methodology (RSM), *Streptomyces sp.*)

Edible freshwater snail (*Paludomas conica*) attenuates STZ-induced diabetic complications by regulating PFK-1 and PON-1 gene expression

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Abstract. Freshwater invertebrates, particularly mollusks, have recently piqued the interest of researchers as a potential new source of protein hydrolysates, as the bioactivity of mollusk protein hydrolysates is higher in some areas than that of other sources, owing to their high taxonomic diversity and distinctive characteristics. This study aimed to develop bioactive protein hydrolysate from freshwater snail *Paludomas conica* via proteolytic enzyme digestion. The resultant protein hydrolysate (PPh) was further evaluated for its effects as an antioxidative and antidiabetic agent. Antioxidative capacity was investigated through the DPPH free radical assay, ABTS scavenging assay, FRAP assay, and superoxide scavenging assay. Antidiabetic effectiveness was assessed using an *in vitro* α -amylase and α -glucosidase inhibitory action, *in vivo* streptozotocin (STZ)-induced diabetic animal model, and gene expression studies. The protein concentration of the *Paludomas conica* (PPh) hydrolysate was 380.25 mg/g dry weight. In all antioxidative experiments, the half-maximal inhibitory concentration (IC₅₀) was less than the cut-off value, 1000 μ g/mL. The inhibitory concentration of PPh was 1.89 mg/mL for α -amylase and 334.70 μ g/mL for α -glucosidase *in vitro* assay. In the STZ-induced model, elevated glucose levels, animal body weights, and biochemical markers such as alanine aminotransferase, aspartate aminotransferase, uric acids, total proteins, lipid profiles (low-density lipoproteins, high-density lipoproteins, total cholesterol, triglycerides) were found to be partially restored with the administration of PPh 250 mg/kg body weight. Molecular assay by the qPCR analysis showed a significant upregulation of the relative mRNA expression of antioxidative and glucose metabolism-regulating enzymes-related genes of superoxide dismutase (SOD-1), Paraoxonase-1 (PON1), and Phosphofructokinase-1 (PFK1). Findings demonstrate that PPh could be used as a potential therapy to manage diabetic complications by regulating gene expression of antioxidative and glycolytic enzymes with further clarification.

Keywords: Freshwater snail; *Paludomas conica*, Protein hydrolysate, Paraoxonase-1, Phosphofructokinase-1, Superoxide dismutase

A novel processing method for enhancing resistant starch content of semolina flour for sustainable glucose release

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Starchy cereals and vegetables are consumed as staple food by the population worldwide irrespective of ethnicity. Semolina (*Triticum turgidum*) is an important such staple starchy food widely prevalent in Indian cuisine. A sharp boost-up of the blood glucose level has been reported upon consumption of food prepared with semolina. Processing of semolina with novel enzyme coupled with gelatinization and retrogradation technique was able to enhance its resistant starch content. Resistant starch resists the action of digestive enzymes and thereby curbing down the glucose level. Thus, in the present study, an attempt has been made to enzymatically modify semolina flour for the production of resistant starch. It is believed that resistant starch can be indispensable in therapeutic food formulation as it is gaining huge acknowledgement not only for its low glycemic index but also for its ability to behave like dietary fibres. Semolina being enzymatically modified by amylopullulanase resulted in increased resistant starch content, amylose content, *in vitro* bile acid binding capacity and crystallinity by $11.12 \pm 1.67\%$, $12.44 \pm 0.89\%$, $6.23 \pm 0.11\%$ and 10.23% respectively. The modified starch exhibited decreased readily digestible starch and oil holding capacity by $12.67 \pm 1.12\%$ and 3.56 ± 0.21 g/g respectively. Furthermore, *in vitro* starch digestibility analysis of the resistant starch rich semolina displayed a sustainable glycemic response along with prebiotic effect. Growth of gut friendly microbial species like *Bifidobacterium brevis*, *Lactobacillus amylophilus* and *Bacillus closti* was found to increase when the modified semolina was subjected to *in vitro* colonic fermentation. These initial findings of the present study are interesting as the results showed elevated potential of the enzymatically modified semolina flour to be used as functional ingredient in cuisines worldwide.

Keywords: Resistant starch, amylopullulanase, glycemic index, dietary modulation, colonic fermentation

Next-generation bioformulations for sustainability in agriculture

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The bioinoculant technology suffers from various limitations, which has obstructed its application. The performance of these microbes in field where multiple stresses act simultaneously is often inconsistent, and also much reduced than that observed under lab conditions. One of the reasons for this is its competition with the native microbial communities, other reasons could be the requirement for specific ecological niches. Besides, it struggles with issues like shelf life, and slower action compared to chemicals. Another important aspect that is majorly ignored is the larger picture of mechanism of action of bioinoculants, which is yet to be elucidated. This includes gaining in depth understanding of how the native microbial community responds to such an amendment, which determines the latter's robustness and efficiency in real-life conditions. The group works on developing bioformulations that can overcome some of these challenges. This is done by employing state-of-art omics tools together with conventional techniques. By employing metagenomic and metabolomics studies, next generation bioformulations have been developed for *Bradyrhizobium* sp. with enhanced survival and efficiency. This was initially tested in a series of plant growth experiments under controlled conditions with model crop *Cajanus cajan*, and then validated in farmer's field subjected to multiple stressors simultaneously. The bioformulations served tripartite functions of plant growth promotion, enhanced nodulation, and stress mitigation. Such a polyphasic approach to design bioformulations can be adopted on a large scale for ushering in sustainability in agriculture.

sgRNA Design And *In Vitro* Nucleolytic Analysis of Cas9-RNP Complex for Transgene-Free Genome Editing of *Capsicum annum* L. *eIF4E1* Gene

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Abstract. Chili (*Capsicum annum* L.) is one of the most sought vegetables for their unique taste and aroma infused to the dishes, especially in Asian and South American cultures. However, the high demand of chili is often unsatisfied by the problematic production due to pathogens infection. For instance, ChiVMV (Potyvirus) is the second most prevalent chili-infecting virus across South, East, and Southeast Asia that decreases yield. Previous studies suggested that chili *eIF4E1* plays an important role in potyvirus gene transcription. Hence, CRISPR-Cas9 based genome editing to introduce premature stop codon or truncated protein is hypothesized to increase resistance of chili. In this study, two sgRNAs were designed to guide editing in the first and second intron of the *eIF4E1* gene, respectively. The recombinant system of Cas9 production was also optimized by testing IPTG at the concentration of 0, 250, 500, 750, and 1000 μ M. Cas9 synthesis was carried out in *Escherichia coli* BL21(DE3) containing 4xNLS- pMJ915v2-sfGFP (www.addgene.com). The expression cassette consisted of NLS-Cas9-sfGFP with 6X histidine and TEV protease cut-site at the N terminal. The results showed that optimal IPTG concentration for inducing Cas9 production was 500 μ M. Purification using the Ni-NTA column confirmed the presence of Cas9 protein in the first 2 mL out of 5 mL of the eluted fractions. Unfortunately, purity was still compromised as indicated by numerous background proteins. However, based on Rapid Agarose EMSA, Cas9-RNP complexes were successfully formed for both sgRNAs. Further, the nucleolytic activity of Cas9 treated with or without TEV protease (carrying MBP site) was confirmed in vitro in the form of RNP complex with sgRNA1 or 2, respectively. The next step is to transfect chili protoplasts with these RNP complexes with the ultimate goal to edit chili *eIF4E* in a transgene-free manner.

Keywords: CRISPR-Cas9, sgRNA Cas9-RNP, endonuclease, recombinant protein

Isolation and identification of salt-tolerant, plant-growth-promoting rhizobacteria: their potential application as biofertilizer for climate-smart agriculture in the coastal areas

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Abstract: The salinity level in the coastal ecosystem and agricultural lands is being increased gradually due to the sea-level rise, one of the many effects of climate change. Consequently, a substantial reduction in crop yields is experienced in some South Asian countries threatening their food security. To bring the salinity-affected lands under agriculture, the application of salt-tolerant, plant-growth-promoting rhizobacteria (PGPR) as biofertilizer could improve salt resistance in plants, thereby augmenting plant growth and production. Here, we isolated 53 PGPR from saline and non-saline areas in Bangladesh where electrical conductivity was measured as >7.45 and <1.80 dS/m, respectively. Bacteria isolated from saline areas were able to grow in up to 2.60 mol/L salt concentration, contrary to the isolates collected from non-saline areas that did not survive beyond 854 mmol/L. Among the salt-tolerant isolates, *Bacillus aryabhatai*, *Achromobacter denitrificans*, and *Ochrobactrum intermedium*, identified by comparing respective sequences of 16S rRNA using the NCBI GenBank, exhibited a higher amount of atmospheric nitrogen fixation, phosphate solubilization, and indoleacetic acid production at 200 mmol/L salt stress. While in soil, rice growth under non-saline condition was comparable between *B. aryabhatai* MS3-fertilized and control pots, the scenario was statistically significant when challenged with 200 mmol/L salts: 42.60% and 8% survival were recorded respectively. Biochemical analyses revealed that *B. aryabhatai* MS3 supported the plants under salinity by increasing the availability of nutrients (Fe, P), accelerating the levels of IAA and chlorophyll content, enhancing proline accumulation and decreasing malondialdehyde formation. Further, rice growth was found to be favoured by enhanced expression of a set of at least four salt-responsive plant genes: *BZ8*, *SOS1*, *GIG*, and *NHX1*. Fertilization of rice with osmoprotectant-producing PGPR therefore, could be a climate-change-adaptation framework to build climate-smart agriculture for the coastal ecosystem.

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Isolation And Selection Antagonistic Bacteria Against *Cercospora arachidicola* Causing Brown Spot on Peanut

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Abstract. This study was conducted with the aim to isolation bacterial strains capable of antagonism to *Cercospora arachidicola* causing brown spot in peanut. From 3 soil samples collected in the rhizosphere of peanut grown in Tay Ninh province, Vietnam, the result that 10 bacterial strains were found having antagonistic action with *Cercospora arachidicola*. The antagonistic efficiency of all isolates ranged from 33.33 ± 1.28 to 60.183 ± 0.927 %. Studying the antagonistic mechanism showed that 5/10 isolates produced siderophore, 5/10 isolates were capable of decomposing chitin, 7/10 isolates capable of decomposing cellulase and 8/10 isolates could proteolytic. Three strongest antagonistic strains were identified as Bacillus based on Bergey classification system. These strains of antagonistic bacteria have the potential to produce probiotics.

Keywords: Antagonistic, Bacillus, bacteria, *Cercospora arachidicola*, isolation, peanut, selection.

Ethnic Fermented Foods Reveal the Probiotic Potentials of Associated Microbiome

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Abstract. Traditional practices of fermented foods preparations have a rich history considering its importance as a source continuous food supply and essential nutrients. As initially it served the pivotal role of food supply during drought and floods, and recently it has been observed to play a crucial role in back-sloping and preservation of native microbial consortia. Successive elucidation of type of starter material and consortia suggested importance of flavor-enhancement and longer shelf-life attracted researchers for the surveillance of microbial communities from diverse fermented foods. The recent advancement in high throughput sequencing and area of genomics has paved the way to characterize the fermented foods for their potential of being probiotic and nutritional supplement. The present study aims to characterize the microbiome and mycobiome of ethnic fermented foods “*Axone*” prepared from fermented soyabean and “*Bastenga*” fermented bamboo shoot from North east region of India using targeted metagenomics and imputed metagenomics approaches. We found predominance of *Firmicutes* (98.82±0.57%) and *Ascomycota* (99.65±0.09%) among both the food samples. The bacterial community composition at the lower taxonomic level depicted differential abundance of *Lactobacillus* (45.45%), *Bacillus* (31.88%) and *Staphylococcus* (20.61%), similarly fungal communities follows the order of *Debaryomyces* (98.96%) and *Saccharomyces* (0.44%). Imputed metagenome revealed that the fermented foods *Axone* and *Bastenga* have potential for the probiotic properties. However, *Axone* shows higher (24.63%) gene abundance associated to probiotic properties compared to *Bastenga*. Moreover, the gene families involved in the biosynthesis of secondary metabolites were found to be 12.92% more enrich in the *Axone* when compared to *Bastenga*. From the composite results of our analysis, we conclude that ethnic fermented foods are potential source of future probiotics and our results demands further validations using comparative genomics and laboratory explorations.

Keywords: Ethnic fermented food, Microbiome, Fermentation.

Comparative morphology, photoperiodism, and yield of KDML105 rice (*Oryza sativa*) and its mutants.

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Abstract. Climate change and decreasing in farmlands threaten global rice production. The productivity and grain quality of KDML105 (*Oryza sativa* 'KDML105'), one of Thailand's famous scent rice are depending on photoperiod and cultivating conditions. Cultivar improvement using induced mutagenesis has previously been performed and screening of candidate mutant lines (MT1, MT3, MT4, MT5, MT6) were then investigated in comparison with KDML105 to gain M6 generation. Comparative morphological features revealed that six mutant lines showed semi-dwarf of shoot length with erected and more greenish leaf, higher tiller numbers, and compact clump when compared to KDML105. Interestingly, the variations of photoperiodism of flowering were observed among mutants and its original. All six lines of candidate mutant showed flowering unaffected by day length even under long-day or short-day that required only 70-85 days after cultivation to reach the flowering date. In contrast, KDML 105 shoed flowering only under short-day conditions. On the other hand, two candidate mutants (MT5 and MT6) after screening for salinity tolerance were further selected to grow for M6 seeds under greenhouse conditions. The tiller numbers, spikelet numbers, total grain yield, and grain weight obtained from MT5 and MT6 mutants were significantly more than those obtained from KDML 105. The mutants obtained in this study are characterized as neutral photoperiodism with shortened production time. It is also suggested to further investigate the grain quality to examine the overall properties of these mutants prior to use in the rice breeding program.

Keyword. KDML 105, rice mutants, photoperiodism, semi-dwarf clump.

Postharvest treatment of ‘Orin’ apples (*Malus domestica*) using 1-methylcyclopropene coated paper-based shellac solution as functional packaging

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1-Methylcyclopropene (1-MCP) is known as ethylene inhibitor agent that effective to delay ripening rate in fruits. Controlled release packaging with 1-MCP as active compound is an alternative method to extend the shelf life of apple. Currently, 1-MCP coated paper has been investigated to develop the functional packaging for fruits. Therefore, this research is aimed to investigate the effectiveness 1-MCP coated paper during preservation of apple.

The formation of 1-MCP coated paper was done by dissolving a weight of 1-MCP inclusion complexes (1-MCP ICs) powder into 0.5 mL of shellac solution (35 %wt. in ethanol). The effectiveness of 1-MCP coated paper were examined in the apple fruits with different weight of 1-MCP ICs powder: 10, 20 and 50 mg of 1-MCP ICs powder for over 30 days. All apples were treated in the expanded polystyrene (EPS) and stored at 4°C for initial 15 days. Thereafter, apples were transferred to 20°C storage temperature for another 15 days. The first analysis of the quality attribute of apple were measured to evaluate the physicochemical properties of apples such as the ethylene production rate, firmness and titratable acidity. After this treatment, the apples were stored at 20°C for another 15 days to evaluate the shelf life of apple and were also analyzed the physicochemical properties of apples as the second analysis. During storage time, ethylene release was measured by gas chromatograph (GC-FID 2014 Shimadzu).

The results showed that apples treated with 1-MCP coated paper inhibited the increase in ethylene production rate during storage time compared to control. During storage time at 4°C, the release of ethylene from apples with treatment of 1-MCP coated paper were less than the control apples. Low ethylene production rate of 5.57 nL/g FW/h could be observed at apple with treated 50 mg of 1-MCP ICs powder in the coated paper. On the other hand, for the control apples without treatment of 1-MCP coated paper, the ethylene production rate was 47.7 nL/g FW/h. The results indicated that 1-MCP coated paper with 50 mg of inclusion complex was effective to delay the ripening rate and maintain the quality of apple during storage time.

Keywords: 1-methylcyclopropene; apple; encapsulation; release; ripening

Effect of sucrose concentration on characteristics of fermented vinegar from corn silk

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This study aims to produce a flavouring agent as a food additive from corn silk. Corn silk has an abundance metabolites, flavonoids, and phenolic compounds that influence antioxidant and xanthine oxidase inhibitors for the treatment of gout. The objective of this study is to divide added sugar between qualitative and quantitative vinegar fermentation. The sweet corn silk (*Zea mays*) was boiled for 20 min, then the fragments were separated. To the corn silk boiled water, 20% sucrose was added to prepare for anaerobic fermentation with *Saccharomyces cerevisiae* who convert the glucose to alcohol. In addition, the alcohol fermentation was co-cultured with *Lactobacillus casei* to obtain lactic acid for enhancing the flavouring agent. In the second step, the alcohol was converted to acetic acid as vinegar by aerobic fermentation with *Acetobacter aceti*. Therefore, the fermented alcohol was divided to add different sucrose concentration (0%, 10%, 12% and 14%) and aerated 0.5 vvm in 100 ml working volume of 250 ml an Erlenmeyer flask at 30°C for 45 days. The vinegar was measured for cell growth and the percentage of acids. Moreover, the activities of substances in the vinegar were determined antioxidant inhibition and xanthine oxidase inhibition including total phenolic and flavonoids. The results showed that the highest initial growth rate of *A. aceti* on 12% sucrose (0.314 h⁻¹) with doubling time of 2.2 h, significantly ($p \leq 0.05$). The culture of 0%, 10%, 12% and 14% sucrose concentration obtained significantly ($p \leq 0.05$) the percentage of acetic acid content which were of 6.95±0.14 %, 11.34±0.0%, 11.66±0.0% and 8.87±0.03%, respectively after 42 days of incubation. The activity of the substance in vinegar showed significant p-value at 0.05 of antioxidant inhibition and xanthine oxidase inhibition. The results indicate that corn silk could be value-added vinegar as a functional food additive by aerated fermentation with 10% sucrose addition.

Keywords: Acetic acid fermentation, Corn silk, Sucrose, Vinegar

Construction of a Novel Continuous Flow Pulsed Electric Field Reactor with Cooling System and Its Application to Sake Pasteurization

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Abstract. The pasteurization technologies are able to prevent decrease of fresh food ingredients during the sterilization process, and the pulsed electric field (PEF) technology is one of the promising technologies for pasteurization of liquid food. To fundamentally eliminate release of metal ion from the electrode, we previously constructed and engineered continuous-flow type PEF reactor with carbon material as the electrodes¹⁾. Carbon electrodes was as effective as metal electrodes in PEF treatment, and the microorganisms in the sake were significantly inactivated. After that, we found that inactivation efficiency had decreased after the long time (totally over 3 h) continuous treatment compare to the early period. This might result from the change of the surface of the carbon material at high-voltage electrode, the surface appearance of carbon that had been contacting with sake looked differ than another place. In the pasteurization of sake by PEF, therefore, avoiding to contact sake with high-voltage electrode is necessary.

In this study, we used a membrane to separate the PEF treatment chamber and the room in contact with the ground electrode was used as the PEF treatment zone. And the room in contact with the high-voltage electrode was continuously supplied with chilled electrolyte solution as the cooling solution to cool the liquid food supplied continuously in the PEF treatment zone by the heat transfer through the membrane. The characteristics of microbial inactivation in the continuous operation of the novel PEF treatment were investigated using the NaCl solution containing *Escherichia coli* as the model treatment solution and the NaCl solution as the cooling solution. The effects of flow rate and electrical conductivity of cooling solution on the PEF inactivation efficiency of microorganisms in liquid food and temperature increase of liquid food in PEF treatment were investigated. And a pasteurization of sake was carried out.

In the PEF treatment at the two different treatment solution flow rates, the lower flow rate could achieve effective inactivation and the temperatures of treatment solutions after PEF treatment were almost same. Increase of cooling solution flow rate was effective to prevent the increase of treatment solution during PEF treatment, but the temperature of treatment solution remained at around 28°C when even the flow rate was increased. The effect of the electrical conductivity of cooling solution was also investigated. It was demonstrated that increasing the electrical conductivity of cooling solution increase not only the inactivation efficiency but also temperature of treatment solution after PEF treatment and considering appropriate electrical conductivity of cooling solution would be essential to obtain effective pasteurization. Finally, inactivation of sake yeast and *Lactobacillus homohiochii* was carried out. Both microorganisms in sake are fully inactivated and the temperature of sake after PEF treatment was able to be maintained below 40°C.

Keywords: pulsed electric field, pasteurization, sake

Detection of DNA Sequences Alteration Rodent Tuber (*Typhonium flagelliforme*) Mutant Plant Pekalongan Accession With Lectin Gene Primers

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Abstract. The rodent tuber (*Typhonium flagelliforme* Lodd.) is also a herbal plant with significant potential as a cancer drug raw material. GC-MS analysis revealed that the rodent tuber plant contains bioactive compounds that demonstrate potential as anticancer agents, including stigmaterol, hexadecanoic acid, oleic acid, and squalene. Rodent tubers reproduce vegetatively, so they have little genetic diversity. Gamma irradiation and somaclonal variation are responsible for changing the genetic variation of rodent tubers in tissue culture. In several mutant clones of rodent tuber Pekalongan accession, high levels of bioactive ingredients are present that are active as anticancer agents. Bioactive compounds related to anticancer genes have not yet been studied in mutant clones of the Pekalongan accession. We examined several mutant clones and wild-type Pekalongan accession plants in order to identify genes related to anticancer compounds. In this study, primers were designed specifically for lectin genes as a marker for the presence of anticancer compounds. A lectin gene was detected at 500 bp in four mutant clones and in wild-type Pekalongan accessions. A 500-bp genome sequence was obtained from four mutant clones and the wildtype. There were three bp differences observed between PM6 mutant clones and the wildtype on 123, 270, and 281 bases. In PM4, the mutant clone differed by 322 bp from the wild-type. The mutant plants of KP 20-1-2-1-2-6 were different from the wild type at 321 bp. This study indicates that there is a lectin gene in the wild-type as well as the modified genomes. A single nucleotide base is changed between the mutant and wild-type plants. According to the result, the alteration gene is a point mutation in the Pekalongan accession.

Keywords: *Typhonium flagelliforme* Lodd, mutant accession, gene lectin, point mutation, specific primer.

Effects of Nanosecond Pulsed Electric Field on the Structure and Function of Various Proteins

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Abstract. The application of pulsed electric field (PEF) on proteins is receiving considerable attraction in medical, agricultural, and food industries. Our group reported the enhancement of the activity of native enzymes [1], the refolding of the thermal denatured enzymes [1], and the solubilization and refolding of the inclusion bodies (IBs) by PEF treatment [2]. The mechanism of these interesting results was not clear. Therefore, more and more detailed experiments focusing on the changes in the structure of proteins should be required to explain these phenomena systematically. In this study, the effects of nanosecond PEF (nPEF) on the structure and function of various proteins were investigated using a batch treatment chamber. Proteins with different molecular weight (MW) and isoelectric point (pI) such as cytochrome *c* (cyt *c*), myoglobin (MYO), horseradish peroxidase (HRP), bovine serum albumin (BSA), and ovalbumin (OVA) were used. Since a nanosecond pulsed power generator was used, it is considered that the influence of heat on the structure of proteins can be excluded. Judging from the circular dichroism (CD) spectrum of each protein, the secondary structure was retained even after nPEF treatment. However, the changes in the surface hydrophobicity of proteins were observed by fluorescence probe method. We consider that nPEF treatment alters the sites connecting α -helix and β -sheet, i.e., random coils, and partially changes the three-dimensional structure of protein. The function of proteins before and after nPEF treatment will also be reported.

Keywords: Pulsed electric field, protein, secondary structure, three-dimensional structure, enzymatic activity

References

- [1] T. Ohshima et al., *J. Electrostat.*, **65** (2007) 156-161.
[2] T. Tanino et al., *J. Inst. Electrostat. Jpn.*, **37** (2013) 28-33.

Genetic Diversity of Gia hac (*Dendrobium anosmum*) Orchids based on DNA Barcoding and Prospects for Commercialization Purpose of Its in vitro Clones in Vietnam

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Abstract. Vietnam, a tropical country, is very diverse in *Dendrobium* species. Gia hac (*Dendrobium anosmum*), an endemic and precious *Dendrobium* orchid with various flower colors and shapes, is distributed from North to South of Vietnam. However, recently, due to the high economic value of the wild Gia hac collections, genetic resources of this species have been over-exploited and are at risk of depleting. Therefore, it is necessary to conserve and sustainably exploit the Gia hac orchids with precious genetic characteristics. In our works, first, based on the DNA barcoding method, we assessed genetic diversity of Gia hac collections from different climates. After DNA barcode screening and evaluating, *matK* and ITS sequences were selected for genetic analysis of the genetic resources. The relationships among the orchids showed that the collected samples from different locations were genetically separated from each other. Significantly, some of the orchids showed unique nucleotide characters in the DNA sequences, also having distinct flower colors and flower shapes. Second, in order to effectively maintain, develop and exploit these collections with no harm to the wild Gia hac genetic resources, micropropagation protocols have been established for some selected ones. PLBs induction and shoot multiplication stages were set up from an original single explant with DNA barcoding genetic indications. Based on this micropropagation, some in vitro clones were rapidly multiplied and produced a large number of shoots/plants. These results indicated that the application of DNA barcoding and micropropagation together can give a chance to evaluate and exploit Gia hac genetic resources effectively, and also give prospects for commercialization of them.

Keywords: *Dendrobium anosmum*, DNA barcoding, *matK*, ITS, micropropagation.

Solid-state fermentation of healthy vinegar from black glutinous rice and corn silk

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Abstract. Black glutinous rice and corn silk provide a unique formula for producing an innovative healthy vinegar. The objective of this study was to evaluate efficacy from a mixture of koji and loog-pang microorganisms. Black glutinous rice (*Oryza sativa* var. *glutinosa*), Leum Pua glutinous rice and fresh corn silk were steamed together. The steamed glutinous rice has been mixed with corn silk strains that were divided to inoculated with koji, loog-pang, and koji plus loog-pang microorganisms. The microbes were then incubated at 30°C for 10 days in anaerobic fermentation to obtain alcohol. *Acetobacter aceti* TBRC 474 was added, which converted continuously the alcohol to acetic acid for 50 days incubation. The results showed the efficacy of koji plus loog-pang produced a low percentage of alcohol (10.5%), which was less than using sole the koji and loog-pang microorganisms, which produced alcohol level of 17% and 21%, respectively. However, the treatment of koji plus loog-pang converted mostly alcohol to acetic acid (6.67 %) after 50 days incubation, while the treatment of koji and loog-pang produced acetic acid of 4.33% and 2.03%, respectively. There was possibility of the higher concentrated alcohol product from the fermentation inhibited *Acetobacter* growth. In addition, the functional benefit of vinegar was determined to function well as an antioxidant and xanthine oxidase inhibition, which include total phenolic and flavonoids compounds. These results indicated that there was significantly high activities at *p*-value, less than 0.05. Therefore, the mixture of koji and loog-pang could be used in a suitable solid-state fermentation needed to achieve a black glutinous rice plus corn silk vinegar product that can be used as a functional flavoring agent.

Keywords: black glutinous rice, corn silk, Koji, Loog-pang, vinegar

Potential of *Acacia crassicarpa* Honey from *Apis mellifera* as a Natural Preservative for Fresh Beef

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Abstract. Fresh beef is a foodstuff food ingredient that is susceptible to microbiological damage and spoilage because it has high water content and nutritional value. Honey can be used as a natural preservative without harming human health. Honey has the potential to preserve beef because of it has antibacterial activity. Acacia 1 honey from Tanjung Jabung Barat (Jambi), acacia 2 honey from Sontang (Riau), acacia 3 honey from Dumai (Riau), and randu honey from Pati (East Java) were tested for SNI and total phenolic content first. In this research, fresh grinding ground beef was used as a sample that was preserved using honey with variations OF concentration of honey and storage time. The objectives of this research were to know evaluate? the influence of addition OF honey to microbiological and physical characteristics of fresh grinding beef. This research used four kinds of analysis, there were such as pH, water activity, Total Plate Count (TPC), and organoleptic. In this research, five treatments were used with various concentration of honey; there were which were 0% as control, 5%, 10%, 15% and 20% and various storage time; there were day-0, day-1, day-3, and day-5. The data obtained were analyzed by ANOVA alpha 0.05. The result showed that variations in honey concentration and storage time of meat significantly affected the pH value, water activity, Total Plate Count (TPC), organoleptic. 10% honey showed the best preservation effect on the sample.

Keywords: Beef, *Acacia crassicarpa* Honey, *Apis mellifera*, Natural Preservative, Antibacterial, Total Phenolic Content.

Molecular Identification and Phytochemical Analysis of Kecarum (*Ocimum* sp.) from Bali

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In Indonesia, *Ocimum* sp. (Lamiaceae) is one of the most economically and medicinally valuable herbs. This species is known on the island of Bali by the local name kecarum. The *Ocimum* genus comprises numerous species and is widespread throughout Indonesia. Consequently, their genetic and phytochemical content may alter in response to different geographical and environmental conditions. Therefore, their species and nature should be determined through molecular identification and phytochemical analysis. This research comprises DNA barcoding using *matK* genetic marker, phylogenetic analysis using the maximum parsimony method, and GC-MS (Gas Chromatography-Mass Spectrometry) examination of kecarum ethanolic extract. DNA barcoding and phylogenetic analysis revealed that kecarum is closely related to *Ocimum americanum* (MF379675.1), as evidenced by a bootstrap value of 78 and a genetic distance of 0.00. Actinobolin, 2-Heptanamine,5-methyl, ethylamine,2-(adamantan-1-yl)-1-methyl, and 2-aminononadecane are the four secondary metabolites identified as the major constituents of kecarum.

Keywords : *Ocimum* sp., DNA barcoding, phylogenetic, GC-MS analysis

Different strain of *Liberibacter asiaticus* produced the same specific protein in the citrus vein phloem degeneration (CVPD) disease in Kintamani, Bali

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Abstract. The main problem in the citrus plantation is citrus vein phloem degeneration disease (CVPD) or citrus greening disease caused by a Gram-negative bacterium, *Liberobacter asiaticus* L. The disease is usually detected using polymerase chain reaction (PCR) with the specific fragment of 16S rDNA as a primer. The amplified DNA fragments were analyzed for their polymorphism, and we found two strains of *L. asiaticus* in the citrus plantation area of Kintamani, Bali, Indonesia. Furthermore, the protein from the citrus leaves infected by the two strains was isolated and then subjected to Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE). The results found that the two strains produced the same specific protein due to infection. The specific protein detected was 16 kDa and 66 kDa. However, there were no specific proteins detected from healthy plants. These two specific proteins can also be utilized to detect CVPD disease, even the infection made by a different strain of *L. asiaticus*.

Keywords: CVPD Disease, PCR, SDS-PAGE, *L. asiaticus*, Specific Protein

High genetic differentiation of the stingless bee *Tetragonula laeviceps* based on *Cytochrome c-oxidase 1 (COX1)* gene in Sumatra and Java

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The stingless bee *Tetragonula laeviceps* (Hymenoptera: Apidae) is frequently transferred from its native regions to many areas across Indonesia for meliponiculture purposes. This species has a high number of morphological variations that made it difficult to identify. Identification at the molecular level based on the standardized sequence of *Cytochrome c-oxidase subunit 1 (COX1)* gene from mitochondrial DNA is needed to support morphological identification. However, there is a lack of *COX1* gene data of these bees in the GenBank. Thus, we aimed to characterize *T. laeviceps* in the native and transferred regions using the *COX1* gene. Stingless bee samples were collected from two native regions: Batanghari (Jambi, Sumatra) and Lebak (Banten, Java), while the transferred bees were from Bogor (West Java). Genomic DNA was extracted from the bee thorax using 0.2% CTAB method. The *COX1* gene primers were manually designed based on the complete mitochondrial genome of *Melipona bicolor* (AF466146). BLAST-N and putative amino acid analysis were applied to the 13 *COX1* gene obtained sequences. Haplotype analysis within *T. laeviceps* was conducted using DnaSP6. The genetic distance and phylogenetic tree of *T. laeviceps* and other *Tetragonula* bees from GenBank were constructed using the maximum likelihood approach implemented in MEGA 6.06. The substitution rates of the putative codon position were analyzed using the Tamura-Nei model. BLAST-N analysis revealed that the closest relative of *T. laeviceps* was *Lepidotrigona flavibasis* (MN747147.1) with 82.09%–82.61% and 99%–100% of similarity and query cover values, respectively. They aligned to *L. flavibasis* due to the absence of *T. laeviceps* *COX1* gene in GenBank. We found a total of 24 nucleotide variations out of 581 bp multiple alignment sequences, with 21 nucleotides belonging to *T. laeviceps* from Batanghari and Bogor. Based on the haplotype analysis, *T. laeviceps* *COX1* genes were further classified into six haplotypes (H1–H6). The genetic distance within *T. laeviceps* ranged from 0.2%–3.7%. The lowest 0.2% genetic distance was observed between all haplotypes except for H1 which showed a high 3.7% genetic distance compared to other haplotypes. The high intraspecific variation of *T. laeviceps* H1 formed a single clade that split from other haplotypes with a 99% bootstrap value in the phylogenetic tree. The high variation in the intraspecific level also showed in the interspecific level which has a high genetic distance ranging from 16.9%–31.5%. Despite a high genetic distance between *Tetragonula*, the constructed phylogenetic tree showed that *Tetragonula* bees belonged to a single cluster. In addition, putative codon position analyses revealed that transition exceeded the transversions, with the highest substitution number observed at the third codon position. Furthermore, the substitution of nucleotide number 157 of the H1 *T. laeviceps* *COX1* gene generated a single putative amino acid mutation from serine to phenylalanine. In conclusion, *T. laeviceps* has a high molecular variation and additional molecular data are needed to explain *T. laeviceps* haplotype variations among wider native and transferred areas. Integrating the molecular and morphological variation of *T. laeviceps* is needed to unravel the possibility of complex species in *T. laeviceps*.

Keyword: DNA barcode, intraspecies variations, Java, stingless bees, Sumatera, *Tetragonula laeviceps*

Haplotype Variations of *Apis cerana* from Sumatra and Molecular Relationship with the Asian Honey Bees based on the *COX2* of Mitochondrial Gene

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Apis cerana is the most widely distributed honey bee in the Asia region with various climate conditions. There are eight subspecies of *A. cerana* and two of them are found in Indonesia. Based on previous studies, subspecies of *A. c. javana* were distributed from Java to Timor, and *A. c. johni* were distributed in Sumatra. A molecular study using the *cytochrome oxidase subunits 2 (COX2)* gene in many regions in Sumatra has not been conducted yet. Thus, this research aimed to analyze the haplotype variation of the *COX2* gene for *A. cerana* in Sumatra and revealed the molecular relationship with the other Asian *A. cerana*. *Apis cerana* from seventeen locations in six provinces in Sumatra were collected and placed in 4°C. DNA extraction was carried out using GenAid DNA Mini Kit and was amplified using primers E2 and H1 from the previous studies. The *COX2* gene haplotype variations were analyzed using DnaSP 5.10. Transition and transversion of the *COX2* gene and the putative amino acid variations were determined. *Apis cerana* sequences from this study were aligned with the *A. cerana* in the GenBank database to analyze genetic distance and to construct Maximum Likelihood (ML) phylogenetic trees. This study found 11 new *COX2* gene haplotypes from a total of 12 haplotypes in *A. cerana* from Sumatra. Haplotype 1 as the common haplotype consists of 37% of the total haplotypes. Moreover, we found specific haplotypes in five Sumatra Provinces, i.e. haplotype 2 in Aceh; haplotype 4, 5, and 6 in North Sumatra; haplotype 8 in Jambi; haplotype 9 in West Sumatra; haplotype 10 and 11 in South Sumatra. In the *A. cerana* Sumatra *COX2* gene, the number of transitions was higher than transversions in the 1st and 3rd codon, while no mutation occurred in the 2nd codon. Furthermore, there was a transition mutation that alters Isoleucine into Valine in amino acid number 97. We found the genetic distance between *A. cerana* in Sumatra and Borneo to *A. cerana* from Java was 4,51-5,63%. This genetic distance was similar to the distance between Indonesian *A. cerana* (*A. c. javana*) and East Asia (*A. c. cerana* and *A. c. japonica*) which was 4,21-5,57%. Based on the molecular phylogenetic tree, *A. cerana* Sumatra clustered with the bees from Borneo (AY587544.1 and AP018149.1) with 88% bootstraps in the ML phylogenetic tree. The clade of *A. cerana* from Sumatra and Borneo was separated from the *A. cerana* from Java, Moluccas, and Papua. This result confirmed the separation of *A. c. javana* and *A. cerana* Borneo based on previous studies. The exception was found in *A. cerana* haplotype 4 from colony 2 Siantar Sitalasari, Pematangsiantar, North Sumatra that was grouped in Java *A. cerana* and separated from Sumatra and Borneo clade. For future study, more molecular data based on other genes and also morphological traits are needed to justify these findings.

Keywords: Asian honey bee, mitochondrial DNA marker, specific haplotype, Sumatra, Sundaland

Induction of resistance against sugarcane mosaic virus by pathogen-derived resistance and RNA interference methods in transgenic sugarcane.

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Sugarcane mosaic virus (SCMV) is one of the most serious diseases of sugarcane and reported causing yield losses of up to 45% in susceptible varieties. The SCMV was also reported causing mosaic disease in other plants such as maize and sorghum. The genome of SCMV contains an open reading frame (ORF) that encodes 10 mature proteins, including coat protein (CP) located at 3' terminus of the ORF. The CP is most widely used method for inducing resistance against virus either using pathogen-derived resistance (PDR) or RNA interference approach. To induce the resistance, the gene encoding for CP was successfully cloned from the susceptible variety of PS-881 cultivar (MH393888.1). Induction of the resistance using the PDR method that also called protein-based resistance by overexpression of the CP gene resulted in the resistance of transgenic sugarcane against SCMV infection. The full sequence of CP gene produced a highest resistance compared to N-terminally truncated the protein. This result indicated that the full sequence of the CP gene is required to disrupt viral assembly and packaging, thereby generating more resistance to SCMV infection. The RNA interference (RNAi), also called RNA silencing, has emerged as an efficient mechanism for controlling invasive virus. The transgenic sugarcane expressing intron-hairpin (RNAi) constructs produced a high number of resistances against SCMV infection. Interestingly, *Ubi* promoter-driven gene expression resulted in higher resistance compared to *CaMV*-35S promoter. The *Ubi* promoter is suggested an effective promoter for producing transgenic sugarcane. Whether the RNAi is more effective method than the PDR to induce the resistance, the two-type transgenic sugarcane were examined their resistances to combat sugarcane mosaic virus. The result showed that the RNAi approach targeting the gene for CP effectively produces more resistance against the SCMV infection in sugarcane compared to the PDR approach.

Keywords: sugarcane mosaic virus, resistance, pathogen-derived resistance, RNAi, transgenic sugarcane.

Molecular Identification and Exploration Phytochemical Compounds of Basil (*Ocimum spp.*) Growing in Bali

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Basil (*Ocimum spp.*) is an aromatic plant *Lamiaceae* that is commonly consumed in Indonesia. In Bali, various types of basil grow but are not yet known and widely used. This study aims to conduct molecular analysis and identify phytochemical content in four types of Basil (*Ocimum spp.*). Both of these things can be influenced by the conditions of the growing environment. The four types of basil that grow in Bali are known as Kecarum (Balinese basil), Selasih (Javanese basil), Tulasi (purple basil), and Ruku-ruku (purple stem basil). In identifying phytochemical content, basil is extracted first using the maceration method with 96% ethanol solvent until a viscous extract is obtained—identification of phytochemical content using GC-MS (Gas Chromatography-Mass Spectrometry). Molecular identification is carried out by the DNA Barcoding method using primers based on *matK* genes. Electrophoresis, sequencing, and amplification are continued and analyzed with BLAST. The analysis results through the construction of Phylogeny showed high homology between Kecarum and Selasih, as well as Tulasi and Ruku-ruku. They found homologs to species that had been registered in GenBank with an average bootstrap value of more than 78 and a genetic distance of less than 0,0010, namely Kecarum (*Ocimum americanum*), Selasih (*Ocimum basilicum*), Tulasi (*Ocimum campechianum*), and Ruku-ruku (*Ocimum tenuiflorum*). GC-MS analysis showed that the four basiles contained different types and amounts of compounds. Phytochemical compounds as secondary metabolites identified as dominant were, Cyclotrisiloxane, hexamethyl-, Actinobolin, Methyleugenol, and Caryophyllene. Various previous studies have proven some biological activity of several phytochemical compounds contained in the four types of basil as useful as anti-inflammatory, pain relief (analgesic), antidepressants, attractants, and others.

Keywords: *Ocimum spp.*, DNA Barcoding, GC-MS analysis, phytochemical, *Lamiaceae*

Extraction of Water-Soluble Nondigestible Nonstarch Polysaccharides from Barnyard Millet (*Echinochloa frumentacea*) Grain

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Abstract. As 2023 is declared as 'international year of millets', the awareness is increasing among the general public about importance of millets and its health benefits. Barnyard millet grains have high ratio of carbohydrate to crude fiber beside its high nutritive value. It has been reported that non-starch polysaccharides have great health implications for humans. After ethanol extraction of sugar from barnyard millet, residue part was treated with water at 1:2 sample solvent ratio for 2 hours at 40° C temperature for isolation of water-soluble non-starch polysaccharides. The yield of water soluble non-starch polysaccharides was 2-3%. Non-digestibility assay of barnyard millet water soluble non-starch polysaccharides were carried out and it showed that percentage (%)of hydrolysis was less compared to positive control starch.

Keywords: Barnyard millet, Crude fiber, Non-starch polysaccharides, Non-digestibility

Digestibility of Water-soluble non-starch polysaccharides produced from Proso millet

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Abstract. Recently, the importance of millet has received attention due to its high nutraceutical value. Millets are a rich source of both water-soluble and insoluble non-starch polysaccharides. The bioactivity of water-soluble non-starch polysaccharides revealed their prospective in the treatment and control of various metabolic diseases as well as enhancing gut health and immunity. WNSP content of 2-3% (w/w) was obtained from the alcohol-insoluble residue of Proso millet grain powder with water in a 1:2 (w/v) ratio at a 40 °C hot water bath for 2 hours. In comparison to inulin, WNSP made from Proso millet displayed higher resistance or low digestion to human-amylase and synthetic human gastric juice, demonstrating accessibility to probiotic bacteria in the human bowel.

Keywords: proso millet, Water-soluble non-starch polysaccharides, probiotic bacteria, Digestibility.

Organic amendment determines the crop yield and rhizospheric bacterial community structure: A three-year field study with *Cajanus cajan*

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Abstract. Excessive use of chemical fertilisers in agriculture to increase soil nutrient status, plant development, and yield has resulted in a decline in soil fertility and health. Minimizing chemical use, as an alternative, is much required for a sustainable ecosystem. Organic farming promotes biological amendments, which help to balance the ecosystem by using various biological or agricultural wastes as additives. Understanding the dynamic patterns of belowground microbial populations and the biological pathways is essential for developing sustainable agricultural systems. Therefore, the study was designed to evaluate the effect of different agri-based practices on rhizospheric bacterial diversity and crop yield in an Indian agricultural system. Three-year field experiment was set up in a randomized block design using *Cajanus cajan* as a model crop. Conventional (CON), organic with animal manure (ORG-M), organic with bio-compost (ORG-C) with control (C) were chosen to compare the effect of agri-based practices. Plants were collected at the harvest stage for assessing various parameters. Rhizospheric soil samples were collected for characterizing bacterial diversity using culture-independent approach. The impact of various agricultural strategies on native soil microorganisms and crop yield parameters was observed. Bio-compost-based organic amendments had a positive impact on crop productivity over the years (2018-2020). In comparison to the control treatment, there was a 2-fold increase in grain yield and a 1.5-fold increase in both stover and biological yields were recorded. Bio-compost amended organic farming practice exhibited more bacterial diversity and higher abundance of *Bacillus* (6.7%), *Candidatus nitrososphaera* (6.5%), and *Nitrospira* (1.5%) along with some exclusively present genera, like *Pseudoxanthomonas*, *Agrobacterium*, and *Bifidobacterium*. The study brings forth the positive aspects of different organic amendments on soil health and rhizospheric bacterial diversity. The bio-compost treatment led to the enhancement in crop productivity, bacterial diversity and abundance, thereby, enhancing soil health for sustainable agriculture.

Isolation and Characterization of *Chalcone Synthase (CHS)* Gene in Variegated-Flower of *Dendrobium* and *Phalaenopsis* Orchids

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Abstract. *Dendrobium* and *Phalaenopsis* orchids are plants which are widely cultivated due to their attractiveness. Variegated flowers are characterized by the presence of different colors in one flower. The uniqueness of variegated phenotype will increase the commercial value of ornamental plants. Research related to genes that play a role in the formation of flower color is interesting to be examined for plant breeding. The main pigments of purple flowers are anthocyanins. One of the essential genes in anthocyanin biosynthesis is *Chalcone Synthase (CHS)*. Analysis of the structure and function of the *CHS* gene may reveal alterations that occur in the process of color formation in flowers supported by data on changes in the content of anthocyanin pigments in normal and colorless flower parts. The objective of this study is to determine the structure of *CHS* gene and the amino acid motifs needed in the formation of flower color in *Dendrobium* and *Phalaenopsis* with variegated-flower. The methods applied in this study are: 1) observation of floral morphology, 2) isolation of plant genomic DNA, 3) amplification of the *CHS* gene by using polymerase chain reaction (PCR) with degenerate primers: *CHS* F1 and *CHS* R1, and 4) analysis the content of anthocyanin pigment. The results of flower morphology observations showed that the appearance of the variegated-flower in *Dendrobium* and *Phalaenopsis* started when the flower was still in bud. *Dendrobium* variegated-flowers have more white areas than the purple ones, while *Phalaenopsis* flowers have wider purple areas than the white ones. There are differences in the length of the *CHS* gene fragment in the purple and the white regions of *Dendrobium* variegated flower, the purple region has a fragment length of 1213 bp and the white region is 1217 bp; mutations at several points in the white zone, differences in the location of the PLN03170 protein motif superfamily in the purple zone at intervals of 118-825 bp and the white zone 156-923 bp; the difference in the size of the amino acids in the purple zone has 507 amino acids and the white zone has 557 amino acids; and differences in the location of the amino acid motif PS00441 CHALCONE_SYNTH (Active site of chalcone and stilbene synthase) in the purple zone at intervals of 295-331 bp and white zone 93-109 bp. Meanwhile, in the *Phalaenopsis* variegated-flower, the difference in the length of the *CHS* gene fragment is that the purple region has a length of 1170 bp and the white region is 1200 bp; the *CHS* gene sequences show mutations at multiple points in the white region; with some changes in protein motifs, in the purple region superfamily PLN03173 and white region superfamily PHA 03247, superfamily PLN03173, superfamily PLN 03168, superfamily PLN03170; the difference in amino acid structure in the purple area has 454 amino acids, in the white area it has 478 amino acids; and differences in the location of the PS00441 CHALCONE_SYNTH amino acid motif in the purple area with an interval of 366-382 bp and the white region is 308-324 bp. In conclusion, the instability of amino acids size, and the mutations at some points may cause the absence of anthocyanin pigments formation in the white region. This was supported by the analysis of anthocyanin pigment levels, that there with significant different in anthocyanin pigment levels between the purple and white zones, which showed significant differences in anthocyanin pigment levels between the purple and white regions, which affected the difference in flower color; the higher the anthocyanin content, the darker the flower color.

Keywords: Anthocyanins, *CHS*, *Dendrobium*, *Phalaenopsis*, Variegated-flower

Characterization of black rice oligosaccharides and evaluation of their prebiotic potential

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Abstract. Non-Digestible Oligosaccharides (NDOs) are the most common prebiotics which are low molecular weight carbohydrates with degree of polymerization (DP) 3-20, that are delivered intact into the large intestine. They facilitate prevention and treatment of lifestyle related disorders. In the present study, black rice oligosaccharides (BROs) were extracted at optimized process parameters. They were purified through charcoal-celite column and further by dialysis membrane (500 Da). The obtained black rice oligosaccharides proved to be non-digestible when subjected to gastric acidity, salivary and pancreatic amylases. The positive prebiotic score of BROs obtained in presence of different Lactobacilli strains is indicative of their prebiotic potential. Keeping in view the pharmacological importance of functional oligosaccharides and their proven efficacy over polysaccharides, BROs could serve as efficient prebiotics and can be further explored as functional food additives.

Keywords: Black rice, Characterization, Non-digestible oligosaccharides, Prebiotics, Functional food.

De Novo Transcriptome Assembly of *Tagetes erecta* and *Tagetes patula* Flowers

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Abstract

Marigolds (Asteraceae) have two popular species namely *Tagetes erecta* and *T. patula*. *T. erecta* generally produce yellow to orange petal color, adversely *T. patula* produce yellow to red maroon or mix of yellow and red maroon color. The yellow color of this plant suggested the carotenoid is the major pigment in these species. However, the red color of *T. patula* may be resulted from the anthocyanin pathway. In this study, transcriptome assembly and profiling were performed to generate expression profiles of *T. erecta* orange (Teo) and *T. patula* variegate (Tpv). A cDNA library was generated from RNA isolated from Teo and Tpv petal flower. Totally 44,500,535 clean reads were obtained from Teo and 45,173,627 from Tpv, and assembled into 164,549 transcripts and 164,545 unigenes. The ratio of Successfully Annotated Genes was 78% in NR, 69.92% in NT, 30.22% in KO, 62.33% in Swissport, 30.86% in PFAM, 19.61% in GO, 22.16% in KOG, 5.36% were annotated in all databases while 80.84% were annotated in at least one database. The expression level of several genes involved in carotenoid and anthocyanin in both species will be elucidated.

Keywords: transcriptome, de novo assembly, annotation, anthocyanin, carotenoid

Comparison of Three Cassava Generations from Irradiation Mutation (M1v8, M1v9, And M1v10) Related Mineral Content in Leaves

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Abstract

Cassava is a nutritious food source in several countries. The distribution of cassava accessions in several Indonesian regions is relatively high with various characteristics. Evaluation of the potential character of cassava diversity resulting from irradiated mutations can be carried out in developing new high-yielding varieties. In contrast, in previous studies in this series of studies, an evaluation of the tuber character was carried out, so in this study, an assessment of the leaf character and mineral content was conducted. This study used twelve cassava genotypes from gamma irradiation mutations in M1V8, M1V9, and M1V10 generations. All the cassava generations were planted in a randomized complete block design with three replications. Cassava leaves characters based on cassava descriptor IITA. The AAS method analyzes mineral content using cassava leaves that have fully opened. The data were analyzed using ANOVA, DMRT, t-test, and stability test. This study is to identify potential and stable mutant genotypes related to mineral content and to compare various leaf characters and mineral content of cassava mutant genotypes in three generations. The result of this study showed all the genotypes in the three generations had about 134 leaves per plant, five lobes per leaf, and a middle lobe width of about 15 cm. The leaf greenness index ranged from 31 to 48 units, of which genotype G3D2-413 had the highest mean leaf greenness index. The magnesium, iron, and zinc content range in the three generations is around 2100 to 3200 ppm, 25 to 160 ppm, and 44 to 517 ppm. Lobe length, lobe width, petiole length, and Fe content between the three generations are not significantly different; therefore, these characters are assumed to be stable. Fe content in the G4D1-222 and G4D3-113 genotypes was stable, and the G4D3-113 has stable Mg and Zn content.

Key words: *iron, mutant potential, stability, zinc*

Early Development of Somatic Embryogenesis Technology for Clonal propagation of the Indonesian Exotic Kopyor Coconut from Rachilla Explants: Initiating the Embryogenic Callus

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Abstract. Kopyor coconut is an exotic plantation commodity from Indonesia, characterized by its broken endosperm, so that the nuts could not be conventionally grown. Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB) has long succeeded in developing in vitro propagation technology for kopyor coconut through embryo rescue technique using zygotic embryo as explant. However, this technique produced seedlings which are not necessarily true-to-type. Therefore, developing a clonal propagation technology, somatic embryogenesis (SE), is in urge to produce clonal seedlings, and also increase seedlings production capacity. Embryogenic callus initiation is the early step of the SE technology. Hence, this study aimed to develop protocol for initiation of embryogenic callus using rachilla explants isolated from the flower of the dwarf kopyor coconut. Media for callus initiation used antioxidant and range of pH treatments to prevent explants browning. The media pH of 4,5 and the addition of antioxidant through double layer system into the media were the most effective in suppressing explants browning. Calli were successfully emerged from explants after 10 weeks incubation in the initiation media, and turned into nodular calli after 14 weeks, which have embryogenic potential. This early protocol is promising for further development of SE technology for clonal propagation of kopyor coconut.

Keywords: kopyor coconut, somatic embryogenesis, callus, rachilla explant, clonal propagation

Enhanced Plant Responses to Drought Through Modulation of Cytokinin-Metabolic Soybean *Isopentenyl transferase* Genes

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ABSTRACT

Drought is one of major external threats that negatively affect crop productivity and sustainable agriculture. It has been known that in response to the adverse conditions, plants will deploy various defense and adaptive mechanisms. In soybean (*Glycine max*), a drought-sensitive crop plant, *GmIPT08* and *GmIPT10* are two genes that belong to the gene family encoding adenine isopentenyl transferase enzymes responsible for biosynthesis of cytokinin phytohormone. Previously, expression of these genes was induced strongly under water deficit conditions, indicating their association with plant responses to drought stress. Here, the aim of this study was to explore the drought-related functions of *GmIPT08* and *10 in planta*, by using transgenic soybean system and performing comparative analyses of various aspects of plant function and activities under normal and drought conditions. At morphological and physiological levels, the transgenic lines harboring either *GmIPT08* or *10* displayed better growth maintenance for the shoot and root, as well as higher relative water content in the plant tissue. Under this unfavorable condition, in addition, the transgenic soybean plants also had stronger antioxidant enzyme activities accompanied with a lower endogenous cellular hydrogen peroxide content, indicating a lower drought-induced oxidative stress status imposed to these genotypes. These results also support for the findings that overall survival rates of the transgenic lines were significantly higher than the wild-type plants. Taken altogether, the obtained data indicate positive roles of *GmIPT08* and *10* in mediating plant defense against drought stress, which pave the way for study their potential utility in crop improvement by genetic engineering.

Keywords: cytokinin, drought tolerance, soybean isopentenyl transferase, transgenic plant

Genome-wide identification and characterization of WRKY transcription factor gene family in *Citrus reticulata* and its response against various biotic and abiotic stress

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Abstract. WRKY is an important group of transcription factors (TrFs) gene family and was identified primarily in plants. WRKY TrFs play vital roles in modulating gene expression during several biotic and abiotic stresses, senescence and different hormone responses. The DNA-binding domain of these proteins is a conserved hepta-peptide sequence WRKYGQK. Although the Mandarin Citrus (*Citrus reticulata*) genome has been published but functional studies are still necessary to understand Mandarin Citrus. The mining of *C. reticulata* genome identify 46 CrWRKY genes that are classified into three main groups (G1-G3) with five subclasses (IIa–IIe) in G2, and all were present on 29 different scaffolds representing numerous segmental duplication (100%) events. Through multiple sequence analysis It is predicted that WRKYGQK domain and metal chelating zinc-finger motif C2H2 is present in 45 genes while WRKYGQK domain is replaced with WRKYGKK only in CrWRKY20.

Cis elements of CrWRKY genes provide information about their significant role in cold stress in *C. reticulata*. Furthermore, transcriptome analysis from previously generated RNA seq data, indicated that CrWRKY gene play critical role in different organs and tissues of plants under various biotic as well as abiotic stresses.

Keywords: *Citrus reticulata*, Expression pattern, Genome wide, Transcription factor, WRKY gene family.

Effect of Citrus Peel Powder in Marinade for Safety, Quality, and Tenderness of Chicken

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Abstract. Consumer awareness about the quality and safety of the food products has been increased in this century and consumer demands the use of natural ingredients to preserve the food for long duration. Citrus fruits are used to manufacture the citrus juice and other related beverages. A significant amount of peel wasted in the citrus processing industry that can be used for many other purposes due to high polyphenol contents in the citrus peel. Food processors are also investing the money in the research areas to find the possible ways to improve the quality, and safety of the chicken during storage. Therefore, the present research was conducted to understand the impact of different percentages of citrus peel extract in marinade of chicken on quality, safety and shelf life of chicken. Chicken was stored at refrigeration storage after treatment of citrus extract and storage study of 30 days was done to understand the impact on shelf life and quality. Physicochemical analysis, microbial analysis, cooking yield, lipid oxidation and sensory evaluation was done. The results of study revealed that among all treatments and storage days, lowest pH was recorded at T0 at 30th storage day with value share of 5.78 ± 0.10 . Means showed the highest moisture (%) value of 76.27 ± 0.05 at T0 at zero day's storage. T0 showed the lowest protein (%) value of 20.33 ± 0.31 , at zero's day's storage. Means showed the lowest TPC (mg GAE/g) value of 1.21 ± 0.02 at T0 at zero day's storage. In contrast, T3 showed the highest TPC (mg GAE/g) value of 4.32 ± 0.23 , at zero's day's storage. Means showed the lowest TFC (mg QE/g) value of 1.69 ± 0.01 at T0 at zero day's storage. In contrast, T3 showed the highest TFC (mg QE/g) value of 8.16 ± 0.05 , at zero's day's storage. Means showed the highest TBARS (mg-MA/kg) value of 0.51 ± 0.01 at T0 compared to other treatments at zero day's storage. Means showed the lowest TVC (log CFU/g) value of 4.37 ± 0.01 at T3 at zero day's storage. In contrast, T0 showed the highest TVC (log CFU/g) value of 4.62 ± 0.02 , at zero's day's storage. After 30 storage days, lowest cooking loss (%) was recorded at T3 with value share of 24.6 ± 0.42 . Means showed the lowest hedonic scale score of overall acceptability 7.33 ± 0.577 at T1 at zero day's storage. After 30 storage days, highest hedonic scale score of overall acceptability was recorded at T3 with value share of 7.33 ± 0.577 . It can be concluded that, with the addition of citrus peel extract microbial load decreased, total phenolic contents increased and overall acceptability of the product also improved.

Keywords: Citrus peel powder, Marinade, chicken meat.

Fast Preparation of *Ganoderma boninense* Sample using Modified Portable Coffee Grinder

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Abstract

The exploration and identification of *Ganoderma boninense* in Indonesia is crucial, considering their ability to cause heavy losses on palm oil plantations in Indonesia. Furthermore, the ideal control to overcome the diseases caused by *Ganoderma* is to use tolerant plants, but to obtain tolerant plants still takes a relatively long time. Therefore, the fast detection of the *Ganoderma* in plantations is needed. Molecular technology has been widely used in various industries both at the research level and its application in the field. Currently, molecular detection using Polymerase Chain Reaction (PCR) techniques has been developed to obtain accurate results. However, the use of PCR instruments has been known for its complexity due to the length of the process and requires laboratory personnel with certain skills. Furthermore, an extra preparation was taken place in the case of palm oil tree to transform the solid sample to liquid phase. Here, a fast preparation of sample using coffee grinder from different parts of palm-tree were investigated. Common practice told that the solid sample was prepared using mortar and liquid nitrogen. This troublesome treatment was proposed to be simplified using a modified coffee grinder that equipped with heating unit. The preparation begun with portable coffee grinding the leaves, stems, bark, and fruit of palm-oil tree with sterile silica sand. Sampling generated from a portable grinder is then verified using polymerase chain reaction (PCR) amplification. PCR products was visually observed in the form of fluorescent tapes using the gel method of electrophoresis. The results showed that the results of preparation using coffee grindier tools have similar qualities with conventional methods.

Keywords: *Ganoderma boninense*, palm oil, molecular detection, polymerase chain reaction, portable coffee grinder, sample preparation

Easy Extraction of *Ganoderma boninense* Liquid Sample using Lab-on-Chip Device

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Abstract

The identification of *Ganoderma boninense* in Indonesia is essential in order to reduce the spreading of infected palm oil in a larger population. At this moment, a tolerant plant is in under development and yet to be invented. Therefore, various method to detect *Ganoderma boninense* in the population is massively investigated. Currently, molecular detection using Polymerase Chain Reaction (PCR) techniques has been developed to obtain accurate results for the *Ganoderma boninense*. However, this detection method shall use laborious work of serial preparation of the sample. One step of them is the extraction of the DNA from the liquid sample that hardly done in the plantation. Therefore, a portable device that known to be used for blood purification was introduced. The portability was mostly brought by a lab-on-chip device that has a size of microscope prepartate. The chip was put in the extraction device using magnetic beads to adsorb the lysed DNA. the process was easy and automated to resulted purified DNA sample. The polymerase chain reaction confirmed that the extraction using the lab-on-chip device was comparable to that conventional extraction method.

Keywords: *Ganoderma boninense*, infected palm oil, molecular detection, polymerase chain reaction, lab-on-chip, magnetic beads

The Effect of Drying Temperature on Phenolic Compounds and Antioxidant Activity of *T. laurifolia* Tea Infusion

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Abstract. *Thunbergia laurifolia* leaf is enriched with polyphenolic compounds which exert several biological functions such as antioxidant, among others and generally incorporated into Thai herbal medicine to moderate alcohol, food poisoning and other health-related diseases. Plant polyphenols are easily degraded into phenolic acid derivatives and other compounds at elevated drying temperatures, hence, reducing antioxidant activity as well as other health-promoting benefits. This study evaluated the influence of hot air drying method (80 °C, 90 °C, and 100 °C) of *T. laurifolia* tea leaf on selected phenolic compounds, total flavonoid (TFC), total phenolic contents (TPC), and antioxidant activity (AOA) of the tea infusion. *T. laurifolia* tea leaf showed a positive respond to all drying regimes. The infused tea exhibited effective AOA (DPPH and ABTS-scavenging activity) based on TFC and TPC which were significantly influenced by drying regimes. HPLC analysis detected rosmarinic acid, caffeic acid, gallic acid, catechin, rutin and quercetin as main phenolic compounds present in tea samples. The results showed that different drying regime affect tea polyphenols and AOA of the infused tea as increase in drying temperature showed increase in TFC, TPC, and AOA. This observation suggests that hot air-drying combination of 90 °C – 100 °C with 30 min could retain phenolic compounds of *T. laurifolia* tea leaf and consequently deliver potent AOA.

Keywords: Antioxidant activity, *Thunbergia laurifolia*, Phenolic compound, Rosmarinic acid

Optimization of Liquefaction and Saccharification Conditions for High Yield of Isomalto-oligosaccharide (IMO) Production from Rice Flour

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Abstract. Low substrate conversion in liquefaction and saccharification steps is one of the main drawbacks which cause for the low isomalto-oligosaccharide (IMO) yield in the IMO production process. Therefore, it is necessary to optimize the conditions for liquefaction and saccharification steps to have a maximum substrate conversion of starch prior to the transglucosylation step. Rice slurries of 20, 25, 30%(w/v) concentrations were prepared and treated with various concentrations of KLEISTASE T10S bacterial α -amylase enzyme, 0.035, 0.055 and 0.075%(v/w) incubated at 95 °C. The samples were taken every 10 min time interval for maximum hydrolysis of starch. The optimum liquefaction conditions were obtained as 20%(w/v) substrate concentration, 0.075%(v/w) enzyme dosage and 40 min reaction time, which resulted in 87% hydrolysis of rice flour. The liquefied solution was further hydrolyzed by BIOZYME LC fungal α -amylase enzyme at three levels of enzyme dosage, 0.5, 0.15 and, 0.2%(v/w). Maltose and glucose concentrations were analyzed in the saccharified solution every 10 min time interval. The optimum conditions for saccharification were found as 0.2%(v/w) enzyme dosage, 30 min reaction time at 60°C. The optimized saccharified solution consisted of 146.81 (g/L) of maltose and, 6.21 (g/L) of glucose. Finally, Transglucosidase L “Amano” enzyme (1%(v/w)) was used to synthesize IMO for one hour of incubation, and its composition was analyzed by HPAE-PAD. The IMO mixture consisted of 24.35 (g/L) of isomaltose, 11.03 (g/L) of isomaltotriose, 0.92 (g/L) of isomaltotetraose, 0.88 (g/L) of isopanose, and 20.98 (g/L) of isomaltohexaose, resulted in 58.16 (g/L) of total IMO concentration, which is approximately 75%(w/w) of IMO yield relative to initial maltose. The optimum conditions developed in the present study showed a significant improvement in substrate conversion, reaction time, enzyme usage, and temperature control.

Keywords: liquefaction, saccharification, transglucosylation, isomalto-oligosaccharide (IMO)

Formulation and Evaluation of a Novel Functional Cereal Containing Avocado Seed Flour

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Abstract. Celiac disease is the most common food-sensitive enteropathy in humans, caused by consumption of wheat gluten and linked to proteins found in barley, rye, and oats. A gluten-free diet is the only known cure for celiac disease. Most low gluten foods on the market are high in starch but low in other nutrients, functional ingredients, and health benefits. Avocado is a tropical fruit with a high annual growth rate (19.09%). A large amount of solid seed residue, approximately 21-30% of the fruits, is commonly discarded. Avocado seeds contain a variety of nutrients, including phenolic compounds (flavonoids, polyphenols), fiber, reducing sugars, pentosan, arabinose, and protein, as well as a high starch content of 29.6%. To reduce the levels of sap in avocado seeds, several pre-treatment stages were carried out. The bitter taste of the sap will affect the taste of the final product. Avocado seeds can be ground into flour with a high antioxidant content that is low gluten. Avocado Seed Flour (ASF) is a low-protein flour that can be used to make dough for dry food ingredients such as biscuits and pastries because low-protein flour absorbs less water, making the dough crisper and more resistant to storage. The goal of this study is to create a nutritious and functional low gluten diet cereal formula by replacing wheat flour with ASF. Cereals with the least amount of ASF were described as having a high grain percentage and crunchiness, whereas cereals with medium and high ASF levels were described as having a darker color and bitterness. Contrary to popular belief, the ASF content of gourmet cereals with the highest ASF content was lower than that of cereals with the lowest content.

Keywords: Avocado Seed, food sustainability, celiac disease, low gluten diet

Preliminary Research on Tea Production from Fruit and Flower of *Annona Squamosa* L. in Vietnam

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Preliminary research on production

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ABSTRACT

In recent decades, the *Annonaceae* family has been recognized for its therapeutic potential; therefore it is highly probable that consumption of the sugar apple fruit could provide potential health benefits, and thus have health importance. Custard apple (*Annona squamosa* L.) is one of the main fruit trees of Ba Ria - Vung Tau province. However, the growing areas have been narrowing and the local varieties have been being replaced by commercial varieties which are the major factors responsible for the genetic erosion of this crop in the province. In order to diversify tea products, bring convenience with many good medicinal properties to consumers, improve economic value, and take advantage of available domestic raw materials, conducting research on the processing of tea from custard apple is very necessary and appreciated by the requirements of society in the present time. The main purpose of this study is to investigate the applicability of hot-air drying technique and the effect of drying temperature agents on the loss of heat-labile natural compounds in custard apple tea production technology. First, this study examined suitable ages of custard apple fruit for tea production. Among the ages of 1, 1.5, and 2 months, the 1.5-month-age custard apple is more suitable for the tea production due to its high concentrations of total acid (0.149 %), vitamin C (2.392 mg/g), and total phenolic compounds (28.167 mg GAE/g). Next, this study investigated the influence of drying temperature on drying efficiency and loss of heat-sensitive natural compounds (vitamin C and total polyphenols), with temperatures of 40°C, 50°C, and 60°C. The results showed that the drying temperature at 50°C can be considered as the appropriate temperature for drying custard apple pulp to observe a minimum reasonable change in vitamin C and total phenolic content (0.935 ± 0.05 mg/g and 22.630 ± 1.744 mg GAE/g). Finally, the effect of the ratio of flowers added to tea has been investigated, with the ratio of flowers and pulp at 20%:80%, 30%:70%, 40%:60% and 50%:50%.... The results showed that the addition of 40% flowers provided the tea product with a golden yellow colour, a harmonious combination of the aroma and slight sweetness of flowers and pulp custard apple, a long-lasting scent, and no strange smell.

In conclusion, a especially green tea from fruit mixed with its flower of *Annona squamosa* was well produced with lab-scale for the first time in Vietnam

Keywords: Tea production *Annona squamosa* L., custard apple, pulp, flower, aroma.

In Vitro Propagation of Some Elite Varieties of Indonesian Sugarcane (*Saccharum officinarum* L.) Using Temporary Immersion Bioreactor

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Abstract. Indonesia has released some elite varieties of sugarcane that are suitable for Indonesian climatic and land conditions. Therefore, large quantities of these elite sugarcane seedlings are needed to support the Indonesia's sugar self-sufficiency programs. The large quantities seedlings can be provided through scaling-up in vitro propagation method using bioreactors, such as Temporary Immersion Bioreactor (TIB). Here, we conducted a study to analyze the response of different plant genetic materials on the in vitro propagation of some elite sugarcane varieties using TIB. The sugarcane varieties used for the study were the rainfed sugarcane varieties (PSKA 942, PS 094) and the irrigated sugarcane varieties (PS 091, PS 881), released by the Indonesian Sugar Research Institute. The plant materials used as explants for in vitro propagation were the young spindle leaves isolated from the plant canes. The in vitro propagation stages were explants preparation and sterilization, callus initiation, callus proliferation and regeneration in TIB, shoot maturation, and acclimatization. Results showed that all sugarcane varieties have been successfully induced to form callus, proliferate and regenerate in TIB. However, in general, the rainfed sugarcane varieties showed better response, growth and regeneration rate than the irrigated sugarcane varieties. The in vitro-derived sugarcane seedlings have been successfully obtained from all varieties except for PS 881.

Keywords: sugarcane, in vitro propagation, temporary immersion bioreactor

Isolation And Selection of Antagonistic Bacteria Against *Cercospora arachidicola* Causing Brown Spot on Peanut

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Abstract. This study was conducted with the aim to isolation bacterial strains capable of antagonism to *Cercospora arachidicola* (*Passalora arachidicola*) causing brown spot in peanut. From 3 soil samples collected in the rhizosphere of peanut grown in Tay Ninh province, Vietnam, 10 bacterial strains were found having antagonistic action with *Cercospora arachidicola*. After 5 days of testing, the antagonistic efficiency of all isolates ranged from 33.33 ± 1.28 to 60.183 ± 0.927 %. Studying the antagonistic mechanism showed that 5/10 isolates produced siderophore, 5/10 isolates were capable of decomposing chitin, 7/10 isolates capable of decomposing cellulase and 8/10 isolates proteolytic. Basing on the results of 16S rRNA gene sequence combined with morphological and biochemical characteristics, TN-TB 4, TN-TB 6 and TN-TB 12 were identified as *Bacillus*, representing the genera *Bacillus amyloliquefaciens*, *Bacillus pasterurii*, and *Bacillus velezensis*, respectively. These strains of antagonistic bacteria have the potential to produce probiotics.

Keywords: Antagonistic, *Bacillus*, bacteria, *Cercospora arachidicola*, isolation, peanut, selection.

Applied Microbiology

The Amount of Oral *Candida albicans* and mRNA Expression HWP1, YWP1 and ALS3 Genes in COVID-19 Patients with and without Non-Invasive Oxygen Supplementation

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Abstract. To evaluate the effect of non-invasive oxygen supplementation on the quantity oral *Candida albicans* and its associated biofilm gene expression. Twenty-four hospitalized COVID-19 patients were included and among them seven patients have been supported by non-invasive oxygen supplementation. Saliva and tongue swab were obtained for RNA extraction for further fungal counts and analysis the mRNA expression HWP1, YWP1 and ALS3 genes the by qPCR. No evidence that the amount of Oral *C. albicans* were affected by the non-invasive oxygen supplementation. The YWP1 and ALS3 expression were higher in saliva of patient with non-invasive oxygen supplementation ($p < 0.05$) whereas in the tongue surface the HWP expression was lower in patient with non-invasive oxygen supplementation. YWP1 and ALS3 genes expression were potential as biomarker of oral ecosystem dynamic due to non-invasive oxygen supplementation use in COVID-19 patients.

Keywords: *Candida albicans*; mRNA; HWP1; YWP1; ALS3 ; COVID-19; non-invasive oxygen supplementation

Production, Extraction, and Characterization of Violacein from *Chromobacterium violaceum* and its Antibacterial, Anticancer, Anti-biofilm, and Synergistic Antimicrobial Profiling

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Abstract. Violacein is a violet-colored pigment molecule and a microbial bioactive compound, produced mainly by *Chromobacterium violaceum* that exhibits several biological activities and has currently gained increasing importance in medicine, cosmetics, and textiles. Here, *C. violaceum* was isolated and identified from different sediment samples of China clay hill lake, Bangladesh (25°9'29"N, 90°38'36"E), based on their production of deep violet colonies on Luria Bertani (LB) agar, followed by microscopic, cultural, biochemical and molecular techniques using 16S ribotyping. The presence of the violacein-coding *vio* gene was confirmed in a polymerase chain reaction using *vio*-specific oligonucleotide primers. The addition of tryptophan (up to 2.3%) in the media (TSB and LB broth), and a sub-lethal dose of tetracycline (2 µg/ml) ensured the enhanced production of violacein from the bacterium. Violacein was purified after cell lysis by sonication followed by ethanol extraction, and its identity was confirmed by UV-Vis spectrophotometry, Fourier-transform infrared spectroscopy, and gas chromatography-mass spectrometry (GC-MS). The latter method revealed that violacein was co-purified with '9, 9- Dimethoxybicyclo [3.3.1] nona-2,4-dione' and 'Cholestan-7-one'. Inhibition of biofilm formation by *Staphylococcus aureus* and its eradication was achieved by applying violacein at 11.3 and 27.3 µg/ml respectively, while growth retardation was attained at 6.5 µg/ml. Other gram-positive pathogens: *Listeria monocytogenes*, *Streptococcus* spp., and *Bacillus* spp. showed profound sensitivity to the purified material. It was observed that violacein works synergistically with several commercial antibiotics like tetracycline, gentamicin, azithromycin, and kanamycin against the previously mentioned pathogens and could be a possible supplementary candidate in combination with other antimicrobial agents. The metabolite showed significant cancer cell cytotoxicity on HeLa cell, while no toxicity was found on the normal cell line (Vero cell). Overall, violacein has the potential to be used as a natural antimicrobial and anticancer agent, isolated and reported first from Bangladesh.

Keywords: *Chromobacterium*, antimicrobial, anticancer, metabolite, violacein

Isolation and Characterization of CuFeS_2 and FeS Oxidizing Bacteria from Acid Sulfate Soil in Kalimantan

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Abstract. Biooxidation in gold pretreatment process in the form of refractory gold is necessary to conduct. The biooxidation of gold-bearing sulphide produces sulfate. The oxidizing bacteria of metal sulfides can be isolated from acid sulfate soil in Kalimantan. This research was conducted to obtain and identify chalcopyrite (CuFeS_2) and troilite (FeS) oxidizing bacteria from the acid sulfate soil in Kalimantan. *The medium for isolation was done using thiosulfate glucose with a pH level of 4.2. Meanwhile, the medium for biooxidation process contained CuFeS_2 of 5.85 g/L or FeS of 5.56 g/L.* From cultivation, eight isolates were selected from the thiosulfate screening. Based on selection process for biooxidation, 1 strain namely tan2 that grow on ferrous-iron and sulfuric compounds was obtained. The concentration of sulfate [SO_4^{2-}] was measured using turbidimetry, while the concentration of total [Cu] and [Fe] were analyzed by Atomic Absorption Spectroscopy (AAS). *The concentration of sulfate [SO_4^{2-}] reached a maximum value of 91.29 ppm in CuFeS_2 biooxidation at hour-18, while within FeS biooxidation reached a maximum value of 21.87 ppm at hour-21.* Furthermore, identification using API 20NE kit and sequencing 16S rRNA were carried out. The results of the sequencing were analyzed using DNA baser and MEGA 6.0 in order to construct a phylogenic tree. Physiological and phylogenetic studies showed that the tan2 obtained a similarity percentage of 99.9% with *Burkholderia cepacia*. This oxidizing bacteria of the CuFeS_2 and FeS from the acid sulfate soil in Kalimantan had the potentials as oxidizing agent for metal sulfide.

Keywords: Biooxidation, refractory gold, pretreatment, acid sulfate soil, CuFeS_2 , FeS.

Metal Biosorption Process under Low pH Conditions for E-waste Recycling and Polluted Water Treatment

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Abstract. Owing to the rapidly developing information technologies, a large amount of electronic and electrical wastes (E-waste) has been produced all over the world. Disposal of E-waste causes not only the loss of metal resources but increases the risk of heavy metal pollution of water and impacts human health. Both E-waste recycling and metal removal from the polluted water are receiving increased attention from the point of view of resource security, environmental protection, and development of sustainable society. Due to the similar conditions (low pH, coexistence of various metal ions), some technologies like chemical precipitation and adsorption are applied to both metal separation from E-waste leachate and removal from polluted water. Biosorption is regarded as an environmentally friendly process and recently many studies have been performed. Although metal leachate and heavy metal polluted water are generally strongly acidified, most of biosorption studies focus on the process under neutral pH conditions. Thus, neutralization process is required. In this study, metal biosorption process under low pH condition was developed using acid-tolerant bacteria. Metal adsorbing acid-tolerant bacteria were isolated from neutral pH environments. Metal adsorption efficiency and selectivity of isolated strains were evaluated under acidic condition. According to the metal adsorption ability, four isolated bacteria and the bacterial type-strain *M. luteus* JCM1464 were applied to develop multiple metal adsorption process from metal leachate. All strains adsorbed multiple metals from simulated metal leachate (pH 1.5) containing 100 mg l⁻¹ each of Co, Cu, Li, Mn, and Ni. The maximum adsorption abilities were as follows: Co: 8.48, Cu: 20.09, Li: 6.20, Mn: 12.64, Ni: 9.45, and total metal ion: 11.47% (w/w), respectively. Although metal adsorption efficiency was lower than the conventional metal recycling process and biosorption process under neutral pH conditions, an E-waste recycling process without neutralization was established. For treatment of metal polluted water, this process was also applied to acidic river water (pH 2.12) supplemented with 100 mg l⁻¹ each of Co, Cu, Li, Mn, and Ni. With five isolated strains, the maximum removal abilities were as follows: Co: 7.25, Cu: 15.97, Li: 4.36, Mn: 7.15, Ni: 7.84%, and total target metal ion: 8.54% (w/w) were removed. The metal adsorption efficiency was lesser than the case of the simulated metal leachate. This result suggests that competitive adsorption occurred between target heavy metals and native metal ions in the river water. In this study, metal adsorption process under acidic conditions was established, and successfully worked on metal recycling and polluted water treatment. Although further improvement of metal adsorption efficiency is required, our process potentially contributes to the sustainable metal resource circulation from both E-waste recycling in metal consuming regions and polluted metal removal in metal producing regions.

Keywords: acid-tolerant bacteria, biosorption, e-waste, heavy metal pollution, metal recycling

Exploring Indonesia Natural Resources for Antibiofilm Agents

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Abstract. Natural resources continue to provide solutions for health problems including microbial resistance. Indonesia's diversity in natural resources and culture is among the highest in the world which offers enormous potential for new promising anti-infective. Considering that most microbes form biofilm in nature, we combine several approaches to explore natural resources as antibiofilm based on ethnomedicinal, ecological, random screening, and database approaches. Activities towards polymicrobial cultures are of interest since naturally existing biofilm consists of multistrain and even multispecies microbes. Biofilm matrix is one promising target for eradication, despite research involving its manipulation being limited. Our research combines the approach of providing suitable bioassays for screening the bioactives from from the terrestrial and marine environments. Several promising compounds are revealed by in vitro assays, but further research on its application by in vivo and clinical aspects are required.

Keywords: Indonesian, natural resources, antibiofilm, antiinfection

A Programmed Cell Death Pathway in *Entamoeba histolytica*

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Abstract. *Entamoeba histolytica* is a unicellular protozoan parasite that is devoid of caspase as well as a caspase-dependent apoptotic pathway. Apoptosis is generally considered to be evolved with multicellularity as a prerequisite for the elimination of aged, stressed, or infected cells that ensure the survival of the organism. Here we report that an Apoptosis-inducing factor (AIF)-dependent but caspase-independent cell death pathway operates in the unicellular parasite *Entamoeba histolytica*. Down-regulation of the EhAIF increases the number of viable cells in the stressed conditions compared to the wild-type cells as well as reduces the apoptotic features evident from trypan blue dye exclusion assay and flow cytometry. EhAIF migrates to the nucleus as observed by confocal microscopy and binds with the nucleic acids resulting in intracellular chromosome degradation leading to apoptosis under stress. The occurrence of apoptosis in unicells is an important phenomenon that might signify the altruistic death that improves the population's health.

Keywords: Entamoeba, apoptosis, apoptosis inducing factor, nucleic acids

Microbial Production of Violacein from Co-Utilization of Methanol and Acetate using a Metabolically Engineered Strain of *Methylobacterium extorquens* AM1

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Abstract. The global concerns over environmental pollution and putative health and safety issues are gradually pushing the demand to replace synthetic colorants with safer and more eco-friendly alternatives, such as microbial ones. Violacein, a blue-violet-colored pigment is of particular interest due to its wide range of bioactivities. Therefore, violacein is considered a potential compound for multiple applications in the pharmaceutical, cosmetic, food, and textile industries. Many metabolic engineering strategies have been applied to sugar-based industrial hosts to enhance the production of violacein with various degrees of success; however, the high cost of synthetic growth media is one of the main challenges for the commercialization of violacein production. Therefore, in addition to applying genetic engineering or systematic biology to improve production from sugar feedstocks, the development of host strains that can utilize non-sugar, inexpensive substrates to reduce production costs would enable the commercialization of violacein. To address this issue, we engineered a promising facultative methylotroph, *Methylobacterium extorquens* AM1, to develop a methanol-based microbial platform for violacein production. By optimizing gene expression level via screening promoters and inducer concentrations, 11.7 mg/L violacein production was first demonstrated using methanol as the sole substrate. Then, unidentified bottlenecks for violacein biosynthesis in the shikimate pathway of *M. extorquens* AM1 was addressed by random mutagenesis and site-directed mutagenesis, and a 2-fold improvement in violacein production was achieved. Finally, by co-utilization of methanol and acetate, a remarkable enhancement of violacein production to 118 mg/L was achieved. Our results contribute to the development of an economically efficient large-scale fermentation system for violacein production from non-sugar feedstocks.

Keywords: violacein, methylotroph, random mutagenesis, co-utilization, methanol, acetate

Characterization of Bioactive Compounds Isolated by a Corn Silk Fermentation

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Abstract. Harvesting corn produces corn silk residue which yields a positive waste product through fermentation. However, corn silk contains an abundance of bioactive compounds which possess various health benefits and help to avoid numerous chronic diseases, which included edema, cystitis, and gout. Its bioactive compounds have been used as a therapeutic attribute amongst Asian communities. This research aims to assess the bioactivity of the compounds that were isolated from corn silk strains by using a biological extraction, which used hydrolytic enzymes from the microorganisms of the fermentation. The fresh corn silk (*Zea mays*) from sweet corn and feed corn were fermented with a mixture of microorganisms, *Saccharomyces cerevisiae*, *Bacillus* sp., and *Lactobacillus* sp. The solid-state fermentation (300g fresh corn silk: 30g sucrose: 100g water in 1.6 L bottle); and the immersion fermentation (300g fresh corn silk: 300g sucrose: 900g water in 1.6 L bottle) processes were compared in this study. The solid-state fermentation corn silk samples were collected on day 7 and day 20 of the experiment, the fermented corn silk was then dried in a hot-air oven at 60°C for 24 h. The fermented dried corn silk was boiled (5g dry wt./150 ml water) for 20 min. While the immersion fermentation was incubated at 30°C for a few months, an aliquot was extracted and examined every month to test acidity, inhibition of xanthine oxidase, inhibition of antioxidant using the ABTS assay method; and in so, the amount of total phenolic content and flavonoids content were determined. The results showed that the highest phenolic content (5.17 ± 0.74 $\mu\text{g GAE/g dry wt.}$) and flavonoids content (20.14 ± 1.56 mg QE/g dry wt.), which was carried out using solid-state fermentation, was responsible for its antioxidant activity. The solid-state fermentation process of the fermented corn silk antioxidant activity was 9.91 ± 0.17 TEAC mg/g dry wt. of ABTS; equally important, process also produced antioxidant inhibition at 59.05 ± 0.95 %. The fermented corn silk product had a higher flavonoid content than unfermented product. In addition, The fermented sweet corn silk had the highest percentage of xanthin oxidase inhibition (81.06 ± 12.16 %) after one month incubation. The bioactive compounds isolated from both types of corn silk was fermented immersion for one month. This phase had the most significantly inhibition impact on the percentage of xanthine oxidase at p-value, less than 0.05. While the highest antioxidant inhibition using ABTS method was found in the fermented sweet corn silk using solid-state fermentation without incubation time effects. The results indicated that the fermentation could be used to isolate the bioactive compounds. The sweet corn silk was used to amalgamate the appropriate conditions used for the immersion fermentation process of 1 month. Therefore, due to the potential bioactivity and fermentation suitability, corn silk can be scaled up for industrial production levels.

Keywords: (antioxidant, biological extraction, corn silk, fermentation, xanthine oxidase inhibition)

Comparison Test of Extract Activity from *Syzygium aromaticum* L., and *Alpinia purpurata* as Anti-*Candida krusei*: Bioactivity Test

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Abstract. *Candida krusei* is the most common cause of candidiasis found in the bloodstream with a high mortality rate due to treatment failure. Intrinsic resistance to fluconazole is one of the causes of treatment failure of *Candida krusei* candidiasis. The development of natural ingredients extracts of *Syzygium aromaticum* L., and *Alpinia purpurata* which contain lots of phenolic compounds is one of the appropriate alternative solutions in dealing with the antifungal resistance of *Candida krusei*. This study used *Candida krusei* ATCC 6258 with ethanol extract *Syzygium aromaticum* L., *Alpinia purpurata* and fluconazole as positive controls. Growth control and negative control were included in each test to find the minimum concentration that could inhibit 50% of the growth of *Candida krusei* (MIC₅₀). The MIC₅₀ scores of fluconazole, ethanol extract of *Syzygium aromaticum* L. and *Alpinia purpurata* were 92,43 µg/mL, 0,031 µg/mL dan 1,435x10⁵ µg/mL. The percentage of inhibition of the ethanolic extract of *Syzygium aromaticum* L. was greater than that of the ethanolic extract of *Alpinia purpurata* and fluconazole with statistical results in general showing a significant difference (p<0.05).

Keywords: *Candida krusei*, ethanol extract of *Syzygium aromaticum*, ethanol extract of *Alpinia purpurata*

Homologous recombination of rumen *Escherichia coli* CCU-8 for the production of optically pure D-lactic acid

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Abstract. *Escherichia coli* CCU-8 is a novel bacterial strain isolated from bovine rumen that produces D-lactic acid with high optical purity. We used the λ -Red homologous recombination system to knock out some genes in chromosomes to increase the production of D-lactate. Genes in metabolic pathways that directly lead to byproduct formation, including, *pta*, *mgsA*, *gldA*, *ppc*, *lldA*, and *adhE*, were selected for deletion. After deletion of the acetate-forming gene, the recombinant strain *E. coli* CCU-8 (Δ pta) was able to produce more lactate than the wild-type strain. Lactic acid production by fermentation on glucose increased from 0.33 \pm 0.01 g/g to 0.70 \pm 0.07 g/g. After further removing the *mgsA* gene, the yield and productivity of lactic acid were further improved. The deletion of *mgsA* gene (methylglyoxal synthase) eliminated the low-level production of racemic lactate, resulting in a slightly improved optical purity of the product. When the bifunctional aldol dehydrogenase gene *adhE* was further removed, the ethanol production by the resulting recombinant strain *E. coli* CCU-8 (Δ pta Δ mgsA Δ adhE) was not significantly reduced compared with the strain without *adhE* removal, *E. coli* CCU-8 (Δ pta Δ mgsA). This study shows that removal of *pta* and *mgsA* genes from the chromosome of *E. coli* CCU-8 was enough to yield an ideal D-lactic acid production strain.

Keywords: rumen *Escherichia coli*, D-lactic acid, homologous recombination.

Engineering robust yeast strain for sustainable lactic acid production

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Abstract. Utilizing sustainable raw materials to produce bio-based chemicals requires a microbial host with high resistance to various by-products generated before and during the fermentation stage. This study harnessed a robust yeast strain from the Indonesian Culture Collection, identified as *Saccharomyces cerevisiae* BTCC3, displaying a strong tolerance against numerous pre-treatment-related chemical stressors to biosynthesize lactic acid. This non-lactic-acid producer strain was metabolically engineered to adjust its pathway by introducing an exogenous *L-LDH* gene from *Lactobacillus casei* and disrupting the *PDC1/5* genes to reduce the metabolic flux to ethanol, the most predominant competing product. Through this strategy, lactic acid titer could improve from 0 to 43.2 g·L⁻¹ (productivity = 4.8 g·L⁻¹·h⁻¹) and to 33.2 g·L⁻¹ (productivity = 3.7 g·L⁻¹·h⁻¹) under semi- and non-neutralized conditions, respectively. This engineered strain could also accumulate lactic acid from non-detoxified sugarcane bagasse at a productivity of 1.69 g·L⁻¹·h⁻¹, which was relatively similar to other studies that still performed detoxification steps to remove chemical inhibitors prior to cultivation. This study accentuates an approach to developing an industrial strain from naturally robust microorganisms using minimalistic genetic modifications rather than refining lactic-acid-producer hosts by extensive tolerance engineering steps.

Keywords: lactic acid; *Saccharomyces cerevisiae*; robustness; metabolic engineering

Long-Term Impact of Antimicrobials on the Efficiency and Microbiome of Anaerobic Digestion

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Abstract. Antibiotics are widely used in human and veterinary medicine, and they are accumulated in various types of waste, including sewage sludge (SS) and cattle slurry (CS), thus inhibiting the anaerobic digestion (AD), which is a method enabling the stabilization of these substrates before evacuating to the environment. The study was undertaken to evaluate the long-term impact of growing concentrations of a mixture of antibiotics on the AD of SS and CS. Due to the most popular classes of antibiotics used in agriculture and human medicine include beta-lactams, fluoroquinolones, and nitroimidazole derivatives, therefore, the experiment involved these three classes of antimicrobials that can potentially influence microorganisms participating in the entire anaerobic treatment process. The main aims of the study were investigation of the effect of selected antimicrobials on: 1) methane fermentation efficiency; 2) quantitative and qualitative changes in microbial consortia that participate in anaerobic digestion; 3) the fate of antibiotic resistance genes (ARGs) and the spread of antibiotic resistance (AR). The experiment with two replicates per bioreactor lasted 417 or 268 days for CS and SS, respectively. Methane (CH₄) yields, which is the main indicator of the efficiency of AD, were determined in substrates exposed to antibiotics and in control substrates without antimicrobials. Biogas quality was analyzed in a gas chromatograph equipped with a thermal conductivity detector (GC-TCD, Agilent Technologies 7890A). Metagenomic sequencing (Illumina Inc.) was performed to identify the influence of antibiotics on changes in microbiome and resistome of AD biomass. Antibiotic exposure significantly decreased CH₄ production only in SS. The domain *Archaea* was represented mainly by methanogenic genera *Methanotherix* and *Methanosarcina* (9 ppm) and the order *Methanomassiliicoccales* (15 ppm). Their abundance was considerably higher in AD samples collected from the process bioreactor than from the control bioreactor. These results indicate that the prevalence of *Archaea* in the process digester increased steadily in response to growing concentrations of the tested antibiotics. The copy numbers of the *mcrA* gene, a functional marker of methanogenesis, were not reliable indicators of CH₄ yields in either substrate. At the end of long-term AD, methanogens belonging to the family *Methanosarcinaceae* were more prevalent than methanogens of the family *Methanosaetaceae* in SS samples, whereas the reverse was noted in CS samples. This experiment demonstrated that the extent to which long-term exposure to antibiotics influences the effectiveness of AD is dependent on the type of processed substrate. The metagenomic analysis revealed that prolonged mesophilic AD of biomass in the presence of all three tested antibiotics inflows the structure of microbial populations and can modify the resistome profile and increase the relative abundance of individual ARGs in digestates. The exposure to antibiotics significantly increased the number of OTUs characteristic of *Archaea* in AD samples, but these changes did not affect the efficiency of biogas production. These findings suggest that higher concentrations of antimicrobials in biomass can substantially compromise the efficiency of anaerobic digestion.

Keywords: anaerobic digestion, antibiotics, methane production, sewage sludge, cattle slurry

Isolation and Identification of Thermophilic Bacteria from Cisolong Hot Springs Banten

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Abstract. The exploration and identification of thermophilic bacteria in Indonesia is crucial, considering their ability to produce widely used thermostable enzymes for industrial applications. Indonesia as a country situated atop the Ring of Fire has a lot of volcanoes, which often times have multiple hot springs surrounding the area. Hot springs are ideal environments for the growth of thermophilic bacteria. This study aims to isolate and identify thermophilic bacteria. Cisolong Hot Springs is an unexplored thermal spring situated near Torong Mountain, Banten. Two water samples from two different sources were collected from Cisolong Hot Springs, Source (S) and Pool (K). Bacterial cultures for the two samples were done using Gellan Gum agar media, single colonies were then selected and cultured in Luria Bertani broth. DNA extraction for both cultures was carried out using GeneAll® Exgene™ commercial kit. Extracted DNA are then amplified and sequenced for identification. Based on the 16S-rRNA bi-directional sequencing results, two different sequences of thermophilic bacteria from the two samples are identified. The Source (S) bacteria have a sequence length of 1518 bp while the pool (K) bacteria have a length of 1422 bp. Phylogenetic tree results show that the source bacteria is closest to *Geobacillus kaustophilus* strain BGSC 90A1 while the pool bacteria is closest to *Geobacillus thermoleovorans* strain V0.

Keywords: thermophilic bacteria, 16s-rRNA, DNA purification, *Geobacillus kaustophilus*, *Geobacillus thermoleovorans*

A Review of the Relationship between CD4 T-lymphocytes and COVID-19 Vaccination Efficacy in People Living with HIV (PLWH)

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Abstract. Due to weakened immune system, immunocompromised patients like HIV patients are one of the most vulnerable people during COVID-19 pandemic. Therefore, vaccination is very important to reduce the morbidity and mortality caused by SARS-CoV-2 infection among people living with HIV (PLWH). However, the interaction between SARS-CoV-2 vaccines and cellular immunity, such as CD4, is often times are neglected, despite CD4 cell count importance in HIV viral load control. This review aims to describe some of the findings related to the immune response of HIV patients to SARS-CoV-2 vaccines with regard to their CD4 cell count. The data search was performed on databases such as PubMed, Google Scholar, Embase and Scopus from 2019 to 2022. Our review found that the CD4 cell count in PLWH is responsible for the efficacy of SARS-CoV-2 vaccination and the CD4 cell count could decrease in response to SARS-CoV-2 vaccination, causing the HIV load to increase. In conclusion, CD4 cell count should be taken into consideration when administering vaccination to PLWH and careful supervision must be carried out in PLWH after vaccination to prevent adverse immune responses.

Keywords: HIV, COVID-19, Vaccine, Cellular immunity, CD4 T-lymphocytes

Potential of Crude Protease from *Bacillus tequilensis* HSFI-5 as Meat Tenderizer with Anticoagulant Activity Based on Protein Profile Analysis and Lee-White Test

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Abstract. Proteases are a family of enzymes that can hydrolyze proteins reasoning their potential as meat tenderizers in the food industry. On the other hand, a number of proteases also have potential as anticoagulant agents in the case of cardiovascular disease (CVD) caused by thrombus. Thus, proteases having both abilities as meat tenderizing agent as well as blood anticoagulation agent are important health food ingredients to be discovered. This study aimed to explore the potential of the bacterial crude protease of *Bacillus tequilensis* HSFI-5 isolated from intestine of sea cucumber in degrading proteins of meat samples and in preventing human blood clotting *in vitro*. The ability of crude protease in degrading meat proteins was determined using the Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) method through protein profile analysis with beef, chicken and milkfish meat soaked with crude protease of *B. tequilensis* HSFI-5 for 4 h as samples. The anticoagulant activity of crude enzymes on human blood was determined by the Lee and White method. The results showed that the crude protease *B. tequilensis* HSFI-5 had the ability to denature beef protein sized 100 kDa (correlated with α -actinin). In addition, bacterial crude proteases also showed anticoagulant activity (with clotting time of 16 mins 30 s) compared to negative controls (only 5 mins clotting time). It can be concluded that the crude bacterial protease HSFI-5 has potential as a beef meat tenderizing agent with blood anticoagulation activity.

Keywords: Anticoagulant, *Bacillus* sp. HSFI-5, bacterial protease, meat tenderizer, SDS-PAGE

Muconic Acid Production from Glucose and Xylose in *Pseudomonas putida* via Evolution and Metabolic Engineering

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Abstract. Muconic acid is a bioprivileged molecule that can be converted into direct replacement chemicals for incumbent petrochemicals and performance-advantaged bioproducts. In this study, *Pseudomonas putida* KT2440 is engineered to convert glucose and xylose, the primary carbohydrates in lignocellulosic hydrolysates, to muconic acid using a model-guided strategy to maximize the theoretical yield. Using adaptive laboratory evolution (ALE) and metabolic engineering in a strain engineered to express the D-xylose isomerase pathway, we demonstrate that mutations in the heterologous D-xylose:H⁺ symporter (XylE), increased expression of a major facilitator superfamily transporter (PP_2569), and overexpression of *aroB* encoding the native 3-dehydroquinate synthase, enable efficient muconic acid production from glucose and xylose simultaneously. Using the rationally engineered strain, we produce 33.7 g L⁻¹ muconate at 0.18 g L⁻¹ h⁻¹ and a 46% molar yield (92% of the maximum theoretical yield). This engineering strategy is promising for the production of other shikimate pathway-derived compounds from lignocellulosic sugars.

Keywords: muconic acid, adaptive laboratory evolution, *Pseudomonas putida* KT2440, shikimate pathway, performance advantaged bioproducts

Halophiles-Based Synthetic Biology and its Applications in Biomaterials and Pharmaceuticals

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Abstract. *Halomonas bluephagenesis* TD01 is one of ideal chassis for the low-cost industrial production based on “Next Generation Industrial Biotechnology”, yet the limited genetically regulatory parts have hampered the engineering and wide applications of the strain. By bioinformatics mining and synthetic biology design, a series of genetic parts with gradient activities especially the transcriptional terminators were developed for the tuned gene expression and product synthesis in this chassis. Based on the metabolic engineering and synthetic biology approaches, the environmental-friendly biomaterials polyhydroxyalkanoates (PHAs), were proven to be produced in *H. bluephagenesis* in high yield. Meanwhile, a chiral drug L-DOPA and a healthy sweetener D-piscose were efficiently synthesized by a robust biocatalyst-the functional PHA nano-granules immobilized with the target proteins, expanding the applications of *H. bluephagenesis*. Our studies will contribute to the extensive engineering and applications of *H. bluephagenesis* TD01 in the low-cost production of various chemicals.

Keywords: *Halomonas bluephagenesis*, Transcriptional terminators, Polyhydroxyalkanoates, D-piscose, Low-cost production

Enhanced Production of Triterpenoids in Engineered *Saccharomyces cerevisiae*

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Abstract. Triterpenoids have lots of important physiological and pharmacological activities and have been widely used in medicine, health care, industrial and agricultural production. Ursolic acid (UA) is a kind of plant-originated high-valued triterpenoid with α -amyrin as the precursor, exhibiting diverse functions such as anti-inflammatory, anti-oxidation, diabetes treatment, and anti-bacteria. The synthesis of α -amyrin and UA have been achieved in engineered *Saccharomyces cerevisiae*. However, the low yield highly limits their industrial applications. The formation of α -amyrin from (3S)-2,3-oxidosqualene is catalyzed by α -amyrin synthase (α AS). And the low catalytic activity of α AS and the toxic effect of α -amyrin have been considered key elements. In this study, MdOSC1 from *Malus domestica* as well as some other α ASs were identified. MdOSC1 had significantly higher specific activity of α -amyrin. Therefore, MdOSC1 was introduced into *S. cerevisiae* combining with the increased supply of (3S)-2,3-oxidosqualene to achieve the encouraging α -amyrin production, and the yield of α -amyrin achieved 11.97 ± 0.61 mg/L. After that, MdOSC1 was remodeled and the yield was increased to 11-fold higher than that of the control by the triple mutant MdOSC1N11T/P250H/P373A obtained based on the modeling analysis. Furthermore, key genes of MVA pathway were overexpressed to provide sufficient precursors, and the intracellular storage capacity was expanded. As a result, the highest yield of α -amyrin was obtained in engineered *S. cerevisiae* with 213.7 ± 12.4 mg/L in the shake flask and 1107.9 ± 76.8 mg/L in fed-batch fermentation; the fermentation yield was 106-fold higher than that of the original strain under the same conditions, representing the highest α -amyrin yield in yeast reported to date. Based on this, CYP450 and CPR were mined and introduced into *S. cerevisiae* to synthesize UA. The production of UA was increased via cofactor engineering and acetyl-CoA optimization. Finally, the yield of UA was improved to 2.3 g/L, which is the highest reported yield to our knowledge.

Keywords: triterpenoids, microbial cell factories, *Saccharomyces cerevisiae*, metabolic engineering

Genome Mining Approaches for New Antimicrobial Agents Discovery

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Abstract. Microorganisms are known to be a rich source of unique bioactive compounds, which are valuable for the search for drugs and lead compounds. For example, many commercially available antibiotics and more than 120 essential therapeutic agents known to date are derived from microorganisms. Advances in high-throughput sequencing methods have led to various in silico genome mining strategies being developed and applied to identify natural product biosynthetic gene clusters (BGCs) in microbes. Genome mining studies reveal that microbes may have different putative BGCs. Furthermore, the various types of putative BGCs in these microbes indicate that these microbes have the potential to produce new compounds. Thus, linking BGC to the encoded product is critical in discovering new secondary metabolites. In this mini-review, we discuss the steps required in utilizing genome mining to search for novel antimicrobial agents in microorganisms. In addition, the successful uses of this method are shown here.

Keywords: microorganisms, genome mining, biosynthetic gene clusters, secondary metabolites

Lignin Valorization by Metabolic Engineering of *Pseudomonas taiwanensis* VLB120

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Abstract. Lignin is the most underutilized part of the lignocellulosic biomass that can be exploited for producing bio-based chemicals. But the major challenge of lignin valorization is its high recalcitrance and the toxicity exhibited by lignin-derived aromatics (LDAs). In this work, we have engineered *Pseudomonas taiwanensis* VLB120 to efficiently utilize LDAs such as p-coumarate, ferulate, 4-hydroxy benzoate, vanillate, and caffeate. This was achieved by metabolic engineering approaches that included genomic integration of ferulic operon for utilization of non-native substrates, p-coumarate, ferulate, and caffeate. Also, the tolerance towards these LDAs was achieved by adaptive laboratory evolution that resulted in improved growth rates. Sequencing results have revealed several mutations mainly in two genes *actP* (acetate transporter) and *paaA* (phenyl acetyl-CoA oxygenase) that can be the potential targets for reverse engineering to obtain platform strains with high tolerance. Also, the growth of the metabolically engineered strain on LDAs was coupled with the biosynthesis of rhamnolipids which are high-value biosurfactants. We were successfully able to demonstrate the production of rhamnolipids using all the LDAs as the sole carbon by the engineered strain. To valorize the lignin-rich part of the biomass leftover after saccharification of the pre-treated rice straw and corn cob will be utilized. This will contain a solid lignin-rich fraction which will be subjected to depolymerization using mild base catalysis. The depolymerized lignin obtained will be used to produce rhamnolipids by the engineered strain. Overall, this study will form a basis for circular bioeconomy by recycling waste to useful products using an eco-friendly approach.

Keywords: lignin valorization, lignin-derived aromatics, metabolic Engineering, *Pseudomonas taiwanensis* VLB120, adaptive laboratory evolution, rhamnolipids

Metabolic Engineering of *Methylovimicrobium alcaliphilum* 20Z for Production of Alkaloid and Sesquiterpenoid from Methane

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Abstract. Indole alkaloids and sesquiterpenoids are subgroups of natural products normally present in plants. These compounds have been used in many industrial applications. Thus, the production of natural products using microorganisms with short-term cultivation has emerged recently. Among carbon substrates to produce value-added compounds, methane is considered a promising source with low price and abundance. Herein, *Methylovimicrobium alcaliphilum* 20Z was metabolically engineered to produce two representatives of alkaloids and sesquiterpenoids, indigo and α -farnesene. Expression of tryptophanase (TnaA) from *Escherichia coli* and flavin-containing monooxygenase (Fmo) from *Methylophaga aminisulfidivorans* enabled the engineered strain to produce 1.3 $\mu\text{g/L}$ of indigo. To unravel the bottlenecks in the shikimate pathway, *E. coli*-originated feedback-resistant enzymes (AroG and TrpE) were additionally expressed. As a result, indigo titer was enhanced 2.7-fold to 3.5 $\mu\text{g/L}$, but the growth of the engineered strain was impaired. Initially, α -farnesene was produced with a titer of 42.61 mg/L in the engineered strain expressed a codon-optimized apple α -farnesene synthase (Afs) and native farnesyl diphosphate synthase (IspA). To overcome the limitation in α -farnesene biosynthesis pathway, we employed a ribulose 5-phosphate-interconnecting methylerythritol phosphate shortcut route, resulting in the production of 48.98 mg/L of α -farnesene and defective cell growth. xylose was used as a co-substrate to restore the growth of recombinants expressing the bottleneck-bypassing enzymes. Finally, 6.3 $\mu\text{g/L}$ of indigo and 91.55 mg/L of α -farnesene, which were 4.8-fold and 2.1-fold higher than the initials, were gained. This work offers prospective ways to increase the production of natural compounds from waste and renewable sources.

Keywords: methane, xylose, indigo, α -farnesene, methanotrophic bacteria, metabolic engineering

Biocatalysis and Protein Engineering

Improved PET-Hydrolyzing Enzymes Activity by Fusion to Class I Hydrophobin

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Abstract. Polyethylene terephthalate (PET) fibers could be hydrolyzed by PET hydrolases, however with a slow rate. Some strategies have been developed to overcome the limitation including pretreating PET with hydrophobin protein. Hydrophobin, a cysteine-rich fungal protein, can generate hydrophobic-hydrophilic interface thereby facilitates strong binding between hydrophobic PET and PET hydrolases. Class I hydrophobin HGFI was designed to be fused with *Ideonella sakaiensis* PETase and *Thermobifida fusca* cutinase (Cut_2) to accelerate their PET hydrolysis rate. HGFI fused Cut_2 could be successfully expressed as a soluble protein in *Escherichia coli*. However, soluble expression of HGFI fused PETase (HGFI-PETase) could not be achieved. As a solubility enhancing partner, cellulose binding domain (CBD) was fused to HGFI-PETase (CBD-HGFI-PETase) and its soluble expression was obtained. Both PET hydrolases maintained their esterase activities against p-nitrophenyl acetate after fusion with HGFI and CBD. Cut_2 demonstrated a 7 fold higher esterase activity than that of PETase. On the contrary, PETase showed 2.5 fold higher PET fibers hydrolysis rate than Cut_2 based on a 5 day hydrolysis endpoint analysis. The stimulatory effect of HGFI and CBD fusion on PET hydrolysis was demonstrated by 1.8 and 2.6 fold enhancement on the amount of PET monomers released by PETase and Cut_2 hydrolysis.

Keywords: Hydrophobin, PETase, cutinase, fusion protein, PET recycling

Identification and Expression of Novel Cytochrome P450 Monooxygenases from *Thamnidium elegans* and Its Biocatalytic Potentials in the Bioconversion Reaction of Various Substrates

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Abstract. Cytochrome P450 monooxygenases (P450s) are probably the most abundant enzymes and diversely distributed in all kingdoms including in fungi. Fungi provide a diverse and complex array of P450s, which are essential for numerous secondary metabolic synthesis and biodegradation of xenobiotic compounds. In addition to their physiological effects on fungi, P450s are attractive for use in cutting-edge biotechnology applications due to their versatile properties. Herein, we report the gene identification and functional characterization of P450s from the zygomycete *Thamnidium elegans* (TeCYPs). From the entire genome sequence of *T. elegans*, we identified 48 TeCYP genes, including two putative pseudogenes. In addition, 46 TeCYPs were heterologously expressed in *Saccharomyces cerevisiae* to prepare a functional library of TeCYPs. The recombinant strains of *S. cerevisiae* were then applied as whole-cell biocatalysts for the bioconversion of numerous compounds. Through a functionomic investigation, the catalytic potentials of various TeCYPs for converting a variety of compounds, including steroidal substrates, were demonstrated. Interestingly, we identified a novel P450, CYP5312A4, which capable to catalyze a rare hydroxylation reaction of testosterone: 14 α -hydroxylation. This is the first report to identify a previously unknown fungal P450 that catalyzes the 14 α -hydroxylation of testosterone. In addition, we investigated the latent potentials of other TeCYPs by utilizing a variety of substrates. This study serves as a foundation for future research into the potential use of TeCYPs as catalysts in the pharmaceutical, agricultural, and biotechnology industries.

Keywords: cytochrome P450 monooxygenase, *Thamnidium elegans*, heterologous expression, whole-cell biocatalysis, testosterone.

Functional Modulation of Biopolymers by Biocatalysts: From Bioconjugation to Sustainable Bioproduction

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Abstract. Biocatalysts have been widely utilized in various fields related to bioscience and bioengineering. In particular, the range of applications of enzymes that recognize biopolymers and synthetic polymers as substrates has attracted wide attention in recent years. For example, enzymes that degrade cellulosic biomass such as cellulases and their accessory enzymes have shown their potential in the sustainable production of biochemicals and bioenergy. More recently, a hydrolytic enzyme that can degrade a synthetic polymer such as polyethylene terephthalate (known as PETase) has shown to be a potent biocatalyst for the decomposition of plastics. By contrast, enzymes that catalyze the formation of cross-links between (bio)macromolecules have also shown a range of applicability. For example, peroxidase has been utilized to form hybrid hydrogels comprising of natural biopolymers and a synthetic polymer such as polyethylene glycol (PEG). Transglutaminase, a protein-crosslinking enzyme, has also been utilized in the preparation of functional foods as well as various types of novel bioconjugates. In this talk, potential approaches by focusing either on bond-breaking or bond-forming reactions catalyzed by enzymes will be presented with a special focus on the design of biomaterials and bioconjugates for biomedical applications.

Keywords: Chitinase, Hydrogel, Lipid, Membrane, Peroxidase, Silkworm, Transglutaminase

Enzymatic protein crosslinking under macromolecular crowding conditions yields highly polymerized protein assemblies

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Abstract. The cytoplasm consists of biomacromolecule such as proteins and nucleic acids with high concentration (300-400 mg/mL). This crowded environment is one of the characteristics of solutions in vivo. Different protein behavior has been reported in such molecular crowding solutions, compared with that in an aqueous buffer solution, owing to the excluded volume effect. A number of studies to understand protein behavior in vivo, especially enzymatic reactions have been reported in model molecular crowding environments prepared by synthetic polymers. However, previous reports have mainly focused on reactions using small molecules as substrates. Microbial transglutaminase (MTG) catalyzes acyl transfer reaction between the side chains of glutamine (Q) and lysine (K) residues and is capable of cross-linking a wide range of substrates from small molecules to peptides and proteins. MTG has thus been widely used in the preparation of bioconjugates and hybrid materials that consist of proteins and the other kind of biomaterials. Although MTG has been widely applied for the preparation of functional foods and biomaterials, its catalytic behavior in molecular crowding environment has not been investigated. Herein, we report our recent findings on the MTG-mediated crosslinking reaction behavior under crowding conditions prepared by dextran (Dex) with various molecular weights and their monomer unit, glucose. Previously, we reported a versatile strategy to obtain protein polymers consisting of a single protein as a monomeric unit by MTG reaction. We prepared green fluorescent protein (EGFP) mutant fused with a designed peptide tag including MTG reactive sites (PolyTag, HKRWRHYQRGG) at its N-terminus (PolyTag-EGFP) as a substrate in this experiment, and confirmed the formation of EGFP polymers by the MTG treatment. A typical reaction conditions of this research are as follows. PolyTag-EGFP (10 μ M) and MTG (0.5 Unit/mL) were mixed in 1 \times PBS solution (pH 7.4) including 0~15 wt% of glucose, Dex 60k and Dex 500k. The reaction solution was incubated at 37°C for 1, 5, 10, 15, 60 min and EGFP polymer products were evaluated SDS-Poly-Acrylamide Gel Electrophoresis (SDS-PAGE) and the band intensity was measured by Image J software. After an hour incubation PolyTag-EGFP with MTG in crowding environment containing Dex 60k and 500k, the formation of huge EGFP polymers were observed, which was not observed in PBS. To better understand the details of the polymerization behavior, kinetic analysis of MTG-mediated PolyTag-EGFP was performed in both PBS and 15 wt% Dex 500k solution. As a result, an increase in the initial rate of cross-link product formation was observed in the crowding environment, which can be attributed to the excluded volume effect of Dex 500k in the reaction media.

Keywords: Molecular crowding environment, microbial transglutaminase, protein polymer

Active site remodeling of amine transaminases guided by computational design

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Abstract. Amine transaminases are important toolkits in the industry to synthesize chiral amines that are valuable building blocks of a number of pharmaceutical drugs and fine chemicals. Amine transaminases have attracted great attention for the synthetic applications owing to their unique capability affording the enantioselective transfer of an amino group to prochiral ketones from cheap amino donors such as isopropylamine. However, the restricted range of the acceptable substrates has been challenging to expand a product pipeline using the amine transaminases. Therefore, engineering of the substrate specificity of wild-type enzymes is highly demanded to allow the production of diverse chiral amines with enhanced variants that fit the industrial process setup. Here I present recent progress in the engineering of amine transaminase guided by computation modeling. Mechanistic analysis of the Michaelis complex and the critical reaction intermediates was carried out to explain the catalytic properties of the amine transaminases. The computational model could explain how active-site mutations affect enzyme activities for a given substrate. Eventually, active-site redesign based on the computation prediction enabled the creation of engineered variants displaying desired catalytic properties.

Keywords: chiral amines, machine learning, transaminase

Machine learning techniques for functional annotation of enzyme sequences

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Abstract. Enzymes are characterized by functional classification methods such as enzyme commissions (ECs) numbers based on the reaction mechanism, in addition to sequence information encoded in the genome. Functional annotation of many enzyme sequences depends on sequence homology based on local alignment, etc., but it is also true that there are unexplained sequences that cannot be annotated. Recent advances in machine learning technology are changing the perception of the sequence similarity itself. I will introduce an example of machine learning technology for functional annotation of enzyme sequences.

Keywords: bioinformatics, machine learning, enzyme

Production of Tyrian purple indigoid dye from tryptophan in *Escherichia coli*

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Tyrian purple, mainly composed of 6,6'-dibromoindigo (6BrIG), is an ancient dye extracted from sea snails and was recently demonstrated as a biocompatible semiconductor material. However, its synthesis remains limited due to uncharacterized biosynthetic pathways and the difficulty of regiospecific bromination. Here, we introduce an effective 6BrIG production strategy in *Escherichia coli* using tryptophan 6-halogenase SttH, tryptophanase TnaA and flavin-containing monooxygenase MaFMO. Since tryptophan halogenases are expressed in highly insoluble forms in *E. coli*, flavin reductase (Fre) that regenerates FADH₂ for the halogenase reaction was used as an N-terminal soluble tag of SttH. A consecutive two-cell reaction system was designed to overproduce regiospecifically brominated precursors of 6BrIG by spatiotemporal separation of bromination and bromotryptophan degradation. These approaches led to 315.0 mg/L 6BrIG production from tryptophan and successful synthesis of regiospecifically dihalogenated indigos. Furthermore, it was demonstrated that 6BrIG overproducing cells can be directly used as a bacterial dye.

Keywords

Halogenated indigoids, Tryptophan halogenase, Flavin-containing monooxygenase, enzyme solubility, whole-cell biotransformation

The Production and Characterization of Penicillin G Acylase from *Bacillus thuringiensis* BGSC BD1 Wild Type

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Abstract. Penicillin G Acylase (PGA) is the responsible enzyme for hydrolysis of β -lactams antibiotics. It became important since the majority process to produce these antibiotics are enzymatical. One which is amoxicillin. In this study, a wild type *Bacillus thuringiensis* BGSC BD1 among three other isolates from Bioindustrial Lab, LAPTIAB, BRIN Culture Collection are selected as the best producer of the enzyme. The PGA production in LB media was tried for five times with the PGA activity stable around 3 U/ml with harvesting time at around 20 hour of fermentation. The characterization of the enzyme showed optimum in a pH range of 7-8 with maximum temperature up to 60 °C. The SDS-PAGE result showed two bands with molecular weights around 26 and 41 kDa that represent the α and β units of PGA.

Keywords: Amoxicillin, Penicillin G Acylase (PGA), *Bacillus thuringiensis*, Wild Type, Characterization

Better understanding of protein functions by using deep learning for successful metabolic engineering

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Abstract. Successful metabolic engineering requires accurate understanding and optimal engineering of a production host's metabolism. Metabolic engineering has now been better facilitated by the increasing availability of bio big data and the use of machine learning methods. Here, our recent deep learning models will be presented, which were developed to better understand protein functions. First application of deep learning is to predict enzyme commission (EC) numbers with high precision in a high-throughput manner by taking a protein sequence as an input. Prediction of EC numbers is essential for accurately understanding enzyme functions and metabolism, especially during genome annotation. Second application is to examine the feasibility of a large number of retrobiosynthesis-derived enzymatic reactions. Retrobiosynthesis helps systematically design novel biosynthetic pathways for the production of a target chemical, but often generates a very large number of candidate reactions, which makes experimental validation challenging. Deep learning can facilitate the implementation of retrobiosynthesis by substantially reducing a large number of candidate enzymatic reactions. Third application is to predict changes in the expression level of genes in a microbial cell in response to environmental conditions, for example medium composition. Continued efforts in developing computational models along with generation of meaningful biological datasets will innovate our approaches to metabolic engineering.

Keywords: Metabolic engineering, Bio big data, Deep learning, Protein function

Determination of Kinetic Parameters of Cholesterol Oxidation using Cholesterol Oxidase from *Streptomyces* sp

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Abstract. Cholesterol oxidase (CO) can be used as an enzyme-based cholesterol biosensor application. However, the enzymatic behavior of cholesterol oxidation still cannot be defined clearly. In this research, the kinetic of cholesterol oxidase was studied by using the first order irreversible reaction. The catalytic activity of the crude enzyme was compared to the commercial enzyme. In order to reach the optimum conditions for enzymatic cholesterol oxidation, the effect of initial cholesterol and enzyme concentration were investigated. The cholesterol concentration was rapidly measured by HPLC. The kinetic model was derived and verified according to the experimental data. The enzyme was successfully produced by submerged fermentation method of *Streptomyces* sp and obtained 69 U/mL of enzyme activity. The produced enzyme was diluted into 15×10^{-1} , 7.5×10^{-3} and 3.75×10^{-3} U/mL. In oxidation reaction, the cholesterol was prepared in three different concentration, 3.23, 6.46 and 12.93 mM, respectively. The enzyme was able to oxidize substrate up to 84% within 240 minutes. Further, both the commercial CO (EC 1.1.3.6) and crude CO showed slightly different in oxidation reaction for 60 minutes. However, if it was compared to the commercial enzyme with the oxidation time for 240 minutes, the crude CO was slower in oxidizing the substrate. The fitted result of the kinetic model showed that the irreversible reaction model can describe the cholesterol oxidation behavior. The kinetic rate constant was reached 0.0141/min for 0.15 U/mL commercial CO and 0.010/min for 0.15 U/mL crude CO.

Keywords: cholesterol, cholesterol oxidase, enzyme, Kinetic Model, *Streptomyces* sp

Harnessing the designability and evolvability of enzymes for producing valuable compounds

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Enzymes find wide applications in many areas due to their high catalytic efficiency and specificity, and single or multiple enzymes are used for synthesis of valuable compounds. In many cases, microbial metabolic pathways comprising a number of different enzymes are exploited for producing diverse metabolites through sequential biocatalysis. Despite great potentials and crucial roles in the industrial biotechnology, some drawbacks of enzymes in terms of catalytic efficiency and specificity have limited practical applications. Many efforts have been made to improve the enzyme property by various approaches including structure-based rational design, directed evolution and computational method, showing notable successes. Over the years, my lab has focused on the design and evolution of enzymes with greater potential for producing aromatics and amino acids. We improved catalytic efficiency and substrate specificity of enzymes by rational approach and directed evolution. In addition, a key enzyme in a biosynthetic pathway was engineered to construct a simpler and more efficient metabolic pathway for production of valuable chemicals. In this lecture, I will introduce our recent works on enzyme engineering for producing valuable products.

Keywords: Enzyme, design, evolution, metabolic pathways, chemicals

Accessing Secondary Alcohol Oxidation with Galactose Oxidase for API Manufacturing

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Abstract. Alcohol oxidation is a key chemical transformation in synthetic routes for specialty chemicals including active pharmaceutical ingredients (APIs). Carbonyl (C=O) functional groups introduced through alcohol oxidation are convenient reactive handles amenable toward further modification. However, chemical alcohol oxidation typically involves elevated temperatures, stoichiometric oxidants, and toxic by-products. In contrast, biocatalytic alcohol oxidation possesses advantages such as high selectivity, mild reaction conditions, benign by-products, and sustainable operation in water instead of organic solvents.

In this work, we combined the power of prediction models with a systematic and comprehensive saturation mutagenesis survey of the key domains of the galactose oxidase enzyme to create an industrially relevant enzyme panel for secondary alcohol oxidation. We first significantly improve its activity against a challenging bulky secondary alcohol substrate. Subsequent engineering further expanded the galactose oxidase's substrate scope to include new substrates not accepted by the parent, improved its stability, and increased its solubility, thus improving the overall properties of the enzyme for biomanufacturing.

Keywords: Enzyme engineering, Galactose oxidase, Enzyme panel, Alcohol oxidation, Enzyme modelling

Optimization of Culture Medium of Cellulolytic Enzyme Isolated from Termite Gut in Indralaya Peatlands, Indonesia

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Abstract. Bioethanol is an energy source that has the potential to be developed as an eco-environmentally energy. One of the technologies used to produce bioethanol from cellulosic biomass is enzymatically. Termite is the organisms that consume cellulose as a food source. In this study, cellulolytic bacteria from termite gut were optimized to increase the cellulolytic enzyme production. Several factors can affect the growth and production of cellulase enzymes. In this research, optimizing the culture medium followed by different carbon sources (glucose, fructose, sucrose, galactose, and maltose); various concentration of carbon sources (0,5%; 1%; 1.5%; 2%; 2.5%; 3%; 3.5%; 4%; 4.5% w/v); diverse nitrogen sources (peptone, yeast extract, urea, sodium nitrate, and ammonium sulphate); various concentration of nitrogen sources (0,5%; 1%; 1.5%; 2%; 2.5%; 3%; 3.5% w/v); pH (3-9); temperature (27, 33, 37, 45, 50, and 55°C); and agitation (100; 120; 150; 200; 250 rpm) were examined. A basal medium containing [(g/L): 1.8 K₂HPO₄; 1 NaNO₃; 0.5 KCl, 0.9 MgSO₄.7H₂O; 2 yeast extract; 10 pepton; 10 CMC; pH 5]. The result showed that medium optimal with higher enzyme activity was obtained in the addition of a carbon source of fructose with concentration of 3.5%; nitrogen source of sodium nitrate with a concentration of 2%; pH of 5; temperature of 45°C; and agitation of 150 rpm.

Keywords: bioethanol, cellulolytic enzyme, termite

Engineering Cascade Biotransformations for Chemical Synthesis

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Abstract. Biocatalysis is non-toxic and chemo-, regio-, and stereo-selective, thus being a useful tool for green and sustainable chemical synthesis. Moreover, biocatalysis is an attractive tool for one-pot multi-step synthesis via cascade biotransformations, avoiding the expensive, yield-reducing, and waste-generating isolation of the intermediates in conventional multi-step synthesis. Great progress has been achieved in this field. Nevertheless, new types of cascade reactions and practical cascade biotransformations are highly wanted for the manufacturing of chemicals. In this presentation, some representative examples of our work on development of new types of cascade biotransformations for practical synthesis of several important classes of fine chemicals from easily available substrates (simple chemicals and bio-based chemicals) will be addressed, including a) enantioselective conversion of racemic alcohols to chiral amines[1], b) enantioselective conversion of simple alkenes to chiral 1,2-vicinal diols, 1,2-aminoalcohols, α -hydroxyacids, and α -aminoacids, as well as, acids, alcohols, and amines[2-7], and c) synthesis of some of these chemicals from bioderived L-phenylalanine.[8,7].

Keywords: Biocatalysis, Biotransformation, Cascade reactions, Chemical synthesis

Bioenergy and Biorefinery

Increasing Cellulosic Ethanol Production by Enhancing Phenolic Tolerance of *Zymomonas mobilis* in Adaptive Evolution

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Abstract. Cellulosic ethanol fermentability of ethanologenic strain *Zymomonas mobilis* is severely inhibited by phenolic aldehydes generated from lignocellulose pretreatment. Here, a 198 days' laboratory adaptive evolution of *Z. mobilis* 8b in corn stover hydrolysate was conducted to increase its phenolic aldehydes tolerance and ethanol fermentability. The obtained *Z. mobilis* Z198 demonstrated a significantly improved conversion of the most toxic phenolic aldehyde (vanillin) by 6.3-fold and cellulosic ethanol production by 21.6%. The transcriptional analysis using qRT-PCR revealed that the gene ZMO3_RS07160 encoding SDR family oxidoreductase in *Z. mobilis* Z198 was significantly up-regulated by 11.7-fold. The overexpression of ZMO3_RS07160 in the parental *Z. mobilis* increased the ethanol fermentability to that of the adaptively evolved strain *Z. mobilis* Z198. This study provided a practical method to obtain a robust cellulosic ethanol fermenting strain, and a candidate gene for synthetic biology of biorefinery strains with strong phenolic aldehydes tolerance.

Keywords: *Zymomonas mobilis*, Lignocellulose, Adaptive evolution, Phenolic aldehydes inhibitors

Enhanced Carbohydrate Accretion via Discarded Potato Peels Utilization in Marine Cyanobacterial Isolates: A Sustainable Approach for Bioethanol Production

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Abstract. Global energy demand has prompted the quest for alternate fuel sources. Due to the depletion of existing fossil fuel supplies, alternative energy sources are sorely needed. Global shortages of energy resources have collapsed monetary policy. It is anticipated that if fossil fuels burn at the present pace, all fossil fuels will be consumed by the year 2060, and biofuel may help in this regard. At present, there are many established approaches to make biofuel, like as using plant-based products. Our goal is to reduce pollution while producing biofuels that are eco-friendlier. Bioethanol from cyanobacteria has potential as a sustainable biofuel, as some cyanobacterial species exhibit extremely high carbohydrate storage capacities under a variety of nutritional combination. A major obstacle to commercially feasible production of cyanobacterial fuel and value-added products is the high cost of nutrients required for algal cultivation. Currently, potatoes are commonly used at large-scale in the food processing industry worldwide. However, as a by-product, these industries produce enormous quantities of potato peels as wastes (PPW) which is often discarded. PPW are zero-value wastes containing essential nutrients and elements which could be explored for the development of carbohydrate-rich cyanobacteria. In this investigation, PPW was used in the growth medium as a low-cost nutrient source for the development of marine cyanobacterial cells. The PPW was collected, dried and crushed to peel powder. Different concentrations of peel powders were subjected to physical pre-treatment followed by autoclaving and then supplemented to novel seawater medium as a mixotrophic nutritional source and biomass yield and the carbohydrate accretion in marine *Synechococcus elongatus* were investigated. PPW were found to be a rich source of essential nutrients like lysine (an imperative factor in cellular metabolism and participates in carbon fixation) and sugars. The carbohydrate content in the PPW was recorded to be more than 50% after physical pre-treatment. Growth and carbohydrate accumulation were found to be increased significantly in cultures supplemented with PPW. Also, the maximum carbohydrate productivity was found to be ~2-fold higher than control. The results clearly showed that supplementation of PPW resulted a surge in carbohydrate pool of more than 35%. This increased carbohydrate output owing to the addition of PPW as a nutritional component to the test organism cultures resulted in more than 40% bioethanol conversion. Consequently, it was shown in this study that these discarded food wastes may be used as a naturally occurring nutrition source for the growth of cyanobacterial species that is cost-effective, environmentally friendly, and economical.

Keywords: Bioethanol, carbohydrate, cyanobacteria, nutrients, potato waste

Influence of Indoor and Outdoor Culture Condition on Bioethanol Production from Marine Cyanobacterium *Leptolyngbya valderiana*: A Comparative Study

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Abstract. Continuous exploitation of energy has jeopardized fossil fuel resources, which also influences the environment and the global economy. Bioethanol production by marine cyanobacteria is one of the sustainable, renewable resources which can potentially provide constant appetite of the nation. Moreover, marine cyanobacteria can also easily thrive on seawater. Thus, a combination of seawater together with the effective application of marine microalgae and cyanobacteria would lead to a noble approach towards cost-effective bioethanol production. Yet, very few reports have been available on bioethanol production under outdoor and indoor conditions to date. Therefore, the objective of the study was to evaluate bioethanol production from *Leptolyngbya valderiana*, a marine cyanobacterium. It was cultured under outdoor shade (24 to 38°C, 140- 158 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 60-85% RH) and laboratory conditions (control condition, 25°C, 75 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 80% RH) and obtained data were compared based on biomass yield, carbohydrate accumulation followed by bioethanol yield. Outdoor cultures of cyanobacterium have significantly higher biomass yield (~1.1) than those grown under indoor conditions. Carbohydrate yield was considerably higher in outdoor conditions (505.4 mg L⁻¹), whereas carbohydrate content was low in outdoor conditions (32% dcw). In carbohydrate composition analysis, no significant difference was found in reducing sugar and glycogen accumulation under two different situations. In addition, bioethanol production exhibited that *L. valderiana* attaining more carbohydrate yield under outdoor conditions, producing more bioethanol yield (227.4 mg L⁻¹) compared to indoor conditions (218.9 mg L⁻¹). This study illustrated that outdoor cultivation of cyanobacteria could be feasible despite the fluctuating environmental regime.

Keywords: bioethanol, biomass yield, carbohydrate accumulation, *Leptolyngbya valderiana*, culture condition

Effects of Sodium Bicarbonate Supplementation on Lipid Accumulation of *Chlorella sp.* Cultivated in Artificial Medium

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Abstract. Microalgae are promising sources for biofuels, food products, and secondary metabolites for pharmaceutical purposes. *Chlorella sp.* is one of the microalgae strains known to have relatively high lipid and protein, carbohydrates, and a wide range of secondary metabolites. However, high operation costs and relatively low biomass production are challenges in the mass cultivation of this microalgae. In this study, the effect of sodium bicarbonate on *Chlorella sp.*'s growth, including biomass, lipid and carbohydrate content, and fatty acid compositions, were studied under the combination of urea and Walne nutrient as the cultivation medium. Sodium bicarbonate in the concentration of 1.0, 2.5, and 5.0 g/L was supplemented to the culture in the batch cultivation method with continuous illumination and constant aeration. The control sample was treated using the same medium without sodium bicarbonate supplementation. As the result, the addition of sodium bicarbonate for all concentrations significantly affected the growth profile of *Chlorella sp.*, shown by its cell and biomass productivity compared to the control culture without sodium bicarbonate ($p < 0.05$). The microalgae cultivation with 1.0 g/L sodium bicarbonate had the highest biomass concentration, reaching 0.7600 ± 0.0278 g/L and 0.1623 ± 0.0020 specific growth rate. Fatty acid compositions of microalgae oil from *Chlorella sp.* supplemented with sodium bicarbonate were found dominated by unsaturated fatty acid from C₁₄-C₂₀ with linoleic acid in the highest FAME concentration (23.43%). As a comparison, fatty acids of microalgae oil from the control culture were mainly composed of saturated fatty acid with palmitic acid in the highest concentration (21.91%). From these results, microalgae oil extracted from wet biomass of *Chlorella sp.* grown in sodium bicarbonate has more potential to be essential lipids that can be consumed as a food supplement.

Keywords: *Chlorella sp.*, lipid, fatty acid methyl esters, sodium bicarbonate, urea-walne

Evaluation of Various Sodium Bicarbonate Supplementations in the Lipid Accumulation of Marine Microalgae *Nannochloropsis oculata* for Biodiesel Production

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Abstract. Microalgae are unicellular microorganisms with a fast growth rate and potential raw materials for biofuels, nutritional food, and pharmaceutical products. Amongst all microalgae species, *Nannochloropsis oculata* is a species that has a promising composition of biomass which mainly consists of carbohydrate, lipid, protein, and high-value components, such as β -carotenoid. Modifying the strain cultivation medium can impact the yield of those components, particularly the supply of additional carbon as a macronutrient. In this study, sodium bicarbonate supplementation as an additional carbon nutrient in the range 0.1-0.5 g/L was applied to investigate its effect on the *Nannochloropsis oculata* growth profile and the biomass content. As a result, the increment in biomass and cell productivity improved due to the induction of sodium bicarbonate compared to the control medium without additional carbon nutrient. The highest biomass and cell productivity was indicated in 0.5 g/L supplementations. The carbohydrate content of biomass showed an opposite trend, with the highest percentage found in the culture without sodium bicarbonate ($52.87\% \pm 4.81$) and the lowest ($29.07\% \pm 8.70$) in the culture with 0.5 g/L addition. This result was also followed by lipid content analysis which the highest lipid extracted from microalgae wet biomass was obtained from the culture with 0.5 g/L sodium carbonate addition. Fatty acid compositions of the microalgae oil from all extracted wet biomass were also analyzed to evaluate the implication of sodium bicarbonate addition on the distribution of fatty acid methyl ester compounds. The study showed that the cultivation of *Nannochloropsis oculata* in F/2 Guillard standard medium with sodium bicarbonate addition is suitable for enhancing lipid production and can be applied in large-scale cultivation for biodiesel production.

Keywords: biodiesel, lipid, microalgae, *Nannochloropsis oculata*, sodium bicarbonate

Extraction and Isolation of Cellulose Fiber from Ulva Seaweed using Green Deep Eutectic Solvent

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Abstract. Macroalga, known as seaweed, is a potential aquatic biomass for the production of sustainable biochemical products. The simple aquatic plants grow rapidly and can be cultivated in a short period of time. In addition, the cultivation of seaweed does not need fresh water and land area like terrestrial plants. Therefore, in the future, seaweed will become a sustainable biomass feedstock resource. Seaweed contains cellulose 1.5-21.6% (dw) and more than 34% in the by-product fraction of seaweed processing. The cellulose feedstock can be processed for various cellulose derivative products, which are used in the fields of wastewater treatment, electronic sectors, food packaging, and biomedical engineering. However, the cellulose processing needs some sequential steps such as delignification, bleaching, and hydrolysis with high concentration reagents, high temperature, and a long processing time. The process will need high energy input and high-cost operation to treat the waste generated. Currently, deep eutectic solvents (DESs) have great attention as a new medium for biomass pretreatment due to their significant merits, including easy to prepare, stable chemical property, low cost, recyclability, and eco-friendliness. In this study, choline chloride-urea DES was used to treat cellulose of Ulva seaweed obtained from by-products of ulvan processing. The concentrations of choline chloride-urea were 10, 20, and 30% (w/w), and hydrochloric acid as one of the common methods was used as the control. The structures of pretreated biomasses were characterized by SEM, XRD, FTIR, and TGA. Ulva seaweed was dominated by carbohydrate fractions mainly ulvan (26.5%), hemicellulose (23.8%), and cellulose (10.4%). The yield of biomass from ulvan extraction by-product was 7%. The biomass may contain high cellulose. XRD analysis shows that seaweed biomass had more amorphous structure than that of crystalline Avicel. The cellulose was pretreated with DES (Choline Chloride-Urea) and HCl. SEM images show that the biomass pretreated by DES formed a small fiber structure whereas pretreated with HCl showed a swelling structure. The pretreated biomass with HCl showed cellulose II whereas the biomass pretreated with DES showed an amorphous structure. The results were supported by FTIR analysis results. TGA analysis showed that seaweed biomass was more degraded than Avicel with microcrystalline cellulose, and the treatments with DES caused the lowering of their thermal stability.

Keywords: biorefinery, extraction, DES, macroalgae, seaweed by-products

Advanced Bio-Production Using Designer Microbial Cell Factories

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Abstract. Biosynthetic production such as porphyrins, heme, and their proteins not only play critical roles as an essential component in natural systems but also have attracted much attention as a high value specialty chemical in various fields, including renewable energy, cosmetics, medicines, and foods. Recently, our studies on the biotechnological production of porphyrins, heme, and their proteins using microbial cell factories from renewable carbon sources have been reported to replace petroleum-based production. These results provide recent advances in their biotechnological production using engineered microbes developed by various engineering techniques such as systems metabolic engineering, synthetic biology, protein engineering, and membrane engineering and up-to-date consolidated information on biosynthesis pathways and cellular regulations. In this talks I would like to introduce their applications in various fields and then discuss challenges and future perspectives.

Keywords: biosynthesis, enzyme complex, metabolic engineering, synthetic biology, microbial cell factory

Continuous Biodiesel Synthesis from Vegetable Oil Using Ion Exchange Resin as Heterogeneous Catalyst in Packed Bed Reactor

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Abstract. Ion exchange resins as heterogeneous catalysts can be used for the synthesis of biodiesel for alternative fossil fuels. The use of ion exchange resins in the solid and activated phase can make the separation process easier, can be reactivated, and used repeatedly, thereby reducing the need for catalysts. In this study, the ion exchange resins used Lewatit MP-64, Amberlite IRA410Cl, and Diaion PK208LH. This biodiesel production is carried out in a packed bed reactor with a transesterification process using vegetable oil and methanol as substrates. Then, regeneration of the resin used in the first cycle will be carried out, then this process will be tested regarding the stability of the resin and the yield of biodiesel produced. The results showed that under the optimum conditions; dry weight of resin was 4 grams, synthesis time was 3 hours, the anion exchange resin activation time was 3 hours; Lewatit obtained biodiesel yield of 94.06%, Amberlite obtained 90.00%, and Diaion obtained 73.88%. The stability test of the regenerated Lewatit showed that this resin still had good activity after being used for 4 cycles of biodiesel synthesis, namely is still capable of producing biodiesel with a yield of more than 80%.

Keywords: ion exchange resin, continuous, biodiesel, packed bed reactor

Bioconversion of Biomass in Thailand for Biorefineries Production

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Abstract. At the Plant Biomass Utilization Research Unit, Chulalongkorn University, we explored the potential of biomass in Thailand as feedstock for bioethanol and biorefineries production. A number of biomass feedstock were collected including agricultural residues, tropical weeds and industrial wastes, and then evaluated for their potential for bioethanol production. The lignocellulolytic microbes were successfully isolated from various habitats in Thailand. Cellulolytic bacteria and fungi were obtained and investigated for cellulase production and bioconversion. Hemicellulolytic fungi, mainly black yeast *Aureobasidium* spp., were isolated and explored for their potential in biotechnology. Biodegradation of xylan, extracted from various biomass, by beta-xylanase and beta-xylosidase were studied. The enzymes produced from *Aureobasidium* spp. were characterized and applied for biotechnology. This black yeast also produce a variety of commodities including biopolymer (pullulan and beta-glucan), biosurfactant, siderophore and antibiotics. We successfully optimize the production of these products and also investigated their potential in food application. Utilization of lignin and liginin-degrading fungi were also investigated.

Keywords: biomass, enzymes, bioconversion

Probing the Current Advances in Biodiesel Production Utilizing Oleaginous Consortia

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Abstract. The energy crisis is becoming a very significant problem, especially with regard to the concern of mineral oil depletion. The world's total primary energy consumption is gradually increasing with urbanisation as well as increase in population leading to a huge rise in energy consumption that eventually results in excessive greenhouse gas emission and environmental pollution thus the major effort remains to go for addressing such issues through eco-friendly and sustainable manner. In order to mitigate the alarming energy crisis, the scientists and engineers work together on such acute issues on biodiesel production utilizing algae as the major livestock. Moreover, a hybrid method of production *via* phycomyco co-cultivation or open source reservoirs for biodiesel production is considered to be a good alternative that may bring a potential economic shift in the area of energy sector. The present article emphasises the strategic attempt on the production of biodiesel from co-cultivation/ open culture condition and its appropriate characterization which may turn to address the rapidly growing energy consumption and high energy intensity level. For the present study, different microorganisms including *Chlorella minutissima* MCC27, *Aspergillus awamori* and oleaginous bacteria have been used for the production of lipid.

Keywords: biodiesel; de-novo and ex-novo lipids, *Aspergillus awamori*, *Chlorella minutissima* MCC27, myco-phyco cultivation

Optimization of Furfural Liquid-Liquid Extraction from Oil Palm Empty Fruit Bunch Hydrolysate Solution with Solvent Variations

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Abstract. Furfural is a compound that is widely used in the pharmaceutical, resins, and petroleum industry with continuous increase of price and demand every year. Liquid-liquid extraction is a purification method with great potential as a more sustainable option for furfural purification to overcome high energy consumption in furfural production process. Research on furfural purification using liquid-liquid extraction method is conducted with the aim of increasing yield and purity of furfural product from OPEFB hydrolysate, which is a lignocellulosic biomass with abundant amount in Indonesia. In this research, solvent with high affinity towards furfural such as 1-butanol, MIBK, and toluene were used as solvent at extraction temperature of 65°C, 85°C, and 105°C, with extraction time of 30, 60, and 90 minutes. Determination of furfural concentration in the extract was carried out using HPLC. Optimization of the operating condition was done with Response Surface Methodology (RSM) to obtain optimum value of each parameter and investigate the effects of extraction time, temperature, and solvent selection on furfural yield. This study has shown that the optimum operating condition for furfural liquid-liquid extraction is at 105°C for 30 minutes using MIBK as extraction solvent, which resulted in 49.103% furfural yield. The optimum condition was selected in order to provide the most efficient condition that produces the highest yield of furfural.

Keywords: liquid-liquid extraction, furfural, HPLC, hydrolysate model solution, RSM, OPEFB

Catalytic Depolymerization of Technical Lignin in Supercritical Ethanol over A Series of Copper-Based Catalysts to Produce Aromatic Monomers

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Abstract. Nowadays, the utilization of renewable resources to replace fossil fuels is extremely attractive and urgent due to the increasing environmental issues as well as the inevitable depletion of petroleum resources. Among the renewable alternatives, lignin—the second most abundant biopolymer and the richest source of aromatics in nature is considered a promising candidate due to its widespread availability, high abundance, remarkable functionality, and inexpensive price. In terms of lignin valorization, catalytic depolymerization via hydrogenolysis has been reported as an outstanding process owing to its great efficiency in producing aromatic monomers. In this study, a series of copper monometallic catalysts supported on MIL-101(Cr), prototypical metal-organic framework support, were synthesized as non-noble metal-based catalysts and used for the catalytic hydrogenolysis of technical lignin. Also, supercritical ethanol was employed as the solvent, hydrogen donor, and capping agent for the hydrogenolysis reaction of lignin. At first, the optimal copper catalyst for lignin hydrogenolysis was firstly selected by evaluating its catalytic performance, especially the resultant monomer yield. To increase the overall monomer yield, the reaction conditions for catalytic hydrogenolysis of technical lignin were systematically optimized to maximize the total monomer yield. Herein, the optimal conditions were determined to be 6 h of reaction time, 20 min of sonication pretreatment, 50% catalyst loading, and 5% lignin loading. Indeed, an aromatic monomer yield of 38.5% was successfully achieved under these conditions. Moreover, this depolymerized lignin stream, which is mainly composed of G-type monomers, can serve as a promising aromatic feedstock and carbon source for further microbial upgrading and bioconversion to produce various value-added products such as biodegradable plastics.

Keywords: catalytic depolymerization, lignin hydrogenolysis, technical lignin, aromatic monomers, supercritical ethanol, copper monometallic catalyst, metal-organic framework

Formulation of Herbal Solid Soap with Illipe Oil as Raw Material with Ginger Extract as Antibacterial

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Abstract. The world market for natural cosmetic products reaches a value of \$34.5 million in 2018 and \$54.5 million in 2027. Due to increasing consumer awareness of natural cosmetic products, this research makes soap formulations made from Illipe oil with ingredients. organic ginger extract. With the addition of ginger extract, it can be an antibacterial alternative to solid soap. This study aims to determine the optimal composition of Illipe oil and herbal active ingredients and have the appropriate specifications to make formulations made from Illipe oil with quality, moisture effect, and skin health with the presence of better antibacterial activity. The observed characteristics were pH, foam antibacterial, moisture, hardness, and the test against *Staphylococcus aureus* and *Escherichia coli*. The results of the transparency test showed that the soap with formula 2 had optimal conditions, namely with a pH of 9.75, foam inhibitor 86%, hardness 9.5 mm, humidity 76.9 AU, and the ability to test bacteria against *Staphylococcus aureus* and *Escherichia coli* are 15 mm and 12 mm with a soap sample concentration of 500 mg/ml.

Keywords: antibacterial, ginger, Illipe oil, herbal soap, antibacterial test

Biochemical Reaction Kinetics of Biobutanol Production from Pineapple Waste Using Coupled Fermentation- Pervaporation System

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Abstract: In this study, biobutanol production from pineapple waste hydrolysate using *Clostridium acetobutylicum* as microorganism in coupled fermentation-pervaporation setup as Acetone-Butanol-Ethanol (ABE) fermentation reactor was investigated. Biochemical reaction kinetics rate equation and its kinetic parameters were determined for the fermentation of pineapple waste hydrolysate to butanol in (ABE) fermentation system. The acetone, butanol and ethanol fermentation products were simultaneously removed as soon as they are produced by coupling the fermentation with pervaporation system. The effect of temperature and circulation flowrate on butanol permeate flux, separation factor, and butanol yield was determined. It was found that at higher temperature and circulation rate enhanced butanol flux, but overall butanol yield was lower as higher temperature negatively affected the fermentation process. It was further observed that the coupled system still produced more butanol compared to fermenter only, with 28.87% increase of butanol yield from the best combination at 35°C and 0.15 L/min. Compared to fermentation only, the application of pervaporation into the fermenter allowed 10 times higher butanol concentration in the permeate mixture, which was clear and almost free of biomass. Additionally, kinetics of the coupled system was analyzed, and under the effect of pervaporation, higher butanol tolerance was achievable with 4 times higher, interpreted from kinetic parameters. The kinetic model of the coupled system also showed good data fitting with R² higher than 0.81. Overall, it can be concluded that the coupled system has great potential for ABE fermentation, and the study of its biochemical kinetics can be useful for process design and optimization.

Keywords: butanol fermentation, pervaporation, PDMS, pineapple, *Clostridium acetobutylicum*

Black Soldier Fly Larvae Nanochitin-Stabilized Pickering Emulsions for Encapsulation Development by Response Surface Methodology

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Abstract. Nowadays, environmental issues prompt us to reconsider our eating and living habits. Black Soldier Fly Larvae are among the most promising alternative sources of chitin that have emerged. Chitin, the primary polysaccharide in insect exoskeletons, has considerable potential and may be treated into compounds with high value-added. In addition, there is a rising interest in manufacturing biopolymer-based Pickering emulsions due to the worldwide need for bioproducts and a suitable replacement for synthetic surfactants. In order to generate a stable emulsion, an ultrasound-assisted Pickering emulsion stabilized with Nanochitin was formulated and refined using response surface methodology (RSM). Nanochitin concentrations of 0.2% to 0.4%, vitamin E concentrations of 1% to 3%, and oil/water ratios of 1:9 to 3:7 were proposed as independent variables in the emulsion. This study was examined a number of potential applications of these emulsions: to encapsulate and deliver bioactive ingredients; to control the digestion of emulsion lipids; and to measure the antioxidant activity. The physicochemical composition of the newly synthesized formulation was evaluated using a variety of characterization methods. The results show that stable Pickering emulsions can be prepared using insect-based chitin nanofibrils. These emulsions are highly stable against droplet coalescence during storage, which have the ability of the nanofibers to adsorb onto the surface of the oil droplets and form a thick particulate layer that protects them from coalescence. It is able to adsorb lipid droplets on the surface firmly during homogenization and able to stabilize them with droplets size of 766.40 ± 12.30 nm. The results revealed that 0.3 NCh/3 VE/1:9 O/W were feasible to obtain the desired emulsion. It exhibited high encapsulation efficiency of 97.56 ± 0.20 %, and high antioxidant activity of 1.7 ± 0.09 g/mL. These insect-Nanochitin may be useful as edible particle-based emulsifiers to form more sustainable pharmaceutical and food emulsions.

Keywords: black soldier fly larvae, nanochitin, pickering emulsion, RSM

Green Synthesis of Multiwalled Carbon Nanotubes Bifunctionalized with Amine and Sulfonated Organosilane and Determination of its Catalytic Activity for One-Pot Conversion of Oils to Biodiesel

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Abstract. This study focuses on green synthesis of multiwalled carbon nanotube (MWCNT) bifunctionalized with amine and sulfonated organosilane, this functionalized multiwalled carbon nanotube was used as catalyst for transesterification of oils extracted from coconut and non-edible seed. The catalytic activity for one-pot conversion of high free fatty and oil was determined. Previous study was on an amine-functionalized MWCNT was prepared for use as a basic heterogeneous catalyst for the conversion of *Cocos nucifera* oil and *Hibiscus cannabinus* oil to biodiesel. The 3-aminopropyltrimethoxysilane was chosen to form an amine-reactive surface to bind with hydroxyl and carboxyl groups of oxidized MWCNT. Silanization took place using a green surface modification method in which supercritical CO₂ fluid was utilized under the following conditions: 55°C, 9MPa, and 1 h. The synthesized catalyst was characterized using Thermogravimetric analysis, Fourier transform infrared, Field emission scanning electron microscopy–energy dispersive x-ray, Time-of-flight secondary ion mass spectrometry, X-ray powder diffraction, and Brunauer–Emmett–Teller. Transesterification of *Cocos nucifera* oil using 10 wt% NH₂-MWCNT catalyst (3 wt% APTMS), 12:1 molar ratio of methanol and oil at 63°C for 1 h resulted in a >95% conversion. On the other hand, the same catalyst was used in the transesterification of kenaf oil, and formation of ammonium carboxylated salt was observed. In another previous study, a green surface modification method was also applied to produce a solid acid catalyst for biodiesel production. It was prepared from MWCNT and 3-mercaptopropyltrimethoxysilane oxidized in H₂O₂. The CO₂ under supercritical condition with ethanol solvent allows swift transportation and promotes uniform distribution of organosilane groups on randomly entangled and layered orientation of MWCNT. The catalyst was characterized by using Field emission scanning electron microscopy–energy dispersive x-ray, Thermogravimetric analysis, Fourier transform infrared spectroscopy, X-ray powder diffraction, Brunauer–Emmett–Teller and Time-of-Flight secondary ion mass spectrometry. The catalytic activity of the catalyst was tested using a high free fatty acid *Hibiscus cannabinus* oil and its fatty acid methyl esters were identified after simultaneous esterification and transesterification reactions. An optimum conversion of 93.1 % was recorded using an supercritical CO₂ synthesized catalyst at the following transesterification conditions: temperature = 63°C, methanol:oil ratio = 14:1, 10 wt. % catalyst and time = 240 min. In this study, the amine functionalized carbon nanotubes were mixed with sulfonated organosilane carbon nanotubes and was used as bifunctionalized carbon nanotube catalyst. In another case, the carbon nanotubes are functionalized by both amine and sulfonated organosilane to form a bifunctionalized catalyst. The coexistence of these incompatible species, nearby on the same solid particle surface was also characterized and tested its catalytic performance. In both cases there is only a slight improvement of catalytic activity, but this study has shown the potential of having bifunctionalized carbon nanotube as transesterification catalysts for biodiesel production.

Keywords: biodiesel, carbon nanotube, organosilane, supercritical carbon dioxide, transesterification

Supercritical Fluid Reactive-Extraction of Mahogany (*Swietenia Macrophylla*) Seed Lipid Using Subsequent (*Trans*)esterification Process for Biodiesel Production

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Abstract. This study investigated the cheap, indigenous, and non-edible Mahogany (*Swietenia macrophylla*) seed in response to feedstock sustainability and economic viability challenges in biodiesel production. Subsequent (*trans*)esterification (STE) process was employed in the experiments opting to increase the extraction efficiency and the biodiesel yield. The first step is that the methanol-macerated seed is subjected to uncatalyzed *in-situ* (*trans*) esterification followed by a catalyzed (*trans*)esterification all with the aid of supercritical carbon dioxide. This process was used to create fatty acid methyl ester by combining supercritical carbon dioxide, methanol, and a catalyst. The activated multi-walled carbon nanotubes (MWCNT) catalysts MWCNT-H, MWCNT-OH, and the bifunctional catalyst MWCNT-H + MWCNT-OH were examined in this work to see how temperature, pressure, and carbon dioxide flowrate affected them. The Taguchi technique was used to design the experiment and analyze its optimization. The analysis revealed that the reaction temperature affects the FAME yield the least. Using MWCNT-H (level 1), the maximum conversion and biodiesel yield was achieved with the following settings: 90°C (level 3), 30 MPa (level 3), and 15 ml.min⁻¹ CO₂ flowrate (level 3). It follows that since saponification is prevented, MWCNT-H is the best kind of catalyst to utilize, according to the main effects plot for means. However, upon analyzing the main effects plot for signal-to-noise ratios, it came out that the maximum conversion and biodiesel yield can be achieved at the following optimum conditions: MWCNT-H (level 1), 90°C (level 3), 30 MPa (level 3), and 7 ml.min⁻¹ CO₂ flowrate (level 2). As these factors are crucial to the simultaneous (*trans*)esterification and diffusion process, increasing reaction temperature and CO₂ flowrate will result in a proportionate rise in biodiesel production. The study has given producers of biodiesel inexpensive feedstock and a more straightforward STE process.

Keywords: subsequent (*trans*)esterification process, Taguchi, *Swietenia macrophylla*, *in-situ* (*trans*)esterification

The Synergistic Effect of the Carbon Dioxide-Ethanol-Water System on the *In-situ* Fatty Acid Ethyl Ester Production from Wet Mahogany (*Swietenia Macrophylla*) Seed at Supercritical Conditions

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Abstract. This study involves *in-situ* (trans)esterification process of seed lipid from Mahogany (*Swietenia macrophylla*) involving the synergy of *in-situ* formation of the catalyst, carbonic acid, and carbon dioxide-expanded ethanol-water system. This technique provides cheaper production cost since it eliminates the energy-intensive process which is the drying of seeds and utilizing cheap solvent, azeotropic ethanol and the recoverable co-solvent, carbon dioxide. Upon investigation, it was observed that higher water loading provides better extraction efficiency and conversion when operated low conditions. Pressure increase has also provided good effect on the oil recovery. This is attributed to the increased expansion of the solvent by compressed CO₂, which not only enhanced the transport properties of the solvent, but also provided extrusion and swelling power towards the seed matrix. As pressure is increased, more CO₂ is dissolved in oil, and the volume of oil expanded as well, resulting in a significant drop in the viscosity of oil and an increase in oil recovery. In addition, miscibility of oil to CO₂ were greatly improved under the effect of supercritical ethanol. Even if a better yield is achieved at high conditions, this can be complimented by less process steps and cheaper alcohol. In addition, miscibility of oil to CO₂ were greatly improved under the effect of supercritical ethanol. Improvement of oil recovery was observed which is associated to the increased amount of H⁺ in the solution from the dissociation of the acid promoted by the hydrogen bonding and affinity between the oil and the solvent. Finally, the formation of carbonic acid brought by the reaction of CO₂ and water played a vital role as a cheap *in-situ* catalyst that led to the increase of conversion and eventually, the yield. In conclusion, the novel and simpler technique employed in this study transpired significant potential in producing fully renewable and cheap biodiesel which is due to the positive synergistic effect of the carbon dioxide-ethanol-water system.

Keywords: mahogany, *in-situ* (trans)esterification, azeotropic ethanol, synergistic effect, supercritical carbon dioxide

Seawater as a Medium for Lignocellulolytic Enzyme Production by Halotolerant Fungi using Oil Palm Empty Fruit Bunch (OPEFB) in Solid State Fermentation

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Abstract. Oil palm biomass is widely known for its potential as a renewable resource for lignocellulolytic enzymes due to its lignocellulosic content and availability. The use of OPEFB for lignocellulolytic enzymes reduce the risk of environmental pollution from open burning activities of OPEFB. The process of lignocellulolytic enzymes production from EPOFB involved high consumption of freshwater. As an alternative to freshwater, sea water which contain macro and micronutrient can be used as a fermentation medium. The potential application of sea water and EPOFB for lignocellulolytic enzyme production by halotolerant fungi isolated from mangrove area at Tanjung Piai was conducted in solid state fermentation (SSF). The sea water consisted of Magnesium, Potassium, Sodium, Chloride, Zinc and other trace elements which contribute as a nutrient for fungi growth and metabolism. Four halotolerant fungi isolated from sediment with decayed wood (SSE) mangrove area at Tanjung Piai and the eight fungi from different location were screened qualitatively and quantitatively for cellulolytic, xylanolytic, and ligninolytic enzymes activities under solid state fermentation process. Out of 12 strains, the highest lignocellulolytic enzymes producer was showed by strain SSE.PUZ. The phylogenetic analysis of fungi strain SSE.PUZ shows it has 100% similarities with *Aspergillus oryzae*. Medium development for cellulolytic enzymes using combination of Mendel medium and sea water was first carried out using conventional method of one-factor-at-a-time (OFAT). The optimum conditions for cellulolytic enzymes production based on OFAT are 50% v/v of seawater, 800 µm size of OPEFB, 60% moisture content, initial pH 6.0 and temperature was 30°C. The medium was further optimized using Central Composite Design (CCD) in which response surface was generated later from the derived model. An experimental design of four variables including initial pH, sea water concentration, moisture content and temperature were created using Design Expert® Software, Version 12.0. The design consists of 30 run of experiments, which include 6 replicates at center points. The optimal value for cellulolytic activity for each variable are seawater concentration 51.29% v/v; moisture content, 64.363%; pH 5.2; and temperature 29.67°C with predicted value of 17.37 U/g. These predicted parameters were tested and the final cellulolytic enzyme activity obtained was 19.303 U/g, which is slightly higher than the predicted value. The optimal condition of Mendel medium with sea water showed an improvement of cellulolytic activity by 46.46% as compared to cellulase activity from the OFAT with 13.18 U/g.

Keywords: sea water, EPOFB, lignocellulolytic enzymes, halotolerant fungi, mangrove

Techno-Economic Analysis of Bioethanol Production from Palm Oil Empty Fruit bunch

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Abstract. Bioethanol has become more attractive as an alternative to fossil-based fuel: a biofuel and fuel additive to gasoline. Almost ethanol production is produced by the fermentation process. Therefore, people are interested in ethanol from a feedstock that does not compete with the food supply. Oil palm empty fruit bunch are major biomass by-products from the palm oil industry. This study proposes commercial-scale bioethanol production from EFB to produce ethanol 99.5 wt.% and 10,000 L/day. A bioethanol production process from EFB was modeled using the Aspen Plus commercial simulation program divided into four stages: pretreatment, hydrolysis, fermentation, and purification. EFB is pretreated using hot water, hot-compressed water, and alkaline hydrogen peroxide approaches, with a simultaneous saccharification and fermentation approach chosen to convert the raw materials into ethanol. In steady-state conditions, it can conclude that the ethanol production rate was 13,950 liter per day by using an empty fruit bunch of 47,208 kg per day. Moreover, the economic feasibility is also evaluated using techno-economic analysis. From the economic perspective, the net present value (NPV), internal rate of return (IRR), and payback period (PBP) equate to 9.016 M USD, 15%, and 7 years, respectively, based on 20 years of life and a total capital investment of 12.32 M USD. The results show that bioethanol production is profitable.

Keywords: bioethanol production, process simulation, empty fruit bunch, techno-economic analysis

Effect of Delignification and Bleaching Process in NaOCl Solution Under Hydrothermal Conditions Suppressed with Supercritical Carbon Dioxide on Cellulose Extraction from Sengon Sawdust

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Abstract. Sengon wood is a type of wood that is widely used in the Indonesian Furniture Industry. The sawdust wood contained high cellulose content, so it has great potential to be reused as a more useful product. This research aims to extract the cellulose contained in Sengon sawdust. This research was carried out by an environmentally friendly process such as the biodelignification process using white rot fungi (*Trametes versicolor*) and hydrothermal combined with a low concentration of NaOCl solution. The raw material was pretreated by ultrasonication to reveal the lignocellulosic structure, so it can maximize the penetration of *Trametes versicolor*'s enzymes. Biodelignification using *Trametes versicolor* can degrade lignin content to 1,04%. Lignocellulose content measured by chesson analysis. The crystallinity of the product measured from X-Ray Diffraction analysis (XRD). Scanning Electron Microscopy analysis (SEM) will be done to know the effect of each process on the morphology of the product and also measure the particle size of the product. The results shown that the material still has a brown color. Hydrothermal combination with NaOCl can destroy the amorphous region of cellulose, so the crystallinity of the product increase to 66-79%. The addition of NaOCl in the hydrothermal process is expected to do bleaching in low concentrations. However, the addition of NaOCl showed that the hemicellulose content did not change significantly.

Keywords : Sengon sawdust, cellulose, *Trametes versicolor*, hydrothermal, NaOCl

Comparative Account of Different Homogenous and Heterogenous Base Catalysts for Biodiesel Production

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Abstract. The unending use and exploitation of fossil fuels have resulted in serious environmental concerns like greenhouse gas emissions, carbon dioxide accumulation and global warming. Abatement of these concerns would require the replacement of fossil fuels through alternative and renewable energy sources like biodiesel and bioethanol. Microalgae being a third-generation feedstock has reported to serve as a substantial source for biodiesel. The reaction for biodiesel production is termed as transesterification. Although homogeneous base catalyzed transesterification through NaOH and KOH has been considered to be standard protocols, there are several disadvantages as well like high energy expenditure, release of high amount of wastewater and no catalyst recovery. These disadvantages are lesser with heterogenous base catalyst like CaO and there is catalyst recovery as well resulting in less wastage and a cost-effective protocol. The current work compares three base catalysts: NaOH, KOH and CaO of which the NaOH and KOH are homogenous and CaO is heterogenous in nature. A marine microalga *Picochlorum soloecismus* biomass was harvested followed by lipid extraction. Transesterification was carried out under different concentration of all 3 catalysts: NaOH and KOH (0.3, 0.5, 0.7, 0.9, 1.1 and 1.3% wt./wt.) and CaO (2%, 2.5%, 3%, 3.5%, 4%, 4.5% and 5% wt./wt.). The biodiesel yield under homogenous catalysts reached up to 80% while it was found to be 82% under heterogenous catalyst. CaO showed a catalyst recovery and reuse up to 3 more runs of transesterification whereas NaOH and KOH were completely lost in the reaction. The fatty acid methyl ester profile revealed a higher abundance of saturated fatty acids and lower abundance of poly-unsaturated fatty acids. Therefore, the use of heterogenous base catalysts for transesterification was found to be high yielding and efficient protocol for biodiesel production.

Keywords: biodiesel, heterogeneous catalyst, homogenous catalyst, transesterification

Multi-regional Analysis of Biomass Agriculture Waste Potential and Bio-pellet Development for Electricity in Indonesia

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Abstract. The potential of renewable energy sources in each region would be different from each other hence it requires multi-regional analysis in energy management, including the inter-regional energy distribution. Biomass resources such as agricultural waste are one of the strategic energy sources in many countries. The purpose of this study is to map the biomass potential based agricultural waste that is converted into bio-pellets and its utilization for electricity generation. Calculation and mapping of biomass potential were carried out through the primary and secondary agricultural residues. The biomass conversion into bio-pellet refers to Austrian standards (ONORM M 7135). The amount of waste estimated using the Product Ratio Residue (RPR) by the International Renewable Energy Agency (IRENA) and the Asian Handbook of Biomass. The results showed that the total energy potential of agricultural waste for bio-pellets in Indonesia was 2,707,779,555 GJ/year, which is equivalent to electrical power potential was 23,693 MW. In a view of multi-regional, the biomass resource locations are distributed in Sumatera (4,010 MW), Java-Bali (11,406 MW), Kalimantan (1,647 MW), Sulawesi (1,471 MW) and Eastern Indonesia (1,279 MW). The percentage of biomass material available in Indonesia was in the form of rice (67%), corn (19%), oil palm (8%) and others 6%.

Keywords: agricultural waste biomass, archipelagic nation, bio-pellet, Indonesia, multi-regional analysis, power generation

Bioindustry Promotion and Bioeducation

Manufacturing Process Evaluation and Performance Test of Cellulose Acetate-based Body Scrub from Empty Fruit Bunches

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Abstract. Microplastic is usually used as a personal care product's material for exfoliant purposes. Nevertheless, due to their smaller size, some particles will pass subsequently through wastewater treatment and polluting the aquatic environment. In the need for an alternative exfoliant material that is safe and biodegradable, the search for another material with the same characterization as plastic has reached natural fiber. One of the organic raw materials with high cellulose content is empty fruit bunches (EFB) which are waste from the palm oil industry. However, the main problem of cellulose to be used in personal care product is its shelf life because cellulose can be fermented easily with a slight microbial activity. Cellulose acetate emerged as one of the most important esters of cellulose which could offer more stability with the same exfoliant effect as its main compound. This research was focused on analyzing the cellulose acetate production process and its characterization in the variation of body scrub formula. Cellulose acetate-based body scrub's process evaluation was divided into beads production from EFB, while the second phase was the body scrub performance test. The result of process evaluation gives a stable production yield of cellulose acetate at (33.74 ± 0.19)% and it seemed to have a stable solution in the formula of 10% concentration of propylene glycol and virgin coconut oil which could be seen from organoleptic and viscosity test. SEM/EDX analysis also had been conducted and saw cellulose acetate's exfoliant ability from 1000 and 2500 magnification. It is hoped with this finding, it can encourage cosmetic manufacturers to utilize EFB as affordable raw material to produce biodegradable microbeads for its scrub product.

Keywords: body scrub, cellulose acetate, empty fruit bunches, microbeads, process evaluation

Adaptation of Recircular Economy with the Fish and Shellfish Co-Products: An Overview and Perspective in Bangladesh

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Abstract. Fish and shellfish as a whole “blue food” is being raised at least 10-40% of volume of co-products (CPs) of total yield through traditional fish processing methods. The raised CPs can be utilized as a key source of chitin and chitosan, natural multifunctional polymers. Experiment showed that the percentage of CPs found in black tiger shrimp is 49% and in freshwater giant prawn is 67% of whole weight. Our recent study evaluate both the quantitative and qualitative of CPs arising from 14 fish and 2 crustacean species in local fish markets and household levels in Bangladesh. Among the by-products, shrimp, and prawn heads (36.33 ± 1.41 - $38 \pm 3.12\%$), and fish intestine (2.77 ± 1.02 - $10.74 \pm 2.21\%$) contributed the large volume followed by fish gill, fin, and scale. we investigated the nutritive content (protein, lipid, Ca, Fe, Zn, and Se) of co-products (fish: fin, scale, gill, and viscera; shrimp and prawn: head, shell, brain, and claw) of fish and shellfish as a future indication in industrial sectors for potential applications. The results showed 9.34 ± 3.57 - $18.78 \pm 7.61\%$ by-products, and 3.68 ± 0.53 - $19.75 \pm 7.28\%$ plate waste are raised through the traditional fish processing in Bangladesh. The nutritive content (protein, lipid, Ash, Carbohydrate, Moisture; Ca, Fe, Zn, and Se, fatty acids, amino acids) of co-products (fish: fin, scale, gill, and viscera; shrimp and prawn: head, shell, brain, and claw) of fish and shellfish species and it was estimated that both edible portion and the co-products portion are providing almost similar nutrient content. Our findings revealed that CPs can provide essential nutrients and that can be applied as direct food, pet animal feed, and as raw materials in agriculture, pharmaceutical, and cosmetic industries. Furthermore, the estimated byproducts from the existing fish production of 14 fish species can result in 2,81,161-5,65,332 MT by-products on a wet weight (ww) basis and potentially can contribute to fish oil (FO) and fish meal (FM) replacement. It is estimated that derived by-products can contribute 6.49-13.06% fish meal development for sustainable aquaculture in Bangladesh, which value is almost 32.56-65.46 million USD that render to help in economy. We also tried to standardize the chitosan and chitin extractions from shellfish species. Chitin and chitosan were extracted by using different concentrations of HCl (1M, 1.25M, 1.5M) in the demineralization step. The purity of chitosan was tested by the ash content, moisture content, solubility test and biuret test. The improvised method that we developed seems to time sparing and efficient than the existing methods. Production of chitosan from co-products will reduce the dependency on import for chitosan and may create employment and, exporting opportunities. As the aquaculture industry moves into a new era of production and processing, the research identifies that Bangladesh could increase its foreign currency 19 times greater than conventional one by alternative utilization. Modern cleaning and processing techniques are needed to incorporate for the strategically use of underused by-products for sustainable aquaculture.

Keyword: fish, shellfish, recircular economy, Bangladesh, processing

Development of Microbial Biotechnology Processes for Industrial Enzymes to Bio-industry Growth

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Abstract. Microbial biotechnology process refers to the creation of useful compounds and services by the use of microorganisms for the benefit of human welfare. The development of molecular biotechnology, cell cultures and protein engineering has aided the output of microbial products by bio-industries contributing to bio-economy and sustainable development goal. Prior and judicious commercial exploitation of microbial processes on local cheap raw materials could deliver agro-industrial products such as bio-fertilizers, bio-pesticides, microbial inoculants, probiotics, biofuel, including industrial enzymes, diagnostics, biopharmaceuticals and vaccines etc profitably with impact on saving foreign currency, cleaner environment, food security, reduction of GHG and development of critical man power. Of the bio-products, microbial enzymes unlike chemical catalysts are biodegradable, having high specific catalytic activity under conditions of mild temperature and pressure rendering it to be eco-friendly. We have developed several potential mutants from *Bacillus licheniformis* MZK05 capable of secreting commercial level of serine proteases which include alkaline protease, keratinase, thrombolytic-streptomycin like protease. The wild strain was improved through mutation (by both chemical and UV radiation) and gene cloning aided by genome sequencing. The selected mutants were cultivated for high level enzyme production on Soybean-molasses medium at 37°C under cascade control of dO₂ (30 % saturation) in bioreactor. The cell free enzymes were characterized and tested for technical applications in leather manufacturing and detergent industries. Three types of serine proteases were tested and worked successfully in bating, soaking and un-hairing processes of skin and hides in leather manufacturing industries. The bating enzyme from the mutant (BIMZKM9) was capable of hydrolysing selectively the non-structural proteins (albumin, globulin and mucin) embedded in the collagen structure of hides and skins allowing the finished leather soft and pliable with desired qualities. The enzyme while tested with commercial grades, found quite suitable and thus transferred to industrial production and uses. The high-level protease producer by BIMZK10 showed a wide pH stability ranged between pH 6.0 to 11.0, was found stable in the commercial detergents in presence of calcium and/or magnesium ions. This enzyme aided good cleansing efficiency of proteinaceous stained clothes and utensils showing its high potentiality as commercial application. The keratinase encoded gene from BIMZK05 was cloned and its high level expression was obtained by the recombinant strain. The BI MZK05 mutant also showed encouraging results as thrombolytic protease (as plasmin or streptomycin like). The genome sequencing of the two strains to pinpoint the probable cause of high-level expression of serine proteases in BIMZKM9 mutant, the number of proteases, and other industrially useful enzymes, genes responsible for extracellular protease secretion, genome variation in between the strains studied shall be included in the presentation. Thus the techniques of fermentations and molecular biology used in the present studies for industrial enzymes will be a useful basis for bio-industry promotion and bio-education as well.

Keywords: industrial enzymes, *Bacillus licheniformis*, industrial growth, serine proteases

Industrial Application of Simulated Moving Bed Chromatography (SMB) Coupled with Supercritical Fluid (SF) to Separate of Ω -3 PUFA from Esterified Fish Oil

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Abstract. The omega-3 polyunsaturated fatty acids (ω -3 PUFA) fish oil is expanding rapidly while scientific researches show its key role in preventing and treating many diseases over the past ten years. The ω -3 PUFA in fish oil consists mainly of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In Taiwan, the separation of ω -3 PUFA from esterified fish oil was conducted on an industrial scale simulated moving bed (SMB) coupled with supercritical fluid, which is called as SF-SMB. A six-column SF-SMB with 200 mm in diameter designed by JOPE Co. (Kaohsiung, Taiwan) was employed and divided into three sections with 2/2/2 configuration. It is expected to process 20 tons of fish oil annually with 2.2 KKD (kg-feed/kg-adsorbent/day) of adsorbent productivity. The feedstock is an ethyl ester fish oil, contained about 50~60% of EPA and DHA which is fractionated by molecular distillation, and 98% of EPA and 90% of DHA can be obtained by SF-SMB. The SF-SMB produces EPA with 98.0% of purity and 90.0% of recovery. Since supercritical carbon dioxide and ethanol as cosolvent were used, the solvent recycling can be simply finished by two evaporators independently, one for the recycling CO₂ at high pressure and another for ethanol at low pressure. Therefore, the fourth section in the SMB was eliminated and productivity was increased. Compared with the reverse phase SMB by liquid solvents or SFC (Supercritical Fluid Chromatography) with a single column, the SF-SMB has higher productivity, lower solvent and energy consumptions. It should be also noted that only CO₂ and ethanol were used for the separation, the separated products were relatively safe for human consumption and fully complied with food regulation. A larger production line handling 100 tons of fish oil is also under designed and expected to commence in the future. The successful application of the SF-SMB in industry represents that the next generation of chromatography with greener and more efficient is feasible. More applications of supercritical fluid to the continuous chromatography are undergoing, and this will further extend the use of supercritical fluid in industries.

Key words: simulated moving bed, supercritical fluid, fish oil

Biopharmaceutical and Medical Biotechnology

Frankincense Leaves' (*Stryrax paralleloneurum*) Protease and Its Thrombolytic Activity Assay

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Abstract. Thrombotic disease is considered to be one of the leading causes of human death in the world and thrombolytic therapy is still the best way to achieve recanalization. Thrombolytic therapy is performed by enzymes known as thrombolytic agents. A thrombolytic agent is a drug used to dissolve a thrombus or blood clot that forms in a blood vessel and reopen the artery or vein. All the thrombolytic agents in use today have undesirable side effects such as gastrointestinal bleeding, allergic reactions, and resistance to thrombolytic agents, and some of them are very expensive. Therefore, the search for thrombolytic agents derived from plants is carried out, because some plants are reported to have protease enzymes with thrombolytic activity and drugs from plants are known to be safer. This study aimed to obtain protease enzymes from frankincense leaves (*Stryrax paralleloneurum*) and to perform its thrombolytic activity assay. This research was started by extracting protease enzymes from frankincense leaves. The results of the caseinolytic radial assay revealed that the crude enzyme extract of frankincense leaves contained protease which was indicated by a clear zone around the paper disc dripped with enzymes. The enzyme was partially purified using ammonium sulfate precipitation and dialysis. Enzyme concentration was determined by Bradford method and the results showed the highest enzyme concentration obtained was 1.6812 g/μL at 40% ammonium sulfate fraction. A quantitative assay of protease activity in the 40% fraction using *Folin & Ciocalteu's Phenol* reagent and casein as a substrate showed that 1.2609 mg enzyme hydrolyzed 32.5 mg casein and released 0.0980 mg L-tyrosine. Furthermore, the protease fibrinogenolytic activity against human fibrinogen tested using SDS PAGE showed that the protease from frankincense leaves could hydrolyze the fibrinogen γ chain within 0 minutes and the β chain within 60 minutes, while the α chain could not be hydrolyzed until 720 minutes. The inhibitor effect assay on protease activity showed that the protease from frankincense leaves' was a serine protease. Based on the results obtained in this study indicate that the protease enzyme from frankincense leaves has the potential to be used as a thrombolytic agent.

Keywords: *Stryrax paralleloneurum*, Thrombotic, Thrombolytic agent, Thrombolytic therapy, Serine protease.

Molecular docking simulation of copper oxide and zinc oxide nanoparticles toward target enzyme SARS-CoV-2 RdRp

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Abstract. The COVID-19 pandemic caused by the SARS-CoV-2 virus has infected over 2 million people in Indonesia and has killed over 60 thousand. The urgency of the situation drove researchers to prevent the spread of SARS-CoV-2. One method to disinfect surfaces contaminated by SARS-CoV-2 is by attacking RdRp, an enzyme crucial to the virus's life cycle. This *in silico* research has simulated molecular docking on copper oxide (CuO) and zinc oxide (ZnO) nanoparticles to determine the best nanoparticles for inhibiting the SARS-CoV-2 RdRp enzyme. The results of molecular docking were compared. The CuO nanoparticles obtained a docking score of -5.9 kcal/mol, whereas the ZnO nanoparticles obtained a docking score of -5.5 kcal/mole. Both nanoparticles show potential as antiviral agents against the SARS-CoV-2 RdRp enzyme. However, CuO nanoparticles have a better potential than ZnO nanoparticles. This study may serve as a reference to identify an antiviral agent suitable for disinfecting surfaces from SARS-CoV-2 contamination.

Keywords: Metal oxide nanoparticles, COVID-19, RNA-dependent RNA polymerase, Molecular docking

Thrombolytic Protease Characterization from Leaves and Fruit Flesh of Jernang Rattan Plant (*Daemonorops draco*)

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Abstract. This research aims to investigate the proteolytic activity of protease isolated from leaves and fruit flesh of jernang rattan plant (*Daemonorops draco*). The proteases were isolated from the leaves and the fruit flesh, then partially purified by ammonium sulphate precipitation. Caseinolytic radial assay showed that proteases in 60% ammonium sulfate fraction gave a clear zone with diameter of 1.4 cm and 1.8 cm for the protease isolated from leaves and fruit flesh of jernang rattan respectively. The Follin-Ciocalteu assay showed that the enzymes isolated were able to hydrolyze casein and release L-tirosin with activity of 0.158 U/mL and 0.174 U/mL for the protease isolated from leaves and fruit flesh of jernang rattan respectively. Fibrinogenolytic assay showed that the protease from fruit flesh of jernang rattan hydrolyzed the A- α , B- β , and γ chain of human while the protease from leaves of jernang rattan hydrolyzed the A- α and γ chain. Both of the proteases were inhibited by 56% by phenylmethylsulfonyl fluoride (PMSF) indicating that the enzymes are serine proteases. Based on the assay results obtained, it can be concluded that proteases isolated from leaves and fruit flesh of jernang rattan have a potential as a thrombolytic protease.

Keywords: *Daemonorops draco*, protease, serine protease, fibrinogenolytic, thrombolytic.

Rapid Screening of Monoclonal Antibodies against G Protein-coupled Receptor: Case Studies with Kisspeptin Receptor (GPR54)

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Abstract. G protein-coupled receptors (GPCR) are a potential drug target since they control many cells signaling processes such as neurotransmission, hormone, etc. Among the drugs being developed, one of them is monoclonal antibody (mAb). There are several challenges when developing mAbs against GPCRs, mainly due to their hydrophobicity and difficulty of isolation and purification, and the lack of immunogenic region, which make GPCRs less recognized as an antigen for development of specific mAbs. Here, we reported the attempt to isolate mAbs against Kisspeptin receptor (GPR54) using a platform called Ecobody technology that enables screening and selection of mAbs in typically two days using single cell RT-PCR to amplify light chains and heavy chains gene, and cell-free protein synthesis (CFPS) for producing fragment of antigen binding (Fab) format of isolated antibodies. This technology has been applied to find mAbs against several antigens from bacteria to virus, but it has not yet been applied for finding mAbs against difficult-to-isolate proteins like GPCRs. Combining DNA Immunization and magnetic beads-based B cells isolation, antibody genes were successfully isolated from nine B cells from spleen of immunized rabbit, each had IGKV and IGHV genes pair. Preliminary results showed that some of the selected antibodies could bind specifically to the GPR54 based on immunoassay and immunostaining data.

Keywords: mAbs, Ecobody technology, single cell RT-PCR, cell-free protein synthesis (CFPS), GPCR

Phytochemical Screening, Chromatographic Evaluation and Antioxidant Activities of Different Solvent Extracts of Brazilian Cherry Fruits (*Eugenia uniflora* L.) Grown in NSTU Campus, Bangladesh

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Abstract. The main aim of this work is to investigate the presence of phytochemicals, detection of bioactive compounds and antioxidant activities of water, methanol and ethanol extracts of Brazilian Cherry Fruits. Phytochemical present in water, methanol and ethanol extracts of Ripe and Non-Ripe Brazilian Cherry Fruits in Red and Green Colors, respectively are Alkaloids, Tannins, Saponin, Flavonoids, Cardiac Glycosides, Carbohydrates, Proteins, and Amino Acids. The result also showed the purple color compound present in water, methanol and ethanol extracts of both Ripe and Non-Ripe Brazilian Cherry Fruits in Red and Green Color, respectively in TLC may be Vitamin C or unknown nitrogenous compound as their R_f value is same as L-Ascorbic acid and the white color compound is absent in water, methanol and ethanol extracts of Ripe Brazilian Cherry Fruits in Red-Color, it is only present in water, methanol and ethanol extracts of Non-Ripe Brazilian Cherry Fruits in Red Color which may be another important unknown phytochemicals. Unknown phytochemicals present in water, methanol and ethanol extracts of Non-Ripe Brazilian Cherry Fruits in Green Color that detected in TLC by UV light. This result also showed that all the extracts of Ripe and Non-Ripe Brazilian Cherry fruits are a good source for antioxidant ability and phenolic compounds, but among the analyzed fruits of all extracts, in both Ripe and Non-Ripe, the water extract of non-ripe Brazilian Cherry Fruits is the good source for strong antioxidant ability (73.66%) and it showed the lowest IC_{50} of DPPH scavenging activity (1.01 $\mu\text{g/ml}$) which may be due to the presence of high content of Vitamin C. From the above results, it can be concluded that Brazilian Cherry Fruits in both Ripe and Non-Ripe stage contain an enormous amount of phytochemicals that will be useful as an additive or as a raw material in food and pharmaceutical industries to prevent oxidative damage and also to prevent and fight against infection recently caused by COVID-19 in worldwide and other infectious diseases by increasing our body immunity in the long term by including this fruits in our diet chart.

Keywords: Brazilian Cherry Fruits; Phytochemicals; Antioxidants; DPPH; Phenolic compounds

Embolization of Cerebral Aneurysm by in situ Photocrosslinking of Alginate Hydrogel Microfibers

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Abstract. Continuously spun hydrogel microfibers obtained via spatiotemporally controllable in situ photocrosslinking exhibit great potential in embolizing vascular malformations (e.g., aneurysms) without resulting in complications like premature gelation or non-crosslinking of hydrogels. However, accomplishing this process is challenging due to the absence of biocompatible and morphologically stable hydrogels and the difficulty in continuously spinning the microfibers via in situ photocrosslinking in extreme endovascular environments, such as a tortuous geometry and high absorbance. This study develops a double-crosslinked alginate-based hydrogel with tantalum nanopowder (DAT) that employs the synergistic effect of covalent crosslinking through visible light irradiation and ionic crosslinking by Ca^{2+} in the blood. Furthermore, an effective strategy using microfluidic device capable of continuously spinning hydrogel microfibers by in situ photocrosslinking in endovascular environments. As an embolic material, DAT exhibits promising characteristics, such as non-dissociation, non-swelling, and constant mechanical strength in blood, including excellent cyto- and hemo-compatibilities. DAT microfibers can safely, uniformly, and completely fill aneurysms within endovascular simulators without generating microscopic fragments or clogging demonstrating itself as an effective embolization strategy.

CRISPR Target-Based Single-guide RNA (sgRNA) for Diagnostic of Hepatitis B Virus in Indonesia

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Abstract. Indonesia is the second-highest country with hepatitis B cases in the South East Asian region. CRISPR-Cas12 could be developed as a diagnostic tool to detect Hepatitis B infection. CRISPR target-based single-guided RNA (sgRNA) was designed for the development of a diagnostic method for the Hepatitis B Virus that circulating in Indonesia. The Core-gene sequences of the Hepatitis B virus were collected from the NCBI. The selected conserved sequence was submitted to Cas Designer and CRISPOR tools to design sgRNA. The expression vector was constructed using SnapGene software. The 27-nucleotide sequence 5'-AATCTAGCCACCTGGGTGGGAAGTAAT-3' had 56.5% GC content, 67.4 out of frame and 72 predicted efficiencies. This sequence had no mismatch based on analysis. This preliminary study will help design a CRISPR-based diagnostic kit for the detection of Hepatitis B Virus in Indonesia. However, further studies in vitro and in vivo are required to demonstrate its potential and efficiency.

Keywords: CRISPR-Cas12, diagnostic, HBV, sgRNA

Design of CRISPR Target Sequences for the Development of a Sequence-Specific Antimicrobial for ESBL-producing *Escherichia coli*

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Abstract. Antimicrobial resistance is growing rapidly and spreading globally due in part to the overuse of antibiotics. Many of the currently available antibiotics have become ineffective and lack specificity, killing indiscriminately both harmful and beneficial bacteria. In addition, the discovery of new antibiotic molecules is limited. Therefore, a new approach is needed to cope with this global health problem. CRISPR-Cas systems can be developed as new generation antibiotics using RNA molecules to specifically target and kill pathogenic bacteria. In the present study, we designed CRISPR-Cas9 target sequences for the development of CRISPR-Cas-based antimicrobial against extended-spectrum β -lactamase (ESBL)-producing *Escherichia coli*. Sequences for SHV- and TEM-type ESBLs were collected from the Beta-Lactamase DataBase (BLDB) and NCBI database. The sequences were aligned to identify conserved regions using a multiple sequence alignment program. Each selected sequence of SHV and TEM was submitted to CHOPCHOP, Cas-Designer, and CRISPOR to design the CRISPR-Cas9 target sequences. The same target sequences generated in at least two different design tools and showed optimal parameters were selected. An expression vector was then constructed using the SnapGene program. The target sequence for SHV-type ESBL is 5'-GTTATCGCTCATGGTAATGGCGG-3' and for TEM-type ESBL is 5'-TCGTAGTTATCTACAYGACGGGG-3'. The target sequences have 70.03% and 73.22% efficiencies, respectively. Both sequences have 45% GC content without self-complementarity and mismatches. These results will help in designing CRISPR target sequences for the development of CRISPR-based antimicrobials. However, to evaluate its efficiency in specifically killing ESBL-producing *E. coli*, this *in silico*-designed construct needs to be tested *in vitro*.

Keywords: Antimicrobial resistance, CRISPR-Cas, *Escherichia coli*, extended-spectrum β -lactamases (ESBLs), sequence-specific antimicrobials.

Extracellular Metabolites of Endophytic Fungi from *Azadirachta indica* Inhibit Bacterial Superbugs and Phytopathogenic Fungi

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Abstract. Endophytes are endosymbiotic microorganisms found ubiquitously in all plant species without causing side effects to the plant. The objective of this study is to evaluate antimicrobial activity of extracellular metabolites (EM) of endophytic fungal isolates (EFI) from *Azadirachta indica*. Seventeen EFI were obtained from leaves, fruits and stems of *A. indica*. The EM was produced from EFI at different temperatures under shaking fermentation. The EM produced during axenic culture of eight of the seventeen EFI showed strong antibacterial activity against six multidrug resistant (MDR) clinical bacterial superbugs, which were resistant to commercially available antibiotics. Biosynthesis of the antimicrobial EM by the eight EFI was optimum at 34 °C. These eight EFI were identified based on internal transcribed spacer (ITS) region of the ribosomal DNA. The EM from two of the EFI were both peptide and non-peptide types. Furthermore, the EM produced by some isolates under dual induction, showed antibacterial activity against the MDR bacteria. In addition, the EM of some of the EFI produced through axenic culture showed antifungal activity against phytopathogenic fungi isolated from diseased tomatoes and potatoes. The phytopathogenic fungi were identified based on the ITS sequence. The minimum inhibitory concentration of the EM against MDR bacteria and phytopathogenic fungi ranged from 0.125 to 1.0 and 0.5 to 1.0 µg/µL, respectively, and the minimum bactericidal and fungicidal concentrations were 0.5 to 4.0 and 1.0 to 4.0 µg/µL, correspondingly. Results of the study indicate that the EM of the EFI has promising antimicrobial activity against bacterial superbugs and phytopathogenic fungi, and might have novel antibiotics.

Fabrication of Microcapsules-based Chitosan from Combination of *Ruellia tuberosa* L. and *Cosmos caudatus* Kunth Extracts for Pharmaceutics Application

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Abstract. This study aims to co-microencapsulate the extracts of *Ruellia tuberosa* L. and *Cosmos caudatus* K., with chitosan-Na-TPP (sodium tripolyphosphate) as coating materials. Alpha-amylase inhibition and antioxidant assays were carried out to determine the potential of microcapsules used as antidiabetic agent. The fabrication of microcapsules was conducted under the influences of pH, concentration of Na-TPP, and stirring time. The optimum conditions for microcapsules were selected based on the highest percentage of encapsulation efficiency (% EE). The optimum conditions were obtained at pH 4, Na-TPP concentration of 0.15% (w/v), and 60 min of stirring time. The microcapsules resulted in IC₅₀ values of 223.64 ± 0.81 µg/mL and value of 104.05 ± 0.88 µg/mL for alpha-amylase inhibition and antioxidant activity, respectively. The bioactive compounds released from microcapsules were carried out in HCl pH 1.2 and phosphate buffer pH 7.4 for 30-120 minutes. The bioactive compounds were released at pH 1.2 and 7.4 was 5.99% and 58.96%, respectively, in 120 min. The FTIR spectra showed the P=O functional group from Na-TPP at 1213.71 cm⁻¹ and C-N from chitosan at 1155.23 cm⁻¹. Characterization with SEM and PSA indicated the microcapsules were spherical in shapes and had a mean diameter of 132.08 µm. The current study demonstrates that co-microencapsulation is a promising multi-approach for enhancement of pharmaceutics application of plant extracts combination.

Keywords: *Ruellia tuberosa* L., *Cosmos caudatus* K., co-microencapsulation, chitosan, Na-TPP

Ferulic Acids of Rice Pigmented: Potential Therapeutic Agents as Anti-Oxidant and Anti-Aging

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Abstract. Introduction: At the cellular level, aging is characterized by an increase in senescent cells in organisms, caused by several factors, including oxidative stress, deregulated nutrient sensitivity, autophagy dysfunction, and systemic inflammation. In the aging process, proinflammatory cytokines also inhibit preadipocyte differentiation and maturation and promote adipocyte senescence. Natural bioactive compounds can act as highly diverse structural scaffolds with potential antiaging effects through antioxidants. Pigmented rice ferulic acid has the potential to have a variety of biological functions that support its health benefits with particular emphasis on the antiaging effects of this compound. These studies focus on determining the potential therapeutic agent of pigmented rice ferulic acid as an anti-oxidant and anti-aging at the cellular and molecular levels. Methods: extraction of ferulic acid (FA) rice pigment, measurement of total phenolic content, analysis of FA functional groups using Fourier-transform infrared spectroscopy (FTIR), antioxidant activity test using iron-reducing antioxidant potential (FRAP), inhibition test of enzyme activity causing aging and biological function activity using in silico analysis. Result: Pigmented rice ferulic acid has bioactivity as an anti-aging candidate, which is indicated by the presence of antioxidant activity, inhibition of enzyme activity that causes aging, and anti-inflammation. The purple rice FA showed very strong antioxidant activity and the ability to inhibit tyrosinase enzyme activity. In silico analysis showed the FA has high activity as a TNF expression inhibitor and moderate activities as an anti-inflammatory in general. The proinflammation cytokines are suitable targets for FA anti-aging properties. Conclusion: These results indicated that FA function as key mediators might become beneficial to inhibiting cell senescent and preventing aging.

Keywords: aging, antioxidants, cell senescent, ferulic acid, health benefit

COX-2 and CHI3L1 Gene Polymorphism of Colorectal Cancer Patients in Bali

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Abstract. The pro-inflammatory condition has a critical role in promoting the progression, invasion, and metastasis of colorectal cancer (CRC). Several studies revealed various pro-inflammatory genes in CRC carcinogenesis, including cyclooxygenase-2 (COX2) and Chitinase-3-like protein 1 (CHI3L1). COX-2 affects cell proliferation, apoptosis, and angiogenesis by catalyzing the prostaglandin E2 (PGE2). CHI3L1 plays in the tumor inflammatory processes, such as stimulating angiogenesis and remodeling the extracellular matrix. This study aimed to determine COX-2 and CHI3L1 gene polymorphism of CRC in Bali. To this end, DNA was extracted from 39 paired FFPE samples (tumor tissues and its adjacent normal tissues). To identify the microsatellite instability (MSI) subtype, pentaplex polymerase chain reaction (PCR) was performed. SNP genotyping of COX-2 (rs20417) and CHI3L1 (rs4950928) was identified using real-time quantitative PCR (qPCR) using allele-specific primer. Data were analyzed by SPSS ver. 21 and statistical significance was defined as a p-value ≤ 0.05 . Our results showed that the age was mostly ≤ 60 years old (n=22/56.4%) and gender was mostly male (n=24/61.5%). Furthermore, MSI-H subtype and left tumor was the predominant cases (n=21/53.8% and n=24/61.5%, respectively). We found no significance difference of COX-2 polymorphism, however, there was a significance difference of CHI3L1 polymorphism between tumor and normal samples. Therefore, further studies are important to identify the role of CHI3L1 polymorphism in CRC pathogenesis.

Keywords: CRC, COX-2, CHI3L1, SNP

Production Plant Design and Economic Feasibility Test of Fast Release Fluoride Varnish with Antibacterial Agent Using Pharmaceutical Ampoule Packaging

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Abstract. In Indonesia, about 93% of early childhood experience dental caries. One of the prevention of dental caries by using fluoride therapy. Fluoride therapy in the form of fluoride varnish has been shown to inhibit the growth of *Streptococcus mutans* bacteria and is able to remineralize teeth with an effectiveness of up to 77%. The use of fluoride varnish in early childhood is expected to prevent dental caries. Currently, fluoride varnish has been developed which has fast release capability. Fast release fluoride varnish can release more fluoride ions within 4 hours. The purpose of this research is to design a factory of fast release fluoride varnish product with antibacterial agent using pharmaceutical ampoule packaging and analyze its economic feasibility. The fluoride varnish formulation used consisted of hydrogenated rosin, 99.7% ethanol, sodium fluoride, tween 80, peppermint flavoring oil, DCPD-Xylitol, and extracts of natural antibacterial agents based on the results of previous studies. The antibacterial agents used were red betel leaf extract and holy basil leaf extract. Based on the production test carried out, it was found that in the first 4 hours, fluoride varnish added with an antibacterial agent had more fluoride ion release than other fluoride varnishes. In this writing, the research process begins with the design of the production process and then continues with a pilot scale production test. After that, an economic calculation will be carried out to determine the feasibility of constructing a fluoride varnish factory. In this work we present the results of the process design and production test which will include fluoride ion release test and pH of fast release fluoride varnish along with an analysis of its economic feasibility.

Keywords: Caries; production plant design; fast release fluoride varnish; pharmaceutical ampoule

Advances in the Current Understanding of How Radiation Hormesis Affects the Immune system

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Abstract. Several studies supposed Low-dose irradiation enhancement and stimulation of immune system and referred its action “radiation Hormesis”. This evidence could improve several medical problems. Cytokines are important regulators of the immune response that are involved in maintaining the balance of the immune system. Aim: Monitor the effect of Hormesis, very low, low and moderate ionizing radiation doses on regulating immune system. We used total body Gamma irradiation using the Cesium 137 cell with exposing doses ; 0.25Gy, 0.5Gy, 1Gy, 2Gy, 5Gy, (dose rate 0.66rad/sec) .Blood samples were collected after 24, 48, 96 hours to measure and compare levels of MDA , INF- γ , IL-2 and IL10 .Results confirmed an obvious significant immunological changing in activation of lymphocytes secreted cytokines related to dose of radiation compared to control. A significant positive correlation was obtained between MDA & INF- γ , MDA & IL2 and INF- γ & IL2 (p value<0.001, r=0.964), (p value<0.001, r=0.936), (p <0.001, r=0.91) respectively. On the other hand, the correlation between MDA & IL10, INF- γ & IL10 and IL10 & IL2 were significant inverse correlation. There was no significant change in the Complete Blood Picture. Data proved disputed facts of radiation Hormesis provided from low doses of ionizing radiation (LD-IR) in stimulating mechanisms to enhance and regulate immune system.

Keywords: Low-dose Ionizing Radiation, Hormesis, Gama Rays, Cytokines

The CRISPR/Cas9-Mediated Base Editing (BE) Design of HBV Specific gRNA: A Computational Approach

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Abstract. Therapy to cure due to persistence infection of HBV is needed to suppress viral replication. Current therapy with nucleoside analogues does not destroy HBV covalently closed circular DNA (cccDNA). A new strategy using Base Editing required to tackle this viral latent infection. We designed guide RNAs (gRNAs) to target the pre-S and S genes of HBV for development of antiviral, based on the dominant HBV genotype in Indonesia. The conserved regions of HBV pre-S and S genes were used to determine selected target of Cas9-mediated base editing system. Variant of SpCas9 used furthermore in expanding the candidate protospacer sequences. Base Editing (BE)-Designer web-based software to designed gRNA and SnapGene for designed the expression vector. We identified 8 candidate sequences, including 2 sequences in the pre-S and 6 sequences in S gene. These target sequences showing no mismatches and GC contain between 45-65%. In addition, the gRNA sequences were checked in Nucleotide BLAST tools (NCBI) to prevent the offtarget effect and assured the differences with human genome. This study provides the candidate target sequences to be developed in antiviral therapy. The utility and efficiency of the candidate sequences need to be evaluated in viral gene expression by in vitro HBV infection system.

Keywords: bioinformatics, CRISPR/Cas9, genome editing, gRNA, HBV

Immunomodulatory prediction of *Cinnamomum burmanni* compounds; In silico study

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Abstract. *Cinnamomum burmanni* is a native plant from southeast Asia, especially in Indonesia. *Cinnamomum burmanni* was reported to contain around fifty oil compounds extracted from leaf, bark, and root. In this study, we performed immunomodulatory identification of fifty aromatic oil compounds from *Cinnamomum burmanni* using in silico approach. Fifty aromatic compounds were collected their 3D structures and canonical SMILES at PubChem Database and Knapsack Family web server. The canonical SMILE of aromatic compounds predicted the structure-activity relationship (SAR) with anti-inflammatory, cyclooxygenase, and lipoxygenase activities as parameters. The SAR prediction was conducted by using the PASS Two-Way Drug web server. Six of fifty compounds, including nerolidol, elemene, caryophyllene, nerolidol, elemol, and caryophyllene oxide, showed high potential activity as an anti-inflammatory agent. Docking study performed that nerolidol, elemene, caryophyllene, nerolidol, elemol, and caryophyllene oxide bound to cyclooxygenase-2 at several active sites. Those active sites identified as substrate binding sites. Therefore, we predicted the six aromatic oil compounds *Cinnamomum burmanni* potentially substituted arachidonic acid as a native substrate of COX-2 and might be potentially as anti-inflammatory agent.

Keywords: anti-inflammatory agent, aromatic oil compounds, *Cinnamomum burmanni*, Cyclooxygenase-2

3D Structure Protein, Validation, and Its Function of Human ZiP2 and ZiP4 Based on Molecular Modelling Approaches

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Abstract. Homology modeling predicts the 3D structure of a protein-based on the sequence alignment with one or more template proteins of known structure. This study aimed to analyze the structural protein perspective of drug targets and their molecular interactions. Predicting the 3D structure of protein ZiP2 and ZiP4 from their amino acid sequence is one of the most critical ways to investigate their function from the protein secondary structure. The proteins ZiP2 and ZiP4 reported that they are essential to the influx and efflux of zinc transport in membrane cells. Both of these functions are the maintenance of zinc homeostasis in cellular mechanisms. For future research, the proteins ZiP2 and ZiP4 are necessary to study targeted drugs in deficiency diseases, uncover the mechanism of action in therapy for zinc deficiency, and in silico methods for molecular simulations. Homology modeling, validation, and its function investigation with molecular modeling approach with in silico methods. This research explains the 3D structure of human ZiP 2 and ZiP4 with Ramachandran Plot analysis, physical and chemical characteristics, and binding site prediction with structural biology.

Keywords: 3D structure, zinc transporter, molecular modelling, in silico, structural biology

Antimicrobial Compounds from Intracellular and Extracellular Secondary Metabolites of Actinobacteria InaCC A759

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Abstract. Antimicrobials discovery and development is one of the efforts to overcome the problem of Antimicrobial Resistance (AMR). World Health Organization has determined a list of pathogens that require the development of new antimicrobials, including *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. In addition, *Mycobacterium smegmatis* was used for antimicrobial discovery in an effort to address the increasing burden of tuberculosis. This study conducted an antimicrobial exploration of sixteen Actinobacteria from Indonesian Culture Collection, Cibinong, Indonesia. The secondary metabolites of the intracellular and extracellular extracts of Actinobacteria were tested for antibacterial activity using the Resazurin Microplate Assay method, and the potential strain of Actinobacteria InaCC A759 was found. Intracellular and extracellular extracts of Actinobacteria InaCC A759 had different activities, especially in inhibiting the growth of *M. smegmatis*. The Minimum Inhibitory Concentration (MIC) values of the extract to inhibit the growth of *M. smegmatis*, *E. coli*, and *P. aeruginosa*, respectively, were 50, 25, and 100 µg/mL (intracellular), and 25, 25, and 100 µg/mL (extracellular). Meanwhile, both extracts were unable to inhibit the growth of *S. aureus*. Metabolite profiling using High Resolution-Mass Spectrometry (HR-MS) was also carried out, and there was a difference in the major compound between the two extracts of Actinobacteria InaCC A759. The major compound of the intracellular extract was n-acetyltyramine (C₁₀H₁₃NO₂/179.0945) (24.24%), while the major compound from the extracellular extract was palmitic acid (C₁₆H₃₂O₂/273.27034) (86.92%). Molecular analysis of the 16S rRNA gene was performed on Actinobacteria InaCC A759, yielding 100% similarities to *Streptomyces olivaceus* strain FoRh46. Furthermore, the Actinobacteria InaCC A759 NRPS gene was detected to have similarities with the amino acid adenylation domain-containing protein gene of *Streptomyces olivaceus* (99.57%), while the PKS gene detection analysis identified similarities to the type I modular polyketide synthase of *Streptomyces malaysiensis* (57.65%).

Keywords: Actinobacteria, antibacterial agents, metabolite profiling, n-acetyltyramine, palmitic acid

Genetic Modification of Mesenchymal Stem Cells with Adenovirus: Up-regulating Bone Morphogenetic 2 Protein for Secretome Production

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Abstract. Bone malformations are still a major health problem in Indonesia. For bone defects, bone implantation is a common procedure to improve patient mobility. However, this method requires lifelong maintenance and is quite expensive. Interestingly, mesenchymal stem cell (MSC) therapy is a prospective approach to bone regeneration. MSCs are multipotent stem cells that can differentiate into several types such as osteoblasts, chondrocytes, myocytes, and adipocytes. MSC-derived secretome contains valuable growth factors, eg. IGF-1 and VEGF, however, do not contain bone morphogenetic protein-2 (BMP2), a crucial growth factor for osteogenic differentiation. Our current research is to develop recombinant MSCs harboring foreign BMP2 gene which is expected to produce secretome containing BMP2. With this system, the dependency on bacterial-derived BMP2 for bone regeneration is no longer necessary. An adenoviral system was used to deliver the target gene into umbilical cord-derived MSCs. BMP-2 and viral-packaging plasmids were produced in DH5 α bacteria. The combination of BMP2 and viral-packaging plasmids were then transfected into HEK293 cells to produce the whole BMP2-harboring adenovirus. The chloroform-based method was used to purify the virus and the purified virus was then transduced into UC-MSCs to upregulate BMP2 gene expression.

Keywords: Bone morphogenetic protein-2, Mesenchymal stem cells, Adenovirus, Bone regeneration, Genetic engineering, Secretome, Growth Factor

Modeling of Virus-Like Particle (VLP) Vaccine Production Process Using Chinese Hamster Ovary (CHO) Cell Culture

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Hand, foot, and mouth disease (HFMD) is a viral illness that usually occurs among children. It is a self-limiting disease, but severe complications or even fatality may occur in some cases. HFMD has caused many outbreaks worldwide over decades posing a threat to public health. HFMD is caused by enteroviruses, which can be classified into different serotypes. Coxsackievirus A6 (CVA6), a serotype of enteroviruses, is gaining more attention due to the increasing incidence. Currently, there is no prophylactic vaccine for CVA6 available. Virus-like particle (VLP) vaccine is a novel type of vaccine, which can trigger a strong immune response by resembling the protein coat of the specific virus. It outperforms conventional vaccines in terms of safety because VLP lacks genomic material. Therefore, CVA6 VLP vaccine is a promising candidate for the HFMD prevention measure.

To produce CVA6 VLP vaccines, Chinese hamster ovary (CHO) cell expression platform is selected because CHO cells can produce complex post-translational modifications. In order to produce CVA6 VLP vaccines efficiently using CHO cells, culture conditions such as pH have to be optimized. However, this optimization process is time-consuming and costly. Modeling method, which describes the process mathematically, is commonly used to accelerate this optimization process in the biopharmaceutical industry. Nevertheless, there is no model available for the VLP vaccine production process now. To solve this problem, we constructed the VLP production model.

In this study, gene-modified CHO cells were cultivated with two lab-scale bioreactors in a fed-batch mode to produce CVA6 VLPs. Two batches of cell culture were performed with pH downshift on day 10 and without pH downshift, respectively. Samples were taken from bioreactors every day. Measurements of viable cell density, dead cell density, lysed cell density, VLP yield, and major metabolite concentrations were conducted. Based on the data collected, a mathematical model revealing the relationship between live cell, dead cell, lysed cell, VLP, and main metabolites was constructed. It was found that VLP yield was closely related to cell death and cell lysis. Besides, pH downshift was discovered to accelerate the accumulation of ammonia in the dead phase, which results in an increased specific cell death rate. Sensitivity analysis was carried out to determine the importance of different model coefficients. Subsequently, simulation was conducted to predict the experimental performance if pH is shifted on different days. Finally, with the help of the model simulation, desirability optimization methodology was applied to make the decision for the tradeoff between product yield and quality.

Key words: Hand foot and mouth disease; vaccine production; Virus-like particle; Chinese hamster ovary cell culture; Bioprocess modeling; Process optimization.

Responses of Humoral and Cellular Immune Mediators in BALB/c Mice to LipX (PE11) as a Tuberculosis Vaccine Candidates

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Abstract. A member of the *pe/ppp* gene family, *lipX* (*pe11*) is capable for directing persistent *M. tuberculosis* and avoiding host immune responses. Previous study has also indicated that LipX (PE11) could detect humoral antibodies of TB patients. Hence, information on immune mediators' response to this protein is essential to understand its protective efficacy from TB infections. The aim of the present study is to examine the response of immune mediators to pCDNA3.1-*lipX* expression in vivo. In this experiment, the pCDNA3.1-*lipX* was injected into mice BALB/c strain, male, aged between 6-8 weeks compared to groups were injected with pCDNA3.1 and without injection. The injection was carried out three times intramuscularly every two weeks. Blood was taken retro-orbital and used for humoral response analysis by western blotting against LipX-His protein. Simultaneously, the spleens were cultured and induced with LipX-His protein for cellular immunity analyses. Our study showed that recombinant DNA of pCDNA3.1-*lipX* induced a humoral and cellular immune response, especially IL-4, IL-12, and IFN- γ , that were primary cellular responses to *M. tuberculosis* infections. In conclusion, pCDNA3.1-*lipX* we constructed in this research can induce primary cellular immunity for TB infections and promising to be developed as a tuberculosis seed vaccine candidate.

Keywords: Cellular, Hummoral, Mice, LipX (PE11), Tuberculosis

Effect of *Averrhoa bilimbi* Leaf Extract on Blood Glucose Level, Hepatosomatic Index (HSI), and Liver Histology of Diabetic Mice

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Abstract. *Averrhoa bilimbi* leaf extract (EDBW) is known to contain high level of antioxidant. Antioxidant can suppress reactive oxygen species (ROS) produced during hyperglycemic condition in diabetes mellitus (DM), which is able to damage liver tissue. This study aimed to determine the effect of EDBW on fasting plasma glucose (GDP), Hepatosomatic Index (HSI) and liver tissue damage. This study used 24 mice which were divided into 6 groups, including N (normal mice), K- (DM), K+ (DM + glibenclamide 0.013mg/20gBW), E1 (DM+ EDBW 6.3mg/20gBW), E2 (DM + EDBW 8.4mg/20gBW), and E3 (DM + EDBW 10.5mg/20gBW). Mice were induced DM using alloxan dose 120mg/kgBW. Bilimbi leaf extract treatment was given for 14 days after DM was induced. GDP was measured using glucometer before and after EDBW treatment. Liver tissue damage was evaluated through Hepatosomatic index (HSI) and Hematoxylin-Eosin (HE) stained histological slides. Data was analyzed statistically. The results showed that EDBW had a significant effect on the percentage of GDP and liver tissue damage compared to the K- (diabetes) group, while HSI didn't show significant difference. The E2 group had the highest HSI score of 6,18%, while the K- group had the lowest HSI score of 4,41%. The most optimal dose of EDBW was 6.3mg/20gBW with GDP decrease of 26.44%, normal cell percentage of 88.56% and HSI score of 6,18%. It can be concluded that EDBW was able to lower blood glucose and improve liver histology of diabetic mice but didn't affect HSI significantly.

Keywords: Hyperglycemic, antidiabetic, hepatoprotector, natural ingredient.

Functional 3D Structure Analysis of Quasispecies Variants of Hepatitis B Virus Surface and Core Protein in Advanced Liver Disease and Chronic HBV Infection Patients in Indonesia: In Silico

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Abstract. Hepatitis B Virus (HBV) has a high mutation rate and overlapping open-reading frame which could promote the formation of quasispecies variants. HBV mutations in the surface (S) region have been observed to significantly affect advanced liver disease and chronic HBV infection, while mutations in the core (C) region have been observed to increase viral immune escape ability. These problems might lead to hepatocellular carcinoma of the liver tissue. Thus, this research aims to study and perform 3D modelling of C and S proteins based on 12 in-house HBV genome sequences isolated from Indonesian patients infected with advanced liver disease (8 sequences) and chronic HBV infection (4 sequences) from previous research. The result of our 3D structure prediction is intended to be used as a reference for further research related to the discovery of antivirals and natural remedies against HBV. First, we aligned the sequences with ClustalW. Next, phylogenetic analysis was performed by using MEGA11, continued with translation of nucleotides into protein sequences using the ExPASy Translate portal. ProtParam Portal was further used to perform physiochemical analysis. Furthermore, 3D structure modelling was done by using Phyre2 and SWISS-MODEL. We further identified and analysed the active site of the viral protein using FTMap. As the result, we identified some mutations in the S region which often occur at the amino acid (aa) position of 5, 21, and 44. The mutation is sporadic but rare in the 'a' determinant region (aa 120-147). Moreover, hotspot mutations were found in the C region at the aa position of 79 and 87, which is believed to be the domain responsible for decreasing antigenicity of HBc protein. In addition, we discovered the binding site of C protein at A15 residue (leucine) which has the highest averaged nonbonded interactions of 8.52%, and it could further be developed for the antiviral block. As for S protein, its 3D structure was defined as *de Novo* protein by Phyre2 and identified as tumor necrosis factor receptor superfamily member 4.

Keywords: Hepatitis B Virus, 3D structure modelling, quasispecies, S gene, C gene, advanced liver disease, chronic HBV infection

Biological Activity Potential of *Acacia crassicarpa* Honey as Indonesian National Honey (Immunomodulatory Activity Study of *Acacia crassicarpa* Honey)

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Abstract. Honey is well-known for its various biological activities, one of which as an immunomodulator, which are substances that can affect the response of the immune system. In Indonesia, one type of honey that is widely cultivated recently is the *Acacia crassicarpa* honey. This research was conducted by testing lymphocyte cell proliferation with MTS assay to determine the effect of various concentrations on immune cell response, identification of polyphenolic compounds using Liquid Chromatography Mass Spectrometry (LCMS/MS), as well as measurement of total phenolic content in which polyphenolic compounds in honey are suggested to have immunomodulation effect on human body. In addition, physicochemical tests were conducted based on the SNI 8664:2018 standard. The test results showed that *Acacia crassicarpa* honey able to increase the cell viability of LPS-induced lymphocytes thus showed good immunomodulating activity. This result is supported by the findings of phenolic compounds in honey that vary in large quantities therefore suggests it can affect the immunomodulating activity. *Acacia crassicarpa* honey from West Jabung Province, Jambi showed the highest total phenolic content of 494.03 ppm, thus may have higher biological activity potential compared to similar honey from other regions. The results of physicochemical test showed that *Acacia crassicarpa* honey has good quality and safe for consumption, but with a note on several parameters where the test results exceeded the established standards.

Keywords: Honey, *Acacia crassicarpa*, biological activity, immunomodulatory activity, immunomodulator, Indonesian national honey

Characterization of pearl millet oligosaccharides and evaluation of their prebiotic potential

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Abstract. In the present study Pearl millet oligosaccharides (PMOs) were extracted at optimised process parameters and further purified by passing through charcoal column and dialysis membrane (500 Da) to obtain a purity > 90% with a yield of 4.6% (w/w). The molecular weights of the mixed oligosaccharides ranged from 342 to 1316 Da and was confirmed by MALDI-TOF. The non-digestibility assay was performed by acid hydrolysis, salivary and pancreatic α amylases. The results showed a maximum hydrolysis of $0.2516\% \pm 0.0173$, $2.8\% \pm 0.023$ and $3.1\% \pm 0.11$ by gastric acidity, salivary and pancreatic α -amylases respectively. A positive prebiotic score, enhanced biofilm formation and capability of producing SCFAs (i.e. acetate, propionate and butyrate) by different strains of lactobacilli in presence of purified oligosaccharides hints towards their prebiotic potential. From the observations we propose that the PMOs can be explored further as nutraceuticals and functional food additives.

Keywords: Pearl millet, Oligosaccharide, Prebiotic, Short chain fatty acids, Biofilm

Deciphering bioenergy stimulation and electron transport characteristics to screen traditional Chinese medicine (TCM) for COVID-19 drug development

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Abstract. As electron-shuttles (ESs) were electrochemical “catalysts” to enhance rates of redox reactions, they very possibly owned both potentials in cellular bioenergy extraction and clinical disease treatment. In particular, ES-polyphenolics are appropriate to increase power-stimulating capabilities for energy utilization and catalyze disease-treating efficacy for clinical remedy. From the perspective of chemical structure, chemicals with *ortho*- and *para*-dihydroxyl substituents-bearing aromatics exhibit convertible characteristics of non-renewable antioxidants and reusable electrochemically catalytic ESs. Serial studies disclosed that medicines with *ortho*-dihydroxyl substituents on the benzene ring as “medating centers” of ES (e.g., dopamine, L-DOPA, epinephrine) could treat brain disorder-associated disease (e.g., Parkinson’s disease (PD)) due to electron transfer-steered characteristics. In addition, refreshing medicinal herbs *Lonicera japonica*, *Syzygium aromaticum*, *Camellia* green tea were found to own bioenergy-stimulating capabilities, persistently implementing electron-mediating catalysis. Moreover, the efficacy of PD medicines and antiviral flavonoid herbs was strongly governed by their bioenergy-stimulating proficiency for disease treatment. Among 18 TCM herbal extracts in common for the prevention of SARS and H1N1 influenza, considerable total flavonoid contents were found at least for 11 herb extracts. The findings indicated that these herbal extracts were not only rich in phytonutrient contents, but also plentiful in anti-oxidant and anti-inflammatory activities. Herbs with high polyphenol content had higher antioxidant activity. *Forsythia suspensa* extract expressed the highest inhibition against nitric oxide production for anti-inflammation. MFC studies clearly revealed that top ranking efficacious herbs for anti-COVID-19 were both bioenergy-driven and electron mediated. Electron transfer-controlled bioenergy extraction was very likely the dominant driving force to catalyze antiviral characteristics for anti-COVID-19 expression. **Keywords:** Microbial fuel cells, Electron transfer, Bioenergy extraction, TCM, COVID-19

Molecular Simulation Approach for Screening Bioactive Compounds Using HerbalDB Database as Potential Candidate for Alzheimer's Inhibitor

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Abstract. Alzheimer's disease is one of the neurodegenerative diseases that afflict the elderly. One of the symptoms is a loss of cognitive ability due to neuronal death caused by amyloid plaque accumulation. Alzheimer's disease is one of the diseases with high treatment costs. Drugs for Alzheimer's treatment only treat the symptoms, not the disease itself. According to NIH guidelines, several pathways, such as the mitochondrial cascade, can be used to develop drugs for Alzheimer's disease. Caspase3 is a protein that plays an important role in the mitochondrial cascade, particularly in apoptosis. It is hoped that by targeting caspase3, Alzheimer's therapy will be more effective. Indonesia is a rich country, particularly in medicinal plants. The medicinal plants are consumed by the locals by converting them into a drinkable solution known as jamu. Jamu is thought to help with immunity, stamina, and disease prevention. Medicinal plants also contain bioactive compounds. We used the Ligand-Based Drug Design and Structure-Based Drug Design approaches to screen bioactive compounds in Indonesia to find the best compound candidate. As a protein target, we chose caspase3. In addition, we performed ADMETOX prediction, molecular docking, and molecular dynamic simulation on forty 3D structures of bioactive compounds that have been screened and donepezil as an FDA approved Alzheimer's drug. We discovered that Miraxanthin-V had a higher binding affinity than donepezil using molecular docking and molecular dynamic simulation. As a result, we can conclude that Miraxanthin-V has a high potential for inhibiting apoptosis via caspase3.

Keywords: bioinformatics, molecular simulation, Alzheimer disease, bioactive compound, Indonesia

Anti-Alzheimer Potential of *Citrus hystrix* DC. Peel, Leaf, and Essential Oil Bioactive Compounds by Network Pharmacology Study

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Abstract. Alzheimer's Disease (AD) is the most known neurodegenerative disease which prevalence is predicted to increase significantly in the middle century. Currently, there are only five AD pharmacological drugs approved by The US Food and Drug Administration (FDA), but all of them could only alleviate the symptoms without any significant effects on inhibiting the progression of disease. Thus, there is an urgent need to discover novel drugs and treatments for AD. On the other hand, drug discovery by natural products have been preferred lately due to its high potential and low toxicity. *Citrus hystrix* DC. (kaffir lime; jeruk purut; CH) is one of herbal plants abundant in Southeast Asia with many known biological activities. In this study, we explored the potential of CH peel, leaf, and essential oil bioactive compounds as an anti-Alzheimer agent with network pharmacology approach. First, we identified the compounds with KNApSAcK database and related literatures. Next, we identified protein targets of each corresponding compound with SEA Search Server and Swiss Target Prediction, and proteins associated with AD with OMIM, GenCLiP3, and DisGeNET. Next, we constructed protein-protein interaction (PPI) network and compound-target interaction (CTI) network to identify the most crucial proteins and compounds in the network with Cytoscape v.3.9.1's application, Cytohubba v.0.1. We continued with pathway enrichment analysis using STRING v.1.7.1 and molecular docking with PyRx to further confirm the results. Our results showed that some kinases (EGFR, MAPK14) and transcription factors (STAT3, PPARA) might play the most significant roles in the mechanism of CH in fighting AD. EGFR is the identified target of CH compounds such as umbelliferone and oxypeucedanin, with oxypeucedanin showed the highest docking score of -8.2. STAT3's and MAPK14's most fitted compound is citrusside A which scored -5.0 and -7.7 respectively. Moreover, PPARA has the most compounds out of all protein targets which includes alpha-thujene, alpha-pinene, limonene, beta-myrcene, alpha-terpinolene, alpha-terpinene, beta-caryophyllene, copaene, delta-3-carene, delta-elemene, sabinene, (E)-ocimene, cosmene, neoalloocimene, alpha-myrcene, cadinene, phellandrene, and germacrene D with beta-caryophyllene as the most fitted compound (-8.3). Our results suggest that all three CH parts might alleviate AD symptoms and/or progression through various mechanisms such as inflammatory mediator regulation of TRP channels, parathyroid hormone, and metabolic pathways.

Keywords: Alzheimer's Disease, *Citrus hystrix*, PPI network, CTI network, molecular docking, network pharmacology

Computational Studies in Identifying *Xanthine Oxidase* Inhibition Mechanisms by New Compounds of *Tetragonula Sapiens* Propolis as Potential Anti-Gout Drugs

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Abstract. Gout is a disease in the form of inflammatory arthritis that is closely related to purine metabolism disorders that trigger increased levels of uric acid in the blood (hyperuricemia). A common treatment to reduce uric acid levels in blood serum is by inhibiting the activity of the Xanthine Oxidase (XO) enzyme with Allopurinol compounds. Allopurinol is known to have side effects from nausea to liver and kidney damage. Based on previous research, a new compound from propolis *Tetragonula Sapiens* can inhibit the XO enzyme with an IC₅₀ value close to Allopurinol and has the potential to be an active substance in anti-gout drugs. In this research, computational studies in the form of molecular anchoring and molecular dynamics simulations will be carried out to understand the type and mechanism of XO inhibition by the new compound *T. sapiens* propolis. Molecular docking is complemented by performing the MPS approach and protein side-chain flexibility to consider protein flexibility with AutoDock Vina software. The molecular docking results show that the lowest binding energy between the ligand and the receptor occurs in the intermediate domain and not at the active site, which has the potential for inhibition to occur in a non-competitive manner. These results were further identified by molecular dynamics simulations at both the active site and the docking allosteric site, using the Gromacs program. The RMSD of both systems started to stabilize at the 50 nanoseconds. The RMSD of the system without an inhibitor was in the range of 0.3-0.4 nm and with an inhibitor in the range of 0.2- 0.3 nm. The value of the gyration radius for the system without the inhibitor is 3.18 nm and with the inhibitor 3.19 nm. Occupancy of hydrogen bonds formed reaches 100% with Asp1170₍₁₁₇₁₎. Based on these parameters, the simulation results show that the XO complex with propolis has a structural expansion compared to the complex without inhibitor, and the inhibition has a stable interaction with residues that are overall the same as molecular docking.

Keywords: *Xanthine Oxidase*, anti-gout, new compound of propolis *Tetragonula Sapiens*, molecular docking, molecular dynamics simulation

Molecular Docking and Pharmacophore Analysis of Trisindoline 1 Against Human Topoisomerase II and Human Derived Growth Factor

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Abstract. Liver cancer is currently one of the leading causes of deaths worldwide and its incidence is ever-increasing. This has prompted, in the last decade, an increase in design strategies of computational drugs with the utilization of molecules for anti-cancer candidates. DNA topoisomerase II (topo II) enzymes regulate essential cellular processes by changing the topology of chromosomal DNA. As a multitarget combination with DNA topo II enzymes, human platelet derived growth factor (PDGF) is an appropriate receptor as an anti-cancer target and is used as a prognostic marker for liver cancer. Trisindoline 5'-nitro-[3,3':3',3''-terindoline]-2'-one is an indole trimer alkaloid compound that is cytotoxic in the HepG2 cell line and induces apoptosis. In this study, molecular docking of trisindoline 1 was carried out against topo II and PDGF as an alternative drug for liver cancer. The binding energy value against human topo II for trisindoline 1 and doxorubicin were -10.1 Kcal/mol and -9.2 Kcal/mol respectively. Pharmacophore analysis of trisindoline 1 showed that trisindoline 1 was within the optimal range of the drug-like properties and possessed a lower toxicity compared to doxorubicin. Trisindoline 1 can inhibit PDGF and human topo II enzymes in liver cancer through the apoptotic pathway.

Keywords: Liver cancer, Pharmacophore, Molecular docking, PDGF, Trisindoline 1, Topoisomerase-II

Cell Engineering Targets for CHO Cells

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Abstract. Therapeutic antibodies used for the treatment of cancer and rheumatoid diseases are representative biopharmaceuticals, and the market size is steadily expanding with the diversification of products. The antibody manufacturing process using recombinant Chinese hamster ovary (CHO) cells as producer cells consists of cell culture and purification processes. Efficient antibody production processes have been developed for improving antibody productivity and reducing costs. In the 1980s, the antibody productivity was less than 100 mg/L, but is now 1–10 g/L, which is typical of antibody concentrations in fed-batch cultures. In order to increase the antibody concentration, it is important to acquire a good cell line having a high specific production rate, which has been achieved mainly by improving the cell line development process, to adopt as the producer cells in the manufacturing process. The construction of high-producer cells is an important and first step in the industrial manufacturing of antibodies. Antibody producer cells are constructed by gene transfer of expression vectors containing expression cassettes of target antibody genes into host CHO cells, followed by screening for high-expressing cell lines from stably transformed pool cells. To obtain high-expressing cell lines, optimizations of host cells, expression vectors, gene transfer and screening methods are required. Generally, producer cells are established by introducing plasmid vectors encoding expression cassettes of target antibody genes along with drug resistance genes into host cells, followed by drug selection culture. Since the transgene is usually integrated randomly into the genome of CHO cells, the stable pool cells obtained after drug selection culture contain a variety of producer cells with different levels of target antibody production and passaging stability. A labor-intensive and time-consuming process is required to obtain a small number of highly expressing and stable cells from the stable pool cells. Therefore, various screening methods have been developed to efficiently select cell lines suitable for industrial manufacturing. In contrast, a site-specific gene transfer method into a specific site in the host cell genome has been developed and is expected as an efficient method for obtaining highly stable cells in a short period of time. In this presentation, the cell engineering technologies related to the construction of producer cells, such as engineered host cells, expression vectors, and techniques for obtaining cells that produce target products at high levels, will be reviewed.

Keywords: Cell engineering, CHO cells, Antibody production

Antibacterial Activity of *Adenium Obesum* Flower Extracts Against *Escherichia coli* and *Staphylococcus aureus*

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Abstract. The uncontrolled use of antibiotics to treat secondary bacterial infections has the potential to cause bacterial resistance to antibiotics. Therefore, researchers are aggressively exploring the potential of plants as renewable antibacterials. In this study, the antibacterial activity of *Adenium obesum* flower extracts was explored against *E. coli* and *S. aureus*. *Adenium obesum* flowers were extracted by maceration using four different solvents (ethyl acetate, acetone, ethanol, and aquadest). The extract obtained was then tested for phytochemical content and antibacterial activity against *E. coli* and *S. aureus* by disc diffusion and microdilution methods. From the phytochemical content assay, it was found that all extracts contained flavonoids and alkaloids but did not contain steroids and terpenoids; saponins were only found in acetone and ethanol extracts; tannins were only contained in acetone, ethanol, and aquadest extracts. As for glycosides, only contained ethyl acetate, acetone, and ethanol. Through the disc diffusion assay, it was found that the ethyl acetate extract of the *Adenium obesum* flower had the best activity to inhibit the growth of *E. coli* and *S. aureus*. The minimum inhibitory concentration (MIC) values were determined using the microdilution method. For *E. coli*, the MIC value was reached at a concentration of 93,55 mg/mL. For *S. aureus*, the MIC value was reached at a concentration of 41,18 mg/mL.

Keywords: Antibacterial, *Adenium Obesum* Flower Extracts, *E. coli*, *S. aureus*

Metal Oxide Nanoparticles Modified Glass Ionomer Cement: A Short Review

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Abstract. Glass ionomer cement (GIC) is used as restorative material for the management of dental caries. GIC has fluoride release, biocompatible, and adhesion to tooth structure. Despite its favorable properties, GIC has low mechanical properties. Several studies reported that the addition of metal oxide nanoparticles to GIC enhanced its compressive strength, diametral tensile strength, and shear bond strength. This short review aims to describe the properties of metal oxide modified GIC, including their mechanical and antibacterial properties. This review is expected to provide the information on the current metal oxide nanoparticles used to modify GIC.

Keywords: Glass ionomer cement (GIC), modification, metal oxide, nanoparticles

Transformation and Cloning of Dengue Virus's *rE* Gene using pEGFP-C1 as Vector in *Escherichia coli* DH5 α

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Abstract. Dengue Virus (DENV) infection now has become a major public health threat, with nearly majority of population around the globe at high risk of infection. Effective treatments and drugs against DENV infection have not been established yet. With the emergence of the first licensed vaccine, Dengvaxia® (CYD-TDV), became a start to the development of several vaccine candidates. Hence, with the advent of recombinant DNA technology, DNA vaccines are such a promising approach compared to conventional vaccine methods. The recombinant multivalent protein envelope of DENV, known as *rE*, was constructed based on protein envelope domain III of four serotypes DENV, and was reported structurally stable as well as potentially immunogenic. This research is conducted as an early stage in the development of DNA vaccine candidates against DENV, by transforming optimized *rE* gene mediated with plasmid vector pEGFP-C1 using heat-shock treatment in *E.coli* DH5 α as cloning host. Competent cells were produced using the combination of Magnesium Chloride and Calcium Chloride solution. The results showed growth signs of transformant colonies on a selective media containing Kanamycin 100 μ g/ml, relatively. Colonies was confirmed carrying the target gene by colony polymerase chain reaction (PCR) screening. Observation on agarose gel under UV Transilluminator indicate bands that appeared in the product size of 157 base pairs.

Keywords: cloning, dengue virus, DNA vaccination, pEGFP-C1, transformation

Genome mining reveals the biosynthetic gene cluster of novel lantibiotic 'homicorcin' from *Staphylococcus hominis* strain MBL_AB63

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Abstract. Lantibiotics are antimicrobial peptides produced by different bacterial species and considered as potential agents that can help filling the current void of effective antimicrobial therapies. Due to the accessibility of good quality genomes from a wealth of different bacterial species, presents an opportunity to identify more novel lantibiotic agents and their biosynthetic machinery. We have isolated a novel strain from jute seed as an endophyte named *Staphylococcus hominis* strain MBL_AB63 that was reported to have substantial plant growth promoting activity. Whole genome sequence (WGS) was annotated using BAGEL4 and AntiSMASH 5.0 to predict the gene cluster for secondary metabolites and antimicrobial compounds. Both the *in silico* tools predicted a novel antimicrobial peptide from the WGS of MBL_AB63 that belongs to class I group (lantibiotic) and its associated genes for biosynthesis. Predicted lantibiotic homicorcin was found to have quite similar to a reported peptide antibiotic epicidin 280 and has seven amino acid differences in several positions. We have purified the peptide using RP-HPLC and then subjected to LC-MS/MS analysis to identify the molecular mass of the peptide. Mass data reveals the mass of homicorcin is 3048 Da. Complete sequence of the peptide, structure and detailed biosynthetic mechanisms has been identified. The efficiency of bioinformatics applications such as BAGEL4 and antiSMASH 5.0 to effectively identify novel antibiotic candidates from the genome data is very high. Therefore, this *in silico* approach will provides a unique opportunity for the discovery of potentially novel antimicrobial agents effective against MDR pathogens.

Keywords: Genome mining, BAGEL4, antiSMASH 5.0, lantibiotic, MDR pathogen, Homicorcin

Sensitive and specific microRNA hybridization using partially methylated phosphotriester antisense DNA probes

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Abstract. MicroRNAs (miRNAs), a class of short non-coding RNA, play important roles in regulating gene expression. However, current miRNA hybridization methods for miRNA detection/inhibition using antisense oligonucleotides, including In Situ Hybridization (ISH), RT-qPCR, and miRNA microarray profiling technology, are lacking hybridization specificity and affinity. As such, to ameliorate these problems, the neutralized DNA, an emerging class of DNA oligonucleotides chemically synthesized with site-specific internucleoside methyl phosphotriester linkages was used as a hybridization probe instead of DNA. The reduction of inter-strand charge repulsion of nucleotide duplexes results in stronger binding between nDNA and other nucleic acids. This study suggested that partially methylated antisense nDNA (N4 nDNA, with four modifications) probes inhibited miRNA more efficiently (higher Ct value) than DNA probes in the human plasma-like medium, as demonstrated by the RT-qPCR assays. Subsequently, we performed in situ hybridization analysis using a miR-21-expressing colorectal cancer cell line (HCT116). HCT116 cells stained with N4 nDNA antisense probes revealed a greater detection intensity and specificity than cells stained with DNA probes. Consistently, enzyme-linked immunosorbent assays (ELISA) suggested that the miRNA hybridization efficiency of N4 nDNA antisense probes was greater than that of DNA probes. In addition, the cell-based immune response of the N4 nDNA antisense probe was immune-negative (cooperated with Dr. Ya-Hui Chi at National Health Research Institutes) and the N4 nDNA antisense probe was also inhibited in vitro GFP mRNA translation (cooperated with Prof. Kosuke Fujishima at Earth-Life Science Institute). As N4 nDNA probes are resistant to DNase I and do not elicit a strong immune response in cells, future development of nDNA probes as therapeutic nucleic acid drugs for in vivo gene expression modulation is possible.

Identification of Bioactive Compounds in Indonesian Traditional Herb and Efficacy in healing patients with symptoms of Corona Virus Infection

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Abstract. Coronavirus disease 2019 (COVID-19) is currently affecting humans almost all over the world. People infected with Covid 19 are indicated to be able to cause Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Its mutates very quickly The speed of this virus mutates and infects humans and transmit an acute outbreak of pneumonia. Until now, no specific drug has been found to treat the disease covid 19. This virus is a pathogen and is closely related to the human immune system. Researchers around the world are looking for alternative medicines that can be used to treat the disease covid 19. In Indonesia, an alternative treatment for covid 19 is using herbal medicine. Traditionally, typical Indonesian herbs that have often been used to increase the immunity of the human body and treat diseases have become alternative treatments to reduce symptoms and infections caused by COVID-19. Indonesian herb for Immune are *Andrographis paniculate*, *Curcuma Domestica Rhizoma*, *Curcuma Xanthorrhiza Rhizoma*, *Kaempferia Galanga Rhizoma*, *Zingiber Officinalis*, *Coriandrum Sativum Fructus Extract*, *Lilicium verum flos extract*, *Ammomum compactum fructus extract* and palm sugar. These are formulated called *E-Immune* formulations. Traditionally, *E-Imunne* has also been consumed by the public peoples and has shown a significant effect on healing from the COVID-19 disease. In order to fulfill the scientific study of the medicinal use of Indonesian herb plants, this research was carried out. The purpose of this study was to analyze the bioactive compounds in the *E-Immune* formulation and complement the study with the use of *E-Immune* to treat patients with COVID-19 symptoms in residents in two locations in Indonesia. The results showed that the bioactive compounds in the *E-immune* formulation were, *Phenolic phthiocerol*, *Androstenedione*, *Propylene glycol dioleate*, *2-methyl-2[2-(methyl)-6-xanthonoxy]-propionic acid*, *Valine*, *Carnosic acid*, *Pectolarigenin*, *Microcin SF608*, *Pheophytin a*, *Eramanthin*, *Nabumetone*, *Piptamine*, *Santonin*, *Drimenin*, *Isopentyl cinnamate* and other bioactive compounds.

Keywords: Indonesia, Herb, compound, bioactive, covid 19

Protein Profile Analysis and Lee-White Test on Crude Protease of *Bacillus* sp. HSF1-9 to Study Its Potential as A Meat Tenderizer and Anticoagulant Agent

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Abstract. The use of protease in meat may hydrolyze meat's proteins into simple amino acids causing the meat to become tender. In addition, the enzyme may also destroy the abnormal blood clot in the body called thrombus, a mortality cause of cardiovascular disease (CVD). In this study, crude bacterial protease isolated from marine bacterium HSF1-9 (HSF1 = *Holothuria scabra* Fermented Intestine-9) was investigated for its potential use as a meat tenderizer and blood anticoagulant. Protein profiles of beef, chicken and cob were analyzed before and after immersion in the crude protease of bacteria HSF1-9 at a concentration of 30% v/v for 3 h based on Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) method. Next, the anticoagulant test of crude protease HSF1-9 on venous blood samples was performed by measuring the blood clotting time compared with that of control (10% EDTA) by the Lee-White method. The SDS-PAGE results indicated that soaking beef and chicken using crude protease HSF1-9 could hydrolyze the meats' proteins by denaturing them into smaller molecules indicated by the generations of new minor protein bands yet removing old major bands of the meat proteins. This shows that crude protease of HSF1-9 bacterium has the potential as a meat tenderizer of beef and chicken meat. Meanwhile, results of the anticoagulant test screening showed that the crude protease of isolate HSF1-9 was able to prolong the blood clotting time even though in the current crude form, its anticoagulant activity was still less than that of 10% EDTA (a commercial anticoagulant agent used as positive control).

Keywords: Anticoagulant, *Bacillus* sp. HSF1-9, bacterial protease, meat tenderizer, protein profile

Evaluation of Physical and Biological Performances in Single-use 50 mL Scale Multi-bioreactor System for Mammalian Cell Culture

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Abstract. A variety of biopharmaceuticals such as antibodies, proteins, and gene/cell therapeutics have been developed in recent decades and demands for them have increased dramatically every year. To meet such demands, it is essential to cultivate cells that produce biopharmaceuticals in large-scale bioreactors and it is very important to optimize culture conditions for massive production of biopharmaceuticals. Scale-down model, using multi- and small size (less than 100 mL) bioreactors, has been an effective tool for applying DOE (Design of Experiment) and QbD (Quality by Design) for process optimization of large-scale cell cultures.

In this study, a newly developed single-use multi-bioreactor system that can control pH and DO (Dissolved Oxygen) with optical sensors, and culture temperature of each bioreactor which is expandable to 24 bioreactors is introduced. And the evaluation of biological and physical performances of this single-use multi-bioreactor system are explored. Ultimately, this research implies that the newly developed cell culture system can be a practical tool for optimization of cell culture process in the biopharmaceutics industry.

Keywords: Multi-bioreactor system, scale-down model, single-use, optical sensors, cell culture system

Development of Chemically Defined Media for CHO Cell Culture

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Abstract. The development and optimization of cell culture media are crucial steps in mammalian cell-based manufacturing process for higher growth and productivity. In the industrial market, the demand for chemically defined media(CDM) is increasing due to good reproducibility and elimination of the risk of contaminants. In this study, we showed the development process of CDM through optimization of the composition and screening key components using Plackett-Burman design(PBD). First, we optimized the concentration of amino acids and energy sources in SJ-PFM 2.0, an in-house medium. Based on this medium, a CDM was developed. Then, PBD was adopted to screen significant components for higher growth and productivity. Finally, we found that vitamin B and polyamine groups were the most critical factors. With the optimization of concentration about key factors, the results showed that IVCD was 3.3-fold and IFN- β productivity was 6.3-fold improvement over the control culture. This study suggested that the optimization of glucose and amino acids was crucial. Moreover, PBD was an efficient way for screening effective composition to develop CDM for CHO cells.

Keywords: CHO cell, chemically defined media, Plackett-Burman design, DOE

Engineering UTR(Untranslated Region) of Expression Vectors to Enhance Recombinant Interferon- β 1a Productivity in CHO Cells under Low Temperature

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Abstract. The well-characterized Cold Shock Proteins(CSPs), Cold Inducible RNA binding Protein(CIRP) and RNA Binding Motif3(RBM3) are highly expressed in mammalian cells under mild hypothermia. They are known as RNA Binding Proteins (RBP) interacting with specific sequences in untranslated region(UTR) and increasing their expression level by controlling mRNA stability and translation efficiency.

Here, we hypothesized that production of recombinant protein could be increased when the motif of 3'UTR interacting with CSP are inserted into the 3'UTR of the expression vector. The expression vector containing the specific untranslated region(UTR) of the genes, which are reported to increase gene expression by interacting with CIRP or RBM3, were constructed and transfected into CHO cells. The results showed that the specific motif in 3'UTR of thioredoxin(TXN) increased the IFN- β 1a production in CHO-K1, and that of eIF5A(eukaryotic initiation factor5A) increased the production of it in CHO-S. Moreover, the productivity of IFN- β 1a was further enhanced under mild hypothermic culture condition.

Keywords: Untranslated region, CHO cell culture, Mild hypothermia, Cold Shock Proteins

Recombinant human G-CSF Recovery from Inclusion Bodies of *Escherichia coli* and its Characterization

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Abstract. Expression of recombinant human Granulocyte Colony Stimulating Factor (rhG-CSF) in *Escherichia coli* may lead to the formation of inclusion bodies (IB's) due to the failure of disulfide bond formation. In order to retain biological activity of the recombinant protein, many procedures of isolation, solubilization and refolding are developed. In our previous study, we already improving the expression of rhG-CSF by reducing GC content and improving mRNA folding free energy. By using this codon variant of rhG-CSF, we compared two methods of solubilization and refolding protein. In the first method, refolding process was performed by dialysis with the addition of β -mercaptoethanol. While in the second method, 6x glutathione buffer was used to refold solubilized IB's. The method using β -mercaptoethanol gave better solubilization results than the second method, and produced 580,214 $\mu\text{g/ml}$ of refolded protein. Protein characterization result showed that refolded rh-GCSF was similar with the native G-CSF. Finally, simple purification using two steps of ultra-centrifugal filters produced 345,63 $\mu\text{g/ml}$ pure hG-CSF.

Keywords: rh-GCSF, inclusion bodies, solubilization, refolding

Development of DMSO-free, Serum-free and Chemically-defined Cryopreservation Media for Mammalian Cells

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Abstract. Many studies have shown that cells are likely to experience genetic or phenotypic variations over generations. And cryopreservation is believed to be the only way to keep cells unchanged in their properties (-80°C to -196°C). However, this cryopreservation method can have a negative effect on cells because it provides cells with an extreme environment (high osmotic pressure, physical and chemical stress, etc.). To reduce this negative effect as much as possible, CPAs (cryoprotective agents) are used. Currently, the most commonly used cryoprotective agent is DMSO (dimethyl sulfoxide). However, the use of DMSO is somewhat controversial as studies have shown that it may reduce cell viability or affect gene expression systems.

Therefore, we conducted research to develop preservatives using substitute substances other than DMSO. This study systematically selected cryopreservative candidates that can respond to expected cell damage. And candidate substances were largely classified into main-effect cryopreservatives polymers, and antioxidants. These can prevent ice formation and inhibit physical damage to cells by removing water from the cells. We used Plackett-Berman design (PBD), and general full factorial design (GFFD), which are statistical techniques such as design of experiments (DoE) to develop serum-free, chemically defined formulations with similar performance to DMSO.

In conclusion, we identified cell-permeable substances that have the key effects to replace DMSO, and additionally designed cryopreservation compositions by adding functional additives. By further developing this, we aim to eliminate controversial DMSO when freeze-preserving cells for biopharmaceutical production, and to develop products with equal or better freeze-preservation capabilities without using DMSO.

Keywords: Cryopreservation, CPAs(cryoprotective agents), DMSO(dimethyl-sulfoxide), Antioxidants, DoE(design of experiment)

The Effectiveness of *Red Grape Seed Extract* on *Kydney Organ* of *White Rats Exposed to Formaldehyde*

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Abstract. Even though it is acidic, formalin can harm the body and even cause cancer when it is consumed through food. Continuous consumption of formalin, which causes an excessive synthesis of (ROS) and activates oxidative stress, might harm the kidneys. MDA levels that are higher or histological alterations in the renal tissue might both indicate changes in kidney parameters. Using proanthocyanidins to prevent kidney damage, grape seed extract contains flavonoids such proanthocyanidin oligomers, which are 50 times more abundant than vitamins C and E. 10% of the grape flesh extract contains polyphenols, compared to 60%–70% in the seeds and 28–35% in the grape skin. Effective secondary metabolites, such as polyphenols, have positive effects on human health, including anti-inflammatory, antiviral, anticancer, and antioxidant capabilities. Water makes up 28-44% of the weight of grape seeds, which have a dry matter content of 71.5%. Grapeseed oil benefits human health, notably in the treatment of acute and chronic illnesses. One sign of kidney damage is adjustments in the histological structure of the kidneys, such as tubulointerstitial damage in the form of dilatation, interstitial inflammation, fibrosis, and necrosis. Kidneys are organs that function in removing toxic or toxic substances and maintaining a balance of fluids and substances that are useful for the body. Degeneration, hyperplasia, necrosis, and inclusions are indicators of kidney damage, as are tubular lumen dilation, accumulation of debris cells in the lumen, tubular lumen vacuolization, enlargement of Bowman's gap, and inclusions. formalin exposure of 25 mg/kg BB rats/day for 22 days versus no formalin exposure. Knowing the MDA concentrations and histological characteristics of the kidney tissues of rats treated for 22 days with 0.035 grape ethanol seed extract, 0.070 and 0.140g/BW rat/day, and subjected to 25mg/kg BW of formalin. Find the optimal dose of grape seed ethanol extract to lower MDA levels and enhance the histological picture of the kidney organs of rats that were given formalin exposure. With a post-test only control group design, this study is an experimental lab research. The 30 white rats used as the sample population were separated into 5 treatment groups, each containing 5 rats and 1 reserve rat. The data on the findings of the MDA levels and the results of the histopathological score were then examined using the computer program SPSS 26.0. The study's findings revealed that MDA levels in rats subjected to formalin were higher than those in the negative controls, while the histopathological findings in the formalin-exposed group revealed necrotic damage. Therapy Increased concentrations of grape seed extract reduced MDA levels by an average of 7.3732, 4.8664, and 4.5605 as well as percentages of 30.27%, 53.98%, and 57.98%. They also improved the histological features of the rat kidney, particularly the glomerular features. The greatest daily dose was 0.140g/BW rat/red grape seed ethanol. The concentration of red grape seed ethanol extract that was successful in lowering MDA levels was 57.98%, and it also improved the histopathological picture of the rats at 0.140g/BW rats/day.

Keywords: red grape seed extract, formalin, kidney, histopathology, MDA

The Effectiveness of Grape Seed Extract on the Liver and Heart Organs of White Rats Exposed to Formaldehyd on Malondialdehyd and Histopathology

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ABSTRACT. Although it is illegal to use formalin unrestrictedly, it is nonetheless utilized as a food additive. Overexposure to formaldehyde can raise oxidative stress and harm human cells. The liver is the body's metabolic hub and a sizable digestive organ with activities that are extremely complex. It weighs between 1.2 and 1.8 kg. Hepatocytes can account for up to 70% of the total number of cells or 80% of the liver's volume. The pericardium is a protective membrane that surrounds the heart, which is located in the chest cavity. Cardiovascular muscle and dense connective tissue constitute the heart wall's fibrous skeleton. The purpose of the study was to assess how well grape seed ethanol extract (EBA) reduced levels of malondialdehyde (MDA) and histopathology in the liver and heart of rats after oral formalin exposure. The study's objectives were to determine the liver histology and MDA levels of white rats given formalin exposure, as well as the effect of grape seed extract on reducing MDA levels in the heart and liver of exposed white rats and enhancing liver histopathology. Understanding the concentration of red grape seed extract, which can effectively cut MDA levels and improve the histological picture of white rats given formalin. In this experiment, 30 male Wistar rats (*Rattus norvegicus*) aged 2 to 3 months, weighing 200 grams on average, were separated into five groups. All groups except the negative control group were treated to formalin 25 mg/kgBW/day for 21 days. 35, 70, and 140 mg/kgBW rats/day of grape seed ethanol extract were administered to the three treatment groups. On day 22, surgery was done, and the heart and liver were removed for MDA and histological examinations. In the positive control liver, the average MDA levels were 9.4280, 7.8898, 5.4851, and 3.1983 whereas in the heart, they were 6.1949, 5.7011, 4.1323, and 4.8064 in white rats, according to the data. MDA levels in the liver were greater in positive control rats (exposed to formalin 25 mg/kg BW rats/day) than in negative control rats. MDA levels dropped when grape seed extract at concentrations of 35, 70, and 140mg/kg BW rats/day was added to the treatment, although the heart organ of white rats in the positive control group (exposed to formalin at a rate of 25mg/kg BW rats/day) was larger than that of the negative group. MDA levels reduced with the addition of grape seed extract at dosages of 35 and 70 mg/kgBW rats/day, but rose at 140 mg/kgBW rats/day. The rat liver's histology revealed necrotizing damage, parenchymatous degeneration, and hydropic degeneration, while pyknosis, karyoeksis, and karyolysis were determined to be signs of damage to the heart. demonstrated that giving EBA to the liver after it has been exposed to oral formalin can improve it. Using Roenigk faulty scoring, improvements in liver histology and a decrease in MDA levels, a sign of oxidative stress, were seen. The administration of EBA had no immediate impact on MDA levels or the histology of cardiac organs subjected to oral formalin.

Keywords: Grape seed ethanol extract, liver, heart

Comparative Study of Bioactive Compound Content and Antioxidant Capacity in Different Extraction Method of *Syzygium Polyanthum* Leaf

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Abstract. The use of extraction methods in plant become one of the most crucial step in the use of phytochemicals drug. This study aims to compare the bioactive compounds contained and antioxidant capacity of two different extraction methods, such as maceration and Ultrasound-assisted extraction (UAE), of *Syzygium polyanthum* leaf. The maceration and UAE extraction methods of *S. polyanthum* used 96% ethanol for the extract solvent. Both extract were then screened the bioactive compounds using Liquid Chromatography–High Resolution Mass Spectrometry (LC-HRMS) analysis. Besides, the total phenolic and flavonoid content also measured by comparing with Gallic acid and quercetin as the standard. The antioxidant capacity was then evaluated using 2,2-diphenylpicrylhydrazyl (DPPH) assay. The LC-HRMS analysis result showed there were several different bioactive compounds contained in the two extracts (maceration and UAE extract of *S. polyanthum* leaf). Besides, the phenolic content determination showed UAE has higher phenolic content (122.87 mg GAE/g) compared to maceration (94.63 mg GAE/g), where the flavonoid content was similar (60.074 and 60.194 mg QE/g). The DPPH assay showed UAE has very strong antioxidant capacity indicated by 41.23 µg/ml of IC₅₀, where maceration showed strong antioxidant capacity with 88.21 µg/ml of IC₅₀. This study showed that UAE extraction was better than maceration extraction method of *S. polyanthum* leaf.

Keywords: antioxidant, flavonoid, phenolic, *S. polyanthum*, ultrasound-assisted extraction.

The Cytotoxic Activities of *Xylocarpus granatum* A Coastal Mangrove Plant Ambon Region East Indonesia Leaves Ethanol Extract Against MCF-7 and HEK293 Cell Lines

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Abstract. Historically, Southeast Asian coastal communities have used *Xylocarpus granatum* (XC) to treat malaria, diarrhea, cholera, fever, and cancer. In order to develop new anticancer plant therapies, it is important to come up with bioactive substances and highly effective cytotoxic agents that cause minimal side effects. Thus, this study seeks to identify and evaluate the major and minor bioactive compounds as well as the possible cytotoxic effects of XC leaves ethanol extract origin from Ambon Region, East Indonesia against MCF-7 breast cancer and HEK293 human normal kidney cells. In order to characterize the bioactive compounds in the XC leaves ethanol extract, GC-MS analysis was employed. The Prestoblue assay was used to determine the cytotoxicity of XC leaves ethanol extract toward MCF-7 and HEK293 cell lines. IC₅₀ values were found for MCF-7 and HEK293 cells for XC leaves ethanol extract at 3.90 µg/mL and 360.10 µg/mL, respectively. Based on the GC-MS analysis, thiazole was found as the major compound, followed by fumaric acid as a minor compound. MCF-7 cancer cells were observed to exhibit morphological changes following exposure to leaves XC ethanol extract for 24 h, indicating a decrease in cell numbers. A decrease in cell number was not observed in the morphology of HEK293 normal cells. Based on these findings, it can be concluded that the leaves XC ethanol extract induces pre-apoptotic cell death, exerts anti-cancer activity, but does not interact with normal cells. New anticancer plant therapies may be developed using bioactive compounds in XC.

Keywords: Thiazole, Fumaric Acid, *Xylocarpus granatum*, breast cancer, human normal kidney.

Comparison Spray-Dried and Freeze-Dried Propolis from *Geniotrigona thoracica* as an Anticancer

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Abstract

Propolis, a product of the stingless bee, *Geniotrigona thoracica*, has high polyphenol content and displays anticancer, antibacterial, antifungal, antioxidant, anti-inflammatory, and immune system enhancement properties. Despite its beneficial properties, the use of propolis as an anticancer bio-supplement remains limited because of its low solubility in water and the existence of perishable volatile chemicals. Encapsulation using freeze drying or spray drying was thought to be a promising solution for these addressed issues. The study focuses on making propolis powder and evaluating its anticancer activity. An extract of propolis was encapsulated using various encapsulate agent (pure chitosan, pure casein, and chitosan-casein mixed), then dried using freeze-dried or spray dried. The success of encapsulation was evaluated by yield, solubility, moisture content, loading capacity, encapsulation efficiency, Fourier-transform infrared spectroscopy (FTIR) analysis, scanning electron microscopy (SEM) analysis, total phenolic content, and total flavonoid content. Anticancer activity was also measured to evaluate which drying method yielded a more effective product. Both drying methods were almost equally capable of producing a stable powder. However, propolis powder produced by spray drying using chitosan/casein encapsulant was most promising in several aspects. Yield, solubility, moisture content, and encapsulation efficiency were 44.47%, 92.20%, 3.59%, and 94.47% respectively. Chitosan/casein-encapsulated propolis from spray drying displayed higher total flavonoid content (968.746 mg QE/g of extract) but lower total phenolic compounds (766.082 mg GA/g of extract) compared with freeze dried propolis. *HeLa cell* viability showed the lowest percentage (72,19%) after treatment with chitosan/casein-encapsulated propolis from spray drying

Keywords: Anticancer agents, Encapsulation, Propolis, Casein, Chitosan, *Geniotrigona thoracica*.

Bioprocess and Bioseparation Engineering

Techno-Economic Evaluation of Novel SARS-CoV-2 Vaccine Manufacturing in the Insect Cell Baculovirus Platform

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Abstract. Vaccination against SARS-CoV-2 virus successfully lessens the impact of COVID-19 globally, however low to middle-income countries may encounter issues in procuring the vaccines. It is necessary to increase the vaccine manufacturing capacity especially aimed at low to middle-income countries. Novavax (NVX-CoV2373) is a protein subunit vaccine that is manufactured using a baculovirus and insect cell system (BICS) platform. This vaccine offers thermostability, practicality, adaptability to be modified against upcoming COVID-19 variants, and feasibility for rapid large-scale production. This study aims to conduct a techno-economic analysis to assess the BICS platform of vaccine manufacturing and compare it to the existing mRNA (messenger RNA) platform and the novel saRNA (self-amplifying RNA) platform. Novavax released its patent for the SARS-CoV-2 vaccine formulation and the manufacturing steps, these data are then used to simulate the vaccine production in SuperPro Designer. From the techno-economic analysis, the productivity of all platforms is compared in terms of doses/day per L production scale. The saRNA platform is about 1,000 times more productive than BICS platform and 20 times more productive than mRNA. The saRNA and BICS platforms are economically up to par, shown by their similar cost per dose, while the mRNA platform's cost per dose is 7 times higher compared to the BICS and saRNA platforms. However, since the saRNA platform is less clinically developed it is best to focus on developing the BICS platform as a route for SARS-CoV-2 vaccine manufacturing in low to middle-income countries.

Keywords: baculovirus, COVID-19, insect cell, manufacturing, SuperPro Designer, techno-economic analysis, vaccine.

Modeling and Simulation of an Industrial-scale Fed-batch Penicillin Fermentation: Improving the Model for Mixed Substrates

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Abstract. This paper focuses on modifying a benchmark model for industrial-scale fed-batch fermentation of *Penicillium Chrysogenum*, primarily developed by Goldrick et al. [1]. The adopted model has been modified to apply to mixed substrate fermentations, with the carbon sources being glucose and lactose. The structured model is based on the heterogenous compartmental structure of a *Penicillium Chrysogenum* strain and includes equations for four regions, namely growing regions, non-growing regions, degenerated regions, and autolysed regions. Whereas, being a macroscopic approach, vacuoles have not been modeled, although their concentration has been quantified using a population balance equation. The model provides a comprehensive approach to studying complex fermentations and includes the effects of nutrient consumptions like dissolved oxygen, nitrogen, and phenylacetic acid, along with the impact of pH, viscosity, temperature, and pressure. Mechanical parameters for vessel dimensions and impeller specifications were also integrated into the model as these affect certain nutrient consumptions and environmental parameters like dissolved oxygen content, foaming, and kinematic viscosity of culture. The kinetic parameters have been adopted from literature while manipulated variables like substrate and phenylacetic acid feed rates have been adopted from peer-reviewed industrial data. The algorithm has been developed and simulation performed in MATLAB R2020a using variable order stiff solver because of equations' inherent non-linearity and diverse nature. The algorithm has made provisions to account for pH maintenance using acid-base addition, automatic discharge for volume control, and cold/hot water addition for temperature control. These inclusions help imitate the true nature of the fermentation and pave the way for the algorithm to be used as a process simulator. The results obtained have been extensively validated against both numerical and experimental data using results from the literature [1-3]. The model provides acceptable fits for the experimental data and is adaptable for both single and mixed substrate fermentations. The model was also evaluated for single substrate fermentations and a comparative analysis was undertaken on the effects of vacuole concentration on single and mixed substrate fermentations. The effects of primary substrate feed concentrations on both single and mixed substrate fermentations were also studied. The results support the hypothesis that mixed substrate fermentations provide better conditions for Penicillin growth than single substrate ones. Finally, sensitivity analysis has been performed to study the effects of uncertainties in the process inputs on the simulation results. Thirty different parameters have been studied and ranked based on their significance quantified by a sensitivity measure. Finally, parameters crucial to the optimum fermentation process have been identified and their optimal ranges determined.

Keywords: *penicillium chrysogenum*, mechanistic model, simulation, industrial fermentation, sensitivity analysis.

Optimizing S-Allyl-Cysteine (SAC) Content And Antioxidant Activity in Extract Black Garlic (*Stamilic*) for Health Supplement

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Abstract. S-Allyl-Cysteine (SAC) is a bioactive compound in black garlic. It can inhibits and decrease the value of Plasminogen Activated Inhibitor (PAI-1) in comorbid COVID-19. This work aims to optimized the SAC content and antioxidant activity in extract black garlic which used for health supplement (*Stamilic*). In this study, black garlic was crushed with milling process by colloid mill, which lead to extraction with water solvent and separation with centrifuge. Extraction variables including solvent composition (1:2 ; 1:4; 1:8; 1:10 ratio) and speed of centrifuge were investigated for the highest SAC content and antioxidant activity. Liquid chromatography mass spectrophotometry (LCMS) method was used to analyze the SAC content and antioxidant activity analyzed with DPPH (*,2-diphenyl-1-picrylhydrazil*) method (UV-VIS Spectrophotometry; 517 nm wavelength). The result showed that SAC in extracted BG increased from 305,91 ppm (fresh BG) to 335,46 ppm, also antioxidant activity increased 1,5 times. SAC content enhancement will increase the quality of *Stamilic* product and cost reducing in industrial process.

Keywords: black garlic, extraction, S-Allyl-Cysteine (SAC).

Effect of Substrate Additives and Extraction Conditions on Cellulase Production through Solid State Fermentation

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Abstract. In this study, the production of cellulase enzyme was performed by solid state fermentation technique using oil palm empty fruit bunches (EFB) as the main substrate. *Trichoderma viride*, which was previously identified as the superior organism, was used as the producer. Strategies to enhance crude enzyme activity was studied by focusing on two different aspects, the fermentation steps and crude enzyme extraction step. For the fermentation, various concentration of glucose and Tween 80 were added together with the EFB substrate and other nutrients. The addition of glucose in the beginning of fermentation increased the crude enzyme activity, as quantified using filter paper assay. The highest crude enzyme activity was achieved when 3 mg of glucose was added, while the addition of higher amount of glucose (6 and 9 mg) did not increase the activity further. In contrast, the addition of Tween 80 did not result in any significant change on the enzyme activity at all concentration tested. For crude enzyme extraction, variation of solid to liquid ratio and number of extraction stages were studied. Higher enzyme activity was achieved when lower solid to liquid ratio were used, as higher buffer volume allowed for more enzyme to be extracted from the system. On the other hand, increasing the number of extraction steps did not have any significant effect on the resulting cellulase activity.

Keywords: cellulase, solid state fermentation, oil palm empty fruit bunches.

Overexpression of Sar1A in CHO cells and its effects on antibody production and localization of intracellular antibodies

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Abstract. Chinese hamster (*Cricetulus griseus*) ovary (CHO) cells are mammalian host cells commonly used for therapeutic antibody production. This is because CHO cells are capable of post-translational modification similar to human cells and can be easily cultured in serum-free medium, making large-scale industrial production possible. The process of therapeutic antibody production by CHO cells consists of three steps: cell line development, culture, and separation and purification. In cell line development, genetically modified CHO cells are used to increase the productivity of the cell culture process by increasing the specific production rate, which is the rate of protein production per cell per day. Approaches to improving productivity can be categorized within three processes related to protein synthesis and secretion, including transcriptional, translational, and secretory processes. We previously analyzed the secretion process in antibody-producing CHO cells and found that folded and assembled antibodies were accumulated in the ER. The transport of antibodies from the ER to the Golgi apparatus via COPII vesicle transport could be one of the bottlenecks involved in antibody cellular secretion¹⁾. COPII vesicle formation is initiated when Sar1A binds to the ER membrane and then detaches through the hydrolysis of GTP binding to Sar1A. It is hypothesized that an increase in Sar1A expression promotes COPII vesicle formation and increases antibody transport from the ER to the Golgi. This study aimed to analyze the effect of Sar1A overexpression on antibody production and the localization of intracellular antibodies in CHO cells. Sar1A overexpression vector was constructed and then transfected into CHO-TU1 cells. A stable expression cell line was established, and the antibody productivity of Sar1A-overexpressed cells and mock cells (negative control) were investigated. A Chase assay was performed using cycloheximide, a translation inhibitor, to investigate the effects of Sar1A overexpression on antibody accumulation in the ER. Localization of intracellular antibodies was investigated by detecting antibodies and *cis*-Golgi marker protein using immunofluorescence. Specific antibody production rates were 1.5-fold higher in Sar1A-overexpressed cells than in mock cells. The expression levels of heavy and light chain mRNAs did not change significantly between Sar1A-overexpressed and mock cells. The increased specific antibody production rates could be caused by improved antibody transport from the ER to the Golgi via overexpressed Sar1A. Intracellular antibody accumulation was decreased, suggesting improved antibody transport in Sar1A-overexpressed cells. Antibodies and *cis*-Golgi were more colocalized in Sar1A-overexpressed cells. It was speculated that some of the accumulated antibody in the ER was transported to the *cis*-Golgi by Sar1A overexpression.

Keywords: antibody, chinese hamster ovary cells, COPII vesicle, endoplasmic reticulum.

Design of efficient chromatography processes of bio-nanoparticles based on linear gradient elution data

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Abstract. Bio-nanoparticles (BNPs) such as viruses, virus-like particles, exosomes (extracellular vesicles), RNAs, DNAs, PEGylated proteins and large proteins such as IgM or Factor VIII are important materials as drugs, vaccines or vectors for gene and cell therapy. Although chromatography is needed for purifying BNPs, it is not easy to design the efficient chromatography process due to the large size of BNPs. Monolith chromatography is best suited for BNP separations as the pore diffusion mass transfer does not exist. Because of this advantage, linear gradient elution (LGE) of very shallow gradient slopes is possible at high flow-rates in order to achieve fine separations. However, when we look at this separation as an industrial process, it is not feasible as the buffer consumption is so large. In this study methods for designing efficient ion-exchange chromatography (IEC) of BNPs with monolithic supports and porous particle packed beds were developed. Optimization of LGE can be carried out by using numerical simulations in order to reduce buffer consumption and separation time while the same resolution is maintained. Another method, stepwise-elution (SE), in which the salt concentration is changed stepwise. SE is preferred for the process chromatography as the operation is simple and the buffer consumption can be reduced when the process is optimized. Flow-through chromatography (FTC) is also an efficient separation method, in which the target is not retained while impurities are adsorbed. The critical process parameter in SE and FTC is the salt concentration of the elution buffer, I_E for SE or the equilibrium buffer I_0 for FTC. We have already proposed a method for determining I_E or I_0 with the data obtained by LGE experiments. The most important difference between SE and LGE is the process stability. As the number of binding sites is large (>10), the distribution coefficient K depends on the salt concentration I very strongly. It is not easy to choose the proper I_E for this type of K . We have carried out numerical simulations in order to develop a method for determining I_E or I_0 for large B values. In this presentation we will show how SE and FTC of BNPs can be properly designed based on LGE data in comparison with the separation of proteins.

Keywords: chromatography, proteins, bionanoparticle.

Evaluation of Optimum Conditions for Induction of Anti-NS1 Nanobodies Expression in *E. coli* BL21(DE3) Using Response Surface Methodology

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Abstract. Dengue virus (DENV) is a virus in the class Flaviviridae that causes dengue fever. The global DENV infection rate is estimated at 390 million people per year, making it one of the top 10 global health threats. Dengue is transmitted by the Aedes mosquito in tropical and subtropical urban centers, including Indonesia. Nanobodies that recognize the NS1 DENV antigen become a potential dengue diagnostic tool because they are more stable and easier to manipulate and produce than conventional antibodies. In this study, anti-NS1 nanobody variant DD7 was expressed on *E. coli* BL21(DE3). To determine the optimal induction condition, response surface methodology based on Box-Behnken experimental design was employed. A total of 15 experiments were carried out to investigate the effect of induction temperature, IPTG concentration, and induction duration on dry biomass weight and nanobody concentration. The optimal conditions for the expression of anti-NS1 nanobodies were achieved at an induction temperature of 32,156 °C, IPTG concentration of 0.535 mM, and induction duration of 3,556 hours with a predicted dry biomass weight of 233,253 mg and nanobody concentration of 0.437 mg/mL. The optimization results in this study can be considered in selecting the operating parameters for large-scale production of anti-NS1 DD7 nanobodies.

Keywords: nanobody, *E. coli* BL21(DE3), optimization of expression, response surface methodology, NS1 antigen, dengue virus, recombinant protein

Isolation and Antibacterial Activity of Honey Bee Venom Bioactive from *Apis cerana*

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Abstract. *Apis cerana* is one of the honey bees found in Indonesia. Many bioactive compounds, including melittin and phospholipase A2 (PLA2), are found in the honey bee venom. These are known to have the ability to form pores on the lipid membrane and hydrolysis of the phospholipid membrane. Those bioactive abilities are expected to be an alternative treatment for bacterial infection. However, the study of the antibacterial activity of melittin and PLA2 from *Apis cerana* is still unknown. To do so, honey bee venom was harvested using an electric shock method. Harvested venom was then purified using Fast Protein Liquid Chromatography and analyzed by SDS PAGE and Lowry assay. The antibacterial activity of melittin and PLA2 against *Salmonella typhi*, *Staphylococcus aureus*, and *Escherichia coli* was assessed by disc diffusion method. In this study, the average inhibition value of 45 µg/ml PLA2 is 7.76 mm against *E. coli*. This result showed that PLA2 from Indonesian *Apis cerana* has antibacterial activity and may be a promising candidate for the antibacterial agent on gram-negative bacteria.

Keywords: antibacterial, *Apis cerana*, honey bee venom, melittin, PLA2

Continuous Protein Purification using Microfluidic-Based Valve Controlling System

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Abstract. Continuous protein purification is an emerging bioprocess that potentially provides consistent quality of high-value products, such as therapeutic proteins and antibodies. This process can avoid human contamination and increase the purification efficiency, enabling it to potentially become a next-generation manufacturing process applied to the biopharmaceutical industry. However, the challenges associated with system complexity and lack of real-time process information remain when dealing with the sequence of the continuous purification process. Using the microfluidic networks and fluid control techniques, we can fabricate interconnected and integrated microfluidic-based controlled valves and pumps within one-processing system, allowing to continuously and cyclically perform affinity chromatography procedures including sample loading, washing, elution, and regeneration steps. The results demonstrate that the continuous protein purification system developed in this study can automatically and continuously purify the target protein from cell lysates. The total process time was dramatically reduced from 32 to 8 min per affinity column compared with labor-intensive traditional affinity chromatography. Furthermore, the purity and concentration of protein kept maintaining a similar quality after 10 purification cycles. We believe that the successful development of this system can not only apply to the biopharmaceutical industry but also other biotechnology and medical applications in the future to enable the practice of personalized precision medicine, emerging agricultural biotechnology, and regenerative medicine.

Keywords: protein purification, microfluidics, continuous, automation, downstream process.

Formulation Of Anti-Oral Mouthwash Nanoemulsion Biofilm Based on Propolis Extract *Heterotrigona Itama*, *Tetragonula Sapiens*, and *Tetragonula Clypearis*

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Abstract. Research conducted by Basic Health in 2010 concluded that there were 31.4% of the population who had dental and oral problems with an increase of 70% from the same suffering. Many dental and oral problems often arise due to the formation of oral biofilms. The use of mouthwash is one of the actions against biofilms that is often used. However, commercial mouthwash has a fairly high alcohol content, which is around 26.9% of the total volume, which is considered to have a prolonged impact where high alcohol content with direct contact of the oral mucosa can cause lesions or abnormalities. Propolis with antibacterial properties was formulated using the nanoemulsion method, which was initiated by extracting propolis through drying and then varying the formulation from 3 types of bee propolis *Heterotrigona Itama*, *Tetragonula Sapiens*, and *Tetragonula Clypearis* along with the addition of Tween 80, propylene glycol, glycerin and then seeing the effect on gram microbial growth. positive for *S. mutans* and compared with antimicrobial agents in *Brazilian propolis* with the identification and comparison of antibacterial activity using *in vitro* method and evaluation of the stability of the *organoleptic* formula. Where the active ingredient content of propolis is most commonly found in the range of $97.4 \pm 0.2\text{mgAGE/g}$ - $20.22 \pm 2.45\text{mgKE/g}$ so it is hoped that the formulation designed with the highest propolis content at 90% can be considered effective as an anti-oral biofilm agent.

Keywords: propolis, antibacterial, oral biofilm, *S. mutan*.

Intensification of Curcuminoids Extract from *Curcuma longa*, *Curcuma mangga*, and *Curcuma amada* Using Ultrasound-Assisted Natural Deep Eutectic Solvent Based Extraction

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Abstract. Curcuminoids, a hydrophobic polyphenol derived from the plant of the ginger family (Zingiberaceae), have been shown in many pharmacological studies to have diverse therapeutic potential, including anti-inflammatory, antioxidant, anticancer, and antiviral properties. Therefore, curcuminoids have the potential to be used as a raw material for herbal medicines. The most frequently used method to extract curcuminoids is Soxhlet, which gives high yields. However, this method requires a long extraction time, large amounts of organic solvents, and a heating process that can damage the phytochemicals. This study aims to establish the process intensification with ultrasound-assisted natural deep eutectic solvents (NADES) based extraction. Choline chloride-lactic acid (CCLA-H₂O = 1:2) was used to investigate the impact of various process parameters such as % (v/v) water in NADES, % (w/v) solid loading, temperature, and extraction time. The maximum yield of 79,635 mg/g was achieved based on extraction in 20% water content NADES with a 4% solid loading in 35°C temperature for 1 hour. Peleg's and mass transfer models were used to describe the kinetics of the optimized UAE method, and the results were found to be compatible with experimental data. The optimum conditions obtained from Turmeric extraction were then used for the extraction of Mango Ginger and Black Turmeric, yielding 31.322 mg/g and 19.730 mg/g, respectively. Based on the yield obtained, solvent requirement, extraction temperature, and time, the optimized UAE method can be chosen as an alternative Soxhlet method. Furthermore, hexane as an anti-solvent was utilized in the purification process of curcuminoids from Turmeric, Mango Ginger, and Black Turmeric, which gave curcuminoid recovery of 39%, 27%, and 7%, respectively. Solidification of curcuminoids was also carried out by crystallization method using n-hexane and isopropanol as solvents. However, the solution of CCLA and curcuminoids formed a homogeneous mixture with isopropanol. Hence, the curcuminoids could not be solidified due to the presence of NADES in the extract solution.

Keywords: curcuminoids, ultrasound-assisted extraction, natural deep eutectic solvents, kinetics, anti-solvent precipitation.

Optimization of Solanesol Extraction from *Nicotiana tabacum L* Virginia Leaves

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Abstract. Solanesol is a long chain terpenoid alcohol found in Solanaceae family plants. Solanesol is mainly used as an intermediate compound to form Q10 co-enzyme and vitamin K2. Several extraction methods such as Microwave-Assisted Extraction (MAE) have been developed to extract solanesol. However, existing MAE methods require long extraction times and complex equipment with low yield gains. Different ratios of Hexane: Ethanol or Petroleum Ether: Ethanol as organic solvents were tested. Tobacco leaves collected from East Java were oven-dried and ground until 80 mesh. The extract obtained was further purified through rotary and column chromatography. Silica gel was used in the column as the stationary phase and PE: Ethanol as the mobile phase. The results show that the optimum MAE method to extract solanesol from tobacco leaves is using the ratio of tobacco weight per volume of water was 0.25 gr/ml, microwave power at 200 Watt, and extraction time of 1.5 minutes. The optimum maceration condition to extract solanesol from tobacco leaves was using PE: Ethanol with the ratio of 3:2 for 6 hours by using a column with a D/L ratio of 1:5. Analysis with HPLC shows the highest solanesol obtained from the optimum condition was 1.3% w/w with microwave operating conditions at 1,5 minutes and 200 watts. This operating condition varies from the existing research. It also held a longer microwave duration with lower power usage. The process of MAE helps the lysis of molecular walls, with additional NaOH helps the release solanesol from other compounds inside the tobacco. Solanesol then extracted with non-polar solvents which have the same solubility as solanesol. The extract then goes through a separation process by column chromatography which separates solanesol based on differences in polarity, and capabilities of the stationary and mobile phases. Referring to previous studies with appropriate comparisons, this study obtained an optimum operating condition for more solanesol extraction with less time and simpler equipment

Keywords: solanesol, tobacco, maceration, microwave assisted extraction, column chromatography.

Purification of Anti Spike SARS-CoV-2 Monoclonal Antibodies Using Ion Exchange Chromatography with Different pH Conditions

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Abstract. The development of antibody purification is challenging since the process aims to obtain abundant yield with low cost, while maintaining the level of product purity and quality. Purification of antibodies by ion exchange chromatography is widely used in monoclonal antibodies manufacturing. Several factors influence the binding characteristics of IgG such as pH, conductivity or salt concentration, and linear velocity of sample loading into the column. The net surface charge of immunoglobulins, which are consist of many different amino acids containing weak acidic and basic groups, will change gradually as the pH of the solution changes. In this study, we performed a simple method of anion-exchange chromatography to selectively remove impurities, using capturing and elution buffer with three different pH. DEAE columns were used as weak anion exchanger to obtain high purity levels for IgG antibodies with a broad range of isoelectric points (PI). Increasing pH during an anion exchange chromatography is to increase the concentration of the dissociated form of weak acidic groups as eluant. SDS-PAGE gel electrophoresis were used to visualize the protein profiles and monoclonal antibodies activity were determined with indirect ELISA. The result showed that anion-exchange DEAE resins were able to remove protein impurities compared to unpurified mixture. DEAE column with Tris-Cl buffer pH 8 provided optimal conditions compared to pH 8.5 and 9. We carried out gradient salt elution and obtained a high peak with 2ml volume of fractions from 250ul of BALB/c mice ascites fluid. However, additional steps for downstream process of antibody purification are needed to meet industrial's standard.

Keywords: antibody purification, monoclonal antibodies, ion-exchange chromatography.

Optimization of Ultrasonic-Assisted Extraction Using Natural Deep Eutectic Solvent (NADES) to Increase Curcumin Extract Yield from Turmeric, Javanese Turmeric, And Ginger

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Abstract. In this study, the UAE method is optimized to increase the yield of curcumin extracts from turmeric, Javanese turmeric, and ginger. The results of the optimized UAE method are compared to the soxhletation method. Separation and crystallization methods are then used to increase the curcumin extract yield. Optimization is done by making several variations: water content in solvent, ratio of sample and solvent, temperature, and extraction time. Yield of curcumin extract is calculated using UV-Vis spectrophotometer. The highest yield of curcumin extract from turmeric is 84.325 mg/g (8.43% w/w), which is slightly lower than the soxhletation yield, which is 88.476 mg/g (8.8% w/w). For the extracts of Javanese turmeric and ginger, the highest yields are 2.056 mg/g and 0.209 mg/g, respectively. Separation method done for turmeric extract increased its curcumin extract yield by 7%, while crystallization increased it by 3%. However, the same method caused a loss of curcumin extract yield in Javanese turmeric and ginger. Then, the kinetic model of extraction is made using Peleg's model and mass transfer equation.

Keywords: curcumin, extraction, ultrasonic-assisted extraction, natural deep eutectic solvent (NADES).

Potential of *Acacia crassicarpa* Honey from *Apis mellifera* as an Antidiabetic Agent and Effects on Diabetes Mellitus

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Abstract. Diabetes Mellitus (DM) is a chronic disease that occurs due to the failure of the pancreas to produce sufficient insulin or the body is unable to effectively use the insulin produced. So, it is necessary to make efforts to prevent and control Diabetes Mellitus, including by measuring blood sugar. The use of herbs and natural ingredients is a concern in the treatment of this diabetes. Honey is one of the ingredients whose content has many health benefits and to treat diabetes because some have the potential as antidiabetic agents. One of the honeys that is being widely produced is *Acacia crassicarpa* honey which is widely located in the Jambi and Riau areas. To determine the potential of honey as an antidiabetic agent, it is necessary to conduct research and several tests such as physicochemical tests to determine the content in honey, in addition to the total test of phenol compounds using the method from FolinCiocalteu. As well as the test of alpha-glucosidase inhibition activity by the Moradi-Afrapoli method.

Keywords: diabetes mellitus, *acacia crassicarpa*, *apis mellifera*, antidiabetic agent.

Differences in Treatment of Royal Jelly Powder by freeze-drying as a Serum Substitute in Media Culture for Lymphocyte Cell

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Abstract. Fetal Bovine Serum (FBS), which provides nutrients for cell culture has been the most commonly employed serum in proliferation research. It does, however, necessitate screening because it contains unidentified elements, as well as viruses and prions, which pose a risk of infection. *Apis mellifera* royal jelly has the potential to replace FBS as a serum. In this study, a comparison of the effectiveness of three royal jelly treatments on lymphocyte cell proliferation will be seen: untreated royal jelly, soluble royal jelly, and hydrolyzate royal jelly, which is then freeze-dried to generate a powdered product. Lymphocyte cells were cultured with various concentrations (2.5%, 5%, 7.5%, and 10%) and three different treatments of royal jelly. The results of these various concentrations were continued for up to 48 hours with continuous checking every 24 hours measured by the Microtetrazolium (MTT) assay. According to the results, Lymphocyte cells cultured with the addition of a 2.5% concentration of untreated royal jelly *Apis mellifera* had significant differences, with the percent cell viability of 77.15% at 24 hours and 58.44% at 48 hours. This value was higher than that of soluble royal jelly (64.13% and 38.52%) and royal jelly hydrolyzate (71.08% and 54.59%) with the addition of the same concentration.

Keywords: royal jelly, *apis mellifera*, serum, freeze-drying.

High-yield production of poly(3-hydroxybutyrate-co-3-hydroxyvalate) by *Methylocystis* sp. MJC1 in gas bioreactor

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Abstract. Reducing the total production cost of polyhydroxyalkanoate (PHA), an attractive biodegradable plastic that can replace conventional petrochemical plastics, still remains an unresolved problem. Methane could be a suitable substrate for PHA production, which is cheap and abundant as a major component of natural gas and biogas. The type II methanotroph, *Methylocystis* sp. MJC1 is capable of accumulating polyhydroxybutyrate (PHB) using methane as a carbon source. In this study, we tried to produce a poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [PHBV copolymer] with improved mechanical and physical properties compared to PHB while continuously supplying methane and valerate by *Methylocystis* sp. MJC1. The effects of three variables (pH, valerate concentration and feeding time of co-substrate) were investigated for improving PHBV production yield and 3HV mole fraction in a gas bioreactor. Under optimal conditions, cell biomass levels were 20.77 g DCW/L and cells contained 53.92% of PHBV copolymer with a 3HV mole fraction of 19%. Higher yields of PHBV copolymer than previously reported for methanotrophs were obtained under certain conditions.

Keywords: PHBV copolymer, high yield, *Methylocystis* sp MJC1, methane valerate.

Development Of Kinetics Model for Carotene Production from Oil Palm Empty Fruit Bunch Via Solid State Fermentation

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Abstract. There is an increasing market demand of β -carotene, which is related with its potential applications among others as the nutraceutical product pro vitamin A. Production of β -carotene using *Neurospora sp.* has been shown to have the potential to produce a high number of carotenoids with the use of oil palm empty fruit bunches (OPEFB) as the substrate for *Neurospora sp.* fermentation. Studies need to be conducted to scale up this process and further to evaluate the feasibility of this process for industrial/commercial applications. As the first step, having a kinetic model for carotene production would be beneficial to reduce the number of wet lab experiments for process optimization. The purposes of this research were to determine a kinetic model for the fungal growth and β -carotene production, and use the model to determine the optimum condition for β -carotene production. Model parameters were determined from experimental data of solid state fermentation with variations in air humidity and duration of fermentation. These variations were carried out to determine the oxygen consumption data and cell growth profile, the fermentation product was extracted through maceration and analyzed to obtain concentration, yield, and the rate of β -carotene production. The results showed that β -carotene is a mixed-growth associated product, with kinetics constants α and β were 8×10^{-5} and 8×10^{-7} , succeedingly. Model simulations showed that increased in inoculum and medium concentration, and also the duration of fermentation may increase the production of β -carotene. In validation experiment, the fermentation was conducted for 5 days, with the addition of 20 gram of fungal spore inoculum and 5 grams of glucose to the OPEFB medium resulting on 0.014 mg β -carotene/g EFB.

Keywords: β -karoten, kinetic modeling, neurospora, optimization, oil palm empty fruit bunches.

Potential of Polyurethane Waste as Adsorbent Nanocomposite for Oil Separation

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Abstract. Polyurethane waste is reduced to 177 micron for the modification process. Polyurethane (PU) has a hydrophobic compounds that are useful for oil separation. Polyurethane is modified using reduced graphene oxide (rGO) which will increase hydrophobic properties and increase adsorption efficiency. Graphene Oxide (GO) was synthesized using the modified Hummers method with ratio Commercial Graphite : KMnO_4 (1:3) w/w. rGO was reduced using ascorbic acid with a ratio of GO : ascorbic acid (1:2) w/w. Gasoline and water were used to model oil spills with observed contact times of 3 and 5 seconds. The gasoline/water spill model was used to determine the adsorption efficiency. PU/rGO nanocomposite, with the best adsorption efficiency reach as 71.73% with 5 second of contact time.

Keywords: adsorbent, adsorption efficiency, nanocomposite, polyurethane waste, reduce graphene oxide.

Cloning, Expression, and Purification of Anti-NS1 Nanobodies Expressed in *E. coli* BL21 (DE3) for The Development of Dengue Virus (DENV) Diagnostic Kit

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Abstract. A reliable, fast, and early diagnosis of the dengue virus (DENV) is vital for patient care and epidemiological purposes. NS-1 protein is one of the strong candidates as a diagnostic marker for DENV infection. Due to its presence in the body since day 1 of infection, thus supporting the early diagnosis. A diagnostic test based on the lateral flow test (LFT) was chosen due to its simplicity and easiness. The type of antibodies selected for this study is nanobodies because they have advantages over conventional antibodies in terms of greater affinity, specificity, and stability. In addition, their ability to be expressed in *Escherichia coli* systems means that they are easy to scale up and inexpensive to manufacture. In 2019, Shriver Lake et al. conducted a study and succeeded in selecting a library of nanobody protein sequences that can detect DENV NS-1 from all serotypes. This study focused on producing two clones of nanobodies using protein sequence information from Shriver Lake et al., and then codon optimization was carried out to get the DNA sequence. The pet-15b *E. coli* strain BL21(DE3) system performed the cloning and expression process. Gel electrophoresis, SDS-PAGE, and western blot were done to evaluate the cloning and expression. The expressed protein was then purified by combining DEAE and Ni-NTA resin. The purified nanobodies were characterized using SDS-PAGE, western blot, and Bicinchoninic Acid (BCA) Assay.

Keywords: nanobody, NS-1, *escherichia coli*, recombinant protein, dengue virus.

Perfusion Culture for Recombinant CHO cell culture in Tangential Flow Filtration (TFF) system using a hollow fiber (HF)

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Abstract. According to the explosive development of the biopharmaceutical industry, the importance of scalability and productivity is also highly increased. A perfusion system is increasingly given attention on the biopharmaceutical industry as an attractive culture method due to its unique advantages, such as high volumetric productivity, product quality and so on. In this study, a tangential flow filtration (TFF) perfusion system using a hollow fiber (HF) was introduced and evaluated. In this study, Recombinant CHO cells producing recombinant antibody were cultivated with our in-house medium. A Stirred Tank Reactor (STR) was used at working volume 1.2 liters in a TFF perfusion mode using a HF as a cell separation device. For comparison, another STR and Erlenmeyer flasks were used for control groups. The perfusion culture was maintained for a duration of 31 days and started at the day 3 of the cell culture. The minimum glucose level was kept over 3.0 g/L and perfusion rate was up to 3 vessel volume per day (VVD) during the perfusion mode, and culture temperature shifted from 37 °C to 30 °C or 33 °C. From the experiment, the TFF system using the HF showed effective cell separation, resulted in the high cell density up to 4.79×10^7 cells/mL. And volumetric productivity of perfusion culture was higher compared to the control group in STR. Also, the effects of the temperature shift were confirmed at 30 °C and 33 °C, respectively. The TFF system using a HF was successfully performed for long-term perfusion culture of CHO cells, showing potentials for other perfusion systems that require scalability and flexibility although several limitations and improvements were found for a high cell density culture such as sufficient O₂ supply method, pH control range, and so on.

Keywords: perfusion culture, hollow fiber, animal cell culture.

Soft Sensor for Real-Time Estimation of Specific Growth Rate and Implication of Control Strategies

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Abstract. The scarce in availability of reliable online sensors to monitor difficult-to-measure bioprocess state variables, viz., specific growth rate, and productivity, is severely impeding the implementation of Process Analytical Technology (PAT) in biopharma industries. In this present investigation, soft sensors were developed for real-time estimating specific growth rates using the calorimetric signal. A fermentation calorimeter was deployed to measure the metabolic heat rate, which was further used to model the specific growth rate (μ_{est}). Two estimators were devised

for the computation of real-time specific growth rates. The first estimator was deduced from the dissipation of instantaneous metabolic heat rate during the cultivation of microbes. The second estimator was formulated by looping the cumulative metabolic heat and biomass heat yield coefficient to the estimator model. The raw data emanated from the calorimetric signal was processed with digital filters to minimize the noise. Estimator based on cumulative heat resulted in better performance with lower RMSE values (0.031 – 0.046) for yeast (*Pichia pastoris*) and bacterial systems (*Streptococcus zooepidemicus*). Further, this estimator was coupled with three different control strategies envisaged to control the specific growth rate of glycoengineered *P. pastoris* to produce interferon $\alpha 2b$. A pre-determined feeding of limiting substrate (methanol) was performed through a feedforward control strategy at desired setpoint values of 0.04, 0.035, and 0.03h⁻¹, respectively. The second control strategy was enabled with a feedback loop in the form of standard PID control. Finally, an adaptive PID control configured gain scheduler was incorporated to maintain a tight control over optimal specific growth rate (0.035h⁻¹). Robust control of methanol feeding invoked by adaptive PID yielded a 1.5- and 2.2-fold increase in productivity of interferon $\alpha 2b$ compared to the other two control strategies.

Keywords: soft sensor, fermentation calorimeter, pichia pastoris, streptococcus zooepidemicus, adaptive control, interferon $\alpha 2b$.

Citric Acid Production from Tapioca Waste Industry using *Aspergillus Niger*

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Abstract. Citric acid is an organic chemical compound that is found naturally in fruits. Citric acid functions as a sour taste in beverage and food products. Other uses of citric acid as an emulsifier, antioxidant, blood anticoagulant, component of effervescent tablets (can be dissolved in water), plasticizer, and so on. Making citric acid can be done by fermentation using *Aspergillus niger*. Raw materials that can be fermented to produce citric acid are carbohydrates. Potential Materials containing carbohydrates can be converted to citric acid, such as tapioca industrial waste. There are two types of waste from the tapioca industry, namely tapioca solid waste (TSW) and tapioca liquid waste (TLW). This research uses tapioca waste to be fermented into citric acid using *Aspergillus niger*. In this study, optimization of operating conditions was carried out including the addition of water, comparing the production of citric acid produced with two kinds of substrates, and comparing the yield of citric acid from two fermentation methods, namely the solid-state fermentation and liquid state fermentation methods. The results showed that the optimal addition of water in the production of citric acid using the solid-state fermentation method was 80%. The right fermentation method to treat tapioca waste is solid state fermentation. The type of tapioca industrial solid waste substrate (TSW) produces more citric acid with a yield of 0.2263 grams of citric acid/gram TSW, compared to the liquid waste (TLW) with a yield of 0.0746 grams of citric acid/gram TLW. The optimal yield of citric acid is 15.34%.

Keyword: tapioca, waste, fermentation, *Aspergillus-niger*, citric acid.

Clinical and Public Health

ER-alpha and SMAD2/3 Phosphorylation Response in A Pregnant Mouse Model Injected with Lipopolysaccharide

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Abstract. During pregnancy there are physiological changes. One of them is the increase in estradiol (E2) which has a role to activate or suppress gene expression. The dominant biologic effects of E2 are mediated through two distinct intracellular receptors, ER α and ER β . ER α was able to form protein complexes by phosphorylation of SMAD2/3 in human breast cancer cell lines, human embryonic renal carcinoma cell lines, human renal mesangial cell lines and glioblastoma multiforme cell lines. Therefore, we tried to observe the interaction of ER α and SMAD2/3 phosphorylation in hepatocytes of pregnant mice injected with lipopolysaccharide (LPS). This study involved 16 female mice which were divided into four groups, namely healthy non-pregnant mice as control (K1), non-pregnant mice in LPS injection (K2), healthy pregnant mice (K3), and pregnant mice in LPS injection. (K4). Mice were pregnant in the second week and LPS injection was done by ip using LPS *Escherichia coli* serotype O111:B4. ER α expression and SMAD2/3 phosphorylation were examined using western blot. The data obtained were analyzed using Oneway Anova. The results showed that the expression of ER α decreased significantly at K3 (P=0.034). Meanwhile, SMAD2/3 phosphorylation showed no significant difference in all groups (P> 0.050). There was a significant relationship between pregnancy and ER α in the group that was not injected with LPS (P=0.027; r=-0.764) and that was injected with LPS (P=0.005; r=-0.873). Pregnant mice injected with LPS maintained ER α expression and no interaction of ER α with SMAD2/3 phosphorylation was found.

Keywords: ER α , LPS, Pregnancy, SMAD2/3 phosphorylation

Comparison of Custom-Made Insole (CMI) with Neoprene and Ethylene-Vinyl Acetate (EVA) Materials to the Static Comfort and Balance in Children with Flexible Flatfoot

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Abstract. Flatfoot is a condition that can decrease mobility and balance, therefore increasing the risk of falling in children. Custom-made insole (CMI) with Ethylene-Vinyl Acetate (EVA) material is often used to improve comfort and balance with flexible flatfoot patients. However, locally available EVA has known to be lack elasticity in absorbing the shock and less durable. To compare insole with Neoprene and EVA materials in comfort and static balance in children with flexible flatfoot. A cross-over randomized design was used. Fifteen participants (9 boys and 6 girls) with Dennis grade 2 – 3. Subjects were randomly assigned to CMI with Neoprene or EVA materials. Comfort during standing was measured by Visual Analog Score (VAS), while static balances with one leg standing were measured before and after participants used the insole for 2 weeks. The mean of comfort and balance without using any CMI are 4.00 and 4.87 seconds, respectively. There was a significant difference without and with using a CMI with either neoprene or EVA ($p<0.01$). Based on the analysis, there was a significant difference in comfort level between neoprene and EVA CMI ($p=0.022$), and there was no significant difference between neoprene and EVA in improving children's balance with flatfoot ($p=0.195$).

Keywords: custom made foot orthosis, balance outcome, comfort scale, neoprene, ethylene-vinyl acetate

Early Detection of a Possible Outbreak of Multidrug-Resistant *Acinetobacter baumannii* in Tertiary-Referral Hospital by using Antibiogram, Resistant Genes Detection by PCR and Random Amplified Polymorphism DNA Polymerase Chain Reaction (RAPD-PCR)

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Abstract. *Acinetobacter baumannii* is one of the causative agents of healthcare-associated infections (HAIs), that has multidrug-resistant characteristics. It is commonly found in the hospital environment, therefore could be the cause of outbreaks in a hospital setting. Early detection of its transmission from the environment is important to contain the infection. The combination of antibiogram, PCR of *oxa* genes, and RAPD-PCR are suggested as potential tools for assessing the possibility of *A. baumannii* outbreak. Therefore, this study aimed to validate the combination of antibiogram, *oxa* genes PCR, and RAPD-PCR to assess an outbreak of *A. baumannii* in a hospital. Thirty-eight *A. baumannii* samples (32 from clinical isolates, six from environmental isolates in the Intensive Care Unit) were subjected to RAPD-PCR using DAF-4 and ERIC-2 primers. The dendrogram was generated using the GelJ software and analyzed to determine its similarity. All isolates were checked for antimicrobial susceptibility testing using Vitek 2 compact (bioMérieux) and the presence of *oxa*-23, -24, -51, and -58 genes by PCR. The antibiogram showed all isolates were resistant to several antibiotics. Eighty-four percent (32/38) samples were positive for *oxa*-23, and 97% (37/38) samples harbor for *oxa*-51. All samples were negative for *oxa*-24 and -58. The RAPD results showed that amplification using DAF-4 had more bands average (11 vs. 7), and higher Discriminatory Power (81% vs. 76%) compared to ERIC-2. Statistical analysis using DAF-4 Similarity Index showed the possibility of an outbreak in the Intensive Care Unit ($p < 0.001$). Furthermore, bivariate regression analysis of both antibiogram data and the presence of oxacillinase genes could serve as significant predictors to validate the RAPD-PCR patterns. This study shows that the RAPD-PCR application combined with antibiogram and *oxa* genes detection are promising tools to detect the possibility of an outbreak of *A. baumannii* in the hospital setting, especially in a local hospital without a sophisticated microbiology laboratory.

Keywords: *Acinetobacter baumannii*, RAPD-PCR, antibiogram, hospital, outbreak

XRCC1 Arg399Gln and XRCC3 Thr241Met Genetic Polymorphisms Elevate Cervical Cancer Risk among Females of Bangladesh

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Abstract. Cervical cancer is the second most common cancer in women worldwide, and is both a preventable and a curable disease especially if identified at an early stage. Sequence variations in DNA repair genes can cause aberration in cellular functions leading to cancer. Genetic polymorphisms in XRCC1 (Arg399Gln) and XRCC3 (Thr241Met) genes result in individual variation in their DNA repair capacity. The aim of this study was to identify the association between XRCC1 Arg399Gln and XRCC3 Thr241Met single nucleotide polymorphisms (SNPs) and susceptibility to cervical cancer in Bangladeshi populations. The case-control study comprised 124 cervical cancer patients and 148 healthy controls. Genomic DNAs were isolated from peripheral blood and genotyped for candidate SNPs using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. For XRCC1, heterozygous Arg/Gln and combined heterozygous plus variant homozygous Gln/Gln genotypes showed 1.78-fold (95% CI 1.0037 to 2.8771, $p=0.0484$) and 1.8627-fold (95% CI 1.1470 to 3.0250, $p=0.0119$) increased risk of cervical cancer, respectively, when compared with normal homozygous Arg/Arg genotype. The variant Gln allele was positively associated with cervical cancer by 1.68-fold increase (95% CI 1.1732 to 2.3980, $p=0.0046$). Similarly, for XRCC3, Thr/Met heterozygous and combined Thr/Met + Met/Met genotypes were found to be associated with 1.6993-fold (95% CI 1.0398 to 3.0166, $p=0.0354$) and 1.8312-fold (95% CI 1.0890 to 3.0791, $p=0.0225$) higher risk, respectively, when compared with normal homozygous Thr/Thr genotypes. The variant Met allele showed significant association with 1.71-fold increased risk. XRCC1 (Arg399Gln) and XRCC3 (Thr241Met) polymorphisms may be associated with increased cervical cancer risk in Bangladeshi females

Keywords: XRCC1 (Arg399Gln), XRCC3 (Thr241Met), cervical cancer, genetic polymorphisms, Bangladesh

Urinary Leukocytes, Nitrite, and Leukocyte Esterase Correlation with Urine culture in Urinary Tract Infection Patients

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Abstract. Urinary tract infection (UTI) is one of most common bacterial infection in women. This infection cause uncomfortableness, morbidity, lost of work time and high cost for therapies. Gold standard examination for diagnosis of UTI is urine culture. Other common laboratory examination for UTI is urinalysis. Urinalysis is faster and easier to do than urine culture. To know how good the ability of urinalysis to detect UTI, urinary leukocytes, nitrite, and leukocyte esterase correlation with urine culture is assessed. This is an analytic observational study by using cross-sectional design. Data was obtained from Laboratory Information System (LIS) of Clinical Pathology and Microbiology Installation at Dr. Mohammad Hoesin General Hospital Palembang. The population were all of UTI patients whom result of urinary leukocytes, nitrite, leukocyte esterase and urine culture were recorded. By using incidental sampling, 163 samples were collected taken from 1st November 2015 until 31st January 2016 data period. Data was analyzed by descriptive and bivariate (Spearman's rank correlation) and presented in tables and narration. Urinary leukocytes, nitrite, and leukocyte esterase have a very significant correlation with urine culture ($p < 0,01$). Urinary leukocytes has a weak correlation with urine culture ($r = 0,307$), urinary nitrite has a medium correlation ($r = 0,427$), and urinary leukocyte esterase has a weak correlation ($r = 0,329$). This study shows that urinary leukocyte, nitrite, and leukocyte esterase correlate with urine culture.

Keywords: Urinary Tract Infection, Urine Culture, Urinary Leukocytes, Urinary Nitrite, Urinary Leukocyte Esterase, Urinalysis

Humoral Immune Response to Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) Infection and After COVID-19 Vaccination

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Abstract. Coronavirus Disease 2019 (COVID-19) is a pneumonia infection caused by a novel coronavirus that was first reported in December 2019 in Wuhan City, Hubei Province, China. The number of cases of COVID-19 continues to grow and spread in various parts of the world so on March 11, 2020, the World Health Organization (WHO) declared COVID-19 as a pandemic. Until now there is still no specific drug used to treat COVID-19 infection. Most of the therapy given to COVID-19 patients is supportive therapy. The administration of immunomodulators aims to stimulate or suppress the immune system. In natural infection, SARS-CoV-2 enters the host's body through the attachment of the spike protein (S) on the surface of SARS-CoV-2. The virus will recognize angiotensin-converting enzyme 2 (ACE-2) to attach to the host cell membrane. The host immune response has a role in immunopathogenesis that causes an uncontrolled inflammatory response known as a cytokine storm. The immune response can be divided into a humoral immune response that produces antibodies and cellular immune response. The immune response of antibodies against invading pathogens is the main basis for vaccine development. In the vaccination process, it is hoped that an immune system will be formed that can remember and recognize pathogenic microorganisms when they attack the host. The body's defense system that has recognized the pathogen is expected to react immediately after contact with the pathogen. The strategy for achieving immunity to COVID-19 is to induce the formation of virus-neutralizing antibodies. These antibodies will block the interaction between the virus and ACE-2 as its cell receptor. In this literature review, we will describe the humoral immune response in natural infection with SARS-CoV-2 and after COVID-19 vaccination.

Keywords: immunity, infection, prevention, SARS-CoV-2, vaccination

Methyltetrahydrofolate Reductase (MTHFR) C677T and A1298C Gene Polymorphism as Risk Factors for Essential Hypertension

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Abstract. Hypertension has relatively large morbidity and mortality rates throughout the world, including in Indonesia. The prevalence of hypertension tends to be greater in patients who have a family history of suffering from hypertension. This is thought to be influenced by polymorphisms in the MTHFR gene. This study aims to determine the relationship between polymorphism of C677T and A1298C MTHFR gene as a risk factors of essential hypertension. An observational study with case control design conducted involving 37 cases and 30 control people. Data obtained by PCR-RFLP. Data analysis was performed using chi-square and odds ratio calculation. The most common genotype for C677T polymorphism is CC (94.6%) followed by CT and TT with 2.7% each ($p = 0.001$) with OR of 0.099 (CI95% = 0.02-0.49). The most common genotype for the A1298C polymorphism is AC (45.9%) followed by AA (35.1%) and CC (19%) ($p = 0.001$). The C allele is present in 24 subjects in the case group (64.8%) and in 7 subjects in the control group (23.3%). The OR for the A1298C is 6.06 (CI95% = 2.1-17.9). The C677T polymorphism showed statistical significance but does not modify the risk factor of essential hypertension whereas the A1298C polymorphism is statistically significant and has a 6-fold risk factor for essential hypertension. Polymorphism A1298C Methyltetrahydrofolate Reductase (MTHFR) gene as a risk factors of essential hypertension

Keywords: C677T, A1298C, polymorphism, MTHFR gene, essential hypertension

Molecular Study in Identifying Genotypes to Phenotypes Relations of Transfusion-Dependent Thalassemia Patients in Cirebon, West Java

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Abstract. Thalassemias are one of the most common autosomal recessive disorders in Indonesia marked by quantitative defect in globin chain synthesis presenting high burden on the healthcare system. The molecular complexity of thalassemia causes varying spectrum of disease severity and clinical appearance. Molecular characterization is important prior to clinical management as it can provide extra information for clinical management of patients. A total of 30 transfusion-dependent patients were recruited. None of the patients have undergone thalassemia detection before. After informed consent, 5cc of venous blood were collected. Complete blood count was done using Sysmex XN-1000. Hemoglobin electrophoresis done using BioRad Variant II. HBA mutation analysis was done using multiplex GAP PCR. Beta-thalassemia mutation analysis were done using Reverse Dot Blot. Disease severity was assessed with Mahidol Severity Index. Average hemoglobin level was 9.36 g/dL (± 1.73 g/dL) with pre-transfusion average hemoglobin level of 2.2 g/dL (± 1.8 g/dL). HbF results showed an average of 7.3% ($\pm 7.9\%$). Multiplex GAP PCR2 showed 2 α -3.7/aa patients and 28 normal HBA. Most common mutation in HBB allele are IVS1-5 (41,7%) and HbE (23,3%) with homozygous IVS1-5 (23,3%) and IVS1-5/HbE (30%) as the most common genotype. Mahidol Severity Index results showed 22 classified as severe with 8 moderate and 0 normal. Characterizing most common thalassemia mutations in the Indonesian population can make subsequent diagnostic approach simpler, more cost effective, quicker by focusing on the small range of predominant allele instead of a wide range of alleles, and can provide additional information useful for better patient management.

Keywords: thalassemia, HBA, HBB, Mahidol Severity Index

Influence on Knowledge of Covid-19 towards Usage of pedulilindungi app in Depok, Indonesia

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Abstract. Mitigation strategy during Covid 19 pandemic is tracking and tracing for the virus spreads, the vaccination status, and the people's mobilities. In Indonesia, Pedulilindungi is an application to do so. During the period of June-July 2022, the rule of social distancing is eased, and Pedulilindungi is expected as a barrier to retain the virus' low positivity rate. While it is mandatory to use the application prior to entering a public area, not everyone has been using it. Depok is an urban supporting area for Indonesia's capital city, sampled to find whether their people's knowledge of COVID 19 is determining the usage of the Pedulilindungi app in the past month. This research reaches 105 subjects to answer a questionnaire. Data were analyzed using Mann Whitney U test to find whether usage of the Pedulilindungi app is determined by their knowledge of COVID 19. The test results show that most of the subjects use the application, and analyzed that no significant difference in knowledge of Covid 19 between subjects that have been using the app or not. (p-value 0,445). This shows that the application has a good rule in pandemic mitigation and reach peoples with various knowledge on Covid 19.

Keywords: Pedulilindungi, Covid-19, knowledge, mitigation

Design of the Illustration Book “Badan dan Pikiran Nyaman, Nugas Terus Jalan!” As an Educational Media About the Importance of Stretching While Doing Assignments for College Students

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Abstract. The introduction to this Final Project is entitled “Design of the Illustration Book “Badan dan Pikiran Nyaman, Nugas Terus Jalan!” As an Educational Media About the Importance of Stretching While Doing Assignments for College Students The problems discussed in this design are: (1) How to design an illustration book “Badan dan Pikiran Nyaman, Nugas Terus Jalan!” as an educational media about the importance of stretching while doing assignments that easy for college students to understand? (2) How to design an effective promotional media “Badan dan Pikiran Nyaman, Nugas Terus Jalan!” as an educational media about the importance of stretching while doing assignments for college students? College students are busy with activities and assignments. This can make physical activity neglected, coupled with the habit of sitting in the wrong position and for too long when doing assignments. Therefore, with the design of the illustration book “Badan dan Pikiran Nyaman, Nugas Terus Jalan!” it can serve as an educational media that makes college students aware about the importance of stretching while doing assignments. (3) With qualitative research methods, researchers use an approach with illustration book media so students can take their eyes off the gadget screen for a moment while taking a break dan relax from doing assignments. (4) Because the target audience is college students, the visual display is made with a simple, neat, and dynamic layout so that there is enough space for informative things and interesting illustrations so that the information in this book can be conveyed properly.

Keywords: illustration book, college students, stretching

Cognitive Measurement in Vascular Dementia Patients with Prefrontal Cortex Activation Analysis

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Abstract. Stroke is one of the second leading causes of death in the world with a prevalence of 10.9% in 2018. In Indonesia, stroke has increased over the last five years. Epidemiology suggests that small strokes in the prefrontal cortex (PFC) can cause cognitive impairment leading to vascular dementia. Accurate detection or diagnosis becomes important for therapeutic management because at an early stage it is difficult to identify. Therefore, in this study, an analysis of differences in brain activation in healthy elderly (non-stroke) and post-stroke patients with vascular dementia was conducted when performing recall memory work. This study involved seven elderly non-stroke and seven stroke patients with vascular dementia. Brain activity was recorded using a 19-channel clinical electroencephalogram (EEG). The study compared prefrontal cortex activity during an attention test. Standardized low-resolution brain electromagnetic tomography (sLORETA) was used for the analysis of active brain areas. Then the analysis of differences in prefrontal cortex activity between non-stroke patients and vascular dementia used paired T-test. The results of the paired T-test showed that elderly non-strokes produced significant differences in activity when repeating numbers correctly (remember the numbers) and incorrectly (forget the numbers), while in stroke patients with vascular dementia there was no significant difference when repeating numbers correctly and incorrectly. Another implication is that there is a decrease in the activity of the prefrontal cortex in stroke patients with dementia compared to the elderly non-stroke. This study is expected to be a supporting analysis for the early detection of vascular dementia, especially in post-stroke patients.

Keywords: EEG, stroke, prefrontal cortex, vascular dementia, sLORETA

Correlation Between Personal Hygiene with Helminth Eggs Manifestation on the Fingernail of Garbage-Man in Batulicin, South Kalimantan

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Abstract. Helminthiasis is one of the most common diseases suffered by people in Indonesia. Garbage-man was one of the occupations that were at risk to infection due to direct contact with the soil and waste. Direct contact, particularly with the absence of personal protection might increase the chance for helminth egg manifestation. This study aimed to examine the relationship between personal hygiene (washing hands and feet after working with soap and trimming nails once a week) with the manifestation of helminth eggs in the fingernail of garbage-man. Fingernail sample collection and interview were done on 24 garbage-man in Batulicin, Tanah Bumbu District, South Kalimantan Province. The samples were then examined using the sedimentation technique in the Parasitology Laboratory of Tanah Bumbu National Agency on Health Research and Development. All respondents with the habit of washing their hands and feet after working with soap were absent from helminth eggs in their fingernails. Based on statistical tests, there was a relationship between the manifestation of helminth eggs in the nails with the habit of washing hands and feet using soap after work ($p < 0,05$). Helminthiasis will increase in people with helminth eggs in their fingernails, especially when coupled with a lack of personal hygiene. The habit of washing hands and feet with soap after work has a significant relationship with the manifestation of helminth eggs on the nails of garbage-man in Batulicin, Tanah Bumbu District. Thus, it is necessary to increase understanding of personal hygiene and the implementation of standard operating procedures at work.

Keywords: helminthiasis, fingernail, personal hygiene, garbage-man

Analysis Of The Stress Levels On Online Learning During The Transition Of COVID-19 Pandemic To Endemic: The PSS Method

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Abstract. After the emergence of the COVID-19 pandemic, the government implemented a lock down to break the chain of virus spread. The education sector has not been spared the impact. The government has established online learning as an alternative, but this reaped pros and cons. In college, online learning is considered less effective and increases the stress suffered by students. However, after almost 2 years, the government succeeded in tackling the COVID-19 pandemic and the cases of COVID-19 began to subside, therefore the government began to re-enact offline learning. The transition from online learning to offline learning has once again reaped pros and cons. Factors such as students' perceptions of online learning and the stress they experience affect the choice of learning methods that are preferred by students. This study aims to examines the level of stress experienced by medical students transitioning online learning back to offline learning. This is observational research with a cross sectional design and included 560 students in the study. The study was conducted using a Perceived Stress Scale questionnaire distributed to the students and data were analyzed using the Spearman correlation test to see the correlation between the two variables. Among the 560 students studied, 1.5% experienced mild stress, 80.3% experienced moderate stress, and 18.2% experienced severe stress. The spearman rank obtained is 0.026, indicating that the strength of the correlation between the two variables is very weak.

Keywords: COVID-19, online learning, offline learning, stress levels, medical student.

ANN (Artificial Neural Network)-Based Optimization Study for PRP Mediated Therapy for Tendinopathy

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Abstract. Platelet-Rich Plasma (PRP) is one of the biologics which has been generally applied in orthopedic surgery to treat tendon injuries. Tendinopathy is a widespread tendon disorder, that causes extreme pain and results in impaired normal life activities. Due to the highly complex process of the disease development as well as a healing process, the treatment options for tendinopathy remain largely palliative. Studies on PRP have shown the beneficial effects on tendons including cell proliferation, increased expression of synthetic genes and proteins, and reduced inflammation. However, the efficacy and efficacy of PRP treatment for tendinopathy is still doubtful due to the non-linear response of the patients. In this work, we have conducted a comprehensive study to optimize the efficacy of the PRP. Therefore, different factors were considered and the effect of PRP-based therapy was examined, to predict the key factors. The key factor load-based dose optimization was also predicted to ensure the optimized response. The analysis was done with artificial neural network analysis as well as factorial design to compare the optimized condition and dose. For this study, most of the data was collected from the previous study as well as in-house data. The data and study were conducted within the guidelines of the institutional ethical committee. This statistical tool will be further validated by the real-time treatment study on the animal model to ensure prediction accuracy.

Keywords: artificial neural network, tendinopathy, regenerative medicine, factorial Design, dose optimization

Human Prosthetic Limb with Brain Machine Interface (BMI) in Real Applications: A Scoping Review

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Abstract. Brain Machine Interface (BMI) is a relatively new technology which can be applied for limb prosthesis. This technology is potentially useful for clinical conditions, such as neurological disorders and amputees. Currently, there have been several trials to implement this new technology on human subjects. To identify the advancement of brain machine interface technology for limb prosthetic application in terms of technology, clinical application, and ethical issues. Searching was done in the PubMed database. We only included study in human subjects relevant to this scoping review topic. We excluded non-English papers and animal subjects. Data extraction was focused on study characteristics, current progression, application, and ethical problems of BMI technology for limb prosthetic application. Thirteen trials were included in this scoping review. Based on this exploration, we classified the advancement of BMI based on the recording modality used, decoder strategy, the disease or condition that could be managed, and a slight result about safety and risk-benefit analysis on ethics issues mentioned in several studies. Several advancements of Brain Machine Interface (BMI) in limb prosthetics applications are being developed including its recording modality enhancement technologies (EEG, EMG, Intracortical, and fNIRS) and decoder strategy development. Further research is needed for BMI so that it can be used as clinically useful technology.

Keywords: brain machine interface, human studies, prosthetic limb

An elevated plasma TNF- α /IL-10 ratio in response to high fat diet challenge is associated with family history of type 2 diabetes in healthy males

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Abstract. Inflammation and inflammatory cytokines play a significant role in the pathogenicity of insulin resistance (IR) and type 2 diabetes mellitus (T2DM). Non-diabetic first-degree relatives (FDR) of T2DM patients have been reported to have relatively higher insulin resistance and inflammatory markers compared to healthy population. These features predispose them to progress into IR-associated diseases when exposed to inflammation stimuli such as excessive caloric intake. This study aimed to identify potential alteration in metabolic profiles, emphasized on the changes in inflammatory cytokines in response to high-fat diet (HFD) challenge that associate the FDR T2DM subjects with an IR-inflammation profile. Twenty-seven healthy FDR males and 28 age-and-body mass index (BMI) matched of non-FDR males underwent a 5-days HFD challenge. Metabolic profiles and plasma cytokine levels were assessed from participant's blood that drawn before and after the HFD intervention. Our study showed that the 5-days HFD intervention induced increases in HOMA IR and plasma IL-6 in both group similarly. The important finding of our study was that HFD induced a significant increase in the plasma TNF- α /IL-10 ratio in FDR subjects by which promote them in pro-inflammatory environment, while in contrary, a protective response that marked by decrease in the TNF- α /IL-10 ratio was shown by the non-FDR group ($p=0.001$ for differences between group). The tendency of FDR subjects to develop elevated plasma TNF- α /IL-10 ratio in response to HFD were 7-times (overall analysis) and 25-times (stratified on BMI ≥ 25) as likely as that of the non-FDR group ($p=0.001$ and $p=0.008$, respectively). Bigger waist circumference as observed in FDR group might cause their higher propensity for having pro-inflammatory response than the non-FDR group. This study provided evidence that a shift in the homeostatic mechanism between TNF- α and IL-10 towards a pro-inflammatory response has occurred in FDR T2DM individuals, particularly those with BMI ≥ 25 . This alteration in immune profiles might explain why FDR T2DM may progress to IR-related diseases when facing relatively similar HFD intake.

Keywords: family history, diabetes mellitus type 2, high fat diet, pro-inflammatory response, HOMA IR, TNF- α , IL-10, first degree relatives

Urinary Leukocytes, Nitrite, and Leukocyte Esterase Correlation with Urine culture in Urinary Tract Infection Patients

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Abstract. Urinary tract infection (UTI) is one of most common bacterial infection in women. These infections cause uncomfortableness, morbidity, loss of work time and high cost for therapies. Gold standard examination for diagnosis of UTI is urine culture. Other common laboratory examination for UTI is urinalysis. Urinalysis is faster and easier to do than urine culture. To know how well the ability of urinalysis to detect UTI, urinary leukocytes, nitrite, and leukocyte esterase correlation with urine culture is assessed. This is an analytic observational study using a cross sectional design. Data was obtained from Laboratory Information System (LIS) Clinical Pathology and Microbiology Installation at Dr. Mohammad Hoesin General Hospital Palembang. The population were all of UTI patients whom result of urinary leukocytes, nitrite, leukocyte esterase and urine culture were recorded. By using incidental sampling, 163 samples were collected taken from 1st November 2015 until 31st January 2016 data period. Data was analyzed by descriptive and bivariate (Spearman's rank correlation) and presented in tables and narration. Urinary leukocytes, nitrite, and leukocyte esterase have a very significant correlation with urine culture ($p < 0.01$). Urinary leukocytes has a weak correlation with urine culture ($r = 0.307$), urinary nitrite has a medium correlation ($r = 0.427$), and urinary leukocyte esterase has a weak correlation ($r = 0.329$).

Keywords: urinary tract infection, urine culture, urinary leukocytes, urinary nitrite, urinary leukocyte esterase, urinalysis

Drugs Delivery and Development

Investigation of Ramalina farinacea Compounds as Potential Reverse Transcriptase Inhibitor Through Molecular Docking for The Treatment of HIV/AIDS

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Abstract. HIV/AIDS cases in Indonesia continue to increase from year to year and recorded a peak in 2019, namely 50,282 cases. Currently, HIV/AIDS treatment uses antiretrovirals such as efavirenz, tenofovir, and lamivudine, which are the most common ARVs used in Indonesia. However, these antiretrovirals have many drawbacks, including resistance, toxicity and interactions, and poor bioavailability. Previous studies showed that the soluble ethyl acetate fraction of the lichen Ramalina farinacea was shown to inhibit the infectivity of the HIV-1 virus. Therefore, the lichen has great potential, especially in the health sector as an HIV antiviral agent. The purpose of this study was to determine the potential of the active compound from the lichen Ramalina farinacea as a better HIV-1 drug than the ARV efavirenz through an in silico study. In this study, 11 active compounds of the lichen Ramalina farinacea was carried out against the Reverse Transcriptase receptor (PDB ID: 2ZD1). As a comparison, the most widely used ARV that works on the Reverse Transcriptase receptor was chosen, namely efavirenz. The in silico methods used include molecular docking, ADMET prediction, and review of Lipinski's Rule of Five. The molecular docking simulation results obtained two test compounds with bond free energy values (ΔG) and inhibition constant values (K_i) lower than ARV efavirenz with bond free energy values (ΔG) of -7.54 kcal/mol. These compounds have bond free energy (ΔG) and inhibition constants of -9.96 kcal/mol and 50.36 nM for usnic acid, respectively, and -8.93 kcal/mol and 286.26 nM for divaritic acid. In terms of ADME and toxicity, usnic acid has better pharmacokinetic and toxicity properties than the comparison drug. Based on the rules of Lipinski's Rule of Five, it shows that all the active compounds of the lichen Ramalina farinacea have met the rules so that they can be administered orally. Based on this research, usnic acid and divaritic acid compounds can be used as candidates to be new oral ARVs that are better than efavirenz.

Keywords: HIV/AIDS, molecular docking, ramalina farinacea, reverse transcriptase, in silico study.

Extraction of Phenolic Compounds and Anti-convulsant Activities from Indonesian Anti-degenerative Mixed Herb

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Abstract. Convulsions are symptoms that arise from the direct or indirect effects of a central nervous system disease. Convulsions that occur in humans can be treated with anticonvulsants. These anticonvulsant are drugs that can suppress the activity of the central nervous system and increase inhibitory transmission so as to prevent the occurrence of seizures. Indonesian anti-degenerative mixed herb is expected to be an alternative that works to reduce convulsion activity. A herbal concoction that consist of a mixture of several plants, namely cloves (*Syzygium aromaticum* L.), nutmeg (*Myristica fragrans* L.), and red ginger (*Zingiber officinale* var *rubrum*) is trusted empirically as a nerve tension-lowering herb containing phenolic with anticonvulsant activity. Total phenolic testing was carried out by extracting 10 g of herbal ingredients in 250 mL of solvent by reflux. In this study, the highest total phenol was obtained

at 80°C and the solvent composition of water:ethanol 50:50 was 982,083 ppm. Testing of anticonvulsant activity was carried out in vivo on striknin-induced male mice with ddY strain in 6 test groups. The anticonvulsant activity of herbs in 250 mL of solvent with the reflux method was tested based on the ability to extend seizure onset, accelerate the duration of seizures and increase the rate of protection. Data analysis showed that herbal dosage III (1.3 mL / 40 g BB)

is best for extend seizure onset (641.50 ± 59.5 s), accelerate the duration of seizures (17.50 ± 7.41 s) and significantly increase the rate of protection (100%). To conclude, this Indonesian anti-degenerative mixed herb extract is suitable to become an additional medication for nervous tension, particularly to reduce excessive convulsing activity.

Keywords: nervous tension, anticonvulsant, cloves (*Syzygium aromaticum* L.), nutmeg (*Myristica fragrans* L.), red ginger (*Zingiber officinale* var *rubrum*), phenolic.

In-silico exploration of alkaloids found in Indonesia medicinal plants and its potential against HPV and cervical cancer

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Abstract. In recent years, there has been an increasing awareness of cervical cancer in Indonesia. Nearly all cervical cancer cases are caused by the human papillomavirus (HPV). Two HPV serotypes, 16 and 18, are closely associated with cervical cancer. Cervical cancer prevention is done by giving HPV vaccination to children aged 9-12 years. The development of HPV antiviral and cervical anticancer drugs is still being carried out. The exploration to find medicinal ingredients that have these two biological activities is still being actively conducted. Indonesia, as the tropical country with a diverse plant species, hides great potential in the form of natural medicinal ingredients that have not been fully explored. Plant produces metabolites, such as alkaloids which have been reported for biological activities including analgesic, antibacterial, antifungal, anti-inflammatory, anticancer, and antiviral activity. The anticancer and antiviral activities of alkaloids compounds can be a strategic solution when developing candidates for cervical cancer drug. In this study in-silico prediction using molecular docking will be used to explore several types of alkaloids commonly found in Indonesia medicinal plants and its potential against HPV and/or as cervical cancer treatment.

Keywords: alkaloids, antiviral, drug, cervical cancer, human papillomavirus, medicinal plant.

Needle-free insulin delivery using non-invasive transdermal formulations for diabetic patients

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Abstract. Therapeutic insulin for type I diabetes is usually provided subcutaneously using a needle or insulin pen, or catheters attached to insulin pumps. These strategies are cumbersome and mostly result in inadequate enforcement, which is a significant factor in poor quality of life for diabetic patients. Since injection administration for diabetes is invasive, it is important to develop an effective transdermal method for insulin. However, transdermal delivery remains challenging owing to the strong barrier function of the stratum corneum (SC) of the skin. Here, we developed ionic liquid (IL)-in-oil microemulsion formulations (MEFs) for transdermal insulin delivery using choline-fatty acids ([Chl][FAs])—comprising three different FAs (C18:0, C18:1, and C18:2)—as biocompatible surface-active ILs (SAILs). The MEFs were successfully developed using [Chl][FAs] as surfactants, sorbitan monolaurate (Span-20) as a co-surfactant, choline propionate IL as an internal polar phase, and isopropyl myristate as a continuous oil phase. Insulin was loaded into the core of the MEFs by dissolving it in [Chl][C3] and then the safety, efficacy, and stability of the MEFs were investigated both *in vitro* and *in vivo*. Ternary phase behavior, dynamic light scattering, and transmission electron microscopy studies revealed that MEFs were thermodynamically stable with nanoparticle size. This study demonstrated an unprecedented improvement in the transdermal bioavailability of insulin in BALB/c diabetic mice with excellent transdermal efficacy, biocompatibility, and long-term stability. The newly developed MEFs significantly enhanced the transdermal permeation of insulin via the intercellular route by compromising the tight lamellar structure of SC lipids through a fluidity-enhancing mechanism. *In vivo* transdermal administration of low insulin doses (50 IU/kg) to diabetic mice showed that MEFs reduced blood glucose levels (BGLs) significantly compared with a commercial surfactant-based formulation by increasing the bioavailability of insulin in the systemic circulation; and sustained the insulin level for a much longer period (half-life > 24 h) than subcutaneous injection (half-life 1.32 h). When [Chl][C18:2] SAIL-based MEF was transdermally administered, it reduced the BGL by 56% of its initial value. The MEFs were biocompatible and non-toxic (cell viability > 90%). They remained stable at room temperature for 3 months and their biological activity was retained for 4 months at 4 °C. We believe SAIL-based MEFs will alter current approaches to insulin therapy and may be a potential transdermal nano-carrier for protein and peptide delivery. MEFs have the potential to be transdermal delivery carriers for insulin and other proteins/peptides that are presently available in injection form and should be further investigated.

Keywords: insulin, transdermal delivery, drug carrier, peptide drugs, ionic liquids.

Liquid self-microemulsifying drug delivery system of a simvastatin and/ or g-oryzanol

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Abstract. High levels of total cholesterol in the blood can cause hypercholesterolemia and lead to cardiovascular disease. Statin drugs, especially simvastatin, are often used to decrease total cholesterol levels in the blood. However, simvastatin has low water solubility and bioavailability. Hence it used at high doses that can cause dependence and unwanted side effects. g-oryzanol is a natural product known to have anti-hypercholesterolemic properties found in rice bran oil, therefore it can be used as a substitute for simvastatin, although it is not soluble in water but it has low side effects compared to simvastatin. In our previous study, microemulsion (simvastatin/g-oryzanol in oleic acid-water-tween 80-propylene glycol (1/2/4/4 w/w)) has been able to increase the solubility of simvastatin and g-oryzanol of 6.674 and 6.064 mg/mL, respectively. A liquid self-microemulsifying drug delivery system (SMEDDS) was proposed to increase more the solubility and bioavailability of simvastatin and γ -oryzanol, as well as the drug load. A liquid SMEDDS is composed of oil, surfactant, and cosurfactant which are mixed and will form a microemulsion when dissolved in water or gastric and intestinal fluids. In order to obtained highest solubility of simvastatin and g-oryzanol were test in various oil, surfactant, and cosurfactant. The highest solubility of simvastatin in oil, surfactant, and cosurfactant were found in capryol 90 (7.55 mg/mL), labrasol (6.42 mg/mL), and transcitol HP (8.04 mg/mL), respectively. While, g-oryzanol of 5.14 mg/mL, 3.95 mg/mL, and 7.68 mg/mL in lauroglycol, tween 80, and transcitol HP, respectively. Capryol 90 and rice bran oil (RBO), tween 80, and transcitol HP were selected as oil, surfactant and co-surfactant respectively and for preparation of stable SMEDDS, micro emulsion region was identified by constructing pseudo ternary phase diagram containing different proportion of surfactant: cosurfactant (1:1, 2:1 and 3:1), oil and water. Prepared optimised formula of microemulsion with drug load was evaluated for SEM, particle size analysis, polydispersity index, phase separation, viscosity determination, and zeta potential. Based on the results of the analysis, it is expected to show the potential of a stable liquid SMEDDS of simvastatin and/ or γ -oryzanol as anti-hypercholesterolemic agents.

Keywords: γ -oryzanol, bioavailability, hypercholesterolemia, simvastatin, self-microemulsifying drug delivery system.

Design of self-assembly peptides for antigenic protein delivery application

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Abstract. Molecular self-assembly enables a bottom-up fabrication of materials with designable features in their structures and functions. Self-assembly peptides composed of oligopeptides with sometimes artificial substituents are attractive self-assembling building blocks because of their biofunctions and biocompatibility, and therefore have been used for drug delivery carriers and artificial extracellular matrices. We have previously developed self-assembly peptides with a reactive sequence of microbial transglutaminase (MTG). The micrometer-long fibers formed by the self-assembled peptides could be post-modified with folded proteins with MTG-recognizable sequence by an MTG catalytic reaction. In this study, we investigated the applicability of these folded protein-modified self-assembled peptides for vaccine application. Antigenic proteins were post-modified to the self-assembled peptides and the antigen delivery to immune cells *in vitro* and resultant immune responses *in vivo* were investigated. The results showed that the direct conjugation to self-assembled peptides facilitated the antigenic protein delivery to immune cells and the delivery efficiency was depending on the species of the peptides. When these antigenic protein-modified peptidic fibers were administrated to mice, specific antibody production was induced. These results suggested these self-assembled peptides that can hold folded proteins on the structures are potential vaccine delivery carriers.

Keywords: self-assembly, peptide, antigen, vaccine, drug delivery.

Hierarchical co-assembly with peptide amphiphiles shows the non-endocytic delivery of small molecules into membrane-rich organelles

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Abstract. In the development of materials for drug delivery system (DDS) application, the pathway of cellular internalization of materials is important to control because it determines the efficiency of internalization and fate of the materials. Compared with energy-dependent endocytic pathway, non-endocytic, direct permeation of cellular membranes is more beneficial for delivering therapeutical agents because the route can protect cargos from being trapped and degraded in endosomal vesicles. Although several recent studies have reported some materials that directly permeate membranes in the energy-independent manner, deep insight into the physicochemical properties of materials required for the pathway and application for DDS remains insufficient. [1, 2]. We have recently reported a novel hierarchical co-assembly system composed of peptide amphiphiles (PAs) and fluorescent probes (NBD). In this system, a complementary hydrogen bonding pair, cyanuric acid (Cya) and Melamine (Mel), was introduced to PAs and NBD, respectively, to obtain Cya-PA and Mel-NBD. Upon mixing Cya-PA and Mel-NBD, they co-assembled through the complementary interaction to form nanostructures with different shapes and sizes depending on the design of PAs. [3] In this presentation, we report the size-dependent internalization of the co-assemblies by non-endocytic pathway. Real-time observation with confocal laser microscopy revealed the fast, direct internalization of the co-assemblies, which eventually localized with membrane-rich organelles (endoplasmic reticulum, mitochondria). Experiments with artificial lipid membrane structures confirmed the interaction of co-assemblies with lipid membranes, which would work as driving forces for the non-endocytic penetration and localization with membrane-rich organelles of co-assemblies. This co-assembly system with unique direct internalization behavior will be used to deliver functional molecules into the membrane-rich organelles efficiently.

Keywords: drug delivery system (DDS), peptide amphiphiles (PAS), co-assembly.

Transfersome Formulation with Edge Activator Tween 80 for Secretome Encapsulation using Bovine Serum Albumin as a Model Protein

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Abstract. Stem cell-derived secretomes are rich in valuable growth factors and have been proven to give beneficial effects on the human tissues eg. promoting cell proliferation and angiogenesis, immunomodulation, and anti-tumorigenic effect. Up to the present, the delivery method to promote the therapeutic effect of secretomes is still limited. We propose the use of transfersome for encapsulating stem cell-derived secretome due to their superior elasticity and skin permeability in comparison to conventional liposomes, thus making them an ideal delivery method for future topical transdermal applications. This research aims to formulate transfersome composition with dipalmitoyl phosphatidyl choline (DPPC) and Tween 80 as an edge activator. The capability of transfersomes in encapsulating secretome proteins was evaluated with bovine serum albumin as a model protein. The sphere structure of transfersome was made through thin-film hydration and extrusion methods. Among 6 tested formulas, the best efficiency was achieved with a ratio of 97.5% DPPC and 2.5% Tween 80. Using the chosen formula, the encapsulation efficiency was $78.93 \pm 0.32\%$, and the zeta potential was 6.60 ± 0.26 mV.

Keywords: transfersome, protein, liposome, encapsulation, drug delivery, tween 80, dipalmitoyl phosphatidyl choline.

Development of biodegradable and highly crosslinked polyethylene glycol membrane for controlling drug permeation

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Abstract. Biodegradable membranes have received increasing attention for sustained drug release in the body because they do not require removal after application. It is important to prevent burst release and change of release rate in the body for their application because these can cause side effect locally. In previous research, multi arm polyethylene glycol (PEG) hydrogels have ever been used for developing sustained drug release systems due to their biocompatibility and tunable physical property by changing their molecular weight and arm number. In particular, lyophilized biodegradable PEG membranes with dense structure were developed in previous research. Although it is expected that their dense structure can contribute to sustained drug release, there are no research examining their drug permeability. In this study, we developed biodegradable and highly crosslinked PEG membranes by lyophilization. Their drug permeability was evaluated for investigating their potential for sustained drug release in the body. PEG hydrogels were fabricated by crosslinking three-arm thiol-modified PEG (PEG-SH, molecular weight [Mw] = 1300) and four-arm acrylate-modified PEG (PEG-AC, Mw = 1892). They were then lyophilized to prepare PEG membranes. Their drug permeability was evaluated using two chamber cell system composed of feed chamber and permeation chamber. Carbazochrome sodium sulfonate (CCSS, Mw = 322), vitamin B12 (VB12, Mw = 1355), lysozyme (Lys, Mw = 14000) and bovine serum albumin (BSA, Mw = 67000) were used as model drugs. These drugs were dissolved in PBS (pH 7.4, 150mM). After filling feed chamber with drug solution and permeation chamber with PBS, concentration in feed chamber (Cf) and permeation chamber (Cp) were measured, respectively. The ratio of drug concentration (Cp/Cf) was plotted against time. PEG membranes were fabricated by lyophilization successfully. Their SEM image of their cross section indicated that they have dense structure. In drug permeation tests, although Cp/Cf values of CCSS and VB12 increased constantly just after starting experiment, those of Lys and BSA increased from 17 days after starting experiment. These results showed our PEG membranes have solutes permeability depending on the molecular weight of solutes at physiological pH. Cp/Cf value of each drug reached to about 1.0 after complete degradation of PEG membranes. It indicates that PEG membranes have degradability at physiological pH. It is considered that hydrolysis of ester linkage in PEG-SH and PEG-AC contributed to this degradability. In conclusion, our PEG membranes indicated potential for sustained release of drugs with low molecular weight in the body due to their biodegradability and constant amount of drug permeability till their complete degradation.

Keywords: polyethylene glycol, drug permeation, lyophilized membrane.

Phytochemicals Analysis and Antifungal Activity Propolis Lombok Against *Candida sp* and *Cryptococcus sp*

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Abstract. Propolis is a natural resinous substance formed from flower buds, trees, and resinous exudates collected by bees. Propolis has several therapeutic properties such as antibacterial, anti-inflammatory, healing, anesthetic, antioxidant, antifungal, antiprotozoan, and antiviral. Based on previous study, a new compound was found in Propolis Lombok, which is calophylloidic acid A with antimicrobial activity. The aim of this study is to explore antifungal activity of Lombok Propolis against *Candida albicans*, *Candida glabrata*, *Candida Krusei*, and *Cryptococcus neoformans*. Two methods use on antifungal test, which are agar diffusion and microdilution. In addition, phytochemical analysis also carried out with LC-MS/MS, total polifenol content, and total flavonoid content. 21 compounds found in this study with 4 of them were found in three samples Lombok propolis, namely gallic acid, ellagic acid, 18-β Glycyrrhetic acid and maslinic acid. The total polyphenol content of Rempek, Sekotong, and Bayan propolis respectively was 31,85±3,68; 30,99±3,12 and 36,45±12,06 mgGAE/g. Meanwhile, the total polyphenol content was 95,63±13,09; 110,42±7,39; 51,20±4,28 mg QE/g. Based on the antifungal tests, propolis samples showed an antifungal activity in *C. glabrata*, *C. krusei*, and *C. neoformans* species.

Keywords: propolis, phytochemicals, antifungal, candida sp, cryptococcus sp.

Phytochemical Analysis and Anti-Fungal Activity of Brunei Propolis against *Candida sp* and *Cryptococcus sp*

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Abstract. Propolis is a natural resin produced by bees without stinging. The content of chemical compounds from propolis itself depends on the source of the plant, geographical location, environmental conditions, and bee species. The most common types of chemical compounds contained in propolis are polyphenols and flavonoids. Propolis also has a variety of biological activities that can enrich the source of herbal medicines, such as anti-fungal activity. The propolis used in this study is propolis from Brunei Darussalam, including *Geniotrigona thoracica*, *Heterotrigona itama*, and *Tetrigona binghami*. This study focuses on evaluating phytochemicals, including the total content of polyphenols and flavonoids, marker compounds, and the anti-fungal activity of propolis brunei. Until now, research on the compounds contained in propolis is still being carried out. However, the literature on the chemical compound of Brunei propolis is still limited. The results of the research on the content of Brunei propolis using LC-MS/MS found as many as 21 chemical compounds and three marker compounds, namely maslinic acid, D-(-) Mannitol, and 18- β -Glycyrrhetic acid. The total content of flavonoid and polyphenolic propolis in Brunei was obtained using quercetin as a flavonoid standard and gallic acid as a polyphenol standard. It was found that in Brunei propolis the total flavonoid content was greater than the total polyphenol content. Where the total polyphenol content of propolis *G. thoracica*, *H. itama*, and *T. binghami* were 78.79 ± 17.06 ; 70.51 ± 12.93 and 16.37 ± 0.53 (mgGAE/g). while the total flavonoid content was 19.30 ± 1.99 ; 101.10 ± 6.26 and 61.63 ± 4.53 (mg QE/g). The antifungal activity was carried out by agar diffusion and microdilution methods. Brunei propolis extract showed antifungal activity against *Cryptococcus* and *C. albicans*. Where propolis Brunei extract showed anti-fungal activity with intermediate resistance to both fungi.

Keywords: Brunei propolis, *geniotrigona thoracica*, *heterotrigona itama*, *tetrigona binghami*, LC-MS/MS, polyphenols, flavonoids, anti-fungal.

Histopathology analysis of the lung after intratracheal injection of SARS-CoV-2 spike protein in mice: A Preliminary study

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Abstract. SARS-CoV-2 is responsible for inducing COVID-19 disease, which principally generates inflammatory disease of the airways and lungs and leads to severe respiratory problems. Recent research employing animal model mice has been designed and carried out on various methods that could be used to investigate the mechanisms of inflammation and infection in COVID-19 disease. In this study, we used an intratracheal instillation of SARS-CoV-2 spike protein to create an animal model for studying lung inflammation. **Methods:** The trachea was opened by making a small cut over the trachea and pulling apart the muscles and glands underneath. The SARS-CoV-2 recombinant spike protein was intratracheally delivered to mice at a concentration of 15 µg in 50 µl of saline, followed by a 100 µl air injection. The control group was given 50 µl saline intratracheally. Afterward, mice were euthanized with intraperitoneal injections of ketamine and xylazine at 1, 2, and 7-days post-injection (d.p.i.) to harvest the lungs. Hematoxylin and Eosin (H & E) were used to stain lung tissue for histological examinations. **Result:** Lung histopathology of Balb/c mice injected with SARS-CoV-2 recombinant spike protein at 1-, 2-, and 7-days post injection showed mild immune cell infiltration. Neutrophil infiltration was also found at the locations of peribronchiolar, perivascular, and alveolar sites compared with untreated mice. According to our study, SARS-CoV-2 recombinant spike protein has been shown to trigger mild lung inflammation in Balb/c mice.

Keywords: Balb/c, COVID-19, inflammation, intratracheal, pulmonary disease.

Antioxidant and Hepatoprotective Activity of Traditional Liquid Medicine Containing Temulawak, Turmeric and Red Ginger on Paracetamol-Induced Rat Liver Damage

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Abstract. Many factors can cause liver damage, one of which is drug toxicity. The metabolism of paracetamol in the liver can result in liver toxicity. Curcumin and gingerol, two active compounds found in natural products, have been shown to be antioxidants. The purpose of this study was to look into the antioxidant and hepatoprotective effects of temulawak, turmeric, and red ginger in traditional liquid medicine (TLM) on paracetamol-induced liver damage in rats. Thirty-six male Sprague Dawley rats were divided into six groups of six rats each (normal, negative, positive, and three samples' groups). Except for the normal group, all experimental animals, the positive group (silymarin 200 mg/kgBW) and the treatment group (2.52 ml/kgBW; 5.04 ml/kgBW; and 10.08 ml/kgBW), were given paracetamol 3 g/kgBW on the seventh day. MDA, AST, and ALT levels in the blood were all measured. Additionally, the antioxidant activity of traditional medicinal products was measured using the DPPH method. Curcumin marker compounds were analyzed using TLC-Densitometry, while gingerol was analyzed using HPLC. When compared to the negative group, the TLM could prevent a significant increase in MDA. The best prevention was at a dose of 10.08 ml/KgBW, with an 87.80 percent prevention level of increasing plasma MDA levels. The highest hepatoprotective activity resulted in AST and ALT levels decreasing by 35.4 percent and 51.2 percent, respectively, in the treatment group 10.08 ml/kgBW compared to the negative group. Using the DPPH method, the IC₅₀ value of TLM was 74.0658 ppm. TLM's marker compound, contained 1.32 percent curcumin and 0.20 percent gingerol. It can be concluded TLM, which contains temulawak, turmeric, and red ginger, has potential antioxidant and hepatoprotective activity in rats induced by paracetamol by increasing MDA levels and decreasing AST and ALT levels.

Keywords: paracetamol, antioxidant, hepatoprotective, MDA, temulawak, turmeric, red ginger.

Vitamin D constituted and immunomodulatory drug encapsulated pro-drug nanocapsules for host-directed therapy against tuberculosis

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Abstract. Tuberculosis (TB) has emerged as a global health challenge with the advent of extreme and total drug resistant mycobacteria. Recent insights into pathogen–host interactions and inflammatory pathways are leading to development of a wide range of host-directed therapies capable of overcoming the challenges of resistance development. The immune subversion mechanism of mycobacteria renders macrophages alternatively activated (M2) whereas pathogen elimination requires them to be classically activated (M1). In this study we have fabricated a nanocapsule similar to a phospholipid bilayer, constituted of mannosylated hydrophilic oligomeric chain of ethylene glycol (OEG) linked to hydrophobic VitaminD3 (VitD3) to form Man-OEG-diVitD. The nanocapsule was further loaded with Arginase I inhibitor nor-NOHA. These drug-loaded nanocapsules could specifically target infected macrophages and immunomodulate them to M1 state. The pro-drug VitD3 was converted to its active form 123VitD3, which then led to the overexpression of anti-mycobacterial peptide cathelicidin, which is known to induce autolysosome formation and subsequent mycobacterial killing. Such novel nanocapsules with reduced inert material is an effective formulation to combat the drug resistant TB menace.

Keywords: vitamin D3, nanocapsule, immunomodulation, host-directed therapy.

Immunostimulant Activity of Spray-Dried Propolis Microcapsules from *Tetragonula sapiens* on Human Lymphocyte Proliferation

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Abstract. Propolis plays a role in the immune system and promotes the proliferation of lymphocytes. However, the use of propolis as an active ingredient is limited because of the restricted handling of its physical properties, which is hard, sticky, and difficult to dissolve in water. Encapsulation can solve propolis solubility problems, including protecting bioactive substances. The method includes spray-drying microencapsulation with various coaters, such as maltodextrin and gum arabic. The purpose of this study was to determine the immunostimulant activity of propolis microcapsules from *Tetragonula sapiens* bees against human lymphocyte proliferation. In this study, the propolis microcapsules was prepared from the wax propolis extract which was encapsulated using maltodextrin and gum arabic by spray drying with 2 different operating conditions. The wax propolis microcapsules had been tested for total phenolic and flavonoid levels, morphology and their immunostimulant activity against LPS-induced human lymphocyte proliferation at different doses. It was known that the spray-dried propolis microcapsules had total phenolic and flavonoid levels of propolis microcapsules of 334.02 ± 3.08 mgGAE /g and 21.79 ± 0.19 mgQE/g for SD 1, and 158.26 ± 9.5 mgGAE/g and 53.19 ± 1.18 mgQE/g for SD 2. The size of propolis microcapsules of SD 1 is 0.7-7 μ m, while 1-4 μ m for SD 2. The addition of SD 1 propolis microcapsules with all doses (3.125 μ g/mL - 200 μ g/mL) could increase the viability of LPS-induced lymphocyte cells, meanwhile doses of 12.5 μ g/mL - 200 μ g/mL for SD 2. Both propolis microcapsules still showed immunostimulant activity, hence it can be developed as traditional medicine.

Keywords: propolis, microencapsulation, spray-drying, immunostimulant, lymphocyte.

Loading of Methylene Blue as a Drug Model in to Chitosan-graft-Maleic Sponges

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Abstract. Chitosan, the second most abundant natural polymer, is a potential raw material for 3D polymer matrix (sponge) synthesis on biomaterial field. Because It has beneficial properties such as biodegradable, biocompatible, non-toxic, and antimicrobial. This work successfully synthesize sponges from chitosan-graft-maleic anhydride. Chitosan and maleic anhydride at certain mass ratio (1:2; 1:1; and 2:1) are reacted in dimethyl sulfoxide (DMSO). The reaction is followed with constant stirring at 60°C for 24 hours. The gel formation is carried out in the aquadest using dialysis tube. Further DMSO removal from the gels is conducted by immersion of the gels in aquadest for 2 days. After that, the obtained gels is frozen at -25°C over night before lyophilized. The lyophilization is conducted under vacuum at -40°C for 24 hours. The weight of dried sponges after lyophilization are about 5.61% – 6.77% (dry sponges/wet gels). The sponges then characterized by FTIR and TG/DTA. The FTIR band of chitosan-graft-maleic sponges is compared with pure chitosan. A new strong intensity of FTIR band is appeared on the chitosan-graft-maleic sponge at 1560.13 cm⁻¹ which correspond to C=C stretching. The TG/DTA result shows that the pure chitosan and chitosan-maleic sponges have 2 stage of degradation namely water evaporation and pyrolysis of organic compound. Shifting of degradation rate peaks of second stage occur from 300°C for pure chitosan to 340°C for chitosan-graft-maleic sponges. Both results evince that the reaction is successfully conducted. The ability of drug loading is investigated using methylene blue as the drug model. The kinetic of drug loading fit the pseudo-first order model with k₁ ranged 0.287 – 0.317 day⁻¹. The adsorption equilibrium fit the Freundlich model with k_f and 1/n value are 4.923 mg/gram and 2.192 respectively.

Keywords: sponges, chitosan, maleic anhydride, drug delivery system, methylene blue.

In Vitro Study of Alkyl Gallates as a Growth Inhibitor of Cervical HeLa Cancer Cells

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Abstract. Cervical cancer is the third most common cancer in women and the fifth most common in the world. In Indonesia, the prevalence of cervical cancer is 12.8 out of 100,000 women in 2010, in which become the second most frequent cancer. Current treatments of cervical cancer are surgery, radiation therapy, chemoradiation, or combination therapy. However, the high rate of complications and severe side effects of those therapies indicating to the need for the new anti-cervical cancer agent. Gallic acid is known to have potential anticancer effects. Structure modification of gallic acid into its derivatives of alkyl gallates are expected to increase the hydrophobicity of alkyl gallates which led to the improvement of its anticancer activity due to its ability to penetrate cancer cell membrane easily. In this work, we conducted in vitro study of ten synthesized alkyl gallates (methyl gallate, ethyl gallate, propyl gallate, butyl gallate, isobutyl gallate, t-butyl gallate, amyl gallate, isoamyl gallate, heptyl gallate and octyl gallate) against cervical HeLa cancer cells by MTS assay. In vitro cytotoxic activity of alkyl gallates on HeLa cells are expressed in median inhibitory activity (IC₅₀) value. The results showed that heptyl gallate and octyl gallate had the strong cytotoxicity against cervical HeLa cells with IC₅₀ value of 12.32 µg/ml and 51.98 µg/ml, respectively. This result suggesting that heptyl gallate and octyl gallate are promising to be further developed as the new anti-cervical cancer agents.

Keywords: alkyl gallate, in vitro study, cytotoxicity, HeLa cells, MTS assay.

Chlorhexidine Chip for Periodontitis Therapy: A Short Review on Materials

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Abstract. Antibacterial agents have been used in periodontal infection (periodontitis) therapy along with mechanical debridement. Over the past years, the use of local drug delivery system of the antibacterial agents is getting more popular instead of systemic drugs and have been extensively studied to overcome the limitation of conventional therapy. Periodontal chip has gained more interest because its sustainable delivery of antibacterial agent right into the periodontal pocket which hard to reach with other system. Periodontal chip incorporated with chlorhexidine has been fabricated with biodegradable materials like gelatin, synthetic polymer, chitosan, alginate and cellulose derivate. Therefore, the aim of this review is to focus upon materials available and its properties in fabricating chlorhexidine chip conducted from previous studies.

Keywords: drug delivery, chlorhexidine chip, biodegradable materials, periodontitis.

HP446 inhibits the RNA-dependent RNA polymerase of SARS-CoV-2 and its combination with remdesivir exerts synergistic antiviral activity

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Abstract. We investigated whether a synthetic compound HP446 exerts anti-SARS-CoV-2 activity by targeting RNA-dependent RNA polymerase (RdRp) to inhibit viral replication. We used a Vero E6-based anti-cytopathic effect assay to study the antiviral activity as well as the mode-of-action of HP446, a hydroxychloroquine analog comprising a quinoline core. A robust cell-based minigenome RdRp reporter assay was established to investigate the mode of action of HP446. HP446 possesses broad-spectrum activity against different SARS-CoV-2 variants of concern, including omicron. Interestingly, HP446 successfully reduced the expression levels of proinflammatory cytokines in SARS-CoV-2-infected Calu-3 cells. Additionally, molecular docking analysis revealed that HP446 targeted the RdRp channel to block the substrate entry, indicating that its mechanism of action was different but complementary to that of Remdesivir which binds to the catalytic site. By taking advantage of a sensitive and robust cell-based minigenome RdRp reporter assay, we found that HP446 successfully inhibited SARS-CoV-2 RdRp activity. Finally, we demonstrated that the HP446-remdesivir in combination acted synergistically which attributed to the interaction of both drugs with different domains of the unique and highly conserved RdRp. The newly discovered synergy of HP446-remdesivir in combination supports the clinical potential of these two drugs against different SARS-CoV-2 variants of concern.

Keywords: quinoline, RNA-dependent RNA polymerase, SARS-CoV-2.

Initial Study and Chip Characterization of Two-Step Lab-on-Chip Double Layer Stem Cell Encapsulation System

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Abstract. There had been many methods developed to generate microcapsules for stem cell encapsulation purposes. However, microfluidic emulsion is found to be satisfactory as it allows us to generate a controllable even sized droplet efficiently. However, the process comes with a problem, it was noticed that the microcapsules were easily dissolved in a saline buffer solution. The issue shows that the capsules were unstable. Therefore, double encapsulation was introduced, which allows us to add another layer to the capsule with would allow more stability and increase mechanical strength. Here, an initial study of double layer encapsulation is conducted with Lab-On-Chip technology using oil and water. This study explores the use of Polycarbonate (PC) and Polydimethylsiloxane (PDMS) Chip for double layer encapsulation. Polycarbonate Chips with a channel size of approximately 50 μ m x 50 μ m and PDMS Chips with a channel size of 300 μ m x 300 μ m chips were tested and characterized, where suitable parameters for double layer encapsulation were obtained and used to generate a double encapsulation system. The result shows the droplet generation characteristics of a two-chip system design that could generate double layer encapsulations with sizes of approximately 1300 -1700 μ m at a generation frequency of 4-8 Hz. This paper can be used for further studies in controllable double-layer encapsulation using Lab-on-Chip.

Keywords: encapsulation, droplet, microfluidics, stem cells, lab-on-chip, polydimethylsiloxane, polycarbonate, double-layer, two chip, PDMS, PC.

Functionalizing Nanoparticles - Reviewing Barriers for Clinical Applications

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Abstract. Recent advances in applications of nanoparticles in clinical research has shifted focus to overcoming biological barriers and targeting specific cells and tissues. Such barriers include crossing epithelial tissue, navigating the tumor microenvironment, intracellular delivery and the ability to target immune cells specifically by preventing non-specific uptake by healthy organs. By investigating the different obstacles that the nanoparticle faces in reaching its intended target, they can be engineered accordingly by tweaking their surface and material properties or by modifying the surface of the intended target. Different methods of doing so will be discussed in this review, important among them being bioactive components of nanoparticles that react to local pH, degrade in the intracellular environment by ultrasound or are activated by photothermal therapy. Looking beyond the conventional size, shape and charge of nanoparticles to make these entities shape switching as an example, this review discusses novel methods that overcome barriers to ensure increased efficacy in disease treatment.

Keywords: immune cells, bioactive components, nanoparticle shape switching, microenvironment.

Disclaimer : None of the work herein was affiliated with, performed at, or endorsed, sponsored or funded by Sangamo Therapeutics, Inc.

Extraction of Collagen and Hydroxyapatite from Fish for Bone Scaffold: A Review

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Abstract. Bones are one of the most important parts of the human body. Bone disorders and diseases are a matter of concern because of their increasing prevalence. Bone tissue engineering with structural development through a combination of scaffolds, cells, and biological factors is a promising solution for bone regeneration. Collagen and hydroxyapatite, among the most commonly used scaffold materials for bone tissue engineering, can be extracted from natural sources. Indonesia is the largest archipelagic country and the second-largest fish producer in the world, and has abundant marine resources. In this study, the extraction of collagen and hydroxyapatite from fish was discussed. Collagen can be extracted using acid, pepsin, and salt solubilization. Meanwhile, hydroxyapatite can be synthesized using calcination, alkaline heat treatment, enzymatic hydrolysis, and ionic liquid pretreatment. The extraction of collagen and hydroxyapatite is expected to be able to utilize marine and freshwater fish and can be used as bone scaffold material to treat bone disorders and diseases.

Keywords: bone scaffold, collagen, hydroxyapatite, fish.

Environmental Biotechnology

Development of eco-community type water purification system using EPS-forming microalgae

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Abstract. Currently, social interest in eco-friendly and sustainable water purification systems is increasing. Microalgae are attracting attention from many researchers because they purify water quality through photosynthesis and at the same time remove carbon dioxide and produce useful substances. However, because microalgae are small unicellular and float under normal conditions, it is difficult to directly apply them to contaminated water systems, and high energy costs are required to separate cells from water. Some of the microalgae are known to produce extracellular polymeric substances (EPS) to overcome them in dry or polluting conditions. In this study, a microalgae colony was formed by using CC-03 species that produced such EPS extremely, and then applied directly to the contaminated water system. As a result, EPS formation was accelerated in some dry conditions in which humidity was maintained, and it was found that calcium and phosphorus were involved in the formation. When the formed microalgae colonies were put in water to purify the water, it was also confirmed that the colony shape was maintained for more than 10 days. This study will help develop an eco-friendly and sustainable purification system using microalgae.

Keywords: microalgae, CC-03, extracellular polymeric substances

Characterization of Oil Degrading Bacteria Isolated from Oil-Contaminated Soil, Dhaka city, Bangladesh

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Abstract. Oil spills pose a serious threat to terrestrial and marine ecosystems. It may happen where oil is processed, transported, stored, improper disposal or used. Oil spills are a common phenomenon in Dhaka city as users' demands & rushing towards filling stations are happening every time. Natural populations of microorganisms could break down this looming threat by using bioremediation potential. It is eco-friendly, sustainable & cost-efficient. To investigate this idea, oil-contaminated soil was collected & processed for isolating oil-degrading bacteria. An enrichment media, Bushnell Hass agar was used containing diesel oil as a carbon source. Molecular detection of oil-degrading genes (catechol-2,3-dioxygenase, alkane hydroxylase) was screened by polymerase chain reaction. Optimization of bacterial growth was also performed in a range of different physical parameters (temperature, pH, different oil concentration). Exopolysaccharide extraction was also measured for correlating the oil-degrading bacteria in biodegradation. Characterization of biosurfactant producing isolates were identified in a range of ways, including emulsification index, drop-collapsing, hemolysis test, CTAB-methylene blue, and bacterial adhesion to hydrocarbons (BATH). A total of 34 bacteria were isolated & among them, 29 isolates were identified through 16s sequencing and subsequent analysis. A wide variety of bacteria were found, including *Pseudomonas* sp., *Acinetobacter* sp., *Klebsiella* sp., *Cellulomonas* sp., *Cupriavidis* sp., *Stenotrophomonas* sp., *Glutamicibacter* species. Oil degrading genes (catE, alkB) were found in 58%, and 26% bacteria respectively. Bacterial isolates showed high optimal density at 0.75% oil concentration, at pH 7 & 9 respectively. These indicate that those isolates belong to alkaliphilic group. Oil-degrading isolates also prefer to grow at 30°C. Exopolysaccharide (EPS) was analyzed through Fourier transform infrared spectroscopy. Some isolates are showing biosurfactant production potential, including MB 251, MB 253, MB 502, MB 754, MB 755, and MB 757. Therefore, we concluded that EPS & biosurfactant production is directly correlated with oil degrading bacteria that can be used in oil spill remediation.

Keywords: oil, alkane gene, EPS, bioremediation, biofilm

Colorimetric Detection of Heavy Metal Ions Using Silver Nanoparticles Synthesized Using *Alpinia galanga* and *Kaempferia galanga* Extracts

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Abstract. Environmental contamination due to heavy metals is a serious issue worldwide due to their harmful effect on the ecosystem and human health. Colorimetric detection of heavy metals ions is particularly attractive because this technique offers qualitative and quantitative information by naked-eye visibility without sophisticated equipment. Silver nanoparticles (AgNPs) are extensively used for colorimetric detection because they exhibit a high excitation coefficient and specific optical properties in the visible region. In recent years, the green synthesis of AgNPs using plant extract has garnered a lot of attention due to this process being environmentally friendly, cheap, and simple, compared to the conventional synthesizing processes. The phytochemicals present in the plant extracts act as the reducing and stabilizing agents during the synthesis of AgNPs. The current study is the first to report the potential of AgNPs synthesized using *Alpinia galanga* and *Kaempferia galanga* for detection of heavy metals ions in aqueous solutions. The synthesis of the AgNPs at various parameters was characterized by UV-vis while the detection of heavy metals ions was explored via absorption spectroscopy and colorimetric assay. The formation of AgNPs was confirmed by the absorbance peak at 400 – 500 nm for samples prepared using *A. galanga* and *K. galanga* extracts. A significant decrease in the absorbance was observed when AgNPs prepared using *A. galanga* extract was mixed with Cu^{2+} , Hg^{2+} or Cr^{3+} . This is accompanied with solutions' color changes from brown to colorless when mixed with Hg^{2+} , and from brown to pale brown when mixed with Cu^{2+} or Cr^{3+} . On the hand, a substantial increase in the absorbance was recorded and solution's color changed from brown to dark brown when the AgNPs prepared using *K. galanga* extract was mixed with Fe^{2+} . These results demonstrate that AgNPs synthesized using *A. galanga* and *K. galanga* as the reducing and stabilizing agents have potential applications for heavy metal ions detection in aqueous solution.

Keywords: biosynthesis; silver nanoparticles; *Alpinia galanga*; *Kaempferia galanga*; heavy metals; colorimetric detection

Decolonization of Crystal violet by indigenous bacteria isolated from industrial effluents in Bangladesh

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Abstract. Industrial effluent containing textile dyes is a major environmental concern in Bangladesh. The dying and textile industries are mainly located on the river banks in Bangladesh and discharge untreated effluent containing toxic dyes or residues directly into the rivers. Among different dyes, Crystal Violet is widely used, which has a complex structure which resists their removal from water bodies. Persistence of crystal violet in water not only interferes with light penetration and biological oxygen demand, but also adversely affect the aquatic life. Additionally, exposure to this toxic, carcinogenic and mutagenic dye cause different severe health problems in human including death. Therefore, it is essential to design an effective effluent treatment plant that would degrade and hence remove the toxic dye from the industrial effluent. Aiming to develop such effluent treatment plant this study characterized crystal violet decolorization potential of bacteria isolated from industrial effluent contaminated area of Batik polli, Narayanganj, Bangladesh. Five bacteria were isolated based on their ability to grow on Bushnell Haas medium supplemented with crystal violet as the sole carbon source. For identification, partial 16S rDNA gene sequence of each isolate was amplified and the sequence was analyzed with nucleotide BLAST search in GenBank (NCBI). Analyzing the data, the isolates were identified as *Bacillus pumilus* (gene bank accession number MZ676076.1), *Staphylococcus saprophyticus* (MZ676077.1), *Micrococcus endophyticus* (MZ676078.1), *Pseudomonas mendocina* (MZ820116.1) and *Acinetobacter baumannii* (MZ820116.1). Based on preliminary data, *Bacillus pumilus* and *Acinetobacter baumannii* were selected for assaying the decolorization of crystal violet. The decolorization assay was conducted following method described by Karim et al., (2008) with slight modifications. Three different modifications of Bushnell Haas medium supplemented with crystal violet were used: one with no co-substrate and in the other two 0.1% glucose or 0.5% yeast extract was used. After 10 days incubation at 37°C, *Bacillus pumilus* decolorized 32% crystal violet while *Acinetobacter baumannii* decolorized only 5% when grown in Bushnell Haas media without any co-substrates. Addition of 0.1% glucose or 0.5% yeast reduced crystal violet decolorization by *Bacillus pumilus* to 11% and 15%, respectively. In contrast, addition of 0.1% glucose to the growth medium increased crystal violet decolorization from 5% to 28% by *Acinetobacter baumannii*, though yeast extract did not improve the decolorization. This current study therefore reports potential Crystal Violet degrading bacteria from textile industry effluents. More investigation on detail biochemical and molecular mechanism of dye decolorization of these bacteria is necessary for potential use in the industrial bioremediation process.

Keywords: bacteria, textile dye, decolorization

Catalytic Hydration Reaction and Mineralization of Carbon Dioxide by Immobilized Carbonic Anhydrase

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Abstract. In this study, an electrospinning technique was used to prepare polyacrylonitrile nanofiber film (PAN), and the -CN group on the membrane was further modified to hydroxylamine group (namely P-Oxime), and then coupled with bromoacetic acid (BrA). The reaction produced a weak ion exchange membrane (namely P-BrA) with acidic groups. Carbonic anhydrase (CA) was immobilized on these two modified membranes by physical adsorption and chemical covalent bonding methods. In the characteristic analysis of free carbonic anhydrase, the p-Nitrophenyl acetate colorimetric method was used to measure the changes in its activity under different conditions (e.g., temperature, pH, and salinity). The result showed that under the thermal stability analysis, the activity of CA was the highest at 0°C, and the overall trends continued to decrease as the temperature increased, indicating that the stability of CA was significantly affected by the operating temperature. CA at different environmental pH values has the highest activity at pH 9, indicating that CA was more suitable for survival in a weakly alkaline environment. Due to the consideration of capturing CO₂ in seawater and testing the influence of salinity on CA activity, the activity was highest in an environment with a salinity of 1 % NaCl, but it also maintains good stability when the NaCl concentration increased to 6 %. For the determination of CA immobilization activity, two membranes of P-Oxime-CA and P-BrA-CA were selected by physical adsorption and chemical covalent bonding methods, and the immobilization activity of CA was measured by the Walbur-Anderson method. The results showed that the expression activity of immobilized CA by means of the chemical covalent bonding method was better than the physical adsorption method. In the repeatability analysis results, P-Oxime-CA chemical covalent bonding CA still retains high activity. The immobilized CA can convert the saturated CO₂ solution into bicarbonate (HCO₃⁻). As the pH was adjusted to 12 for mineralization tests and calcium chloride was added to the solution. The calcium carbonate (CaCO₃) deposition phenomenon was observed and analyzed by SEM, FTIR, and XRD to verify whether it was CaCO₃. The results showed that the precipitates produced by the immobilization of CA on different membranes are all CaCO₃, and the SEM images showed that the crystal form was irregular particles.

Keywords: carbonic anhydrase, nanofiber membrane, enzyme immobilization, mineralization reaction

Biotransformation of Methylene Blue by Mixed Fungal Cultures of *Gloeophyllum trabeum* and *Aspergillus oryzae*

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Abstract. This study investigated the biodecolorization of Methylene Blue (MB) by mixed fungal cultures of *Gloeophyllum trabeum* and *Aspergillus oryzae*. *A. oryzae* (10 mL) was added into *G. trabeum* cultures (10 mL) and then MB was added until the final concentration reached 95,24 mg/L and incubated at 30 °C for 7 days. All of mixed and single cultures had the ability to decolorize MB on the liquid media of potato dextrose broth (PDB). The addition of *A. oryzae* to *G. trabeum* culture showed the highest MB decolorization of 69,34%, whereas by *G. trabeum* alone it was only 31,50 % and *A. oryzae* alone 36,82 %. $C_{16}H_{20}N_3S$, $C_{19}H_{22}N_3SO_4$, and $C_{31}H_{48}N_3S^+$ were identified as product metabolites of MB decolorization by the mixed culture. This study indicated that the addition of *A. oryzae* enhanced decolorization of MB by *G. trabeum*.

Keywords: biodecolorization, methylene blue, mixed culture, *Gloeophyllum trabeum*, *Aspergillus oryzae*

Self-Assembled Enzyme Complex for Enhanced Degradation of Polyethylene Terephthalate

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Abstract. Polyethylene terephthalate (PET) is widely used due to its good processing and well-documented properties. Its extensive use has also caused its accumulation in the environment, posing a huge threat to the ecosystem. Recently, it was reported that the hydrolases produced by PET decomposing bacteria could catalyze the complete degradation of PET, providing an eco-friendlier technology to treat plastic waste. This study aims to imitate the structure of cellulosomes, which have an excellent degrading activity towards cellulose. By co-immobilizing two enzymes (PETase and MHETase) and an affinity protein (hydrophobin), we develop a two-enzyme cascade system for PET biodegradation. The PETase, MHETase, and hydrophobin form a supramolecular enzyme complex via the interaction between SpyTag/SpyCatcher. The results show that, compared to free enzymes, this biomolecule complex not only promotes the interaction of PETase and MHETase with PET substrates but also protects the enzymes, thereby increasing their stability. Overall, the macromolecular structure of PETase-hydrophobin-MHETase facilitates the two-enzyme cascade reaction, allowing it to degrade PET more efficiently. This technology facilitates the recycling of waste PET in the future.

Keywords: polyethylene terephthalate, PETase, MHETase, hydrophobin, cascade reaction

The Application of an Unusual Biosurfactant

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Abstract. The application of a high throughput analysis method for the screening of potential biosurfactants from natural sources has been developed. The method described here is based on the effect of meniscus shape on the image of a grid viewed through the wells of a 96-well plate. Methods of producing the lipopeptide biosurfactant, surfactin, from cultures of *Bacillus subtilis* (BBK006) have been investigated. A reactor with integrated foam fractionation was designed and used in batch and continuous modes. Neutron reflectometry has been used to study the structure of the biosurfactant, surfactin, at the air/water and at the hydrophobic solid/water interfaces. The study of photoluminescence of single-walled carbon nanotubes wrapped with surfactin was carried out. Synthesis of silver and gold nanoparticles using a borohydrate reduction was performed at three pH levels and two different temperatures in the presence of surfactin that was used to stabilize the formation of silver and gold nanoparticles. Synthesis of brushite particles in reverse microemulsions of the biosurfactant surfactin was investigated. A novel BMSN (biologically synthesized mesoporous silica nanoparticles) material was prepared using a bacteria-mediated biosurfactant. Removal of fluoride from water was studied using the bacterial surfactin mediated novel hydroxyapatite nanoparticle. Low-temperature synthesis of rose-like ZnO nanostructures was synthesized using surfactin and their photocatalytic activity has been checked. Removal of mercury by foam fractionation was also carried out using surfactin, a biosurfactant. This report describes the use of a renewable, environmentally compatible, biodegradable surfactant as a stabilizing agent for the synthesis of nanoparticles and their application for environmental issues.

Keywords: biosurfactants, neutron reflectometry, nanoparticles, photocatalytic activity, removal of mercury

Potential Use of Vibriophage in Association with Antibiotic to Treat the *Vibrio cholerae* Biofilm

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Abstract. In cholera prevalent areas, *Vibrio cholerae* in the aqueous environment can exist in a cell aggregated form covered by an extra polysaccharide matrix called Biofilm. These types of cells are named as VBNC (viable but non culturable cell) or CVEC (conditionally viable environmental cell). The cells in the biofilm can persist in a wide range of environmental conditions by remaining metabolically dormant and can resuscitate into planktonic cells leading to a cholera outbreak. So, it is imperative to treat these biofilms. It has been found that antibiotic treatment is not effective in degrading the biofilm and also can lead to the dissemination of antibiotic resistance genes. So as a safe alternative, this study has shown the use of a bacteriophage to treat the pathogenic *V. cholerae* biofilm. This study first discussed how the antibiotic treatment on the *Vibrio cholerae* biofilm is ineffective since the cells within biofilm are very resistant to antibiotic. Then the alternative method of using a Vibriophage to treat the biofilm is discussed. The Vibriophage secretes polymerase degrading enzyme which breaks the matrix of the biofilm and planktonic cells are released. The planktonic cells then can be treated with antibiotic. Finally, the synergistic effect of both Vibriophage and antibiotic in treating the biofilm cells have been discussed here. This synergistic effect of antibiotics and bacteriophages holds promising results on the elimination of toxigenic *Vibrio* biofilms. This research could be used to develop a phage-mediated cholera control technique.

Keywords: bacteriophage, *V.cholerae*, biofilm, antibiotic

Screening of Polyurethane Degrading Bacteria from Pakusari Jember Landfill

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Abstract. This study describes the isolation of bacteria from the soil of the Pakusari Jember TPA. Bacterial isolates were selected for their ability to degrade polyurethane. The semi-quantitative degradation ability test was based on the ability to grow colonies with a hydrolysis zone on an agar plate medium containing mineral salts containing polyurethane diol as the sole carbon source. The results of ANOVA analysis showed that isolates with code PB15 showed the highest average growth and degradation than *E.coli* BL21 (control). A quantitative test based on the percentage of weight loss on polyurethane film and polyurethane foam was carried out within 30 days of incubation. The results showed a higher weight loss than the control. Scanning Electron Microscopy analysis showed that there were cracks on the surface of the polyurethane film and polyurethane foam. Cracks in the morphology of the PU film and PU foam indicate the degradation of the PU polymer. Isolate PB15 had macroscopic morphological characters of round colonies, flat edges, convex elevation, and white in color. The isolate belongs to Gram-negative bacteria with a round cell shape. Enzymes that are thought to be able to be secreted by isolating PB15 to hydrolyze polyurethane are esterase, protease, and urease.

Keywords: polyurethane, biodegradation, scanning electron microscope

Marine Biotechnology

Recent Updates on Valinomycin and Its Analogues

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Abstract. Valinomycin is a cyclodepsipeptide antibiotic that can be traced back to the 1950s. It is a highly specific potassium-ion transporter. However, the interest in this compound is growing in recent years. Many studies suggested that valinomycin can be used as a broad-spectrum antiviral agent. Valinomycin was also found to be highly active against the SARS-Cov virus, thus it is argued also to be active against the new variant of SARS-Cov2. Screening of bioactive compounds from actinobacteria especially marine *Streptomyces* spp. added the bioactivity information on valinomycin. In addition, improved techniques for isolation and structure identification resulted in the finding of the analogues of valinomycin. Currently, three valinomycin analogues have been isolated and elucidated. They are streptodepsipeptide P11A, streptodepsipeptide P11B, and streptodepsipeptide SV21. Valinomycin and its analogues showed various bioactivities ranging from antiproliferative, antibacterial, antifungal, and antiviral. This article aims to review the information on bioactivities, mechanisms, and future potentials of valinomycin and its analogues. This article also summarizes the possible biosynthesis pathway of valinomycin and its analogues. The bioactivity and toxicity studies of valinomycin and its analogues showed that they have different strengths and toxicity, hence the compound with the optimum benefit with high activity and low toxicity is preferred.

Keywords: SARS-Cov, valinomycin, *Streptomyces*, natural products

Growth, Lipid and β -carotene content of *Dunaliella salina* (Dunal) Teodoresco Cultured in Acadian Marine Plant Extract Powder (AMPEP) Media

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Abstract. Acadian Marine Plant Extract Powder (AMPEP) is an organic fertilizer derived from extracts of brown algae (*Ascophyllum nodosum*) that is commonly used to increase the productivity of agricultural crops. However, it has the potential to be used as a cheap and sustainable microalgae culture medium for the production of lipid and β -carotene. This study aims to determine the effect of variations in the concentration of AMPEP on the growth, lipid and β -carotene content of *Dunaliella salina*, a well-known microalgae species using in food industries. This study used 4 AMPEP concentration that were 0.01, 0.1, 1, 10 ppm and Walne in triplicates. The initial density of the culture was 10^5 cells.mL⁻¹ which was cultured for 7 days using a plastic bottle (v=600 mL) with a culture volume of 300 mL. Microalgae density was calculated every 2 days using a Neubaur haemocytometer and a hand counter under a microscope with 40x magnification. Microalgae were cultured at light intensity 16.2 moles.m².s⁻¹, temperature 28°C, pH 7-8 and salinity 29-30 ppt. Microalgae were harvested using pre-dried, pre-weighed Whatman microfiber filter paper ($\Theta=25$ mm), dried (1 hour, 100°C) and ashed (5 hours, 450°C) then weighed to calculate dry weight and ash-free dry weight. Density data during the culture period were analyzed using Repeated *Measure ANOVA* while data on specific growth rate, *dry weight* (DW), *ash free dry weight* (AFDW), biomass productivity and lipid content used *One-Way ANOVA*. Lipid analysis was performed using modified *Bligh and Dyer* method. The content of β -carotene was analyzed using spectrophotometer UV-VIS at absorbance of 450 and 665 nm. The interaction between day and concentration of AMPEP media affected the growth of *D. salina* (p=0,000). The final density, relative growth rate and the highest biomass productivity were found at the AMPEP media concentration of 10 ppm (p=0,000) with values $418,8 \times 10^4$ cells.mL⁻¹, 1.003 cells.day⁻¹ and 1.35 g.L.day⁻¹, respectively. The highest lipid content was found at a concentration of 0.01 ppm that was 5.26%. The highest β -carotene content was obtained at a concentration of 10 ppm AMPEP medium of 0.3545 ug.mL⁻¹ (p=0,000), whereas in concentrations of 0.01, 0.1, 1 ppm and Walne, the β -carotene content did not differ, ranging from 0.1-0.2 g.mL⁻¹. This study showed that the concentration of AMPEP media of 10 ppm may be used for *Dunaliella salina* culture for the production of β -carotene as a natural antioxidant although the lipid content was considerably low.

Keywords: *Dunaliella salina*, AMPEP, growth, lipid, β -carotene

Valorization of Seaweed *Gracilaria* sp. Biomass Waste into Liquid Organic Fertilizer: Assessment on Growth of Cayenne Pepper *Capsicum frutescens* L.

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Abstract. Seaweed processing commonly generates biomass waste either used as a low-value product or discarded. However, seaweed biomass waste still contains essential nutrients that can be recovered to generate valuable products, such as organic fertilizer. This study aims to valorize seaweed *Gracilaria* sp. biomass waste into liquid organic fertilizer to support sustainable and eco-friendly production. *Gracilaria* sp. biomass waste from Karawang, Indonesia was composted, and two liquid organic fertilizer doses were formulated (containing 0.5 and 1.0% v/v of compost liquid). The fertilizer variants were then applied to *Capsicum frutescens* L. plants and their growth parameters (plant height, relative growth rate, number of leaves, and dry weight) were evaluated. The fertilizer's physicochemical properties (organic-C, total N, P, K, and pH) were also analyzed. The growth assessment and physicochemical characteristic results were then compared to the results from control and commercial organic fertilizer. The present study showed that both seaweed biomass waste-based liquid organic fertilizer variants generated higher *C. frutescens* L. plant growth parameters than the control, with comparable outcomes to the commercial one. Although, the fertilizer's organic-C, total N, P, and K content were below the national technical standard. These findings demonstrate that seaweed biomass waste is prospective and can be studied further for liquid organic fertilizer development.

Keywords: composting, *Gracilaria* sp., plant growth, seaweed fertilizer, zero waste

Tautomer Shift of Oxidized Dopa in Marine Mussel Adhesion

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Abstract. 3, 4-Dihydroxyphenylalanine (Dopa) is a versatile molecule that enables marine mussels to achieve successful underwater adhesion. However, due to its complicated redox chemistry and vulnerability to oxidation, controlling surface adhesion and cohesion has been a challenging issue to overcome. Foot protein type 6 (fp-6), a thiol-rich interfacial mussel adhesive protein, has been reported as a proteinaceous antioxidant for mussels that helps Dopa maintain surface adhesion ability. In this study, we focused on the role of fp-6 in oxidized Dopa. The effect on the tautomer equilibrium of oxidized Dopa was investigated using recombinant fp-6 (*r*fp-6) and Dopa-incorporated foot protein type 3 fast variant (*dr*fp-3F), which were produced in bacterial cells. The redox chemistry of Dopa in *dr*fp-3F and the role of *r*fp-6 were observed using a UV-vis spectrophotometer and a surface forces apparatus (SFA). We discovered that *r*fp-6 shifts the tautomer equilibrium to Δ Dopa as a preferred tautomer for oxidized Dopa in *dr*fp-3F and makes *dr*fp-3F better on underwater surface adhesion.

Keywords: surface adhesion, underwater adhesion, dihydroxyphenylalanine (Dopa), tautomerization, dehydro-Dopa, foot protein type 6

Exploration of Potential Bacteria from Jakarta Bay as a Candidate for Microplastic Agent

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Abstract. Indonesia is one of the countries that produce the most plastic waste in the world. Plastic waste pollution will lead to the ocean and will pollute marine ecosystems and marine biota. Jakarta Bay as the estuary of 13 rivers makes it a place with high plastic pollution, it is stated that 59% of plastic waste enters Jakarta Bay. As an alternative to overcome the problem of plastic waste in Indonesia, research is carried out on the exploration of candidate microplastic degrading bacteria. This study aims to obtain a consortium of bacteria that can optimally degrade Low Density Polyethylene (LDPE) plastic waste. The samples used in this study came from sediments in 3 locations in Jakarta Bay. The bacteria isolates were able to grow on media infused with LDPE. The extents of biodegradability of the polyethylene granules by the isolates bacterial strains were assessed *in vitro* in the medium containing polyethylene as sole carbon source. After 60 days of incubation, the biodegradation of polyethylene granules was measured in terms of weight loss and rate of polymer reduction. This research is still in process, provisional results obtained that the optimum consortium growth curve was obtained on day 5, so the bacterial consortium that would be used for biodegradation test was on day 5. This research is expected to be an initial study to obtain the optimum bacterial consortium and isolates in degrading microplastics.

Keywords: biodegradation, polyethylene, eksplorasi

A Report on the Potential of Marine Sponge *Theonella* from Indonesia as Producer of Antibiotics

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Abstract. Marine sponges are the oldest invertebrates in the world and it was reported as the most prolific marine-derived bioactive compounds. Among all, the Indonesian sponges are reported to contribute as the second highest producers of many new marine-derived bioactive compounds, including antibacterial and anticancer. Present study summarize any available research on Indonesian sponges throughout 2010-2021, including the study on sponge diversity and the investigation on sponge bioactivity, aiming to contribute to marine bioprospecting in Indonesia. In addition to the general review on Indonesian sponges, this study also investigate the potential of sponges *Theonella* from Indonesia as producers of some antibiotics. Three of the sponges materials were extracted using a mixture of methanol and ethyl acetate (1:1). The sponge crude extracts were subjected for HPLC analysis and compared to the available database of antibiotics. The result showed some signals antibiotics detected in the sponge extracts, i.e Angolamycin, Niphithricin B, Epidermin, and Nocardicin A. This result indicating the potential of *Theonella* from Indonesia as antibiotics producers, highlighting the potential of Indonesian sponges for further bioprospecting and biotechnological application.

Keywords: antibiotics, bioprospecting, Indonesia, sponges, *Theonella*

Association of Macroalgae with Coral Reefs in National Marine Protected Areas, Aquatic Tourism Park, Anambas Islands

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Abstract. Most macroalgae communities grow in association with coral reef ecosystems. The distribution of macroalgae in sea waters generally follows the distribution of coral reefs as their habitat. However, locally in coral reef areas, the distribution of macroalgae is influenced by environmental factors and the characteristics of the macroalgae species. This study aims to determine the biodiversity of macroalgae that are associated with coral reefs or which grow attached to coral reef substrates at a depth of 10 to 15 meters. Sampling locations were conducted at twelve (12) stations spread throughout the Anambas TWP area. The distance of the sampling point for each station is about 500 m from the nearest island. The sampling method was carried out using the roaming method around the UPT transect, which spanned 70 m from Point Zero (T0). The identification results show that the presence of macroalgae associated with coral reefs in the Anambas TWP area is quite abundant with 19 species found, consisting of 3 divisions (*Orchophyta*, *Rodophyta*, and *Chlorophyta*), 3 classes (*Florideophyceae*, *Ulvophyceae*, and *Phaeopyceae*), 5 orders (*Corallinales*, *Bryopsidales*, *Dictyotales*, *Fucales*, and *Cladophorales*), 7 families (*Lithophyllaceae*, *Caulerpacaeae*, *Dictyotaceae*, *Sargassaceae*, *Bryopsidales*, *Halimedaceae*, and *Valoniaceae*), and 10 genera (*Amphiroa*, *Caulerpa*, *Dictyota*, *Lobophora*, *Sargassum*, *Turbinaria*, *Halimeda*, *Padina*, and *Valonia*).

Keywords: macroalgae, association, coral reef, Anambas Islands

Distribution and Squalene Accumulation of Thraustochytrids Isolated from the Gulf of Thailand Mangrove

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Abstract. Thraustochytrids, a heterotrophic protist, were isolated from fallen leaves of mangroves on the western coast of the Gulf of Thailand and used to investigate their total lipid profile and squalene contents. Morphological and molecular features revealed that these Thraustochytrids belonged to the genus *Aurantiochytrium*. These isolates were cultivated and their dry-cell weight, fatty acid profiles, and squalene accumulation were determined. For 5 days of cultivation, the maximum dry-cell weights ranged from 2.75 to 5.82 g/L. The total lipid profile showed a broad spectrum of saturated fatty acids including palmitic acid (16:0), 23.27 - 42.15 % TFA, pentadecanoic acid (15:0) 17.57 - 31.34 % TFA, heptadecanoic acid (17:0) 3.79 - 9.16% TFA, lignoceric acid (24:0) 2.68 - 9.21 % TFA, myristic acid (14:0) 2.54 - 3.67 % TFA, stearic acid (18:0) 0.75 - 1.62 % TFA, and arachidic acid (20:0) 0.21 - 0.37 % TFA, respectively. Unsaturated fatty acids, including docosahexaenoic acid (22:6; 8.17 - 36.82 % TFA), clupanodonic acid (22:5, 2.03 - 9.13 % TFA), arachidonic acid (20:4, 0.28 - 0.71 % TFA), eicosapentaenoic acid (20:5, 0.21 - 0.68 % TFA), linolenic acid (18:3, 0.14 - 0.19 % TFA), and Erucic acid (22:1; 0.01 - 0.09 % TFA) were also found. The squalene contents in these thraustochytrids ranged from 37.21 to 93.19 mg/g. The highest squalene-accumulation was achieved from strain P5W, which was identified as *Aurantiochytrium* sp., with a maximum yield of 93.19 mg/g at 4 days of cultivation. We anticipate that the P5W strain can be used as alternative source for large-scale production of squalene in the future.

Keywords: mangrove, squalene, squalene accumulating, Thraustochytrids, *Aurantiochytrium*

Evaluation of the Cytotoxic Potential of the Crude Extracts from Marine Microalga *Isochrysis* sp.

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Abstract. Exploiting marine organisms to produce natural products is getting a promising trend currently. Marine algae are offering a huge array of important metabolites that can be used to treat different kinds of human diseases, especially cancer. Despite having valuable metabolites, there is an inadequacy in exploring the marine microalgae species for their pharmaceutical and nutraceutical importance. Therefore, this project aims to investigate the cytotoxicity of crude extracts of marine *Isochrysis* sp. against a human breast cancer cell line (MCF-7) and to evaluate its mode of cell death mechanism. To achieve these objectives, Freeze-dried *Isochrysis* sp. biomass was extracted with eight different solvents through sonication for 20 minutes and maceration for one hour. The algal extracts were evaluated for their cytotoxic effect at 100 µg/mL concentration against the MCF-7 cell line using MTT assay. Among eight extracts, the ethanol extract of *Isochrysis* sp. reduced the cell viability of MCF-7 cells to $7.24 \pm 0.47\%$ after 72 hours of incubation, at a concentration of 100 µg/ml. The IC₅₀ (half maximal inhibitory concentration) value was 13.37 ± 0.59 µg/ml after 24 hours in MCF-7 cells and >100 µg/ml in non-cancerous human lung fibroblast cells, MRC-5. Ethanol extract of *Isochrysis* sp. was further investigated for apoptosis induction in MCF-7 cells. Morphological observation under a light microscope revealed cell shrinkage, rounded up, condensation of cellular contents, and membrane blebbing in treated MCF-7 cells compared to untreated cells. With the increasing concentration of the extract, a reduction in cell population was observed as well. The Annexin V-FITC and PI staining analysis confirmed that the mode of cell death is mainly apoptosis. Cell cycle analysis revealed the accumulation of cells in the sub-G₁ phase and G₂/M arrest. An up-regulation of the proapoptotic *Bax* gene and tumor suppressor p53 gene was observed through RT-PCR. The data suggest that crude ethanolic extract from marine *Isochrysis* sp. has induced apoptosis in human breast cancer cells, MCF-7, and may have potential therapeutic value for human breast cancer.

Keywords: apoptosis, cytotoxic, marine microalgae, MCF-7, *Isochrysis* sp.

Isolation and Characterization of Cellulase Enzymes from Marine Endophytic Fungi

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Abstract. Cellulase enzymes are enzymes that can degrade the β -1,4 glycosidic bonds of cellulose into simple sugars or glucose. This study was conducted to obtain cellulase enzymes, measure activity, and determine the optimum pH and temperature of cellulase enzymes from marine endophytic fungi which isolated from seaweed *Kappaphycus alvarezii*. Isolation and characterization of cellulase enzymes from EN marine fungi involved several stages of the research, include: preparation of seaweed which includes soaking seaweed with water at warm and room temperature, extraction of cellulase enzymes including the step of culturing marine fungi on production media with pretreatment seaweed, purification of cellulase enzymes includes the deposition of the cellulase enzyme with ammonium sulfate at a saturation level of 30-80%. The results showed that cellulase enzyme extracted from seaweed endophytic fungi (*Kappaphycus alvarezii*) had the highest crude extract activity of FPase enzyme on day 9 which the amount 0.0276 U/mL with seaweed carbon as a source of warm water immersion. The saturation level of ammonium sulfate that is suitable for precipitating the cellulase enzyme in this study is 70% with FPase activity of 0.0381 U/mL and specific activity of 0.7728 U/mg. Cellulase enzyme deposition in this study was optimum at pH 5 and a temperature of 50°C.

Keywords: cellulase enzyme, extraction, *Kappaphycus alvarezii*, marine fungi, seaweed

Characterization of Carrageenan from Seaweed Hydrolysis Using Marine Fungi as Soft Capsule Material

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Abstract. Carrageenan is a polysaccharide that can be used as a raw material for soft capsule. Carrageenan can be produced through biological hydrolysis method by using marine fungi. The material that can be used to modify the polysaccharides are gelatin. The interaction of carrageenan and gelatin are influenced by characteristic and types of carrageenan, and plasticizer. This study aims to determine the characteristic of carrageenan hydrolysis for soft capsule material. Soft capsule was analyzed its properties including dimension, capsule weight, disintegration time, and moisture content. Physical characteristic of the carrageenan produced by hydrolysis were determined including yield, viscosity, and gel strength. The yield was 25%; viscosity 45 Cp; gel strength 175 gf, respectively. While its chemical characteristic contained moisture content of $\pm 13\%$; ash content $\pm 8\%$; cellulose level 8%. Semi refined carrageenan is the chosen treatment in capsule making. Semi refined carrageenan produced a capsule with body length of ± 18 mm; long capsule ± 10 mm; capsule weight $\pm 0,9$ gram; disintegration time ± 10 minutes; moisture content of $\pm 12\%$.

Keywords: capsule, carrageenan, gelatin, hydrolysis, marine fungi

Physical Stability and Skin Irritation Test (SIT) of Face Toner Containing *Stichopus variegatus* Collagen and Chitosan

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Abstract. Toner is a part of cosmetic preparation that can serves to refresh the skin. The aim of this research is to determine formulation, physical stability and effect of the face toner containing of *S. variegatus* collagen and chitosan on the skin. This study consisted of 4 stages i.e., collagen preparation, toner formulation, physical stability and in vivo irritation test. The face toner formulation consists of 0.3% collagen, 3% chitosan, 2% glycerine, 5% polysorbate 20, and up to 100% distilled water. The result showed stability test of facial toner at room temperature has a pH range of 4.98 to 5.83 with a viscosity range of 5.67 ± 0.06 to 6.44 ± 0.02 , while toner at low temperatures has the pH range is 5.8-5.22 and viscosity was 5.93 ± 0.06 to 6.97 ± 0.15 . The results of cycling test and centrifugation tests show that there is no phase separation on facial toner formulation. Facial Toner is stable at low temperature for 8 weeks through centrifugation test, and cycling test, but unstable at room temperature. Facial toner does not cause irritation in vivo irritation test on male Sprague-Dawley rats.

Keywords: irritation, pH, rats, separation, viscosity

Isolation and Molecular Identification of Protease Producing Bacterium Associated with Brown Algae *Hydroclathrus* sp. from Hoga Island of Wakatobi District

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Abstract. Advances in fermentation technology, genetic engineering, and enzyme application technology have led to increased use of enzymes. Enzymes can be produced by utilizing a source of microorganisms such as bacteria. Proteolytic bacteria or protease enzyme-producing bacteria are found in foods or plants that contain protein, such as the brown seaweed *Hydroclathrus* sp. The purpose of this study was to obtain protease-producing bacterium associated with marine algae *Hydroclathrus* sp. from waters around Hoga Island of Wakatobi District and identify the organism based on its 16S rRNA gene sequence. Isolation of bacteria from algae samples was carried out with Nutrient Agar (NA) media, while proteolytic bacteria selection was carried out on Skim Milk Agar (SMA) media. The bacterial isolates producing proteolytic-clear zone on SMA media were then identified targeting the 16S rRNA gene using the PCR (Polymerase Chain Reaction) method with 27F-1492R primers. Based on the isolation results, there were 3 unique colonies of bacteria that could be cultured from algae samples and coded HIHa-1 to HIHa-3 (HIHa stands for Hoga Island *Hydroclathrus* macroalgae). The selection process for protease-producing bacteria on SMA media resulted in 1 isolate of proteolytic bacteria, namely HIHa-1. Molecular identification by PCR on HIHa-1 isolate resulted a single DNA band on the electrophoresis gel sized ~1500 bp. The sequencing results showed a DNA sequence with the size of 1421 bp sharing the highest similarity with the bacterium *Exiguobacterium aestuarii* strain TF-16 (homology level of 99,93%). As conclusion, the proteolytic bacterial isolate HIHa-1 associated with marine brown algae *Hydroclathrus* sp. was obtained and identified as *Exiguobacterium aestuarii* strain HIHa-1.

Keywords: Bacterial protease, brown seaweed, *Exiguobacterium aestuarii*, *Hydroclathrus* sp.

The Characterization of Anti-Tuberculosis Substances from Indonesian Marine Sponge *Haliclona sp.*

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Abstract. Indonesian waters contain bio-active compounds, used as raw materials for medicines such as anti-cancer, anti-virus, and anti-tuberculosis. This study aimed to investigate bioactive compounds from marine organisms in North Sulawesi, Indonesia, with anti-tuberculosis properties. Furthermore, ethanol was used to extract the sponges, then fractionated using a chromatography column with ODS as the stationary phase. The HPLC was used to purify each fraction with an ODS column. Also, the compounds' identification and characterization were obtained using spectroscopic methods, such as 1D and 2 NMR, IR, Mass spectroscopic, and UV. The Anti-Mycobacterium activity was tested using the disc diffusion methods. The results showed a new alkaloid compound (1) and eight known compounds, including the Halicloclamines A (2), B (3), and C (4), Cyclostelletamines A (5), B (6), C (7), E (8), and F (9). *Mycobacterium smegmatis* was prevented from developing by compounds 1 to 9 at a dosage of 10 mg/disc. Furthermore, compound 2 demonstrated an inhibition zone of 17 mm/disc at a concentration of 10 µg/disc. This indicates that the compound has anti-tuberculosis properties.

Keywords: *Mycobacterium smegmatis*, alkaloids, structure elucidation, *Haliclona sp.*

The Environmental Section of EFB and the COST Action Ocean4Biotech Network Activities: A European Perspective

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Abstract. Blue spaces traditionally supporting human life are the world's most heavily exploited environments. Numerous new services and products targeting improving well-being rely on Blue Biotechnology. Technological advances and underexplored marine resources and biomass applications are boosting this sector. Within this context, Europe has a leading world role with a 37% of the global market (EC, 2021). Promising opportunities are foreseen if (i) transdisciplinary collaboration is established, (ii) co-creation design is promoted, (iii) a circular transfer of knowledge is envisaged and (iv) appropriate funding mechanisms are tailored to support critical steps of the ecosystem. At the same time, the need for Responsible Research and Innovation is growing exponentially to ensure societal relevance and ethical, responsible, and sustainable financial returns (Theodotou-Schneider et al, 2022). At the moment, the way of implementing the legislation significantly varies, creating discrepancies between geographical areas within Europe and in various cases even within the same country. Creating and maintaining sustainable networks of researchers, innovators, policy-making and end-users, the key players of this ecosystem, is a stringent necessity. Collaborative networks such as the European Federation of Biotechnology and the Ocean4Biotech network have a leading role in shaping the responsible research and innovation blue biotechnology arena in Europe. First of all, they act as a bridge of communication between stakeholders and accurately map the future needs in the sector and the way that these activities are also sustainable. They can empower young early career investigators and innovators in the field by supporting their research and innovation ideas and promoting knowledge transfer between geographical areas and sectors. Finally, they can increase inclusivity by enabling less represented groups and geographical areas. A selection of best practices already applied will be presented to act as examples for other geographical areas and to promote the dialogue to achieve a common global understanding of responsible research and innovation in the blue biotechnology sector.

Keywords: blue biotechnology, responsible research and innovation, European Federation of Biotechnology, Ocean4Biotech

Medical Devices

Computer Aided Diagnosis for Skin Lesion using EfficientNet B0 & B1 via Transfer Learning

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Abstract. Automatic diagnosis for skin cancer from skin lesions using dermoscopy images is still a challenging task for artificial intelligence, especially in Artificial Neural Networks using deep learning. Using the correct architecture for classification is an important factor in making an accurate automatic diagnosis. However, the classification models that have been made are still not able to perfectly categorize skin lesions. In this research, a replacement of the classification model architecture was made and designed by using the latest architectures such as EfficientNet-B0 and B1 via transfer learning method. This research was conducted by using HAM10000 dataset with 5 varying combination of augmentation, class weight, and normalization within each model. The result showed that for EfficientNet-B0, the best model among the five only used augmentation and has the accuracy, precision, recall, and f1-scores of 91%, 76%, 68%, and 71%, respectively. While for EfficientNet-B1, the best model used augmentation and class weights with the accuracy, precision, recall, and f1-score of 89%, 78%, 73%, and 73%, respectively. The best EfficientNet-B1 model can outperform the existing state of the art model with an increase in recall and f1-score by 2% and 12% from the semi-supervised model, respectively. The model can also be integrated with a graphical user interface for dermatologists to use in dermoscopy examinations.

Keywords: Automatic diagnosis, skin lesions, deep learning, transfer learning, EfficientNet

Analysis of Curcumin Coating Process on 316l Stainless Steel with Electrophoretic Deposition Method

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Abstract – In 2015 WHO stated that the number 1 cause of death in the world is cardiovascular disease (CVD). Every year CVD kills 17.9 million people worldwide. One of the treatments for cardiovascular disease is percutaneous coronary intervention (PCI). In PCI, a stent is placed on a coronary artery that is experiencing narrowing and blockage (atherosclerosis). PCI treatment using Drug Eluting Stent (DES) has been widely used, where DES consists of scaffold, polymer coating and antiproliferative drugs. Long-term use of polymers in DES results in complications such as inflammation and thrombosis. The development of DES is moving towards Polymer-Free Drug Eluting Stent (PF-DES), where this study uses curcumin as the main ingredient for DES coating using Electrophoretic Deposition (EPD) as the coating method. The coating used 3 different concentrations of curcumin, namely 125ug/ml, 250ug/ml and 500ug/ml. The coating was carried out within 16 minutes and was repeated 2 times. The coating results showed that the weight of the stent increased with increasing curcumin concentration. The average increase in deposition weight of the coating results was different for each concentration, where at a concentration of curcumin 125ug/ml there was a weight gain of 0.025gr, at a curcumin concentration of 250ug/ml the average increased by 0.048gr, while at a curcumin concentration of 500ug/ml the average was increased. increment of 0.057gr. The surface roughness test showed that the higher the curcumin concentration, the higher the roughness value. Where the difference between the average surface roughness values at a concentration of 500µg/ml is 1.07 ± 0.97 m, a medium concentration (~125 g) is 0.94 ± 0.76 m, and a low concentration (~62.5 g) is 0.7 ± 0.65 m.

Keywords: PF-DES, DES, Kurkumin, EPD.

Implementation of Sterilization Ozone Box for Medical Equipment

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Abstract. One of the efforts to control the growth of microorganisms is sterilization. The sterilization process can use Ozone (O₃) gas. Ozone is a triatomic form of the element oxygen. Ozone acts as an oxidizing agent capable of destroying the structure of bacterial cell walls and their molecules are unstable and easily decomposed into oxygen (O₂), so that ozone can be applied in sterilization technology for water treatment processes, sterilization of medical devices and air. In this study, a sterilization box with an ozone generator has been designed with switch timer and the MQ-131 ozone sensor as an indicator if there is a leak in the box. The optimum time for sterilizing medical devices in this study was 20 minutes can reduce colony of Staphylococcus bacteria with ozone concentration was 4.94 ppm.

Keywords. Ozone box, Sterilization, Staphylococcus bacteria, Medical Equipment.

Virtual Three-Dimensional Kinematics Comparison between Normal Healthy Knee and Tumor - Suffered Knee Post Resection with Mega Prosthesis Reconstructed

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Abstract. Modular Prosthesis emerges as one of the most promising alternatives to replace the outdated amputation procedures for bone cancer treatment. To obtain a prosthesis with full flexion ability, better understanding of normal and healthy knees are needed. In this study, two novel designs of Modular Mega prosthesis (MDF 1 and MDF 2) are generated with different geometries and dimensions, with a reference to a normal knee. To replicate knee movements, 3 – axis simulations to acquire interference between femoral condyles and tibial base is applied to both designs and normal knee to better compare the range of movements. Volume interference as an indicator of high - flexion in knee rotation movements is established. The result showed that MDF 2 can give a superior result of flexion-extension rotations compared to normal knees and MDF 1. Range of movement in internal-external and varus-valgus rotations of normal knees are still higher than both MDF 1 and MDF 2, although the results of movement for both novel designs is not too distinctive. The simulations in this study showed that both designs, MDF 1 and MDF 2 has a great potential as a solution after limb-salvage resection. Keywords: Osteosarcoma, mega prosthesis distal femur, Knee Kinematics, Interference Volume.

Does Cryoprotectant's modification in Spermatozoa Cryopreservation Can be an Alternative to Improve Embryo Quality? A Review

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Abstract. Spermatozoa cryopreservation is an effective method for maintaining male fertility in humans. Nevertheless, there are some limitations of sperm cryopreservation, which is called as cell injury by cryoprotectant, that can not be avoided. This process will affect embryo quality. Therefore, it is a mandatory to modify cryoprotectant in spermatozoa cryopreservation, in order to improve embryo quality. This review aimed to summarize the modification of cryoprotectant that can damage the cell injury, thereby improving embryo quality. To this purpose, a computerized search of EMBASE, PubMed, Scopus and Google Scholar databases from 2008 to 2022 were performed on the general term such as “sperm cryopreservation”, “cryoprotective agent”, “modified cryoprotectant”, “cell injury”. Of these, 1847 publications were screened and 28 articles were obtained and evaluated. Although no formal conclusions can be drawn regarding the cryopreservation of spermatozoa to obtain good embryo quality, our results suggest that modified cryoprotectants can be an alternative cryoprotectant compared to commercial cryoprotectants. In addition, the use of antioxidant in spermatozoa cryopreservation can also prevent cell damage due to the negative effects of cryoprotectants. However, further researches still needs to be performed to investigate the cellular mechanisms.

Keywords: sperm cryopreservation, cryoprotectant agent, modified cryoprotectant, cell injury

Fabrication of Rigid Polyurethane Foam Lumbar Spine Model for Surgical Training using Indirect Additive Manufacturing

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Abstract. Lumbar model is an artificial bone that is commonly used in surgical training to provide the feeling of working with human like bone to the trainers. However, the current lumbar model is expensive and has limitation in representing the real human lumbar especially in geometry, visual, and haptic. In recent study, additive manufacturing (Three-dimensional (3D) printing) technology becomes the alternative to overcome the price and geometrical issue. The material used in lumbar model is widely known as rigid polyurethane (PU) foam, where the material can represent lumbar structure. However, it does not available in 3D printing and limited to casting method only. Therefore, a method of fabricating lumbar model with rigid polyurethane (PU) for surgical training using indirect additive manufacturing is introduced in this study. Indirect additive manufacturing is a combination of additive manufacturing and casting method. The main process of this method is started by fabricating a mold using fused deposition modeling (FDM) 3D printing and subsequently casting a material into the 3D printed mold. Accordingly, the aims of this study are to design a lumbar mold using fused decomposition modeling (FDM), to fabricate the lumbar model made from rigid polyurethane (PU) foam using indirect additive manufacturing where FDM is combined with casting, and to evaluate the properties of PU lumbar model fabricated using indirect additive manufacturing. The study was performed using two stages Design of Experiment (DoE) using Taguchi Orthogonal Array. The first DoE was conducted to optimize the printing parameters of the mold and second DoE was performed to optimize the casting process. The geometrical measurements of middle end-plate depth (EPDm), upper end-plate width (EPWn), spinal canal width (SCW), spinal canal depth (SCD), and lower pedicle length (PLI) show that the error were in the range of 0.14% to 0.85%. The porosity was found to be nonuniform, ranged from 18.52% to 21.31% on the middle part and 38.98% to 45.57% on the subsurface of lumbar model. The density was increased by 64.89% compared to standard rigid PU. However, the compressive strength and modulus were found to be decreased by 28.70% and 50.39% respectively.

Keywords: Lumbar model, surgical training, rigid polyurethane (PU) foam, indirect additive manufacturing

Wavelet Decomposition and Feedforward Neural Network for Classification of Acute Ischemic Stroke based on Electroencephalography

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Abstract. Stroke is one of the most leading causes of death in the world, including in Indonesia. It increased from 7% to 10.9 % in 2018. There are two main types of strokes which the majority is the Acute Ischemic Stroke (AIS). It is important to detect or diagnose stroke early as the treatment of the stroke is based on the type of the stroke. Currently, the common tools to diagnose stroke are Computed Tomography Scan (CT scan) and Magnetic Resonance Imaging (MRI). However, the mentioned tools including the stroke diagnosis and rehabilitation services are mostly available in type-A hospitals. Electroencephalography (EEG) has been widely studied as an alternative tool to diagnose as the cost is relatively low and a non-invasive device. This study was a development to the previous studies which aims to figure out which epoch length with several features that provides best performance result to classify four classes of stroke: no stroke, minor stroke, moderate stroke, and severe stroke. The study was conducted in Rumah Sakit Pusat Otak Nasional (National Brain Centre Hospital), Jakarta that yielded the 32-channel EEG recordings, CT Scan images, and NIHSS Scores. The total subject participated in this study was 69 subjects: 41 male and 28 female. The features used in this study are Delta-Theta to Alpha-Beta Ratio (DTABR), Delta to Alpha Ratio (DAR), Relative Power Ratio (RPR), and Asymmetry which were extracted using wavelet decomposition. The epoch length was varied by 1, 2, 10, 30, 60, and 120 secs which were in the resting conditions. These features were proceeded using feed-forward neural network algorithms. The best performance was yielded by epoch length 60 sec with 89% accuracy using 15 hidden layers Feedforward Neural Network.

Keywords: Electroencephalography, Delta-to-Alpha Ratio, Delta-Theta to Alpha-Beta Ratio, Wavelet Decomposition, Epoch Length, Feedforward Neural Network

Optimization of Multiplex Polymerase Chain Reaction (PCR) to Detect Human Papillomavirus (HPV) 6 and 11 in Cervical Swab and Urin Samples

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Abstract. Human Papillomavirus (HPV) is a double strain Deoxyribonucleic Acid (DNA) virus that reside in the epithelial cells. The virus caused a sexually transmitted disease, and it divided as Low Risk (LR) and High Risk (HR) HPVs. The HR HPVs infection may cause cervical cancer, while the LR HPVs infection are commonly self-limiting. However, the LR HPVs has a higher transmission rate than the HR HPVs and caused several diseases such as Recurrent Respiratory Papillomatosis (RPP). The RPP are commonly caused by HPVs type 6 and type 11. The present study aims to optimize multiplex Polymerase Chain Reaction (PCR) to detect the HPVs type 6 and type 11. Various in-house primers were tested to detect the infection in cervical swab and urine, with a range of primer concentration from 0.1 to 1 μ M. In addition to that, various annealing concentration also tested, in a range of 60 up to 70°C. The result showed different optimum primer concentration, 0.5 μ M and 1 μ M, to detect the HPVs infection in the cervical swab and the urine, respectively. While the optimum annealing temperature was 66.8 °C for both samples. An amplicon sequencing was performed to validate the method and the Basic Local Alignment Search Tools (BLAST) showed high similarity to the HPVs type 6 and type 11. Therefore, may conclude that the method presented in this study is valid to detect the infection of HPVs type 6 and type 11 in cervical swab and urine.

Keywords: HPVs, Low Risk, sexually transmitted diseases, multiplex, optimization

Development of IOT Enabled PIVOT: Platform In Vitro Testing in Culturing Liver Organoid

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Abstract. Bioreactors are growing in popularity among network engineers. Bioreactors are generally chambers used for cell culture processes with predetermined parameters. Pivot is a bioreactor system for cell culture that is controlled automatically and remotely using multi-chambers that allow inflow of fresh liquid or output for sample collection. The chamber has a capacity of 1.6 mL, and each chamber is set in parallel. This system facilitates gas exchange between the culture medium and ambient gas. The system provides a storage bag for fresh media and a peristaltic pump is used to move the media in a closed loop during perfusion and stirring in the cell culture chamber. The flow rates used range from 250-350 mL/min. The system has a container for direct sample measurement to obtain pH, oxygen, and carbon dioxide parameters. The bioreactor system is also equipped with a mixing vessel which allows the addition of desired nutrients or additives to the system. The system has a microscope that can record video or images for real-time cell observation. This research is expected to increase the number of cells produced without reducing the quality of each chamber and be carried out simultaneously, automatically, and remotely controlled

Keywords: *In Vitro, perfusion, remotely, automated*

Aluminum Thin-Film Stripline-based Pediatric EEG Net Compatible to MRI and CT

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Abstract— Children's encephalopathies are central nervous system disorders that are often accompanied by seizures. Seizures are one of the distinctive clinical manifestations of epilepsy, hypoxia, abnormal delivery, sleep deprivation, and stress. Magnetic Resonance Imaging (MRI) plays a crucial role in diagnosing and understanding children's seizures. However, pediatric MRI evaluation is incomplete in assessing the entire children's neurologic status, especially concerning cortical functioning. Continuous video EEG can be helpful in such circumstances as it provides essential information about changes in frequency, synchrony, distribution, and other characteristics of cerebral cortical activity. EEG is also an essential modality in understanding developmental disabilities from early childhood. State-of-the-art EEG or dense array EEG (HD-EEG – 64 or more channels) has realized EEG's potential as a neuroimaging tool through source localization of normal and pathological brain activity and network dynamics. However, neither conventional EEG nor HD-EEG are imaging (MRI or CT) compatible; hence, EEG electrodes are typically removed prior to any imaging study, negatively impacting patient management because of extra delays and additional costs. This manuscript describes the "NeoNet", an imaging-compatible HD-EEG net for cross-modal pediatric neural monitoring with artifact-free image quality for both MRI and CT. 540mm×540mm sheets of 75 μm thick Polyimide film were vacuum coated on one side with 30nm of aluminum. The traces of each EEG were etched using standard photolithography methods with a width of 100μm and a length of almost ½ meter, extremely long for thin film technology. The traces were pressure-bonded with a 12.5μm thin film of Polyimide overlay film and laser cut. The NeoNet electrodes were based on a silver/silver chloride coating of thin (25μm) pure silver disks of 10mm in diameter. Rigorous MRI safety temperature assessments were performed at 3 Tesla and found within the Food and Drug Administration (FDA) safety limits. MRI data were acquired on a healthy volunteer and exhibited no artifacts. CT images were obtained using a phantom and compared with a commercial HD-EEG. NeoNet in CT has no artifact from EEG electrodes in phantom, whereas the commercial EEG Net CT showed significant hardening artifacts from electrodes. The proposed NeoNet will enable inexpensive, noninvasive HD-EEG and overcome current cross-modal safety and artifact issues that have severely limited the effectiveness of simultaneous HD-EEG/MRI allowing researchers and clinicians to benefit from the high spatial resolution of MRI and the high temporal resolution of HD-EEG. Furthermore, the technology is lightweight and small, taking advantage of advanced manufacturing thin-film technologies. The novel NeoNet will allow studying brain function in healthy children in natural settings and understanding different pediatric neural pathologies, such as epilepsy.

Keywords—antenna effect, hardening artifact, RF loss, safety.

Spike-Field Coherence in Cochlear Implant-stimulated Congenitally Deaf Cats

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Abstract. Previous studies have documented a reduction of interareal couplings between primary and secondary auditory areas in congenital deafness, particularly pronounced in top-down direction. Furthermore, an anatomical study indicated a dissociation in the effect of deafness between supragranular and infragranular layers, suggesting that their interaction is substantially modified in deafness. In the present study we directly investigated spike-field coherence between supragranular and infragranular layers of field A1 of hearing cats under acoustic stimulation and cochlear implant (CI) stimulation. The data between hearing controls and congenitally deaf cats (CDCs) were compared. The stimulation was using a train of three condensation acoustic clicks (50 μ s) or three biphasic charge-balanced pulses (200 μ s/phase) applied through a CI in wide bipolar configuration to the contralateral ear. A1 activity was recorded in the most responsive spot (defined by a functional mapping). Neuronal activity was recorded with Neuronexus 16-channel probes. The spike-field coherence was analyzed using pairwise phase consistency (PPC). Both the resulting magnitude as well as the preferred phase of synchronization was analyzed. PPC was significantly smaller in CDCs than in controls in the alpha and beta bands. In controls, there were no significant differences between the preferred phase of synchronization between supragranular and infragranular layers. In CDCs, however, there was a large difference in the preferred phase between supragranular and infragranular layers. These results suggested a loss of synchrony and thus a decoupling of these layers in congenital deafness. Together with the anatomy of their connections this observation explains why the effects of deafness differ between supragranular and infragranular layers.

Keywords: Cochlear Implant, spike-field coherence, congenital deafness, auditory cortex, brain connectivity

Simulation Of Arrow-Shaped Micromixer With Obstacles In Blood Plasma Mixing

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Abstract. In recent years, several applications of micromixers have been reported to have a number of advantages, including the use of small amounts of plasma, short reaction times, and good reaction efficiency. The samples analyzed in this study were blood plasma and distilled water. The process is carried out by passive mixing which is based on a microchannel structure to enhance the diffusion of molecules for efficient mixing. The arrow shape was chosen as the micromixer design because it has a better blending performance than the T and Y shapes. The types of obstacles also used to improve the mixing process. The simulation was carried out on COMSOL Multiphysics software version 5.6. The results showed better mixing results for the micromixer design with triangle inside obstacles with the highest Reynolds number was 9.4326. Then followed by ellips obstacles with a value of 9.4322, then without obstacles that is 9.4309, after that the triangle outside with a value of 9.4006, and finally the lowest Reynolds number is a design with a diamond obstacles of 9.2514.

Keywords. micromixer, microfluidics, passive mixing, Reynolds number, turbulent flow.

Development of Electrochemical Glutamate Biosensor: A Review

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Abstract. Glutamate is one of the neurotransmitters that play an important role in the formation and stabilization of synapses, awareness, memory, and learning processes. Abnormal levels of glutamate in the body can lead to various risks of neurological diseases. The existing tools for detecting glutamate levels have several drawbacks. They are time-consuming and expensive. Besides that, less research or development has been done on glutamate biosensors than on other types of biosensors. In this review, the development of electrochemical glutamate biosensors was discussed. A review of the analyte, enzymes used, immobilization techniques, materials used, and analysis findings regarding linear range, detection limit, and sensitivity has been performed in order to develop a glutamate biosensor. The non-enzymatic electrochemical glutamate sensor can continue to be developed for promising enzyme-free glutamate biosensors.

Keywords: biosensor, glutamate, electrochemical, neurotransmitter

Fabrication of Screen-Printed Electrode for Biosensor Applications

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Abstract. A biosensor is an analytical device that combines certain biological elements and physical elements. Several types of transducers are used for physical elements, such as optical, electrochemical, thermic, or gravimetric. Nowadays, electrochemical transducers have become widely used for the application of biomedical sensors. Electrochemical measurement devices called screen-printed electrodes (SPEs) are created by printing several types of ink on ceramic or plastic substrates. SPEs enable speedy in-situ examination with high repeatability, sensitivity, and accuracy. In this study, SPEs were fabricated using carbon ink on a polyethylene terephthalate (PET) substrate. The mask, stencil, and screen-printing dimensions were measured. Surface characterization using SEM was also conducted. The fabrication of SPEs could be carried out up to 5 layers but still not get results that match the initial design geometry.

Keywords: biosensor, screen-printed electrode, electrochemical, PET

Annealing Effect on Improving the Mechanical Properties of Zirconium Based Biomaterials with the Addition of Yttrium Elements for Bone Plate Application

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Abstract. To exacerbate the mechanical properties of biomaterials with zirconium-based alloys, especially on tensile strength, an annealing process was carried out. The process was carried out at a temperature of 800 C and holding time for 3 in a vacuum chamber which was flowed by pure argon gas. The composition of the alloy used in this study was Zr6Mo4TixY ($x= 0, 1$ and 3 %wt). Microstructure observations were carried out using Scanning electron microscopy (SEM) and tensile testing. In this study, micro-sized specimens were used and followed the ASTM E8-04 standard with a surface roughness of 1 μ m. The results showed an increase in the tensile strength value from 590 MPa to 662 Mpa. In addition, the grain boundaries of the alloy are getting smaller, this shows the effect of annealing on changes in the properties of the material.

Keywords: Biomaterials, Zirconium Based, Mechanical Properties, Annealing, Micro Specimens

On-Chip Integrating of Dopamine Sensor with Microfluidic Finger Priming Pump

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Abstract. The microfluidic research has contributed in the neural engineering in such many ways such as the observation of cell-on-chip (CoC) in the setting of neural tissue engineering. The widespread of mechanical and electromechanical systems (MEMS) has also enabled this technology to be equipped with external aid for instance detectors or biosensors to show the characteristics of the observed object. The platform that is being developed here is the on-chip microsystem that integrates dopamine detection with microfluidic handlings such as flowing with the finger pumping and the valve as means to terminate the flow. This microenvironment has many benefits to serve as a quicker result in observing the phenomena related to neural cell activities. The relatively small amount of specimen volume, 50-100 μ L, ease the handling of the movement and consequently cut the cost of consumable items. Here, a platform that was developed showed the pump module that also serves as a mixing point able to deliver at a maximum of 121.36 μ L with 2-3 strokes of normal human pressure priming. A series of valves help the flow to be terminated or isolated in a certain zone to be processed further. Ultimately, the chip is also equipped with a portable electrochemical detection module that allows us to measure the dopamine concentration up to 1 mM. This development showed that the on-chip testing of dopamine could be easier and more portable to handle.

Keywords: neural tissue engineering, cell-on-chip, MEMS, on-chip testing, dopamine

Review and Design of a Transforaminal Lumbar Interbody Fusion (TLIF) Spine Cage

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Abstract. Lumbar Interbody Fusion is a technique used to treat various spinal disorders, which has many various types including the Transforaminal Lumbar Interbody Fusion (TLIF) Technique. With TLIF being one of the most well-known techniques, which many spinal surgeons are trained and skilled at, there are various types of TLIF Spine Cages available on the market. In this paper, we designed a TLIF Cage and compared the analysis of the simulation with the experimental testing of the prototype. The design was developed with the reverse engineering method, as well as findings on the jaws profile and other design considerations through a literature review. The design was then analyzed through several simulations such as compression test, bending test, and torsion test using Finite Element Analysis Ansys Software. and experimental testing using compression test.

Keywords: Lumbar Interbody Fusion, Spine Cage, Finite Element Analysis, Experimental Testing, Prototype

Geometry Benchmarking of Transforaminal Lumbar Interbody Fusion (TLIF) Spine Cage Design

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Abstract. Transforaminal Lumbar Interbody Fusion Technique (TLIF) is used by many spine surgeon has been known to treat various spinal disorders. With this technique being one of the most well-known Lumbar Interbody Fusion (LIF) techniques. Various TLIF Spine Cages are available on the market with different designs, technologies, materials, and so on. In this paper, we have benchmarked the geometry of several types of TLIF Spine Cages available on the market and also frequently used. We replicated the design of three TLIF spine cages, followed by the fabrication of the sample by additive manufacturing 3D resin printing using PLA material. Then, we analyzed each design by simulating compression tests using Finite Element Analysis from Ansys Software.

Keyword: TLIF, spine cage, additive manufacturing, benchmark, FEA

Study of Heat-Stimulated Material as Minimum Invasive Structural Implant

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Abstract. Metamaterials are one of the most recent advances in the materials field. With the emergence of metamaterials, a material specifically customized with the intended properties can be developed, it can lead to higher technological advancements in various areas, such as medical devices. Medical devices have complex requirements for implant materials, such as spine cage implants. To develop a more minimally invasive surgical technique, metamaterials are considered to be used for the implant. In this paper, we designed a solid model design with a block scaffold design. Which was then analyzed through simulation using Finite Element Analysis COMSOL Software and experiment by giving heat stimulation and loading to the specimen. For the experiment, three tests were given, where we analyzed the bending of the specimen. Whereas in the simulation, we analyzed the strain and displacement of the material. Finally, we discussed the potential of metamaterials for the use of spinal cage implants.

Keywords: metamaterial, minimally invasive surgical technique, heat stimulation, stress-strain, spine cage

Development of Electrochemical Lactate Biosensor: A Review

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Abstract. Due to the lack of early-stage symptoms, diabetes mellitus is known as the "silent killer." The prevalence of diabetes mellitus is significant and will continue to rise annually. Metformin is one of the treatments offered to people with diabetes mellitus. This medicine may lower blood glucose concentrations. In addition to the advantages of metformin, it causes lactic acidosis and can cause metformin-related lactic acidosis (MALA), which is associated with a mortality rate of 30-50%. This study discusses the determination methods of lactic acid levels in saliva samples of type 2 diabetes mellitus using an electrochemical biosensor. A review of a working electrode, enzyme immobilization methods, sample, the limit of detection, linear range, and stability has been studied in order to investigate the current status of the development of lactate biosensors.

Keywords: diabetes mellitus, lactate, biosensor, electrochemical

Nanobiotechnology, Biosensors, and Biochips

A Comprehensive Study of Flexoelectricity Generation by Bio-Prepared Ultrathin MXene Sheets

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Abstract. Atomically thin Transition Metal Carbides (TMCs) are the fastest growing energy materials with increasing demand due to their superior properties such as high melting point and electrical conductivity conjugated with higher mechanical and chemical stability. 2D TMCs based self-powered electronics, also referred as nanogenerators, are reported to derive energy from day-to-day mechanical activities such as walking, running, moving, pressing, pulling, rubbing, etc. Similarly, a Flexoelectric nanogenerator (FENG) converts mechanical pressure directly into the electrical signal by mechano electric coupling between strain gradient and electrical energy. In the present study, a unique bio-based strategy, utilizing solid-gas reaction, is developed to fabricate ultrathin niobium carbide (NbC) sheets. The bio-prepared NbC sheets were found to be ~15 nm thin with lateral dimension of about 150 nm, as indicated by AFM and TEM micrographs. The prepared sheets were observed to contain disordered atomic assembly surfaces which induces strain in the material leading to flexoelectricity. It was observed that ~5 V was produced for each tap on the FENG cell and ~ 2.64 mW/m² power density was generated. Strain-dependent Raman analysis confirms the flexoelectric behaviour of NbC sheets. Finally, for practical device applications, a temperature induced effect was studied, which showed ~1.6 times gain in the output voltage for a temperature difference of 13°C. The study, thus emphasized on the utilization of TMCs as flexible flexoelectric nanogenerators in wearable electronics and sensing.

Keywords: biosynthesis, ultrathin, MXenes, flexoelectric nanogenerator

3D Neural Network Composed of Neurospheroid and Bionanohybrid on Microelectrode Array

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Abstract. Although various researches have demonstrated the generation of a two-dimensional (2D) neural network *in vitro* utilizing living cells or organic/inorganic materials, there has been no report of the development of a three-dimensional (3D) neural network. Based on previously developed bionanohybrid composed of protein, DNA, molybdenum disulfide nanoparticles (MoS₂ NPs), and peptides for controlling electrophysiological signal of neural cells, here, we developed the *in vitro* 3D neural network composed of the bionanohybrid, 3D neurospheroid and the microelectrode array (MEA) for the first time. The bionanohybrid constructed on the MEA successfully semi-penetrates the neurites of the 3D neurospheroid and develops the 3D neural network after the generation of the 3D neurospheroid derived from human neural stem cells. Through processing the input signal applied by the bionanohybrid, the developed 3D neural network successfully exhibited the electrophysiological output signals from the 3D neurospheroid. Furthermore, the spatial input signal recognition in the neurospheroid of a 3D neural network is realized for the first time through employing the selectively immobilized bionanohybrid on the MEA. The proposed *in vitro* 3D neural network offers a promising strategy for brain-on-a-chip, therapeutic efficacy evaluation for brain diseases, bioelectronics, and bioelectronic medicine.

Keywords: 3D neural network, 3D neurospheroid, bionanohybrid, electrophysiological signal, bioelectronics

Effect of Extrusion on Transfersome Nanoencapsulation: A Study on a Model Protein for Secretome Delivery

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Abstract. Transfersome is nanoscale vesicles made from lipid with useful deformation properties that can pass through a skin layer. With this capacity, transfersome is a prospective delivery tool to promote the therapeutic effect of stem cell-derived secretome, especially for topical and transdermal applications. In this research, the effect of the extrusion process on transfersome was evaluated based on the particle size and distribution. Bovine serum albumin was used as a model protein for secretome proteins. Transfersome was formulated using dipalmitoyl phosphatidyl choline (DPPC) as a phospholipid and Tween 20 as an edge activator. Based on the encapsulation efficiency, the composition of 95% DPPC and 5% tween 20 had the best encapsulation efficiency ($74.77\pm 0.27\%$). The formulation of 97.5% DPPC and 2.5% tween 20 with the extrusion technique produced the best particle distribution with a polydispersity index of 0.19 ± 0.03 and particle size of 131.47 ± 0.79 nm. Based on the present data, it was concluded that the extrusion step is essential to improve the encapsulation efficiency, particle size, and homogeneity.

Keywords: transfersome, nanoencapsulation, dipalmitoyl phosphatidyl choline, tween 20, thin-film hydration, extrusion, bovine serum albumin, secretome

Optimization of a N-terminal Expression Tag to Maximize Olfactory Receptor Production in *Escherichia coli*

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Abstract. Olfactory receptors (ORs), which humans have about 400 different types, play a very important role in the sense of smell. The ORs can be utilized in various industries due to their unique properties. It has been reported that some ORs were successfully overexpressed in inclusion body form in *E. coli* for use as sensing elements in bioelectronic noses. Despite their potential, most ORs show poor production yields in *E. coli*. Here, we present an expression-enhancing tag which shows markedly enhanced expression of different ORs in bacterial cell-free system and *E. coli*. The expression-enhancing tag consisting of a small number of AT-rich gene sequences was designed based on optimization of translation initiation rate, and is attached to the 5-prime end of the OR coding sequences. We were also able to synergistically increase OR production in *E. coli* by co-expressing the effector genes *djlA* or *rraA*, which can suppress the cytotoxicity caused by OR overexpression. Notably, the maximized production system would also be generally applicable to other membrane proteins, including various G-protein coupled receptors, which are difficult to express in *E. coli*.

Keywords: olfactory receptor, translation initiation rate, AT-rich gene tag, *Escherichia coli*, cell-free expression, inclusion body

Lectin-Glycan Affinity in Nanobio Theragnostics: The Specific Capture of Pancreatic Cancer Exosomes and the Targeted Therapy of Tumor Cells

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Abstract. The unique profile of upregulated glycosylation in metastatic cancer cells may form the basis for the development of new biomarkers for the targeting and diagnosis of specific cancers. This study introduces a cancer cell-derived exosome detection and cancer cell targeting technology, which is based on the specific binding of lectins to distinctive glycan profiles on the surface of exosomes and cells. Lectins with a high and specific affinity for sialic acid or fucose were attached to bifunctional nanoparticles, which facilitated interactions with cancer cell-derived exosomes in a microfluidic device. The lectin affinity to surface glycan of cancer cells was also able to deliver functional nanoparticles to target melanoma cells and serve as an immune checkpoint blocker with a photothermal therapy. This study opens the possibility to achieve a new early diagnosis marker and targeting moiety based on the surface-glycan properties of cancer cells.

Keywords: lectin, glycan, exosome, diagnosis, therapy

Light-Triggered In Situ Biosynthesis of Artificial Melanin for Skin Protection

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Abstract. Tyrosinase-mediated melanin synthesis is an essential biological process that can protect skin from UV radiation and radical species. This work reports on in situ biosynthesis of artificial melanin in native skin using photoactivatable tyrosinase (PaTy). The I41Y mutant of *Streptomyces avermitilis* tyrosinase (SaTy) shows enzymatic activity comparable to that of wild-type SaTy. This Y41 is replaced with photocleavable *o*-nitrobenzyl tyrosine (ONBY) using the introduction of amber codon and ONBY-tRNA synthetase/tRNA pairs. The ONBY efficiently blocks the active site and tyrosinase activity is rapidly recovered by the photo-induced cleavage of ONBY. The activated PaTy successfully oxidizes L-tyrosine and tyramine-conjugated hyaluronic acid to synthesize melanin particles and hydrogel, respectively. To produce artificial melanin in living tissues, PaTy is encapsulated into lipid nanoparticles as an artificial melanosome. Using liposomes containing PaTy (PaTy_Lip), PaTy is transdermally delivered into ex vivo porcine skin and in vivo mouse skin tissues, thus achieving the in situ biosynthesis of artificial melanin for skin tissue protection under UV irradiation. The results of this study demonstrate that this biomimetic system can recapitulate the biosynthetic analogs of naturally occurring melanin. It should therefore be considered to be a promising strategy for producing protective biological molecules within living systems for tissue protection.

Keywords: artificial melanosome, photoactivatable tyrosinase, *o*-nitrobenzyl tyrosine, transdermal delivery, skin protection

Comparison of Nanostructure Arrangement with Combination of Thin Film of Gold and Silver as an Optical Leukemia Biosensor: Simulation Study

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Abstract. The combination of various types and structures of materials were developed to obtain highest sensitivity of biosensor. The optical analysis of the simulation results of the electric field distribution was used to determine the best arrangement of thin layer and nanostructures Au and Ag electrodes as an optical biosensor for blood cancer detection. This study used a finite difference time domain (FDTD) to simulate the electric field distribution and analyze the optical properties of the biosensor. This research was conducted by making an anodic aluminum oxide (AAO) as mold of a nanostructure whose hole dimensions will be used as a model of the nanostructure in the simulation. The holes in AAO are circles with a diameter of 90 nm and the distance between holes is 25 nm. The structural model for electric field simulation is a glass substrate coated with an Ag thin film/Au nanostructures, Au thin film/Ag nanostructures, arrays of Ag/Au nanostructures, and arrays of Au/Ag nanostructures. The top side of the nanostructure arrangement and thin film will be deposited with normal blood cells and leukocytes. The electric field distribution shows that the Ag thin film/Au nanostructure is the best combination electrode of nanostructures and thin film of Ag and Au for the detection of blood cancer because of the highest difference of absorbance intensity and absorbance peak wavelength, which is 8.66 nm. These results provide a preliminary interpretation of the arrangement of nanostructures and thin film of gold and silver for optical blood cancer detection as biomedical device.

Keywords: blood cancer; biomedical; detection; thin film; nanostructures

Lab-On-Chip Based Purification for Bacterial DNA for Identification: Study Case Cisolong, Banten

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Abstract. Exploration of thermophilic bacteria in Indonesia is important for various industrial applications. This study aims to identify the 16S-rRNA gene from thermophilic bacteria found in Cisolong Hot Springs, Banten. Purification was carried out by two methods, namely GeneAll® Exgene™ commercial kit and LOC ChipGenie® Edition P. To date, there has not yet been bacteria identification from hot springs using LOC for DNA purification. Therefore, in this study, a bacterial identification test carried out by comparing the two methods. The hope of this research is that in the future, LOC can be directly implemented in DNA purification, making it easier to identify without the need for laboratory procedures. In future research, reverse engineering will also be carried out so that we can manufacture our own LOC. The variables tested were the results of DNA purity, templates concentration, and result of gel electrophoresis bands. Purification was also carried out by varying the number of bacterial cultures based on absorbance to determine the optimum number of bacteria for LOC. It was found that the bacteria were successfully purified using LOC at 4 and 28 hours of culture. The concentration yield of LOC is good and can compete with commercial kits. To find out whether DNA purification was successfully carried out with LOC, the PCR method and gel electrophoresis were carried out. From the gel electrophoresis band results, it was found that the source bacteria were at 1518 bp and the pool bacteria at 1422 bp.

Keywords: 16S-rRNA, commercial kits, DNA purification, identification of thermophilic bacteria, LOC

Recombinant Nucleocapsid Protein Expression for SARS-CoV-2 Antibodies Detection via Lateral Flow Assay & ELISA

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Abstract. Serological testing is an important tool for diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Nucleocapsid (N) protein is the most expressed and immunogenic protein of SARS-COV-2. In this study, we aimed to express recombinant Nucleocapsid (N) antigen to develop lateral flow assay (LFA) for rapid screening and ELISA for lab-based detection of SARS-COV-2 antibodies. SARS-COV-2 Nucleocapsid (N) gene was cloned in peT28+a vector and expressed as a full-length protein in *E. coli* in soluble form and purified via affinity chromatography followed by Size exclusion chromatography (approximately 95% pure). Recombinant N protein expression was verified by immunoblotting with anti-SARS-COV-2 antibodies. Lateral flow assay was developed by conjugating anti human IgG with gold nanoparticles and was used as the detection antibody. Recombinant nucleocapsid protein (1 µg/µl) and human IgG (0.5 µg/µl) were immobilized in test and control lines, respectively, of a nitrocellulose membrane, working as the capture reagents. The developed LFA was used to detect anti-N antibodies in serum of 50 clinically proven COVID-19 patients and 30 healthy controls. The specificity and sensitivity of the LFIA were 75.6% and 84.8%, respectively. The ELISA was developed by coating a microtiter plate with recombinant N protein (1 µg/ml) followed by adding sera of RT-PCR confirmed positive COVID-19 patients (n=33) and healthy controls (n=77). IgG detection sensitivity was 92.6 % and specificity 91.3% and accuracy of the assay was 91.8%, respectively. The preset study showed that the recombinant N protein is suitable to be used as a detection tool for both in equipment free rapid diagnostic tests, LFA and equipment dependent tests such as ELISA.

Keywords: SARS-CoV-2 antibodies, ELISA, lateral flow assay, nucleocapsid protein, COVID-19

Nucleic Acid Detection Using Endonucleases and CRISPR-Cas

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Abstract. Rapid, sensitive, and easy to deploy nucleic acid detection tools have wide ranging applications including disease monitoring and diagnostics, microbial strain identification and detection of food contaminants. In this study, we developed a method comprising CRISPR-Cas and other enzymes for detecting both DNA and RNA. This method can be deployed as a point-of-care test using lateral flow strips, without the need for thermocycling. This method is not constrained by the presence of a protospacer adjacent motif in the target and can be used to probe any nucleic acid pair. We also demonstrated the versatility of our method at virus detection, identification of antibiotic-resistant bacteria and cancer diagnostics. Based on its unique advantages, our detection method can be used in nucleic acid diagnostics.

Keywords: CRISPR-Cas, nucleic acid detection, biosensor

Nanoplasmonic Biosensing of Individual LC3 Autophagy Markers for Discovery of Autophagy Modulators

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Abstract. Autophagy, a fundamental intracellular catabolic process that degrades cytoplasmic constituents to maintain cellular homeostasis, has gained attention for its association with cancer progression and drug resistance. Hence, the need to develop rapid and precise autophagic flux measurement for the evaluation of chemotherapy efficiency is critical. In this work, we present a localized surface plasmon resonance (LSPR) based two-step sensing platform, enabling microtubule-associated proteins 1A/1B light chain 3 (LC3) turnover assay, measuring LC3-I conversion to LC3-II, which indicates autophagic flux. This nanoplasmonic sensor is capable of detecting LC3-I/II individually with femtomolar sensitivity, exceeding the limits of conventional ELISA and Western blot. The sensor successfully analyzed the drug efficacy with high sensitivity under clinical conditions where autophagy-targeted drugs were treated on human cancer cell lines. Our results suggest that this sensor, capable of quantifying individual LC3 forms in a single platform, can be utilized as a rapid, convenient, and cost-effective drug discovery tool in relevance with autophagy-targeted chemotherapies.

Keywords: autophagic flux, localized surface plasmon resonance (LSPR), nanoplasmonic sensor, LC3, drug discovery

DNA-Directed Gold Nanostructure Synthesis for Detection of DNA Point Mutation

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Abstract. Distortions in the binding affinity of site-specific protein to DNA are associated with genetic diseases, but high-fidelity methods for establishing a consensus rank of protein affinity for mutant DNA—particularly single-point mutations—for the purpose of early diagnosis are lacking. Here we report non-labeling endpoint detection and dynamic analysis of the binding of the mismatch repair initiation protein MutS to the eight most common single-point mutations in BRCA1 using a single-nanoparticle microfluidic platform comprising gold-bridged nanoparticles produced by direction-specific synthesis that can directly transduce information on the MutS-DNA interaction. Mutant DNA was classified into four types based on MutS binding affinity for various point mutations. This powerful method can be used to profile mutant DNA, analyze protein-DNA interactions, and elucidate the mechanisms of gene regulation in disease states.

Keywords: DNA-directed, point mutation, gold nanostructure, MutS

Systems and Synthetic Biotechnology

Marine Bacterial Ribosomal Peptides: Recent Genomics- and Synthetic Biology-based Discoveries and Biosynthetic Studies

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Abstract. Marine biodiversity is represented by an exceptional and ample array of intriguing natural product chemistries. Due to their extensive post-translational modifications, ribosomal peptides—also known as ribosomally synthesized and post-translationally modified peptides (RiPPs)—exemplify a widely diverse class of natural products, endowing a broad range of pharmaceutically and biotechnologically relevant properties for therapeutic or industrial applications. Most RiPPs are of microbial origin, yet their marine derivatives have been quite rare investigated. Given the rapid advancement engaged in a more powerful (meta)genomics approach, more biosynthetic gene clusters and pathways of these ribosomal peptides continue to be characterized. Moreover, the genome mining approach in integration with synthetic biology techniques has markedly led to the revolution of RiPPs natural products discovery. Therefore, this present short review focuses on the recent discovery of RiPPs from marine bacteria based on genome mining and synthetic biology approaches during the past decade. Their biosynthetic studies are discussed herein, particularly the organization of targeted biosynthetic gene clusters that linked to the encoded RiPPs with potential bioactivities.

Keywords: marine bacteria, ribosomal peptides/RiPPs, (meta)genomics, genome mining, synthetic biology, biosynthetic gene clusters

Development of “Patrol yeasts” for the Detection of Multiple Toxic Substances in Foods

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Abstract. In this study, a novel yeast-based immunosensor “Patrol Yeast” that detects various targets, especially toxic substances in food, is demonstrated. Split-ubiquitin membrane-based yeast two-hybrid system has been used as a sensitive method for the identification and analysis of protein-protein interaction on the cell membrane. We combined the antigen-dependent interaction of antibody variable regions (V_H and V_L) and the yeast two-hybrid system to generate the antigen-dependent reporter enzyme signals in yeast. The aflatoxin B1 and M1 were successfully detected by this system with the limit of detection at 3 pM and 33 pM, respectively. When an anti-caffeine nanobody (V_{HH}) was integrated, which is dimerized upon caffeine recognition, the caffeine detection by patrol yeast was achieved. Also, using the single-chain antibody for *E. coli* O157 as the detector and Zymolyase to partially destruct cell wall during incubation, specific detection of *E. coli* O157 with low background signal was achieved. Both intracellular reporter β -galactosidase and secreted CLuc luciferase reporters were compatible with the patrol yeast system. The successful detection of aflatoxin and *E. coli* O157 makes the patrol yeast system a valuable and novel tool in the field of food safety.

Keywords: Biosensor, yeast, synthetic biology, transmembrane signaling, antigen, antibody, two-hybrid system

Metabolic Engineering of *Lactococcus Lactis* for the Production of Heparosan

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Abstract. Heparosan is a precursor molecule for the widely used anticoagulant heparin, which also has other uses such as certain drug delivery applications and as a scaffold for tissue engineering in biomaterials. Traditionally, pathogenic bacteria such as *E. coli* have been used as a host to produce heparosan as an alternative to animal and chemoenzymatic synthesis. Using GRAS status organisms like *Lactococcus Lactis* as the host for production of heparosan provides a safe alternative as well as being a well-established organism for genetic manipulation and reengineering. In this study, a functional heparosan synthesis pathway was successfully expressed in *Lactococcus Lactis* by the expression of *E. coli* K5 genes *KfiA* and *KfiC*, along with the overexpression of *ugd*, *glmu* and *pgma* genes present natively in the host organism. The genes were activated using the tightly controlled NICE expression system. The genes were cloned into plasmid p8148 and transformed into two strains, *Lactococcus Lactis* NZ9000 and *Lactococcus Lactis* NZ9020, totaling six different recombinant strains were created using these two hosts and various combinations of the heterologous genes. The recombinant *Lactococcus Lactis* SH6 strain, expressing the genes *ugd-KfiA-KfiC-pgma* yielded a maximum concentration of 754 mg/l in batch bioreactor experiments and the titer was increased to 1263 mg/l in fed-batch fermentation. NMR imaging successfully determined that the structure of the product derived from *Lactococcus Lactis* was indeed similar to *E. coli* heparosan. The molecular weight of heparosan varied from 10-20 KDa, indicating its potential use for chemoenzymatic heparin biosynthesis.

Keywords: Heparosan, *Lactococcus Lactis* NZ9000, Metabolic Engineering, Glycosaminoglycans

***Saccharomyces cerevisiae* chromosome redesigned for biotechnological applications**

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Abstract. Synthetic genomics is a research field in synthetic biology that aims to re-design and synthesize whole customized genomes to confer desired functionalities that can benefit biotechnological applications. In this study, we redesigned a chromosome of *S. cerevisiae*, a biotechnological workhorse. The synthetic chromosome possesses extensive modifications and features that enable the generation of diverse genotype and phenotype libraries. By exploiting the features incorporated, we developed methods that accelerate chromosome reconfiguration for improved cell fitness, thus identifying strains suitable for enhanced biochemical production. Our work demonstrates the potential of a redesigned chromosome for biotechnological applications.

Keywords: Synthetic biology, synthetic genomics, bioproduction

Cochlear Implant Performance in Children Deafened by Torch Infection: Scoping Review

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Abstract

Background: TORCH infection described as perinatal infections caused by toxoplasma (To), rubella (R), cytomegalovirus (C), and herpes simplex virus (HSV). The most common postnatal symptom of TORCH infection is sensorineural hearing loss (SNHL) which may progress to severe and profound SNHL, in which cochlear implant (CI) may be considered. This scoping review is to map evidence on cochlear implantation performance in children deafened by TORCH infection.

Methods: Adhering to Arksey and O'Malley's framework for conducting a scoping review, a systematic search was performed in the month of November 2021 in several databases, including Scopus, PubMed, and Google Scholar. Considering the inclusion criteria, a total of 26 articles were included.

Results and Discussion: Twenty-six articles were reviewed. The majority of the studies included (61.5%, n=16) were CMV studies, implying that CMV infection was still the primary etiology for sensorineural hearing loss associated with TORCH infection syndrome. CI is a viable option in the treatment of congenital cytomegalovirus (cCMV), leading to improvements in hearing and language. However, when compared to Cx26 mutation as control, poorer performance was reported for cCMV. CI is also recommended for congenital rubella due to increased speech production, which results in a higher quality of life and the ability to attend regular school. CI was associated with several positive outcomes in toxoplasmosis, including age-appropriate speech perception and social-emotional development. These positive outcomes, however, are only possible with adequate environmental and parental support. No research regarding CI outcomes on patients of hearing loss for specific HSV infection. Nevertheless, CI was recommended for its rehabilitative outcomes.

Conclusion: Being one of the first reviews in this context, CI is recommended especially if it is done as early as possible. Nevertheless, prevention measures such as TORCH infection screening and ABR testing are required, particularly in low- and middle-income countries.

Keywords: congenital sensorineural hearing loss, TORCH infection, cochlear implant, auditory performance

Tissue Engineering and Biomaterials

CRISPR-based bidirectional gene regulation for improved chondrogenic differentiation and calvarial bone regeneration in osteoporotic animal

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Abstract. Calvarial bone regeneration poses a tremendous challenge in clinical settings due to poor spontaneous healing. Previous studies reported that chondrogenic induction of ASC was able to boost the healing progress via the non-native endochondral ossification pathway. Non-osteoporotic and osteoporotic ASC (OVX-ASC) are inferior in osteogenesis capacity and predisposed to adipogenic commitment, while OVX-ASC chondrogenic tendency remains elusive. CRISPR-mediated epigenetic manipulation has emerged as a robust approach for targeted gene activation/silencing. To activate chondrogenesis, we developed CRISPR-AceTrans harboring a dSpCas9 fusion with the histone acetylation domain p300^{core} with an extra recruitable MCP-VPR (Bac-p300-VPR) targeting the chondrogenic Sox trio (Sox5, Sox6, Sox9). To suppress adipogenesis, we adopted the CRISPRi architecture comprising the dSaCas9 fusion with the DNA methyltransferase 3A (Bac-D3A) targeting adipogenic regulators (C/ebp α , Ppar γ). Bac-p300-VPR-transduced ASC displayed a profound promotion of chondrogenic phenotype as judged by abundant presence of glycosaminoglycan (GAG), while OVX-ASC remained resistant to chondrogenic differentiation albeit the Sox trio activation. We Strikingly, combination of CRISPR-AceTrans and CRISPRi generated a bidirectional platform, or CRISPR-BiD, robustly driving OVX-ASC towards chondrogenic lineage. 3D construct culture of the CRISPR-BiD-engineered OVX-ASC gave rise to significant amount of GAG and collagen type II over the mock-transduced construct. Ultimately, implantation of the transduced construct accelerated and enhanced calvarial defect regeneration in osteoporotic rats. Collectively, our results demonstrated the feasibility of CRISPR-based epigenetic alteration for bidirectional regulation of gene expression in stem cells for regenerative medicine.

Keywords: CRISPR; osteoporosis; calvarial bone healing.

Viability And Differentiation Properties of Freeze-Dried Dental Pulp Stem Cells and Periodontal Ligament Stem Cells

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Abstract. To identify the viability and differentiation ability of these freeze-dried dental pulp stem cells (DPSCs) and periodontal ligament stem cells (PDLSCs). Methods: DPSCs and PDLSCs were isolated from dental pulp from and Periodontal ligament tissues, respectively. The stem cells then were cultured to reach confluent condition, then processed to freeze-dried using trehalose. Furthermore, to evaluate the viability of freeze-dried cells by using MTT assay, while to observe the cell differentiation ability through the expression of collagen type I (Col1) and osteocalcin (OCN) using rt-qPCR. Results: After freeze-drying, PDLSc remained viable up to 80 percent compared to the number of cells before the freeze-drying process, but there was a decrease in cell viability in DPSCs. DPSCs and freeze-dried PDLSCs showed mRNA expression of collagen type I and osteocalcin. Conclusion: DPSCs and PDLSc freeze-dried remain viable and maintain the differentiation ability towards osteoblasts as they can express Col 1 and OCN.

Keywords: dental pulp stem cells, periodontal ligament stem cells, freeze drying, collagen type 1, osteocalcin.

Review: Feasibility of Novel 3D Printed Porous Tantalum-loaded Mesenchymal Stem Cells to Enhance Bone Regeneration

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Abstract. Bone fracture in orthopedics relates to the complex system while selecting implant materials and essential fabrication methods. Titanium (Ti) and its alloy are materials that are widely used in orthopedics surgery over a long period and could promote osseointegration. But there are some concerns, including metal ion release, allergic responses, and biofilm formation. Porous tantalum (Ta) is a novel biomaterial used for bone defect treatment due to its corrosion resistance, low elastic modulus, and high biocompatibility. Besides, the bone-implant fabrication method with additive manufacturing (AM), known as three-dimensional (3D) printing, can overcome the conventional fabrication chemical vapor deposition (CVD) method's limitations. AM technology such as selective laser melting (SLM) and laser engineering net shaping (LENS) could produce a 3D printed porous Ta with the controlled porous structure, pore size, and porosity. 3D printed porous Ta with a pore size of 400 - 600 μm and porosity $\pm 80\%$ become favorable for cell ingrowth and development. Bone marrow-derived mesenchymal stem cells (BMSCs) are multipotent cells widely used in tissue engineering, while Porous Ta has performed an excellent biocompatibility feature for bone ingrowth. This review paper compared the experimental studies of 3D printed Porous Ta biomaterial loaded by BMSCS and thus concluded the potential methodology for applying bone regeneration therapy enhancement.

Keywords: porous tantalum, 3D printing, bone-marrow-derived mesenchymal stem cells, SLM, LENS.

Biaxial Mechanical Characterization of the Porcine Aortic Valve

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Abstract. Cardiovascular disease (CVD) has become a prevalent case in Indonesia. Aortic stenosis is a CVD that refers to the narrowing of the aortic valve (AV) during systole and affects the ejection of the blood flow. In severe conditions, the patients need a prosthetic valve replacement. To ensure the validity and endurance of prosthetic heart valves, a thorough understanding of aortic valve mechanics and mechanisms is essential. Studies have focused on characterizing their mechanical behavior toward understanding the heart valve data. Compared to the uniaxial tensile test, biaxial mechanical testing is a better method to study the heart as the heart tissue possesses nonlinear anisotropic mechanical behavior. Mechanical testing of the left ventricle (LV) has been extensively used to develop the computational model of the entire heart, including AV. We performed biaxial mechanical testing on the porcine heart while studies have shown that the porcine valve was identified as an appropriate model for further investigation of the human valve tissue when considering the rarity of human valve. The following procedure includes AV sample dissection, tissue acquisition, specimen preparation, mechanical testing with BioTester – Biaxial Tester (Cell Scale, Biomaterial Testing), and post-processing of the mechanical data with LabJoy Software, while SigmaPlot and Origins Software for data analysis and Visualization. Instead of separate testing protocols on distinct tissue specimens, the biaxial testing procedure will thoroughly characterize the valve leaflet tissue under one unified testing scheme. Hence, this mini-research paper provides a comprehensive overview of these issues, focusing on the insight into the AV mechanism of porcine. Biaxial testing results show that the AV leaflet tissues generally have a nonlinear, anisotropic mechanical response. This mechanical behavior data analysis of AV can be used to build the computational modeling system that may help understand various CVDs and facilitate the development of new therapies.

Keywords: aortic valve, cardiovascular, biaxial testing, mechanical testing, mechanical properties.

Microstructure and corrosion behavior of bio implant material Fe-Cr-Al alloy developed by the powder metallurgy technique

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Abstract. Synthesis of Fe-Cr-Al alloys by powder metallurgy technique was developed to produce a bio implant materials. The process was selected to produce a low modulus of elasticity to avoid the effect of stress shielding. The alloy was synthesized with composition of 16 wt % Cr and variation of 2 and 5 wt % Al to produce samples of implant material with high strength and high corrosion resistance. The powder metallurgy technique was performed by mechanical milling for pre-alloying then continued with consolidation by cold compaction and plasma sintering process. This paper presents an investigation on the microstructure of the Fe-Cr-Al alloys to evaluate the hardness and corrosion behavior. Morphology and phase identification were performed by the SEM-EDS and XRD test. The corrosion test was carried out with a potential-dynamic polarization method in an environment that simulates human body fluids. The results showed a properly homogeneous microstructure with small porosity. Phase analysis showed the formation of the main Fe-Cr phase that indicates good alloying in the synthesis process. The hardness test showed a high hardness level of around 200 VHN. The corrosion test exhibited high corrosion resistance for bio implant material with corrosion current around 30 $\mu\text{A}/\text{cm}^2$. Evaluation on the microstructure showed that the hardness and corrosion behavior were significantly affected by the quantity of Fe-Cr phase and Al added in the Fe-Cr alloy.

Keywords: implant material, Fe-Cr-Al, powder metallurgy, microstructure, corrosion.

Synthesis and characterization of Zr-Nb alloys for prospective dental implant materials

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Abstract. Synthesis and characterization of Zr-Nb alloys were carried out for prospective dental implant materials. Zirconium alloy Zr-Nb as refractory material is used with the advantages of excellence in strength and has high corrosion resistance. The alloy was synthesized by an arc furnace process in argon atmosphere with various compositions of 3 and 6 wt% Nb. Microstructure characterization was carried out by SEM-EDX test followed by phase identification using the XRD test. The hardness was measured using the Vickers Hardness Number method to evaluate the relationship between microstructure and mechanical properties. The corrosion behavior was analyzed based on the corrosion test in a simulated body fluids environment using dynamic potentiostat method. Homogeneous structure of equiaxed with relatively fine grains was observed in the Zr-3Nb and Zr-6Nb alloys. The hardness increased with the addition of Nb suitable with the finer grains occurred in the microstructure. Corrosion currents of 200 and 300 $\mu\text{A}/\text{cm}^2$ for Zr-3Nb and Zr-6NB respectively showed a decrease in corrosion resistance due to the addition of Nb. This experiment shows a good possibility for Zr-Nb alloys as dental implants material, however several treatments are required to improve the compatibility of the alloy.

Keywords: Zr-Nb alloy, dental implant, microstructure, corrosion resistance.

PhotoCrosslinkable Microgel-Based Bioinks for Cell-Laden 3D Scaffold Construction

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Abstract. In the field of tissue engineering and regenerative medicine, functional 3D cell scaffolds have enormous opportunities that can be applied to construct *in vitro* or *in vivo* tissue models. To fabricate 3D scaffold for tissue engineering, Three-dimensional (3D) bioprinting technology is predicted to overcome the challenge of insufficient organs for transplantation. However, traditional bioinks are limited in their ability to satisfy both cell viability and printing resolution due to their post-crosslinking processes and densely crosslinked structure of bulk. For these reasons, 3D bioprinting technology that can print living cells with biomaterial inks (bioinks) is expected to overcome these problems by spatiotemporal control. Hydrogel-based extrusion-type bio inks are widely used for their controllable mechanical properties. However, traditional bioinks have limitation of cell viability and printing resolution by their post-crosslinking process or densely crosslinked structure of bulk hydrogel. To solve these problems, hydrogel microparticle (i.e., microgel)-based bioink is a novel approach for both printability and biocompatibility. In this research, we proposed novel multiple-cells-laden 3D bioprinting ink based on visible light-crosslinkable alginate microgels with excellent biocompatibility and cell proliferation as well as superior physical properties that can construct stable and high resolution 3D structures.

Keywords: 3D printing bioink, 3D scaffolds, photocrosslinkable alginate microgels.

Chondrogenic Differentiation of MSCs Using Fibrillized Collagen Microparticles as an Intercellular Binder

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Abstract. Mesenchymal stem cells (MSCs) can be isolated from a variety of mesenchymal tissues and have the outstanding ability to differentiate into various cell types including cartilage, bone, adipose, and bone marrow cells. In particular, techniques for differentiating MSC into cartilage cells is very important in the field of regenerative therapy, because the cartilage deficiency significantly degrades the patients' quality of life whereas cartilage has poor self-repair capabilities. Usually, MSCs are cultured in the form of aggregates when they are differentiated into cartilage. However, conventional methods have following problems: (i) apoptosis occurs in the center of the aggregates, causing non-uniform differentiated areas and (ii) proper extracellular matrix components such as collagen are not present in the surrounding area, which is significantly different from that in the in vivo environments. In this study, we propose a new approach to the efficient chondrogenic differentiation of MSCs using fibrillized collagen microparticles as an intercellular binder. Through a previously-reported method of emulsification of an aqueous solution of type I collagen, yarn-ball-shaped microparticles made of collagen fibrils were formed, because of the spontaneous organization of the collagen molecules in the water-in-oil droplets [1]. We confirmed that microparticles with sizes of 20–30 μm and composed of fibers with diameters of several hundred nanometers were formed. The obtained fibrillized collagen microparticles were mixed with MSCs and seeded into 96-well plates to form aggregates composed of the MSCs and particles. After cultivation for 3 weeks in a chondrogenic differentiation condition, a typical oval chondrocyte morphology was observed in the cross section of the aggregates. Toluidine blue staining showed increased accumulation of glycosaminoglycans, an indicator for chondrogenic differentiation. Furthermore, immunostaining for type II collagen, another indicator, also confirmed that the tissue in the presence of fibrillized collagen microparticles showed increased differentiation efficiency. These results suggest that the culture method proposed in this study is useful for efficient and uniform differentiation of MSCs, mostly because of the positive effects of fibrillized collagen as an intercellular binder.

Keywords: biomaterials, cartilage, collagen, mesenchymal stem cell.

Annealing Effect on Improving the Mechanical Properties of Zirconium Based Biomaterials with The Addition of Yttrium Elements

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Abstract. To improve the mechanical properties of biomaterials with zirconium-based alloys, especially on tensile strength, an annealing process was carried out. The process was carried out at a temperature of 800 °C and holding time for 3 and 5 hours in a vacuum chamber which was flowed by pure argon gas. The composition of the alloy used in this study was Zr₆Mo₄Ti_xY (x= 0, 1, 2, 3 %wt). Microstructure observations were carried out using Scanning electron microscopy (SEM) and tensile testing. In this study, micro-sized specimens were used and followed the ASTM E8-04 standard with a surface roughness of 1 μm. The results showed an increase in the tensile strength value from 600 MPa to 800 MPa. In addition, the grain boundaries of the alloy are getting smaller, this shows the effect of annealing on changes in the properties of the material.

Keywords: biomaterials, zirconium based, mechanical properties, annealing, micro specimens.

Fabrication and Morphology Control of Collagen Tubes Using Microfluidic Spinnerets for Culturing Mammalian Cells

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Abstract. Collagen-based biomaterials are widely used for cell culture because they play essential roles in upregulating cell functionalities. Especially, tube-like structures (hollow fibers) made of collagen are supposed to be helpful in morphologically mimicking linear cell assemblies, including nerve fiber, blood vessels, and hepatic lobules. However, it is challenging to form micrometer-sized collagen tubes through the manipulation and gelation of a high-viscosity aqueous collagen solution while maintaining a hollow pattern. Additionally, it is difficult to control the tube morphology and size precisely. In this study, we proposed a new approach to the fabrication of micrometer-sized collagen tubes using multilayered microfluidic spinnerets. We fabricated the microfluidic devices made of PDMS using soft-lithography and replica molding techniques. A flow pattern is formed inside the microchannel of the spinneret with a collagen solution as the shell and a cell suspension as the core. These solutions were pumped through the microfluidic channels into a gelation solution to fabricate cell-encapsulating collagen tubes with diameters of several hundred micrometers. In the experiment, the depth and width of the microchannel of the spinneret were varied from 100-500 μm and from 500-2000 μm , respectively, in an attempt to control the morphology of the collagen tubes. As a preliminary trial, a suspension of fluorescent microparticles was introduced as the core solution to fabricate collagen tubes; we confirmed that hollow collagen tubes were successfully formed with the fluorescent microparticles inside the tube flowing. The cross-sectional shapes of the tubes were controlled, ranging from circular (Φ : 300–500 μm) to flat (h : 100 μm ; w : 2000 μm), using microfluidic channels with a variety of morphologies. Next, cell-introduced collagen tubes were fabricated by using a cell suspension as the core solution. We encapsulated human hepatoma cells (HepG2 cells) and confirmed that high cell viability (>95%) was maintained at day 14 of culture. Furthermore, we cultured primary hepatocytes isolated from rat liver in the collagen tubes and observed that the cells adhered on the lumen of the collagen tubes. These results suggest that the cell-encapsulating collagen tubes proposed in this study may be useful as a versatile cell culture substrate to create linear tissue models.

Keywords: microfluidics, collagen, hepatocyte, cell culture.

Ovine Collagen Type-I (OTC-I) Biomatrix Integrated with Antibacterial Coating for Rapid Treatment in Full-thickness Skin Loss

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Abstract. Currently, one of the aims in tissue engineering is to provide rapid treatment for full-thickness skin loss without the common complications of recurring bacterial infection and low blood vascularisation. This research focuses on evaluating a ready-to-use biomaterial from ovine collagen type I (OTC-I) that can not only provide rapid treatment for full-thickness skin loss, but also have antibacterial properties as well as angiogenesis. The biomatrix is crosslinked with natural crosslinker genipin for strength. Then, peppermint extract, (R)-(-)-Carvone have functional groups with antimicrobial effects that can be made into a suitable precursor to combine on freeze-dried OTC-I monolayer biomatrix via plasma polymerisation treatment. The composite biomatrix is fabricated and evaluated *in vitro* for physicochemical properties, antibacterial, angiogenic and cytocompatibility using human dermal fibroblasts. Plasma polymerised carvone deposited on OTC-I showed robust physicochemical porosity as well as swelling and biodegradation rates as a biomaterial. Carvone deposition indicates more than 60% cell death for both gram positive and negative bacteria. There we more than 90% cell attachment, more than 90% live cells 48hours post-seeded, positive tubule formation for blood vessels, and 20% higher proliferation in day 7 cells seeded on biomatrix. In this work we present that crosslinked OTC-I biomatrix coated with carvone show strong outcome on wound healing as well as synergistic functions of acellular treatments, advancing future therapeutic use for skin loss using tissue-engineered skin substitutes.

Keywords: scaffolds, plasma polymerization, antibacterial, biomatrix, biomaterial.

Water-Adjustable & Biodegradable Double-Side Adhesive Patch for Multi-Organic Tissues

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Abstract. The development of body adhesives is very important because of the need for replacement of sutures or the attachment of electronic devices in the body. Adhesions at the surfaces of diverse internal organs are difficult to achieve successfully due to the wet environment in the body. In addition, to be safely used, it must be biodegradable, and the decomposed products must be non-toxic. To secure these features, we fabricated an alternative tissue adhesive in the form of a dried patch obtained by crosslinking of bioengineered mussel adhesive protein (MAP) to polymer. When it soaked by water, strong adhesion is achieved by attractive forces such as the Dopa residues of MAP and the van der Waals force of the polymer. This patch also had the characteristics that the time it takes for decomposition in the body to be controlled from several days to several weeks. Through these characteristics, it is possible to provide a customized patch that performs successful adhesion under conditions most suitable for various environments in the body. Our developed water-adjustable and biodegradable double-side adhesive patch might be useful as a tissue adhesive and sealant and for attaching sensors to wet tissues.

Keywords: underwater adhesion, biomaterials, adhesive patch, biocompatible, biodegradable

Human and Ovine collagen type I as biomaterials for wound healing

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Abstract. Collagen type I is the one of the major components of skin and would be an optimal scaffolding material for reconstruction of wound skin. This study aims to extract and develop novel Multifunctionalized human and ovine collagen type I 3-dimensional biomatrix incorporated with quercetin for rapid treatment of skin burns. Collagen type I was extracted from human redundant skin and ovine tendons using chemical-based extraction, mixed with (1mg/ml) quercetin, and fabricated via freeze-dry technology. Besides, the evaluation of physicochemical, cellular compatibility, toxicity, growth profile, and cell migration were assessed. The antioxidant properties of quercetin were analyzed by 2,2 diphenyl-1-picrylhydrazyl (DPPH) assay and the antibacterial effect of quercetin was tested on gram-positive (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*). The results demonstrated that the groups of human collagen type I (HC.I) scaffold and human collagen type I with quercetin incorporation (HC.I-Q), have better physicochemical and mechanical properties compared to the two other counterpart scaffolds of ovine collagen type I (OTC.I) and ovine collagen type I with quercetin incorporation (OTC.I-Q). However, all groups showed physicochemical properties within the accepted level. Confocal microscopy revealed that the cells maintained their viability and migration during the entire 7 days of cultivation period on the scaffolds. In addition, all groups showed no toxic effect on cells as it promoted optimum cell attachment and proliferation of human dermal fibroblasts (5x10⁶ cells/scaffold). The antioxidant and antimicrobial properties of quercetin were detected in the groups incorporated with quercetin (HC.I-Q & OTC.I-Q). The findings of this study suggested that HC.I with quercetin cell-free scaffold is a promising biomaterial for rapid treatment of skin burns.

Keywords: human collagen type I, ovine collagen type I, multifunction bioscaffold, quercetin.

Cytocompatibility of Corneal Cells towards Ovine Collagen Type I Hydrogel

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Abstract. Corneal transplantation is a gold standard for corneal disorders treatment, but the shortage of tissue donors remained a critical problem. The presence of ovine tendon collagen type-1 (OTC-1) hydrogel can overcome the shortage of donated corneas. The aim is to evaluate the physicochemical and biocompatibility of the OTC-1 hydrogel with corneal cells. Collagen fibril of OTC-1 hydrogel was aligned by using a rocker and double crosslinked by using genipin and quercetin. The physicochemical are evaluated (microstructure, porosity, biodegradation rate, energy-dispersive X-ray spectroscopy (EDX), Fourier transform infrared spectrophotometry (FTIR), and X-ray diffraction (XRD) and mechanical testing). In vitro biocompatibility is assessed by culturing the epithelial and fibroblast on the scaffold. Results showed the scaffold has a uniform interconnected porous structure with acceptable porosity (>70%). The biodegradation rate of the scaffold was 1 month. EDX identified the main elements of the scaffold, (carbon (C) 50.28%, nitrogen (N) 18.78%, and oxygen (O) 30.94%) based on the atomic percentage. FTIR confirmed the presence of collagen type 1 with functional groups (amide A: 3302 cm^{-1} , amide B: 2926 cm^{-1} , amide I: 1631 cm^{-1} , amide II: 1547 cm^{-1} , and amide III: 1237 cm^{-1}). The XRD reported an amorphous phase instead of crystallinity on the fabricated OTC-1 hydrogel. The ultimate tensile strength for the scaffold was 2.21 ± 0.70 MPa at a strain rate of 0.5%/s. Confluence epithelial cells migrated over the scaffold and keratocytes inside the scaffold. Thus, the properties of this scaffold suggest its potential to be developed into a corneal tissue substitute for human transplantation.

Keywords: collagen hydrogel, corneal cells, biocompatibility.

Bedsore Revitalization By-LLLT, Low Level Laser (LED- Ga-Al-As 660) Therapy

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Abstract. In 1967 a few years after the first working laser was invented, Endre Mester in Semmelweis University Budapest, Hungary wanted to find out if laser might cause cancer. He took some mice, shaved the hair off their backs, divided them into two groups and gave a laser treatment with a low powered ruby laser to one group. They did not get cancer and to his surprise the hair on the treated group grew back more quickly than the untreated group. That was how "laser biostimulation" was discovered. The effects of pulsed monochromatic light, with fixed pulsations and wavelengths, on the healing of pressure ulcers were evaluated in this prospective, randomized, controlled study. A placebo-controlled, double-blind study using low energy photon therapy (LLLT) was performed in ten patients with bedsore on the back. Treatment was given three times a week for 10 weeks, using monochromatic (red) optical sources; diode 660nm (GaAl-660). The patients who were randomized to placebo treatment received sham therapy from an identical-appearing light source from the same delivery system. Ten patients with 10 bedsore were randomized to receive LLLT or placebo therapy. At the conclusion of the study, the percentage of the initial ulcer area remaining unhealed in the LLLT and placebo groups was 24.4% and 84.7%, respectively ($P = 0.0008$). The decrease in ulcer area (compared to baseline) observed in the LLLT and placebo groups was 193.0 mm² and 14.7 mm², respectively ($P = 0.0002$). One patient dropped out of the study, complaining of lack of treatment efficacy; he was found to be randomized to the placebo group. There were no adverse effects. In this placebo-controlled, double-blind study LLLT was an effective modality for the treatment of bedsore which were resistant to conventional medical management. The results are encouraging as pulsed monochromatic light increased healing rate and shortened healing time. This will positively affect the quality of life in elderly patients with pressure ulcers.

Keywords: bedsore healing, soft tissue healing, decubitus ulcer healing, low level laser ,wound healing.

Effective localized cancer therapy based on adhesive proteinous nanoparticles

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Abstract. In cancer therapy, nanoparticles (NPs) have been developed for therapeutic delivery system capable of providing efficient cellular uptake, targeting ability, and controlled release to improve pharmacological activities of various therapeutic molecules. However, systemic approaches of NPs have not yielded significant clinical outcomes due to insufficient accumulation, rapid clearance, and systemic toxicity. Even though local treatment can offer precise distribution of NPs in cancer, harsh microenvironments including flow of body fluids and diverse clearance mechanisms have been major challenges in effective absorption and bioavailability of therapeutics. In addition, peripheral healthy tissues at cancer regions suffer adverse effects arising from off-target release of therapeutic agents from NPs. In this presentation, I will introduce several types of protein-based stimuli-responsive and adhesive nanotherapeutics based on bioengineered mussel adhesive protein (MAP) for localized cancer therapy to provide a prolonged residence time of NPs on wet/mucosal tissue surfaces and facilitate the controlled release of therapeutic agents with desirable pharmacokinetics, thereby enabling highly effective therapeutic responses with excellent biosafety. MAPs have been considered as promising natural sources for biomedical applications due to great biocompatibility, biodegradability, and superior adhesive property suitable for humid microenvironment within the body.

Keywords: localized cancer therapy, nanoparticles, mussel adhesive protein.

ECM-inspired hydrogel of hyaluronan-gelatin crosslinked via a novel Link module with transglutaminase reactive sequence

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Abstract. The extracellular matrix (ECM) is a natural scaffold of cells in the body. It has a complex structure comprising various proteins such as collagen and hyaladherins and polysaccharides such as hyaluronan (HA). In this study, inspired by the crosslinked ECM structure, we designed a genetically engineered Link module—LinkCFQ—by fusing a microbial transglutaminase (MTG)-reactive tag to the Link module, an HA-binding domain of tumor necrosis factor-stimulated gene-6. Although the HA-specific binding property of the Link module was preserved, LinkCFQ demonstrated excellent MTG reactivity with various proteins. Furthermore, an ECM-inspired hydrogel was fabricated from an HA–gelatin mixture crosslinked via HA/Link module interaction and MTG-catalyzed isopeptide bond formation in LinkCFQ. Cell culture and mouse experiments confirmed the hydrogel’s biocompatibility and degradability. Our findings provide new insights into the design of novel biomaterials and proteins for tissue engineering, regenerative medicine, drug discovery and delivery, disease models, biofabrication, and medical devices.

Keywords: biomaterials, hyaluronan, gelatin, link module, microbial transglutaminase.

The Effect of Temperature, Time, and MIL-101 Catalyst Loading for Lactide Synthesis from L-Lactic Acid

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Abstract. Lactide is an important monomer for high molar mass Polylactic acid (PLA) production through ring-opening polymerization. PLA is one of the most well-known biodegradable and biocompatible polymer. This study prepared Crude Lactide from L-lactic acid with MIL-101 as the catalyst. MIL-101 is metal-based catalyst with organic ligands (MOF) that was prepared by reacting Cr_3O and terephthalic-acid (BDC). This study investigated the role of MIL-101 and the interaction between catalyst loading and temperature as well as the interaction of catalyst loading and reaction time on the conversion of lactic acid to crude lactide using the response surface method (RSM). RSM is statistical modelling to determine the relationship between the variables. Crude lactide was analyzed using ^1H-NMR to confirm the presence of lactide. The results found that a temperature of 200°C, 1.5% w/w catalyst loading, and reaction time of 4 h gave the highest conversion of 22.84% and the RSM model shows that the interaction of catalyst loading and reaction time had a significant effect on the conversion of lactic acid to lactide with P value 0.0021 and F value 50.45, while the interaction of catalyst loading and temperature did not significantly affect the conversion of lactic acid to lactide with P value 0.2565 and F value 1.75.

Keywords: interaction variable, one-step synthesis, lactic acid, lactide, MIL-101, PLA, RSM.

Cationic surface charge effect on proliferation and protein production of human dental pulp stem cells cultured on diethylaminoethyl-modified microcarriers

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Abstract. Human adult dental pulp stem cells (DPSCs) are a type of mesenchymal stem cells (MSCs). Recently, DPSCs have been proposed as a new MSC source for treating immune-mediated, inflammatory, and degenerative diseases via cell-based therapy. Hence, large-scale industrial production of the required cell number will be necessary. In this study, to investigate the cationic surface charge effect on DPSC proliferation, we fabricated two types of 2-diethylaminoethyl-modified cellulose porous beads (CPB-DEAE) with different ion exchange capacities. The cationic surface charge effect increased with increase in DPSC proliferation rate for the ion exchange capacity of 0.55–1.82 meq/g. However, the DPSC proliferation rate decreased at 2.50 meq/g. Thus, the optimal ion exchange capacity was determined to be 1.82 meq/g for DPSC proliferation. Since DPSCs secrete a variety of immunomodulatory proteins that can be collected as serum-free conditioned media, DPSC-derived conditioned media also have been considered a novel therapeutic strategy. The total amount of protein produced from the DPSCs on the CPB-DEAE microcarriers was higher than that produced under the conventional monolayer culture conditions. Furthermore, the hepatocyte growth factor, which is one of the anti-inflammatory growth factors, was produced from the DPSCs on the CPB-DEAE microcarriers. Finally, the DPSCs were confirmed to maintain their proliferative and multi-differentiation ability after culture on the CPB-DEAE microcarriers. We anticipate that the CPB-DEAE microcarriers will be useful for large-scale MSC expansion and production of conditioned media.

Keywords: dental pulp stem cells, cationic surface charge, cellulose porous beads, diethylaminoethyl modification.

Improvement in Mechanical Properties of Mg-Li-Al Alloys as Orthopedic Implants by Using Thermomechanical Process: A Review

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Abstract. Magnesium alloy is one type of alloy that is very attractive to the researcher to develop as a bone implant material. Magnesium has good biocompatibility and mechanical properties that are closest to human bones. However, there is still a major problem where the degradation rate of magnesium in the body is too quick and because of that, it needs to be treated to increase its strength and corrosion resistance. This paper will review the effect of thermomechanical treatment with the hot rolling method for improving the mechanical properties of Mg-Li-Al alloys. Furthermore, this review will also discuss the effect of the percentage of rolling reduction and the addition of lithium (Li) and aluminum (Al) as alloying elements on structural changes and strengthening mechanisms that occur in magnesium alloys.

Keywords: magnesium alloys, orthopedic implant, thermomechanical treatment, dynamic recrystallization, hot rolling, mechanical properties.

Improvement in Mechanical Properties of Mg-Li-Zn Alloys as Orthopaedic Implants by Using Thermomechanical Process: A Review

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Abstract. One of the most favourable materials to use as an implant is magnesium. Among many of the reasons why are its biocompatibility, good mechanical property that is close to human's bone, and decayability. The downside of magnesium is the high degradation rate of it in bodily fluid, henceforth magnesium needs treatments to increase its strength and corrosion resistance. This paper will review the effect of thermomechanical treatment using hot rolling method to improve mechanical properties of magnesium alloy Mg-Li-Zn and discuss the effect of the percentage of rolling reduction and addition of lithium (Li) and zinc (Zn) as alloying elements on structural changes and strengthening mechanisms that occur in magnesium alloys.

Keywords: magnesium alloys, orthopedic implant, thermomechanical treatment, hot rolling, mechanical properties.

Effect of Plasticizer on Malleability of Polylactide-Based Craniomaxillofacial Fracture Fixation Plates

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Abstract. In this study, the effect of plasticization on the malleability of polylactide-based implant plates in craniomaxillofacial bone fracture application was studied. Specimens were synthesized by adding a plasticizer with a concentration of 2wt% in a mixture of PLLA and PDLLA (70:30). The mixing process was carried out by rheo-mixing, and the forming process was utilized in a vacuum oven. Specimens were characterized to determine their properties. The addition of glycerol and PEG 400 plasticizer influenced the shift in the absorbance of the infrared spectra. Furthermore, the plasticizer lowered the glass transition temperature (T_g) from 62.3°C to 58.5°C. The bending strength, bending strain, and modulus of elasticity of the specimens were also reduced. Microstructural analysis evaluation showed that the mixture was miscible. The results of the degradation test showed that the plasticized specimen 30PDLLA/glycerol underwent a degradation process with the smallest portion.

Keywords: implant, polylactide, PLA, CMF, plasticizer, PEG400, glycerol.

Effect of Plasma Treatment on the Hydrophilicity of PCL-Gelatin Film for Wound Dressing Application

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Abstract. Poly (ϵ -Caprolactone) (PCL) is a biomaterial that has been widely used in medical applications. However, its application as a degradable wound dressing is still very rare, due to the hydrophobic nature of PCL compared to other biodegradable polymers. The purpose of this research was to study the effect of plasma treatment on the hydrophilicity of PCL films. PCL film was fabricated using solvent casting method with DCM solvent. Plasma treatment was carried out on PCL films with time variations of 1, 2, and 3 seconds to modify the functional groups present in PCL so as to create polar groups containing oxygen. After plasma treatment, gelatin was grafted through crosslinking with Glutaraldehyde (GA). Changes in surface morphology, chemical bonding, and hydrophilicity were characterized using SEM, FTIR, and Contact Angle Test, respectively. The result of this study indicated that plasma treatment has succeeded in increasing the surface hydrophilicity of the PCL film at an optimal time of 1 second with the lowest contact angle value of 47.00°. After grafting with gelatin, the contact angle decreased further to 42.28°. This result showed that plasma treatment can be used as a facile, fast, and inexpensive strategy to increase the hydrophilicity of PCL as a wound dressing.

Keywords: PCL, film dressing, hydrophilicity, plasma treatment

Local Chlorhexidine Delivery for Periodontal Infection Therapy: A Short Review

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Abstract. Oral diseases affected more than half of the world's population. Periodontitis is the second biggest threat to oral health after dental caries. Periodontitis is an inflammation inside periodontal pockets caused by pathogenic microbial colonies which lead to destruction of bone tissue and soft tissue. Chlorhexidine gluconate is an antimicrobial agent that effective to destroy pathogenic microbial and cure periodontitis. Chlorhexidine gluconate is given locally using a carrier material to the infected area. There are several materials that can be used to deliver Chlorhexidine gluconate to the infected periodontal tissue such as gel, liquid (mouthwash), and chip. This short review aims to describe the current methods to deliver Chlorhexidine gluconate to the infected periodontal tissue, including the advantages, disadvantages, and the future trend of the carrier materials used for periodontitis treatment.

Keywords: Periodontitis, chlorhexidine, local delivery, carrier materials

The Photocuring 3D Printed Phantoms with Spontaneously Generated Submicron- to Nano- Porous Structures for Minimally Invasive Ultrasound-Guided Surgeries

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Abstract. Construction of tissue-mimicking phantom has been a flourishing subject in the medical research field. In the present study, the mimicking phantom is fabricated by integrating a commercial high-resolution photo-curing 3D printing process and the phase inversion technique to generate the 3D structures containing submicron and macropores simultaneously. The nonsolvent-induced and polymerization-induced phase separation (NIPS and PIPS) methods are applied. That was, the 3D printing created pores with a diameter of 500-1000 μm , while the phase separation process caused pores ranging from 0.1 to 10 μm . The NIPS appeared in the high-speed blending and caused emulsification. The following evaporation of solvent and nonsolvent in the system would continuously enhance the progress of NIPS. The other was the PIPS demixing induced by photopolymerization in the 3D printing process. In the printing, NIPS and PIPS would take place in the same system, allowing high complexity of micron- and submicron-structures. The drying process after pore formation was important to the integrality of demixing structures. The micropores were highly destroyed if the product was dried at high temperatures, which was possibly caused by surface tension of solvent evaporation. On the contrary, the supercritical CO_2 drying would well maintain the porous structures. According to the results, porous structures produced by applying NIPS were cellular pores with diameters of 10-40 μm . On the other hand, the pores created by PIPS were highly connected and bicontinuous, where the pore size ranging about 0.1-5 μm . The PIPS demixing is slower than NIPS in phase separation process; however, it can be overcome by increasing the printing intensity energy or the density of photoreactive functional groups. Therefore, the printing efficiency would not be influenced significantly. The microporous structures were filled within different gel or liquid materials, allowing applications in the phantom of ultrasonic-guided minimally invasive surgeries. The filler materials adjusted the ultrasonic properties, and the customized and biomimetic outfit was achieved by 3D printing. By water filling, the ultrasonic velocity was changed from 650 to around 1500 m/s which was quite similar to human soft tissues. The attenuation coefficient, acoustic impedance, and density properties were also controllable by changing microporous structures and filling materials. The integration of the phase separation technique to produce the controllable porous structure and the acoustical properties of the printed object demonstrates the feasibility of constructing realistic tissue-mimicking phantoms. This can greatly contribute to the medical field of simulating surgery.

Keywords: 3D printing, phase separation, porous structure, phantom, ultrasound.

Effects of Plasma-Treated Water on Bacteria and Osteogenic Cells

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Abstract. Many researchers supported that plasma-treated water (PTW) inactivates bacteria efficiently. It was believed that the reactive nitrogen or oxygen species (RNS or ROS) suppresses or damages the activity of microorganisms. However, the influences of PTW on mammalian cells were less investigated. The effects of PTW on osteoblasts were thus evaluated in this research, followed by the comparisons with bacteria treated by PTW. The atmospheric pressure plasma was applied in this study to produce highly active RNS and ROS significantly. The pH value of DI water decreased from 6 to 3 after being treated with PTW for 10 minutes. The DI water did not become more acidic if the treatment was prolonged to 20 or 30 minutes. The results revealed that the plasma-generated setup was efficient enough for generating reactive compounds, so the contents of reactive species reached a steady state within 10 minutes. The acidity of PTW was kept for at least 7 days, indicating the excellent stability of PTW. The PTW was added into Dulbecco's modified eagle medium (DMEM) for cell culture, followed by the pH measurement after one day. The results showed that the pH value decreased by 5% but was still

acceptable for cell culture. It was because HCO_3^- generated by plasma played the role as a reservoir to H^+ , so the pH change was limited. Although PTW did not change the pH value of MEM, the effects on bacterial and osteoblast were highly different. The concentrated PTW resulted in a clear antibacterial ring, and the ring diameter increased with PTW concentrations. The OD values of E coli were lowered by 70% due to the addition of PTW. On the contrary, the viability of osteoblasts decreased by 20% with the same amounts of PTW, supporting that PTW was more toxic to bacteria than osteoblasts. When low PTW content was applied, the elimination of bacteria decayed, but the following antibiotic effects were promoted. Meanwhile, dilute PTW did not suppress osteoblast viability; however, the early osteogenic differentiation was delayed. The effective PTW content was distinct for bacteria and osteoblasts. We will continue to investigate the optimized conditions of PTW antibiotic applications without cytotoxicity in humans.

Keywords: atmospheric pressure plasma, plasma treated water, reactive oxygen species, reactive nitrogen species, osteoblasts, osteosarcoma.

Efficient 3D Printing of Chitosan Hydrogels

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Abstract. The novel biomedical materials should have controllable bioresorbability, required bioactivity, and suitable biomechanical strength. All the above properties can be adjusted by porous structures. Thus, 3D printing (3DP) attracts much attention due to its ability to fabricate accurate and highly customized structures on a wide scale. The hydrogel is able to show good biomimetics to extracellular matrix (ECM) and excellent cell affinity. With the structures provided by 3DP, the hydrogel scaffolds would guide and remodel the cell/tissue generation, migration and differentiation more efficiently. The good biocompatibility, hydrophilicity, antibacterial activity, and processibility make chitosan (CS) become a potential hydrogel in 3DP. CS hydrogels can be solidified by ionic interaction instead of chemical crosslinkers, reducing the toxicity of CS in 3DP. However, it also resulted in poor mechanical strength, nonideal stability, inferior reproducibility and low printing efficiency of CS hydrogels. In this study, the natural crosslinker and accelerated ionic reaction were applied to improve the 3DP of CS hydrogel. In the first part, the natural crosslinker was added into CS solution. GE significantly increased the viscosity and intermolecular forces of CS, promoting the printing resolution and gelation speed in 3DP. Then, the static mixing tube was applied to blend two solutions, where one was crosslinker solution with metal ions, and the other was CS solution with pre-crosslinking. The collector was also heated to accelerate the solidification reaction. The results revealed that the curing time was reduced by 99.44 % and the deformation of printed structures were minimized by 98.43 % under optimized temperature and concentrations. The cell culture results supported the biocompatibility of CS hydrogel was unchanged after 3DP process, and the cell proliferation was effectively promoted by 3D structures.

Keywords: 3D printing, scaffolds, chitosan, natural crosslinkers, hydrogel, static mixing tube.

Preparation of Flexible Resin by Adding Plasticizer for 3D Printing

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Abstract. The polymeric resin used in 3D printing (3DP) was usually rigid and tough. The limited range of mechanical strength constrains the applications of 3DP products. In this study, three plasticizers, 2-butoxyethanol (2-Bu), dibutyl phthalate (DBP) and sorbitol (Sorb), were added to polycaprolactone (PCL), a popular biopolymer, and applied for the digital light processing (DLP) and fused deposition modeling (FDM) 3D printing. 2-Bu and DBP were convectional plasticizers with cytotoxicity, and Sorb from glucose reduction was a novel nature plasticizer. The PCL with high molecular weight was used in FDM, while the PCL oligomer modified with di-acrylic groups was printed by DLP. The results show that the glass transition temperature (T_g) of PCL decreased from 5.72 °C to -34.99 °C due to the addition of Sorb, proving the effectiveness of this plasticizer. The T_g reduction caused by Sorb was more efficient than 2-Bu and DBP. When Sorb was combined with 2-Bu or DBP, Sorb/2-Bu presented additivity in plasticizing, but Sorb/DBP showed offset. The possible reason was the high affinity between Bu and Sorb would allow the additive relaxation times of polymers, causing less crystallinity and highly flexible polymer composites. The low Young's modulus supported that the flexibility of 3DP PCL was improved. From the cell culture, the 3D printed PCL with Sorb addition was more biocompatible than those with 2-Bu and DBP. Moreover, the early differentiation was not interfered by Sorb. The development of highly flexible and biocompatible PCL 3D-printed scaffolds was achieved by adding Sorb in this research, enabling the integration with compressive bioreactors for the dynamic culture of bone tissues.

Keywords: scaffolds, plasticizer, 3D printing, biocompatibility, non-toxic resin, biomaterial, osteoblasts, polycaprolactone.

Effects of Photo-Reactive Monomers on Ultrasonic-Guided Surgery Phantoms Created by 3D Printing

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Abstract. 3D printing (3DP) has proved its potential in preparing medical phantoms because the medical imaging, computational analysis and customized lithography of 3DP successfully bridge the gap between medical experiments and clinical surgeries. However, the ultrasonic characteristics of 3DP phantoms were rarely investigated, especially in the photocuring 3DP with high resolution. It was caused by the limitation of photoresin and commercial printers, but the ultrasonic properties were critical to phantoms for minimally invasive surgery, including preoperative training, education and doctor-patient communications. In this study, several photo-reactive monomers with various amounts were respectively blended with flexible photoresin, IC27. The mechanical properties, printing resolution, density, crosslinking degree, ultrasound velocity and attenuation coefficient were then identified. Our experimental results indicated that the Young's modulus and elongation of photocured resin decreased with PEGDA addition. However, the crosslinking density increased by adding PEGDA. The high crosslinking density should lead to rigid or tough qualities, which did not correspond to our results. It was possibly due to the polarity differences between PEGDA and IC27 that induced the swelling. The hydrophilicity of cured resin was significantly promoted by PEGDA addition, revealed by the swelling test. That was to say, the photo-resin with PEGDA would attract water vapor penetration into 3DP cured products, resulting in the decay of post-curing efficiency. Thus, the PEGDA did not enhance the toughness, flexibility and density of 3DP phantoms, so the ultrasonic properties were not significantly changed. On the other hand, the compressed IC27/PEGDA phantom was recovered by 96% after 24 hours, indicating the good recoverability caused by PEGDA addition. In the second part, hydrophobic TMPTA with three photoreactive functional groups was used instead of PEGDA. 20 wt% TMPTA increased the crosslinking density of photoresin increased by 490% while PEGDA only increased crosslinking density by 108%. TMPTA was more effective in crosslinking than PEGDA, revealing the importance of intermolecular affinity in photo-crosslinking. The density and ultrasonic velocity increased with the density; that was, IC27-TMPTA > IC27-PEGDA > IC27. The ultrasonic velocity in IC27-TMPTA was about 2100 m/s which lay between trabecular and cortical bones. By adjusting crosslinking density, controlling microporous structures and integrating with bio-ceramics, the biomimetic acoustic properties of 3D printed phantoms were achieved.

Keywords: 3D printing, phantoms, photocrosslinking, ultrasonic properties

Magnesium-carbonate apatite composites for bone implant applications: A scoping review

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Abstract. Bone implants are an important component in osteosynthesis and joint arthroplasty. Studies on bone implant materials are currently being developed in order to obtain the most suitable material. Magnesium (Mg) alloy is a biodegradable material that is a new generation in the development of orthopedic implants that allow permanent implants. Carbonate apatite has a good osteoconductivity that allows the bone healing process and has similar carbon content with human bone. Incorporation of magnesium and apatite carbonate as a better implant biomaterial is under research. In this review, we aim to investigate the development and prospects of magnesium-carbonate apatite for bone implants including characteristics, biocompatibility, toxicity, advantages, disadvantages, and challenges. The search engine we used is Google Scholar with the keyword (orthopedic implant "magnesium carbonate"). The inclusion criteria are the application of magnesium carbonate as a bone implant in systematic review, article review, randomized clinical trial, or case report. Literature other than English and Indonesian is excluded. The combination of magnesium and carbonate can reduce the corrosion rate and it does not cause toxic effects but can be influenced by several factors such as the physiological environment and mechanical interactions. Carbonate apatite can bind to bone without formation of fibrotic tissue and has a higher thermodynamic stability than hydroxyapatite. The different content of carbonate in composite is related to the corrosion rate. Limitations of magnesium carbonate such as corrosion rate, rarity, and cost can be a challenge in development of bone implant. Magnesium-carbonate apatite has great potential in the development of bone implants by combining the advantages and maximizing safety. However, more research is needed to be able reach the clinical trials stage.

Keywords: magnesium, carbonate apatite, bone implant, biodegradable.

Prospect of *In Vivo* Study Using Rat Model to Evaluate Potential of Protease from *Bacillus* sp. HSFI-10 as Antithrombotic Agent

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Abstract. Cardiovascular disease (CVD) is a non-communicable disease known as the prominent cause of death, not only in Indonesia, but also in Asia. The *in vitro* antithrombotic effects of several thrombolytic agents, including the fibrinolytic serine proteases from *Bacillus* sp. had been evaluated, as had the discovery of bacterial protease from fermented foods worldwide. Recently, the bacteria from the fermented intestines and muscle of sand sea cucumber, *Holothuria scabra*, were reported to produce proteases with high thrombolytic activity *in vitro*. Among these bacteria is a marine bacterial isolate namely *Bacillus* sp. HSFI-10 was found to produce protease with high clot lysis activity *in vitro* based on the gravimetry. This paper aims to analyze the prospect of an antithrombotic agent in the protease extract of *Bacillus* sp. HSFI-10 *in vivo* based on literature study. The results revealed that the most common type of animal used for thrombosis model research is the white *Sprague Dawley* rat with an average age of around 6 weeks and a weight of around 200 g. The next common type of animal models used in thrombosis related study with carrageenan induction are Wistar (*Rattus norvegicus*) and Swiss Albino. The thrombosis induction mainly targets the tail of rats or mice using carrageenan as the induction agent through intra-vena induction. It was also revealed that despite the high thrombolytic activity of bacterial protease crude extract of *Bacillus* sp. HSFI-10, *in vivo* studies for the development of antithrombotic agents have not been reported. As conclusion, *in vivo* study on experimental animals is important, yet promising as a follow-up study to evaluate the potential of protease from *Bacillus* sp. HSFI-10 as a candidate of antithrombotic agent for CVD treatment. The recommended method of thrombosis induction on testing animals is by using carrageenan injected on the tail of Sprague Dawley since the procedure are mostly reported as more efficient in accelerating the formation of thrombus.

Keywords: anti-thrombotic agent, *Bacillus* sp. HSFI-10, carrageenan induction, thrombosis animal model, tail.

A stretchable, self-healing, adhesive and biocompatible polyzwitterionic hydrogel

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Abstract. Hydrogels provide a three-dimensional environment to supports cell growth have received great attention in tissue engineering and regenerative medicine. Although a variety of natural or synthetic hydrogels have been revealed, hydrogels are prepared by chemical crosslinking usually had weak mechanical property. In this study, we used zwitterionic hydrogels be as a candidate for bone regeneration materials. In order to improve the mechanical properties of zwitterionic hydrogels, we used hydrophobic interactions to crosslink hydrogels to approach tough and self-healing properties. Here, sodium dodecyl sulfate (SDS)-based micelle was used as a physical crosslinker to prepare sulfobetaine methacrylate (SBMA) hydrogels, and ethylene glycol dimethylacrylate (EGDMA) was used to prepare chemically crosslinked SBMA hydrogels (control group). We compared the mechanical properties of the two hydrogels and found that the physically crosslinked hydrogels are more flexible than the chemically crosslinked hydrogels. When the hydrogels were subjected to external forces, the micelles acted as dynamic crosslinking sites, allowing the stress to disperse and prevent the hydrogel from breaking. In addition, the physically crosslinked hydrogels also had self-healing properties within 5 minutes, which could repair damage by themselves, greatly extended the service life, and increased the applicability. Finally, the MTT and hemolysis tests showed the hydrogels have excellent biocompatibility and hemocompatibility. We believe that the physically crosslinked hydrogels with micells will have the potential to be employed as drug delivery system.

Keywords: sulfobetaine methacrylate, self-healing, hydrogel, biocompatible.

Effects of Magnesium Substitution on the Morphology of the Hydroxyapatite Layer Using Sol-Gel Dip Coating Technique on the Surface of SS 316L

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Abstract. SS 316L is a bioinert metal commonly used for implants considering its ease of manufacture and affordability. Coating SS 316L with bioactive material such as hydroxyapatite (HA) increases the biofunctionality of its surface for easier binding to body tissues. However, the composition of stoichiometric HA is different from human bioapatite. As an attempt at biomimicry, this study aims to determine the effects of magnesium substitution on the morphology of the formed HA layer above SS 316L surface. The synthesis of 0%, 4%, and 8% magnesium substituted hydroxyapatite (Mg-HA) $\text{Ca}_{10-x}\text{Mg}_x(\text{PO}_4)_6(\text{OH})$ is carried out using sol-gel technique, then deposited on SS 316L with dip coating under three repetitions. X-ray diffraction (XRD) and fourier transform infrared spectroscopy (FTIR) were conducted to obtain Mg-HA characteristics in its powder form: including lattice parameters, crystallinity index, crystallite size, and functional groups. Meanwhile, the morphology and composition of the formed Mg-HA layer were observed via scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDX). The XRD pattern shows a tendency for biphasic HA formation (HA + β -CPP) when Mg is present in the apatite lattice. Mg ion has relatively smaller ionic radii from Ca ($\Delta r = 0.34 \text{ \AA}$), causing changes in average bond length when substituted into HA. Thus, 0.3–1.1% increase of a and c parameters in both HA and β -CPP phases were observed in 4% Mg-HA variation. While c keeps increasing, a value in both phases of 8% Mg-HA decreased 0.1–0.5% relative to COD data. In agreement with various literature, decrease in crystallinity index (X_c) and crystallite size (B) were found along with increased Mg content. FTIR spectrum documents the bond formation of Mg^{2+} ions with hydroxyl groups in HA. Mg tends to occupy the crystal surface and disrupt crystal growth; hence higher Mg concentration results in smaller Mg-HA agglomerates observed via SEM images. This study found that the 4% Mg-HA produced the most homogeneous layer compared to other variations.

Keywords: hydroxyapatite, magnesium, substitution, dip coating, sol-gel .

Optimization Concentration of Irgacure[®] 2959 as Photo-initiator on Chitosan-Alginate Based Hydrogel for Tissue Sealant

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Abstract. Tissue sealant is a material that is used as an adhesive to unite two tissue surfaces that are used during the operation process. Chitosan and alginate are natural biomacromolecules used to manufacture hydrogels as tissue sealants because these two materials can strengthen the bond and mechanical properties of tissue sealants. Irgacure[®] 2959 as a photo-initiator can help strengthen the crosslinking between chitosan-alginate to produce a tissue sealant with good mechanical strength. This study aims to determine the effect of adding Irgacure[®] 2959 with different concentrations of the chitosan-alginate hydrogel as a tissue sealant designed for the colon. The experimental design was completely randomized (CRD) with the addition of Irgacure[®] 2959 0; 1; 2; 3; 4%. The hydrogel characteristics as a tissue sealant observed in this study included swelling degree, water resistance, simulation of adhesion, degree of crystallinity, and ability to inhibit bacteria. The increased concentration of Irgacure[®] 2959 shows good potential in the formulation of a tissue sealant. The addition of 4% of Irgacure[®] 2959 decreased swelling degree, increased hydrogel resistance to water, and had a higher adhesive ability. The interaction chitosan and alginate with the addition of Irgacure[®] 2959 produced crosslinking, as evidenced by the degree of crystallinity of 26.21%. However, the bacterial inhibition ability of all treatments showed that there isn't significant different between all treatment due to the same concentration of chitosan that used.

Keywords: alginate, chitosan, hydrogel, Irgacure[®] 2959, photo-initiator.

The Effect of Chitosan-Gelatin Ratio on Characteristic Tissue Sealant Prepared Using Photo-crosslinking Method

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Abstract. Tissue sealant is a biomaterial with the adhesive properties that can glue the injured body tissue. Chitosan and gelatin are natural polymer that have potential to be used as tissue sealant. The combination of proteins and polysaccharides results in promising applications in the biomedical field because it has biocompatible, biodegradable, and non-toxic properties. This study aims to know the effect of ratio chitosan-gelatin on characteristics of tissue sealant that prepared using the photo-crosslinking method. The photo-crosslinking method using the photo-initiator Irgacure® 2959 can help the cross-links between chitosan and gelatin become stronger. The experimental design used was a Complete Randomized Design (RAL) with the ratio of chitosan-gelatin of 1:0; 1:1,5; 1:2; 1:2,5; and 1:3. The analysis of characteristics of tissue sealant include adhesion simulation, lap shear, swelling degree, water resistant, functional group, and morphology test. The results showed that the chitosan-gelatin ratio of 1:1.5 showed the highest adhesion compared to tissue sealant without gelatin (1:0 ratio) and others higher ratio. The chitosan-gelatin ratio of 1:1.5 produces a tissue adhesive with high adhesion properties, low swelling properties, high water resistance, and indicates the presence of crosslinking with chitosan shown in the results of the functional group analysis. Crosslinking causes smaller pores so that the surface morphology of the tissue sealant looks homogeneous when viewed from the morphological test using SEM.

Keyword: chitosan, gelatin, tissue sealant, photo-crosslinking, Irgacure® 2959

Preparation and Characterization of Chitosan-Gelatin Based Tissue Sealant Using Various Concentration Irgacure® 2959

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Abstract. Tissue sealant is a substance with the characteristic of allowing two substrates to be bonded and used as an alternative in surgical processes for gluing the tissue. Chitosan and gelatin are natural polymers that can be used as tissue sealant hydrogels and have been proven for wound healing. Photo-crosslinking is a method of preparation of tissue sealant that can help improve its adhesiveness by adding the photo-initiator. Photo-initiator is causing a cross-link between chitosan and gelatin to produce better mechanical strength. This study aims to determine the effect of Irgacure® 2959 as photo-initiator on the characteristics of chitosan-gelatin hydrogel as a tissue sealant. The experimental design used was a completely randomized design (CRD) using the addition of Irgacure® 2959 with concentration of 0%; 0.5%; 1%; 1.5%; 2%; and 2.5%. The characteristics of the chitosan-gelatin hydrogel in this study were evaluated including swelling degree, viscosity, adhesion simulation, lap shear, functional group analysis, water resistance, and morphological analysis. The addition of Irgacure® 2959 resulted a tissue sealant with a higher adhesion than without the addition. Irgacure® 2959 caused increasing in viscosity, adhesion, and water resistance and decreasing in the swelling up to the addition of 1.5%. The addition of Irgacure® 2959 with more than the concentration of 1.5% causes a reverse effect. The presence of cross-link between chitosan and gelatin was predicted by the change of functional group that analyze using FTIR. The chitosan-gelatin hydrogel also showed small pores and a smooth surface so it is promising to apply as a tissue sealant.

Keywords: chitosan, gelatin, tissue sealant, photo-initiator, Irgacure® 2959.

Optimization Concentration of Irgacure[®] 2959 as Photo-initiator on Chitosan- κ -Carrageenan Based Hydrogel for Tissue Sealant

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Abstract. Hydrogel is a three-dimensional network of hydrophilic polymers widely used in the biomedical field, one of which is a tissue sealant. Material that can be used in the manufacture of hydrogels is chitosan. The ability of chitosan in the formation of hydrogel is still limited by its swelling and adhesive properties, so it requires other materials such as crosslinkers, one of which is κ -carrageenan which can stabilize and increase the viscosity of the hydrogel. The addition of Irgacure[®] 2959 as a photo-initiator to the hydrogel can produce free radicals that can bind to the active group of the polymer. This study aims to determine the effect of adding Irgacure[®] 2959 in the manufacture of hydrogel based on a chitosan- κ -carrageenan solution formulated as a colon tissue adhesive. The treatments given were the addition of Irgacure[®] 2959 as much as 0%, 1%, 2%, 3%, and 4%. The characteristics of the hydrogel tested qualitatively included the degree of swelling, resistance to water, degree of crystallinity, and the ability to inhibit bacterial growth. The addition of Irgacure[®] 2959 did not show significantly different results from the control that indicating it cannot increase the crosslinking between chitosan and κ -carrageenan. This is predicted due to the presence of steric hindrance from κ -carrageenan, causing very limited crosslinking to occur due to the difference in molecular size between chitosan and κ -carrageenan. For further, it is necessary to optimize the ratio of the chitosan and κ -carrageenan to get the balance ratio that supports the occurrence of cross-linking.

Keywords: carrageenan, chitosan, hydrogel, Irgacure[®] 2959, photo-initiator.