Appendix 12

THE RELATIONSHIP OF OXIDATIVE STRESS LEVEL WITH THE TYPE OF GLOBIN BETA GENE MUTATION IN BETA THALASSEMIA PATIENTS IN DR. HSAN SADIKIN BANDUNG

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ABSTRACT

Ineffective erythropoiesis and multiple blood transfusion cause iron overload leading to high level of ferritin in β thalassemia patients. Iron has ability to catalyze reactive oxygen species (ROS) production, which is harmful in high level. This process can be prevented by adequate superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity as intracellular enzymatic antioxidants. Oxidative stress is a condition of imbalanced amount between ROS and antioxidants, and SOD and GPx activity may indicate the oxidative stress level in β thalassemia patients, e.g. in homozygous IVS1nt5 and IVS1nt5/HbE patients. Aim of this study is to explore the oxidative stress level by measuring SOD and GPx activity, and ferritin level in β thalassemia patients. Blood was collected from 58 patients with homozygous IVS1nt5 and IVS1nt5/HbE mutation, recruited from The Thalassemia Clinic and Hemato- Oncology Clinic at Dr. Hasan Sadikin General Hospital, Bandung. SOD and GPx (Randox Kit) was measured and compared between homozygous IVS1nt5 and IVS1nt5/HbE. Data of ferritin level was collected from medical records. Kruskal- Wallis analysis was conducted to asses relation among ferritin level, both enzymatic antioxidants and the type of β thalassemia mutation. Spearman analysis was conducted to asses relation between both enzymatic antioxidants, and relation between ferritin level with each enzymatic antioxidant in each mutation. Fourty five patients with homozygous IVS1nt5, age ranges between 1-18 years, and 13 patients with IVS1nt5/HbE, age ranges between 2-26 years, were recruited. Patients with homozygous IVS1nt5 showed median (min-max) of ferritin level 3.784 (791-12.340,33) µg/L, SOD activity 172,12 (54,51-276,26)U/ml and GPx activity 227,12 (8,41-1.329,10) U/l, whereas patients with IVS1nt5/HbE showed 3.555 (1.785-8.135) µg/L, 167,55 (94,31-228,94) U/ml and 319,66 (16,82-1.753,04) U/l, respectively, thus, no significant difference among ferritin, both antioxidants level and the type of β globin gene mutation. There is no relation between SOD and GPx activity in homozygous IVS1nt5 (r=0,106, p=0,488) and IVS1nt5/HbE (r=-0,294, p=0,329). There is no relation between ferritin level and SOD activity (r=-0,073, p=0,634) and ferritin level and GPx activity (r=-0,115, p=0,389) in homozygous IVS1nt5. There is no relation between ferritin level and SOD activity (r=0,094, p=0,761) and ferritin level and GPx activity (r=-0,052, p=0,865) in IVS1nt5/HbE. Oxidative stress level in β thalassemia patients tends to be not associated with the type of β globin gene mutation. Co-inheritance of β thalassemia major mutation could aggravate clinical symptoms of patients with IVS1nt5/HbE mutation.

Keywords: β thalassemia, IVS1nt5/HbE, SOD, GPx, oxidative stress.

THE EFFECTS OF BRAZILIN COMPOUND TOWARD ANTIOXIDANT PROFILE IN RATS (*Rattus norvegicus* L.) AGAINTS THE CONDITION OF EXCESS IRON

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ABSTRACT

Brazilin isolated from *Caesalpinia sappan* was antioxidant which potentially prevents oxidative stress in patient with excess iron. This research aims to determine the activity of brazilin as an antioxidant and to know the dose that influence the antioxidant activity in the serum of rats that suffered a condition of excess iron. This research was conducted in two stages, the first stage was brazilin isolation and the second was antioxidant profile test by using 24 females of wistar rats with an average weight of 200 grams. The treatment consisted of negative control (KN), iron dextran dose 60 mg/kg bw (KP), deferipron dose 7,5 mg/kg bw (KD), brazilin dose 7,5 mg/kg bw (P1B), brazilin dose 10 mg/kg bw (P2B), brazilin dose 12,5 mg/kg bw (P3B), brazilin dose 15 mg/kg bw (P4B), and brazilin dose 17,5 mg/kg bw (P5B) that was given orally for 14 days treatment. The observed parameters included the activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehide level (MDA). Based on analysis of variants and Duncan's multiple-range test showed that the giving of iron dextran caused excess iron, indicated by increased of SOD activity 91,62%, GPx activity 226,89% and MDA level 269,81%. The giving of brazilin dose of 12,5 mg/kg bw can decreased antioxidant profile of excess iron–induced rats with a decreased of 44 % (SOD), 66,4% (GPx) and 72% MDA level. Increased of brazilin had a positive effect on rats antioxidant profile to have normal values on a range of dose 12,82 - 28,88 mg/kg bw.

Keywords: Antioxidant, brazilin, and excess iron

HbA_2 PROFILE IN CARRIERS OF BETA THALASSEMIA WITH IVSINt5 MUTATION AND CODON 26 BETA GLOBIN GENE

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ABSTRACT

Beta thalassemia trait screening are one of strategic to reduce the incidence of thalassemia beta mayor. The problem in screening of beta thalassemia trait was complete blood count normal or anemia microcytic hipokrom. The use of variant hemoglobin analysis becomes an important key to the success of screening beta thalassemia trait. This study aims to determine the profile of HbA_2 in beta thalassemia trait with IVSInt5 and codon 26 mutation. This research was conducted in laboratory using non-randomized sampling method. A total of 196 samples performed complete blood tests and meet the inclusion criteria of 117 samples were analyzed variants of hemoglobin with a mini cap Sebia by France. Analyzed mutation done by sequencing method (Sanger). Effect of different mutation toward profil HbA_2 analyzed by independent T-test with significance value ($p < 0.05$). HbE, HbA and HbF variants were not analyzed for influence but described descriptively. The result of the research based on the inclusion criterion was obtained the 30 samples with the level of HbA2 ≥ 4% and samples with HbE variant consist of 28 samples. The result of beta globin mutation consisted of 28 samples mutation heterozygous IVSInt5, mutations codon 26 with 27 samples and 2 other mutations ie codon 8-9 and codon 19 (Hb Malay). Level HbE on beta thalassemia trait codon 26 averaged 24.06 ± 0.95%. Levels HbF in beta thalassemia trait IVSInt5 are between 0.2- 0.9%, whereas at beta thalassemia trait codon 26 between 0.4-1%. The independent T-test results showed that level of variant HbA and HbA_2 on beta thalassemia trait IVSInt5 and beta thalassemia trait codon 26 were significant different ($p < 0.05$). Mutation IVSInt5 influenced process splicing pre-mRNA so that it caused absent of beta globin chain and significant increased level of HbA_2 4.65±0.41% and decreased level of HbA 95.24 ± 0.47%, whereas on genotype of codon 26 altered amino acid glutamic to lysine caused decreasing production of mRNA in beta globin and increased level of HbA_2 3.18 ± 0.31% and significant decrease 72.51 ± 0.98%. Results from this research was concluded that profil HbA_2 beta thalassemia trait with IVSInt5 mutation has level HbA_2 higher than beta thalassemia trait with codon 26 mutation.

Keywords: genotype beta globin, HbA_2, beta thalassemia trait

BIODEGRADATION OF GLIFOSATE HERBICIDE BY BACTERIA CONSORTIUM FROM AGRICULTURAL SOIL OF HATIVE BIG VILLAGE, AMBON CITY

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ABSTRACT

The herbicide chemical glyphosate is the most widely used in weed eradication. This herbicide inhibits the activity of the enzyme 3-enolpyruvylshikimate-5-phosphate synthase which forms essential amino acids. Application of glyphosate in excess amounts to soil, water, and crop yields. Glyphosate bioremediation using microorganisms can be an alternative if the use of glyphosate cannot be avoided. In the early stages of this study, a bacterial consortium IC50 was tested from soil that was often exposed to glyphosate and soil that was not exposed to glyphosate to determine the level of glyphosate concentration in the test which could inhibit the growth of indigenous consortia by 50% compared to media that did not contain glyphosate. The IC50 value was calculated based on the number of live bacteria. The IC50 of the bacterial consortium from glyphosate exposed soil was 2.04 mg/L and from soil not exposed to glyphosate was 263.38 mg/L. low glyphosate concentration increased the growth of bacterial consortium populations from glyphosate open soil (0.01 - 1 mg/L) and from glyphosate non-open soil (0.01 - 100 mg/L). In the next step, the glyphosate degradation test was carried out by a consortium of bacteria. A consortium of bacteria from soil exposed to glyphosate was grown on a medium with various compositions of carbon, nitrogen, and phosphorus sources with the addition of 500 ppm glyphosate and incubated for 30 days. Parameters measured were cell turbidity, concentration of glyphosate, glycine and orthophosphate using a spectrophotometer. The medium treatment with less C and N sources and sufficient P resulted in the largest decrease in glyphosate (94.91%) and the highest orthophosphate concentration (34.94 M), but the lowest glycine concentration (1201.67 ppm). Medium treatment with excess C and N sources as well as sufficient P sources resulted in orthophosphate and glycine in large concentrations of 30.44 M and 1970.00 ppm, respectively, but the lowest decrease in glyphosate (71.71%). Based on the molecular results using the 16S-rRNA method, the species that were isolated from a consortium of bacteria from soil exposed to glyphosate were Stenotrophomonas maltophilia strain MHFENV 20, Bacillus subtilis strain FX4, Bacillus subtilis strain IP18, Lysinibacillus sp. BNPK-15, Staphylococcus sp. strain InS-021-1, Stenotrophomonas sp. Strains DIB76BC2, and Methylobacterium sp. XBGSY9.

Keywords: herbicide bioremediation, indigenous consortium, nature consortium, biodegradation synergism

OPTIMIZATION OF M2e EPITOPE-BASED VACCINE CANDIDATE EXPRESSION USING RESPONSE SURFACE METHODOLOGY AND PURIFICATION

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ABSTRACT

Vaccination is considered as one of effective biosecurity programs to prevent avian influenza (AI). The mutation of AI virus is able to induce HA (Hemaglutinin) and NA (Neuraminidase) variance. This can cause pandemic which leads to new virus making the available vaccine not to be effective. A vaccine based on the ectodomain of influenza matrix protein 2 (M2e) can overcome these drawbacks due to its universal vaccine which is well conserved in both human and avian influenza AI viruses. The previous study showed that fusion protein (M2eKPC) has succesfully been expressed within E. coli ER2566. This success was not measured whether the recombinant protein was expressed or not. If the level of expression which is obtained was not expected, an optimizing expression is needed. The purifying recombinant protein is the next step. The precise purification of the use of kind and concentration non-ionic detergent can raise interaction affinity of futionprotein within chitin. This research is intended to determine the optimum of IPTG concentration, temperature and time induction on futionprotein (M2eKPC) expression in E. coli ER2566 and to know the optimum condition of non-ionic detergent (Tween-20 and Triton X-100) variation, boosting interaction within chitin. The method of research on expression optimum lies on Response Surface Methodology approach. Besides, the reasearch on purification uses IMPACT method. The conclusion of this research explains that the optimum of futionprotein (M2eKPC) expression lies on IPTG concentration 0.33 mM, 18°C degree within 8 hours resulting the fusion protein yield (M2eKPC) 0.15 ± 0.013 mg/mL and the optimum of non-ionic detergent boosting fusion protein interaction within cithin is 0,2 % of Triton X-100.

STRUCTURAL DESIGN OF SACCHAROMYCOPSIS FIBULIGERA R64 -AMYLASE MUTANT TO INCREASE ITS ADSORPTIVITY TO SUBSTRATE BY USING BIOINFORMATICS METHODS

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ABSTRACT

α-Amylase is one of the important enzymes in the starch-processing industry. In that processing, high temperature is needed to break down the starch molecules thus resulting in high cost of production. The high adsorptivity of α-amylase toward substrate is known to decrease the hydrolysis temperature. Carbohydrate Binding Module (CBM) and/or Surface Binding Site (SBS) are two important part which responsible to the adsorption of starch. *Saccharomycopsis fibuligera* R64 α-amylase (Sfamy R64), a locally-sourced enzyme from Indonesia, showed high amylolytic activity but low starch adsorptivity. This study aimed to design a mutant of Sfamy R64 to have better adsorptivity by introducting a SBS on its structure using bioinformatics approach. The structural behavior of Sfamy R64 and positive control (*Aspergillus niger* α-amylase) were studied using molecular dynamics simulation. After that, the mutants of Sfamy R64 were designed to have a stable SBS which mimic the positive control. The substrate affinity was evaluated using MM/GBSA method. Mutant of Sfamy R64 that constracted by S383Y/S386W/N421G/S278N/A281K/Q384K/K398R and insertions of G400_S401ins-TDGS was stable throughout DM simulation and substrate could bound to the SBS over 55 ns. Mutant and positive control have similar structural behavior of SBS and interaction energy of wild-type, mutant, and positive control were -5.2, -8.2, and -17.6 kcal/mol, respectively. The enhanced substrate binding in the mutant suggests the potential to be tested in laboratory.

Keywords: α-amylase, Sfamy R64, surface binding site, starch adsorptivity, molecular dynamics simulation

POTENTIAL OF BIODEGRADATION OF THE YELLOW CONSORTIUM ON THE QUALITY OF TEXTILE LIQUID WASTE PT KAHATEX RANCAEKEK, BANDUNG REGENCY, WEST JAVA

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ABSTRACT

The Ministry of Industry in 2016 stated that in the period January-February 2016, exports of the textile industry and textile products reached USD 2 billion, this figure will continue to increase by three percent when compared to the previous year. However, this activity can cause various kinds of negative symptoms, including the entry of energy and other materials into the environment that cause air and air pollution which will reduce the quality of the environment. According to the provisions, the waste is transferred to the Waste Water Management Installation (IPAL) and processed before being disposed of. However, the community complained about the negative management because of the negative impact, as a result of the waste of industrial products causing the surrounding environment or into the stream causing disruption of the river flow ecosystem, starting from the non-fulfillment of B3 standard water quality (colorless, smelly, and non-toxic). PT. Kahatex is one of the industries that has WWTPs, it is acknowledged by the company's employees that it is very difficult to remove textile color. Therefore, the required microbes are able to break down the carbon chain so as to effectively remove the color of textile waste. Yeast is a microbe that is able to decompose and break down the color of waste by the fermentation process. This study aims to obtain a yeast consortium from samples of liquid waste contaminated with BOD, COD, and TSS and to determine the ability of these yeast species to degrade color. The study was conducted experimentally using a completely randomized design (CRD) with a 3x3 factorial pattern. The treatment consisted of two factors, namely the time of three levels and various amounts of yeast addition which consisted of three levels with three numbers. The results obtained that the potential for the yeast consortium at a concentration of 10% was able to produce the best degradation in reducing BOD levels by 96.79%, COD by 96.78%, and TSS levels by 66%. The Yeast Consortium has the potential to degrade the color of liquid waste at a concentration of 5% within 120 hours with a clarity value of 77.92%, Consortium concentration of 7.5% with a clarity value of 80.11% and the highest clarity value at a 10% concentration Consortium of 89, 18%.

Keywords: Consortium, yeast, textile, degradation, color, BOD, COD, TSS

IN SILICO STUDY OF STRUCTURAL ENGINEERING PT1 STRAIN PELITA III FOR THE DEVELOPMENT OF PERTUSIS RECOMBINANT VACCINE IN INDONESIA

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ABSTRACT

Pertussis (whooping cough) is a disease caused by the bacterium Bordetella pertussis. Pertussis vaccine development is now using chemical detoxification, resulting in changes in the composition of the pertussis toxin epitope that affect immune stimulation. Genetic detoxification is currently being developed. Bioinformatics methods are used for rational vaccine design. This method helps to see the epitope structure and genetic changes after the detoxification process. The aim of this research is to make a model of the structure of PT1 strain Pelita III. Finding the active site of NAD+ in the PELITA III strain structure. effect of PT1 on PT1 structure and effect of PT3 on NAD+ ligand affinity. Looking at the effect of substitution of other amino acids, especially the amino acid alanine on the binding site of the PT1 toxin structure in the binding of NAD+ as a candidate material for rational vaccine design. This study uses the PT1, PT2, and PT3 homology modeling method, evaluates the comparison of PT1 and PT2, MD PT1 and PT2, predicts pocket volume using POVME and molecular docking. The results of this study showed that the conformation of the PT1 protein in the region of the active site bound to the NAD+ ligand was unstable at the residue of the active loop. PT2 affects loop fluctuations thus blocking the active pathway for binding to NAD+. Predicted POVME gain volume yield increase after open loop using this region can be used to bind the NAD+ ligand structure. Prediction of the binding site for NAD+ on PT1 that occurs in areas with an affinity value of -8.8 kcal/mol. PT3 can bind hydrogen from the interaction of the NAD+ ligand with the weak binding affinity value of NAD+ from -8.8 kcal/mol to -7.0 k/mol. The conclusion of this study is that the PT1 structure conforms to the loop section compared to PT2. The active site region is in the inner space of the loop and the volume of the active space is suitable for binding the NAD+ ligand based on the results of molecular docking and POVME. PT3 resulted in a decrease in the affinity of the NAD+ ligand and the loss of hydrogen bonds.

Keywords: PT1, PT2, PT3, conventional MD, conformational structure, molecular docking, loop, NAD+ binding site.

COMPUTATIONAL MODEL OF scFv ANTI RBD OF SARS-CoV-2 FROM S230 ANTI SARS-CoV

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ABSTRACT

The emergence of SARS-CoV-2 caused a global pandemic in early 2020. Developing neutralizing antibodies targeting the RBD domain of viral spike protein is essential to help the infected person. However, antibody production is expensive and relatively inaccessible to many third-world countries. A more efficient yet smaller antibody fragment, such as a single-chain variable fragment (scFv) from a known antibody structure, is interesting to be explored due to the lower cost of recombinant protein production and tailorability of scFv against the circulating viral strain. In this study, we designed a derivate of S230, a known neutralizing antibody for SARS-CoV-1, to be able to bind with that of SARS-CoV-2. The design was based on the S230 interaction analysis with RBD of SARS-CoV-1 and SARS-CoV-2. Five mutations were introduced to improve S230 binding with SARS-CoV-2, i.e., Ser32Thr, Trp99Val, Asn57Val, Lys65Glu, and Tyr106Ile, then evaluated by molecular dynamics simulation. The modified S230 showed a better interaction with SARS-CoV-2 RBD, indicated by the Principal Component Analysis, distance analysis, and MM/GBSA interaction energy. It is suggested that the mutations were not affecting the folding of S230, as indicated by a positive spot test in lateral flow assay against RBD, using human-serum albumin as a negative control. We hope this study could be useful in designing a specific and low-cost therapeutic agent, which will particularly be helpful in the early outbreak when the information on neutralizing antibodies is still limited.

Keywords: *S230, scFv, Structure-Based Design, Protein Design, SARS-CoV-2*

ANALYSIS OF RESISTANT ANTIBIOTIC PROFILES AND PATHOGENESIS OF *Escherichia coli* IN MASTITIS FROM THE ENVIRONMENT OF DAIRY FARMS IN PANGALENGAN AREA, WEST JAVA

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ABSTRACT

Mastitis is an udder inflammatory disease in cattle. Mastitis occurs due to *E. coli* infection which attacks the under due to unclean cage conditions and poor sanitation management. Treatment of mastitis is done with antibiotic therapy. The purpose of this study was to determine the profile of antibiotic resistant *E. coli* as a cause of mastitis originating from cage water, cage floors, and feces along with the type of pathogenicity in dairy cattle in Pangalengan, West Java. The method used was the isolation and identification of *E. coli* from 48 samples of feces, 9 samples of water, and 9 samples of cage floor, then the antibiotic resistance test for Betalactam (Ampicillin), Fluoroquinolone (Ciprofloxanin), Aminoglycosides (Gentamycin) was carried out, Sulfonamide (Sulfamethoxozole/ Trimethoprim), Tetracycline (Oxytetracycline), and Chlorampenicol and gene pathogenicity detection using *E. coli* primers (EPEC, EHEC, ETEC, EAEC, EAEC, EIEC) using PCR and electrophoresis. The result showed the highest level of resistance was found in the antibiotics ampicillin, sulphamethoxozole-trimethropim and oxytetracycline. The pathogenicity types of *E. coli* obtained are *Enterophatogenic Escherichia coli* (EPEC) from feces and water sample also *Enterohemorrhagic Escherichia coli* (EHEC) types from cage floors or bedding materials.

Keyword: Mastitis*, E. coli,* antibiotic resistance

EXPRESSION, SOLUBILIZATION, AND PURIFICATION OF RECOMBINANT DIPHTERY TOXOID (rCRM197EK) IN THE Escherichia coli BL21(DE3) HOST FOR THE DEVELOPMENT OF VACCINE CONJUATES AND DRUG DELIVERY

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ABSTRACT

CRM197 is generally utilized as a vaccine conjugate and drug delivery. However, CRM197 is still toxic to yeast and mammals cells. Recombinant CRM197EK (rCRM197EK) is a new generation of diphtheria toxoid that has three-point mutations (K51E / G52E / E148K). The target protein in inclusion bodies is frequently the dominant product when expressing heterologous proteins in *Escherichia coli*. N-lauroylsarkosine (sarkosyl) can solubilize inclusion bodies proteins, the target proteins produce in one solubilization step. In this study, the expression host E. coli BL21 (DE3), expression vector pET-28a (+), and Ni-NTA resin as a stationary phase in the purification process. This study aims to construct the CRM197EK gene in the pET-28a (+) expression vector and cloned it into the host *E. Coli* BL21 (DE3), expression the rCRM197EK protein in the host *E. Coli* BL21 (DE3) and solubilize it using sarkosyl and purify the rCRM197EK protein using affinity chromatography system. The research method started with the construction and synthesis of plasmid pET-28a (+) - CRM197EK, cloning of pET-28a (+) - CRM197EK plasmid into the host E. Coli BL21 (DE3), isolation and characterization of the plasmid pET-28a (+) -CRM197EK through restriction and sequencing analysis, expression test and solubilization of rCRM197EK with sarkosyl, characterization of rCRM197EK through DNase assay and purification of rCRM197EK using the QIAexpress® Ni-NTA Start kit. The results of this study were plasmid pET-28a (+) - CRM197EK with a size of ~ 6,919 bp was successfully constructed and cloned into the host E. coli BL21 (DE3), protein rCRM197EK with a size of ~ 61.61 kDa was successfully expressed in the form of inclusion bodies by E. Coli BL21 (DE3) and solubilized using sarkosyl, the concentration of rCRM197EK obtained was 10.52 mg / mL, and rCRM197EK was successfully purified using the affinity chromatography system.

Keywords: Expression, E. coli BL21 (DE), rCRM197EK, sarkosyl, solubilization

ANTIBIOTIC RESISTANCE ANALYSIS OF *Staphylococcus aureus* AS A MAIN CAUSE OF MASTITIS IN THE SOUTHERN REGION OF BANDUNG REGENCY

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ABSTRACT

Bandung Regency is one of the regions in West Java Province known as a milk production center. But the milk produced does not have good quality. The average total number of bacteria contained in milk is 2.59 million CFU with the Indonesian National Standard (SNI) of 1 million CFU. The high bacterial contamination in milk causes mastitis with the main cause being *Staphylococcus aureus*. Mastitis is a disease that often arises and is difficult to treat, so it often causes resistance to antibiotics. Therefore, to find out the spectrum of resistance to antibiotics and their virulence level, milk samples were taken from the Warnasari, Babakan Kiara in Pengalengan District and Tarumajaya areas in Kertasari District to be tested on antibiotic discs and PCR examination to determine virulence factors. The results of observations on the antibiotic resistance test of *Staphylococcus aureus* showed that Ampicillin, Oxytetracycline, Sulfametoxazole - trimethoprim, Chloramphenicol and Ciprofloxacin were five classes of antibiotics that had become resistant. The lowest percentage of resistance was Sulfametoxazole – trimethoprim, Chloramphenicol and Ciprofloxacin which reached 3.12% and the highest was Ampicilin which reached 100%. In general, the percentage of resistance of these five antibiotic groups shows the highest number in the dairy farming area in Babakan Kiara and the lowest in the Tarumajaya region. While antibiotics that are still sensitive are gentamicin, in all three areas of animal stalls. While on the PCR examination the virulence factor of the *Staphylococcus aureus* bacteria from milk samples from the three areas of livestock showed that the bacteria had the virulence factor haemolisin alpha (hlα). To prevent an increase in mastitis infections and bacterial resistance of Staphylococcus aureus to the antibiotics used for the treatment of mastitis, real efforts such as the use of antibiotics that are wise and controlled, apply good farm management about concerning to environmental health aspects. Dairy farms use a group / collective system and are far from human settlements to be one of the farm systems to choose from. The antibiotics that can still be used in dairy farms in Babakan Kiara, Warnasari and Tarumajaya members of KPBS, Bandung Regency to treat mastitis infections are Gentamicin antibiotics.

Keyword: Antibiotic, Mastitis, resistances, *Staphylococcus aureus*, virulency factor

NANOEMULSION OF THE EXTRACTED MICROALGAE SPIRULINA MAXIMA AND ITS POTENTIAL AS AN ANTIOXIDANT AND ANTIBACTERIAL

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ABSTRACT

One of the natural ingredients whose potential is being developed nowadays is Spirulina, contains primary and secondary metabolites which can potentially be used as antibacterial, antiviral, antioxidant, anticancer and antifungal agents. This research is aimed to show the potential of *S. maxima* based on its antioxidant substance, to generate the results of IC_{50} both on the extract and nanoemulsion as well as to test its potential as an antimicrobial in E. coli isolate. This research employed laboratory experimental method with descriptive data analysis, where the data obtained were described and compared with the specified requirements. This research yielded an IC50 value of 234.17 ppm and *S. maxima* nanoemulsion resulted in an IC_{50} value of 12.78 ppm - 29.92 ppm. Nanoemulsion of *S. maxima* was characterized by pH, viscosity, and nanoemulsion size using a Particle Size Analyzer. The PSA test results ranged from 1,211 um-1,523 um. The droplet size yielded is relatively larger than the nanoemulsion standard, which is <1000nm. Antibacterial test results of *S. maxima* extract and nanoemulsion *S. maxima* extract and nanoemulsion did not have the potential to be an antimicrobial in the *E. coli* ATCC 25922 test bacteria, which was indicated by no clear zone formation in the test sample.

Keywords: *S. maxima*, nanoemulsion, antioxidant, antibacterial

DEVELOPMENT OF IgY-BASED LATERAL FLOW ASSAY FOR DETECTION OF GUMBORO DISEASE IN CHICKEN

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ABSTRACT

The poultry industry still faces serious problems against the disease in Indonesia. One of the diseases is Gumboro or Infectious Bursal Disease (IBD). Gumboro caused by infectious bursal disease virus (IBDV). IBDV infects the bursa of Fabricius (BF). BF is a lymphoid organ for controlling the B-cell maturation. The virus infection can trigger the vulnerability to the secondary infection and leads to the high mortality and morbidity of the chicken. Moreover, managing of the Gumboro post-outbreaks requires considerable time and costs. Besides vaccination programs, the early detection of Gumboro is important. The most popular diagnostic tool is a rapid test which meets ASSURED criteria. In this study, the rapid test or lateral flow assay LFA was successfully developed using IgY anti-IBDV as a detection molecule, because IgY production is quite simple compared to the hybridoma mAb production. IgY anti-IBDV was successfully isolated and has required affinity against the IBDV sample and nitrocellulose membrane. In addition, the developed rapid test has high specificity against the field samples of IBDV.

Keywords: Gumboro, Rapid test, IgY anti-IBDV

TOXIC EFFECTS OF ALANG-ALANG (*Imperata cylindrica* L.) ROOT ETHANOL EXTRACT ON ORGANS, COMPLETE BLOOD PROFILE AND BLOOD BIOCHEMICAL PROFILE OF WISTAR RATS

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ABSTRACT

Cogon grassroots (*Imperata cylndrica* L) empirically used as medicine for glomerulonephritis, fever, antihypertensive, hepatoprotective, and can reduce lipid and glucose in the blood. The potential of cogon grassroots makes it an herbal medicine. Cogon grass is the one of candidate's Standardized Herbal Medicines by the National Agency of Drug and Food of Republic Indonesia so further study had regarded it activity and safety in it used. This study aims to investigate the toxic effects of cogon grassroots ethanol extract on organs, hematology profile and biochemistry blood profile in Wistar rats. Cogon grassroots extracted with 70% ethanol. There are ten female Wistar rats divided into two groups: the control group and the treatment group. The control group administered 0.5%CMC, and the treatment group administered cogon grass roots ethanol extract with dose 5000 mg /kg Body Weight (BW) at once. Visual observations carried out for 14 days. On the 14th day, rats took blood for the examination of the hematology profile and biochemistry blood profile. After that, rats sacrificed the liver, heart, lungs, and spleen taken for histology examination. Differences in the control group and the treatment group in visual observation had descriptive analysis. Data on differ incomplete hematology profile, biochemistry blood profile, and histology analyzed by using statistical t-test or Man Whitney test. Visual observations did not show any toxicity in the treatment group. Hematology profile did not significantly differ in the control group and treatment group ($p>0.05$). The biochemistry blood profile did not show any significant difference in the control group and the treatment group ($p>0.05$). Meanwhile on the histology examination, the control group and the treatment group did not significantly differ ($p>0.05$). These results indicate that cogon grassroots ethanol extract did not give sign toxic during observation in rats, did not have a toxic effect on the liver, kidneys, heart, lungs, and spleen in rats, did not change in hematology profile and biochemistry blood profile in rats after 14 days.

Keywords: Cogon grassroots ethanol extract, toxic effect, hematology profile, biochemistry blood profile

GREEN SYNTHESIS OF DIFFERENT PHASES OF TITANIUM DIOXIDE (TiO2) NANOPARTICLES USING MANGO PEEL EXTRACT (*Mangifera indica* L.)

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ABSTRACT

Titanium dioxide (TiO_2) nanoparticle is a material that has anti-bacterial, photocatalytic, non-toxic, and high stability properties, thus it is widely used as a raw material in the cosmetic, paint, and textile industries. In general, TiO_2 nanoparticles are obtained by sonochemical, microemulsion, precipitation, hydrothermal, solvothermal, and electrochemical processes. Due to the high demand, it is deemed necessary to develop a green synthesis method for TiO_2 nanoparticle production which is more environmentally friendly. In this study, TiO_2 nanoparticles were synthesized using titanium trichloride as a precursor and mango (*Mangifera indica* L.) peel extract as a hydrolyzing agent. 20 mL of 15% $TiCl_3$ solution with 5, 10, and 15 grams of mango peel extract were mixed at 80°C for 24 hours. The synthesized TiO_2 nanoparticles were characterized using UV-VIS spectroscopy, SEM, TEM, XRD, and FTIR. The results of XRD and SEM showed that the phase composition (rutile to anatase ratio) of the products decreased with increasing the content of the mango-peel extract in the mixture. Using 5 g of the extract, the product contained 100% rice-grain-like rutile (28 nm), whereas 100% of anatase spherical nanoparticles (~17 nm) were obtained by incorporating 15 g of the extract. IR spectrophotometer analysis result confirms the formation of TiO_2 by the observed Ti-O stretching vibration at the wave number of 670-557 cm^{-1}. In summary, the results showed that the phase, particle/crystal size, and crystalinity of TiO_2 nanoparticles can be controlled by varying the ratio of the extract to $TiCl_3$. The higher the ratio, the smaller rutile-to-anatase ratio and the TiO_2 nanoparticles size.

Keywords: Green synthesis, anatase, rutile, $TiCl_3$, ascorbic acid, and sugar

THE EFFECT OF SAPPAN WOOD EXTRACT (*Caesalpinia sappan* L) AS ANTIOXIDANT IN H9C2 CELL LINE CARDIOMYOCYTES INDUCED AMMONIUM FERRIC CITRATE (AFC)

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ABSTRACT

Blood transfusion therapy in patients with blood disorders such as thalassemia can increase free iron in several organs, one of which is the heart. Free iron can catalyze lipid peroxidation through the fenton reaction. Lipid peroxidation can be prevented by using iron chelation or antioxidants to reduce the oxidative stres that is formed. Sappan wood extract (EKS) by invivo test, is known to have potential as iron chelation and as antioxidant which is seen by reducing ferritin levels, increasing levels of Glutathione peroxidase (GPx) and reducing levels of Malondialdehyde (MDA) and Superoxide dismutase (SOD). This study aims to determine the effect of sappan wood extract as an antioxidant on the H9C2 cardiomyocytes cell line induced by ammonium ferric citrate (AFC). The parameters measured in this study included the percentage of cell viability to the addition of AFC and EKS to the H9C2 cardiomyocytes cell line. Then observation of cell morphology after the addition of EKS to H9C2 cardiomyocytes cell line induced by AFC and measuring levels of ferritin, SOD, GPx and MDA using 6 treatment groups including control cells, AFC 20 mM, AFC 20 mM + EKS 5 µg / mL, AFC 20 mM + EKS 10 µg / mL, AFC 20 mM + EKS 15 µg / mL and AFC 20 mM + DFP 10 mM. The 20 mM (5300 µg / mL) level of AFC had shown a decrease in cell viability approaching IC25 ($P = 0.0004$) and addition of 10 µg / mL EKS showed no significant difference with control cells ($P = 0.4856$). The results of measuring ferritin levels showed that the addition of 20 mM AFC had not increased ferritin levels and MDA levels and had not shown a decrease in SOD and GPx levels. The conclusion of this study is the use of AFC 20 mM which is not optimal in increasing free iron so the addition of sappan wood extract has not known its effect on levels of SOD, GPx and MDA in the H9C2 cardiomyocytes cell line.

Keywords: Sappan wood extract, ammonium ferric citrate, H9C2 cardiomyocytes, antioxidant levels

FERMENTATION OF WASTE PATIN FISH (*Pangasius* sp.) BY PROBIOTIC FOR PRODUCING AMINO ACIDS AND ESSENTIAL FATTY ACIDS

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ABSTRACT

The industrial waste of catfish (*Pangasius* sp.) filet cannot be used as animal feed, but its nutritional value is quite high as a medium for the production of amino acids and essential fatty acids. Catfish waste contains 22.96% carbohydrates; 35.81% protein and 12.47% fat that can be optimized through solid state fermentation using probiotics as raw materials for the production of amino acids and essential fatty acids. The probiotics *L. plantarum, L. curvatus, B. subtilis* and their consortium have high proteolytic and lipolytic abilities and are safe to use (Grass). All three are very potential to produce amino acids and essential fatty acids from catfish waste through solid state fermentation. Various fermentation experiments were carried out to analyze the ability of *L. plantarum, L. curvatus, B. subtilis* and their consortium to produce amino acids and essential fatty acids. The results of the analysis of catfish waste fermentation for 84 hours showed the highest increase in amino acid levels by *L. curvatus* by 27.68% (increase in valine 2.43%; L-leucine 4.96%; L-phenylalanine 3.60%; L - arginine 5.89%, L-theronine 3.95%, and L-histidine 2.43%. Catfish Waste Fermentation could not increase the fatty acid levels significantly (not significantly different) although there was the largest increase in fatty acid levels in the fermentation by *L. plantarum* ATCC 8014 by 0.2% oleic acid, 15.03% omega-6 and omega- 3 30.88%. The use of a bacterial consortium is not more optimal than the use of individual probiotics in increasing the content of amino acids and essential fatty acids in the fermentation of catfish waste. The increase and decrease in some amino acids is due to the interconversion of carbohydrates and proteins, and an amino acid can be a precursor in synthesizing other amino acids.

Keywords: Catfish waste, probiotics, solid state fermentation, amino acids, fatty acids

TOXICITY OF DIFFERENT PHASE OF TiO_2 NANOPARTICLES ON *Spirulina platensis* IN THE BRACKISH ENVIRONMENT

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ABSTRACT

Titanium dioxide nanoparticles (NP TiO_2) are materials used in various industrial and consumer applications or products. This extensive use can increase the risk of release of residues into the aquatic environment. Still, its presence in the aquatic environment can disrupt aquatic biotas, such as the microalgae Spirulina platensis. Microalgae are sensitive to changes in the aquatic environment due to the entry of material and are involved in aquatic biota's food chain. Therefore, this study aimed to determine the toxicity effect of titanium dioxide nanoparticles and determine the effect of the type of titanium dioxide nanoparticle phase on the productivity of *Spirulina platensis*. The NP TiO_2 toxicity responses observed were cell biomass, photosynthetic pigments such as chlorophyll, carotenoids, and phycocyanin, morphological changes in algal cells under microscopy, and scanning electron microscopy (SEM) during cultivation. Toxicity of titanium dioxide nanoparticles to *Spirulina platensis*. Showed that the toxicity was influenced by the type of phase and the exposure time. The results of the toxicity test showed that *Spirulina platensis.* Has been shown to interfere with TiO_2 nanoparticle suspension of 100 mg / L during 96 h exposure. Growth of Spirulina platensis. It has been indicated that there is a slowdown after exposure to anatase phase-type of titanium dioxide nanoparticles at 6 hours. mL. Microscopic examination of algal cells interacting with titanium dioxide nanoparticles showed morphological damage. The SEM EDX spectrum shows the attachment of titanium dioxide nanoparticles to Spirulina platensis. This study concludes that titanium dioxide nanoparticles can potentially cause toxicity and disrupt the aquatic environment ecosystem of the microalgae *Spirulina platensis*.

Keywords: Nanoparticles, titanium dioxide, microalgae, *Spirulina platensis*

ANTIBIOTIC RESISTANCE OF LACTIC ACID BACTERIA ISOLATED FROM COW'S MILK

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ABSTRACT

Antibiotic resistance has been one of the threats for the global health because the use of antibiotic to handle bacterial infectious disease has been ineffective again. Nowadays, Non-pathogenic bacteria like lactic acid bacteria have been resistant to antibiotic and it will spread its resistant gene to other bacteria like pathogenic bacteria. The main purpose of this research is to identify resistance lactic acid bacteria isolated from Pangalengan cow milk against antibiotic. Method in this research consisted of isolation and identification of lactic acid bacteria from milk, antibiotic susceptibility test, and gen detection using PCR. Antibiotic used in this research is vancomysin, ciprofloxacine, gentamicin, and ampicillin. The result exhibits if the bacteria isolated from cow milk is Lactobacillus plantarum. The result of antibiotic susceptibility test shows that gentamicin was the antibiotic that was still susceptible to lactic bacteria with the resistance level of just 7%, followed by ciprofloxacine at intermediate level of 35% and resistance at 8%. Vancomycin and ampicillin were antibiotics with the highest resistance rates reaching 57% and 29%, respectively.

Keyword: Antibiotic, lactic acid bacteria, resistance

SIMULATION OF COARSE GRAINED MOLECULAR DYNAMICS TO STUDY THE EFFECT OF PROTEIN STRUCTURE MUTATIONS IN SPIKE SARS-CoV-2 INDONESIAN STRAINS

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ABSTRACT

The Corona Virus Disease 19 (COVID-19) pandemic has attacked all countries in the world including Indonesia and has become a global health issue, after 2 years various types of vaccines have emerged to reduce the spread of COVID-19. However, the virus from COVID-19, namely Severe Acute Respiratory Syndrome Coronavirus 2 or SARS-CoV-2, continues to experience mutations, especially in the Spike protein which plays a role in the entry of the virus into its host. SARS- CoV-2 continues to mutate to give rise to various Variants of Concern such as the Delta variant and the most recent Omicron variant. This study aims to map mutations that exist in Indonesia, to determine the effect of mutations, especially the Delta and Omicron variants on transmibility and neutralizing antibodies. The first step of this research is to model the native Spike protein, delta variant and omicron variant and then simulate the molecular dynamics of Coarse Grained spike protein along with simulation of RBD and ACE2 receptors and neutralizing antibodies from the astrazeneka vaccine. The simulation results show that there is a high fluctuation in the RBD of the Omicron variant due to a mutation in that area which causes increased interaction with the ACE2 receptor but reduces the interaction with neutralizing antibody. The results of this study can provide information regarding the effects of mutations that occur so that they can assist in the development of new vaccine designs.

Keyword: SARS-CoV-2, varian of concern, energy interaction, ACE 2, antibody neutralizing, coarse grained

FERMENTATION OF LACTIC ACID FROM CASSAVA PULP USING COMMERCIAL TEMPE INOCULUMS AS A BIOPLASTIC POLY LACTIC ACID RAW MATERIAL

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ABSTRACT

Lactic acid is an organic acid most widely used in the food, pharmaceutical, cosmetic and chemical industries. One of the important uses of lactic acid is as a raw material for the production of Poly Lactic Acid biopolymers, namely polymers that can decompose by nature in a relatively fast time. Until now, lactic acid production methods are still being developed, including starch fermentation using microbes. Cassava waste is rich in starch, so it can be used as an alternative fermentation medium that is abundant and economical to produce lactic acid using commercial tempeh inoculums. The types of mold in the tempeh inoculum include *Rhizopus oryzae*, *R. oligosporus*, and *R. Stolonifer*. *Rhizopus* has amylolytic properties because it can produce lactic acid from various starch-containing materials without prior saccharification. This study was to see the potential in the input time of lactic acid and determine the optimal conditions for fermentation of tapioca to lactic acid. The reaction kinetics parameters studied were the specific growth rate (μ), the maximum specific growth rate (μm), the yield of biomass to the substrate ($Y_{X/S}$), and the yield of the product to the substrate ($Y_{P/S}$). The analysis was performed using the Response Surface Methodology (RSM) method and the Box Behnken Design (BBD) experimental design with substrate concentration parameters, inoculum concentration, and incubation time on biomass concentration, glucose, and lactic acid. Fermentation was carried out at 30 °C, pH 6.0, and stirring at 150 rpm. HPLC analyzed the lactic acid concentration of the fermentation product. The highest lactic acid yield of 5.04 g/L was obtained under the substrate conditions of 20 g/L, an inoculum with a concentration of 0.30 % w/v, and an incubation time of 72 hours. The specific growth rate (μ) was 0.1218 h^{-1}, the maximum specific growth rate (μm) was 0.1283 jam^{-1}, the maximum specific growth rate (μm) was the highest yield of biomass to the substrate ($Y_{X/S}$) was 0.2429, and the highest yield of lactic acid to the substrate ($Y_{P/S}$) was 0.7026.

Keywords: Lactic acid, cassava waste, tempeh inoculum commercial, RSM-BBD, HPLC

Anti-RBD Single-Chain Variable Fragment (scFv) EXPRESSION IN THE HOST OF *Escherichia coli* BL21 (DE3) AS CANDIDATE BIORESEPTORS FOR COVID-19 ANTIGEN DETECTION

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ABSTRACT

Coronavirus disease 2019 (COVID-19) is a newly emerged human infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In a global pandemic, development of a diagnostic test is necessary to mount an immediate response to this emerging threat. Primary component is diagnostic test is a molecule that specifically recognizes the analyte known as a bioreceptor. Anti-RBD scFv, wich is a bioreceptor candidate produce by this study, acts as an antibody-based immunoassay targets the receptor-binding domain (RBD) of SARS-CoV-2. anti-RBD scFv is engineered to contain cysteine residues on the linker to improve their abilities to be immobilized by metal surface with form well-oriented. In this study, pET-22b(+)-anti-RBD scFv is cloned to Escherrichia coli strain BL21 (DE3), over-expressed to the periplasm as an active protein, purified by an affinity chromatography system, and it is anti-RBD activity is evaluated through Surface Plasmon Resonance (SPR) and Spot Test platform. This study showed that anti-RBD scFv can be cloned onto E. coli BL21 (DE3) and expressed it is protein to the periplasm, characterized by the size of 28 kDa band on SDS-PAGE and dot-blotting. Anti-RBD scFv fused his-tag can be purified using Ni-nanomagnetic beads in denaturing conditions with the highest concentration at 0.01 mg/mL was analyzed by nano-drop spectrophotometer, and has anti-RBD activity to 250 ng/mL and 0.05 mg/mL RBD on SPR system and Spot Test platform.

Keywords: Bioreceptor, anti-RBD scFv, SARS-CoV-2

EARLY DEVELOPMENT OF SELF-ADMINISTERED COVID-19 RAPID TEST BASED ON NUCLEOCAPSID DETECTION IN SALIVA SAMPLE

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ABSTRACT

More than 6,000,000 people died due to the coronavirus (COVID-19) pandemic. This disease has spread quickly due to its highly contagious nature. The transmission of the SARS-CoV-2 virus that causes this disease can be through saliva droplets secreted by infected people when the social distance is less than 1 m. As a result, saliva has been accepted as an alternative specimen for COVID-19 detection by the Centers for Disease Control and Prevention (CDC). Furthermore, WHO recommended using rapid antigen tests based on lateral flow immunoassay when reverse transcription-polymerase chain reaction (RT-PCR) is unavailable. We developed a saliva-based antigen rapid test by optimizing the antibody concentration and optimum pH for the conjugation of antibody and gold nanoparticles. We found the best running buffer formulation consisting of 75 mM sodium phosphate buffer, 1% salt, 1% nonionic surfactant, 0.5% mucolytic agent, and 0.02% preservative. The addition of a mucolytic agent in the buffer can reduce the viscosity of saliva, thus improving sensitivity. The developed rapid test detected the least concentration of nucleocapsid protein at 0.1 μg/mL. Our study discovered 100% specificity against negative COVID-19 saliva and no cross-reaction with avian influenza virus hemagglutinin.

Keywords: SARS-CoV-2; lateral flow immunoassay; self-test; rapid antigen test; Saliva

POLYMORPHISM OF KLF1 GENES IN B-THALASSEMIA AND EFFECTS LEVELS OF HBF LEVELS, HB LEVELS AND BLOOD TRANSFUSION FREQUENCIES

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ABSTRACT

β-Thalassemia is an autosomal recessive inherited red blood cell disorder, a problem that often occurs in cases throughout the world, especially in the 'Thalassemia belt' area. β-Thalassemia is caused by a defect in the β-globin gene due to reduced or absent synthesis of the β-globin chain. This leads to mild or severe symptoms with certain classifications such as dependence on blood transfusions and iron chelating drugs and physical characteristics that cause complications in several other organs. *Genetic modifiers* is an opportunity in the future for the transition of therapy more specifically to people with thalassemia, initiation of progress and clinical trials that are widely triggered there are several candidate genes, one of which is polymorphisms in the *KLF1* gene. The polymorphisms at the *KLF1* were identified the nucleotide positions of c.325C>T and c.304C>T, this may be associated with an increase in HbF levels which in turn can decrease the severity of symptoms in β-Thalassemia. This study is aimed to identify *KLF1* polymorphisms and study their effect on HbF levels and disesease severity in β-Thalassemia patients in Bandung, West Java. Disease severity in this study are presented as level of Haemoglobin (Hb) and frequency of blood transfusion. Fourty two DNA samples of patients with β-thalassemia major and intermedia stored in Pusat Studi Genetik Medis, Faculty of Medicine, Universitas Padjadjaran were used in this study. Allexonsof*KLF1*were amplied and mutation analysis was performed by using Sanger Sequencing. The polymorphisms that were identified were rs117351327 and rs2072597 in nine subjects with MAF values of 0.047 and 0.0059 respectively. In this study we showed that there was no significant difference in HbF levels between b-thalassemia patients with and without *KLF1* polymorphisms. And there is no association of *KLF1* polymorphism with HbF levels, Hb levels and frequency of blood transfusions in patients with -thalassemia with and without *KLF1* polymorphisms. Found Polymorphism *KLF1* RS2072597 and RS117351327 with changes in nucleotides C.325C> T and C.304C> T or (P.Pro109SER and P.Ser102Pro) with a MAF value of 0.047 at RS117351327 and 0.059 in RS2072597. There is no significant difference in HBF levels between people with β-thalassemia and and without *KLF1* polymorphism. In the association value there is no significant difference in *KLF1* polymorphism with HBF levels in people with β-thalassemia. And at the difference in the HB level and the frequency of blood transfusion in people with β-thalassemia with and without polymorphism KLF1 there is no significant difference.

Keywords: β-Thalassemia, *KLF1* polymorphism, HbF level

EFFECT OF TiO_2- ANATASE ON THE VIABILITY CELL OF *Pseudomonas putida* AND *Enterobacter cancerogenus*

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ABSTRACT

TiO_2 nanoparticle (TiO_2 NP) is a promising material commonly used in industrial products, such as paint, medical and cosmetic application. The extensive use of NP products in the industry is inevitable and will also threaten the environment. The releasing NP into the soil and water should be anticipated because of its potential interaction with the organism. This research aims to investigate the effect of TiO_2 NP anatase towards the bacteria cell viability of *Pseudomonas putida* and *Enterobacter cancerogenus*. SEM analysis was used to confirm the involvement of the cell wall in the surface interaction with TiO_2 NP and the accumulation of TiO_2 NP in bacterial biomass. Cell viability was performed with a variety of 3, 6, and 24 hours exposure times. The exposure process was carried out at a temperature of 30^oC with a stirring speed of 150 rpm under light and dark conditions. The TiO_2-anatase NP was used in the 10- 100 ppm concentration range. *Statistical Package for the Social Sciences* (SPSS) software version 22 was used to obtain the effect of time and concentration of TiO_2 NP on the bacteria cell viability. The results showed that the treatment using light during the incubation process affects cell viability of *Pseudomonas putida,* where the exposure time of TiO_2-anatase NP inhibits the growth up to 57.35% within 6 hours while the treatment in a dark environment needs a longer inhibition time which is 24 hours. The cell viability of *Enterobacter cancerogenus*, both on the treatment using TiO_2 NP in light and dark conditions at constant exposure time, has no influence on cell viability.

Keywords: Anatase, bacteria, TiO_2 nanoparticle, cell viability