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Menara Perkebunan as the continuation of De Bergculture published by Algemeen Landbouw Syndicaat/Centrale Proefstation Vereniging since 1926, was published by the Bogor Research Institute for Estate Crops until 1992, based on a letter of the President Director of the Foundation of Research and Education Fund for Estate Crops No.103/JDPP/1967 and a letter of the Head of General Bureau for Research and Planning of the Ministry of Agriculture No.336/Kpts/OP/12/1968. Since 1993 Menara Perkebunan was published by the Indonesian Biotechnology Research Institute for Estate Crops, based on the Decree of the Chairman of the Managing Board of the Indonesian Planters Association for Research and Development No.084/Kpts/ DPH/XII/1992. During the period of 1997-2002 Menara Perkebunan was published by Biotechnology Research Unit for Estate Crops. Referring to a letter of Executive Director of the Indonesian Research Institute for Estate Crops No.05/Kpts/LRPI/2003, since January 2003 Menara Perkebunan has been published by the Indonesian Biotechnology Research Institute for Estate Crops which changed to the Indonesian Research Institute for Biotechnology and Bioindustry in 2015. On August 2022, Indonesian Research Institute for Biotechnology and Bioindustry join with Indonesian Oil Palm Research Institute (IOPRI) become Indonesian Oil Palm Research Institute, Unit Bogor (IOPRI -Unit Bogor)

Menara Perkebunan as a communication medium for research in estate crops publishes articles on original research results, improved technologies, and reviews of biotechnology and bioindustry and its applications in the areas of agriculture, health, environment, and other aspects of biotechnology.

Reviewer Acknowledment of Menara Perkebunan 2024 Volume 92, Number 2 edition

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FOREWORD FROM EDITOR IN CHIEF

Menara Perkebunan Journal as a research communication medium in the plantation sector has entered its 92nd publication edition and continues to present research results that are mandated by the institution, namely biotechnology, both in pre-harvest and post-harvest activities in the plantation industry. In the 2024 edition No.2, the Menara Perkebunan Journal again presents eight research paper titles, 1) Application of lactic acid bacteria to improve the food safety of sago starch, 2) Optimization of the demineralization process of black soldier fly (*Hemertia illucens*) pupa shell maggot chitosan and the physicochemical characteristics, 3) Biosynthesis of silver nanoparticles (AgNPs) from coconut leaf extract and their antifungal activity against *Ganoderma boninense* mycelia, 4) Cloning and expression study of sugarcane (*Saccharum* sp.) sucrose transporter gene (SoSUT4), 5) Callus induction and regeneration of date palm (*Phoenix dactylifera* L.) cv. Zambli through somatic embryogenesis from four layers of young leaves explant, 6) Optimization of fulvic acids production from oil palm empty fruit bunches using microwave extractor, 7) Characterization and morphological development of oil palm transformed-callus on modified culture media, and 8) Extraction of recombinant fatty acid photodecarboxylase-*E.Coli* and its use for biohydrocarbon synthesis.

We hope that with the eight articles presented in this journal, Menara Perkebunan can make a real contribution to the development of biotechnology in the plantation sector in particular and science and technology in Indonesia and International in general.

Editor In Chief

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Menara Perkebunan
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Abstract Sheet

Tryanisa Ridla Amalia, Titi Candra Sunarti & Anja Meryandini

Application of lactic acid bacteria to improve the food safety of sago starch (page 90-102)

Sago starch production in local industries is still carried out traditionally and uses poor-quality water. This production causes sago starch to be fermented spontaneously, resulting in sour sago and possibly contamination by pathogenic bacteria. Lactic acid bacteria (LAB) can produce lactic acid and are suitable for use as a starter. Adding LAB as a starter in sago starch fermentation is expected to reduce the number of pathogenic bacterial growths, thereby increasing food safety in sago starch. This research aimed to obtain LAB and evaluate their use in sago starch fermentation to improve food safety. LAB selection was conducted by testing the LAB tolerance ability to low pH and the adaptability of the LAB growth in sago starch. This study was carried out using and without a LAB liquid starter. The water source during the fermentation originated from drinking water and the sago starch industrial factory. The fermentation was carried out for ten days at room temperature with an observation every two days. The results showed that fermented sago starch using drinking water did not harbor *E. coli*, *Salmonella*, or *Shigella* bacterial contamination. In contrast, sago starch fermented using water from the factory harbored these bacterial contaminations. Adding LAB IL1 isolate as a starter in fermentation showed the ability to reduce the number of pathogenic bacteria in sago starch.

[Keywords: drinking water, *E. coli*, fermentation, lactic acid, *Salmonella* sp.]

Mira Maulidina, Muhammad Rifqi & Siswanto

Optimization of the demineralization process of black soldier fly (*Hemertia illucens*) pupa shell maggot chitosan and the physicochemical characteristics (page 103-111)

Chitosan is a derivative compound of chitin that has undergone deacetylation. Chitosan has three stages of the manufacturing process, including demineralization, deproteinization, and deacetylation. Chitin is also found in the black soldier fly maggot pupae, but maggot pupae contain high minerals content that can affect the purity of the resulting chitosan. Therefore, demineralization treatment is necessary to remove minerals from maggot pupae shells. This study aims to optimize the demineralization process by finding the best type of acid solvent, the best incubation time, and combination treatments. The black soldier fly (BSF) maggot pupa shell was soaked using various formic acid, hydrochloric acid, and nitric acid solutions with incubation times of 60, 120, and 180 minutes. Chitosan characterization was carried out following SNI 7949:2022, including water content, ash content, nitrogen content, pH, deacetylation degree, characterization of functional groups with FT-IR, and as an antimicrobial comparison is formalin. The best demineralization treatment was obtained at 0.5 M nitric acid treatment with an incubation time of 120 minutes. The characterization of chitosan produced 7.81% water content, 0.56% ash content, 4.73% nitrogen content, pH 7.39, and 75.14% deacetylation degree. Characterization of groups on chitosan with FT-IR resulted in the absorption of O-H and N-H groups 3484 cm^{-1} and 3152 cm^{-1}; C-H 2877 cm^{-1}; and C=O 1653 cm^{-1}. The inhibitory power against *E. coli* of the BSF maggot pupa shells chitosan is better compared to chitosan standard but not better than formalin.

[Keywords: antimicrobial, BSF maggot puppa shell, characterization, chitosan, demineralization]

Gusti Ayu Dewi Lestari, I Gusti Ayu Made Megayanti, Komang Ayu Astuti Maharani & Ni Wayan Nieskajanti Kedeh Van Kempen

Biosynthesis of silver nanoparticles (AgNPs) from coconut leaf extract and their antifungal activity against Ganoderma boninense mycelia (page 112-122)

Basal stem rot disease caused by *Ganoderma* is a major problem in palm cultivation in Indonesia, so an appropriate solution is needed to overcome this problem. One of the solutions that can be applied is through silver nanoparticles. Synthesis of silver nanoparticles can use coconut leaves as a source of flavonoids. Flavonoids are one of the phenolic compounds that are widely used in the process of synthesizing silver nanoparticles. Flavonoid compounds have a hydroxyl group (OH), which can be reduced by donating electrons to the Ag^+ ion from $AgNO_3$ to Ag^0. This research aims to synthesize silver nanoparticles (AgNPs) using coconut leaf water extract and its assessment as an antifungal for *Ganoderma boninense*. Silver nanoparticles were synthesized by mixing coconut leaf extract in 1 mM of $AgNO_3$ solution with a ratio of 1:9 (v/v). The concentrations of coconut leaf water extract were 1% and 3% with a heating temperature of 60°C. Silver nanoparticles were characterized using a UV-Vis spectrophotometer, PSA, FTIR, TEM, and SEM-EDS. The AgNPs had a maximum wavelength of 430 nm, with morphologies like a ball, triangle, and square, a mass percentage of Ag of 51.77%, smallest particle size of 63.29 nm, PdI value of 0.3361, and a zeta potential of -16.98 mV. The FTIR spectra show that the functional group that plays a role in the reduction process is the –OH group. The antifungal activity

assay produced the highest percentage of colony growth inhibition (68.37%) at a concentration of 4.9 ppm in the 10-day incubation.

[Keywords: antifugal, Bali, characterization, coconut leaf waste]

Rani Nur Fitriani, Dwi Andreas Santosa & Miftahudin

Cloning and expression study of sugarcane (*Saccharum* sp.) sucrose transporter gene (SoSUT4) (page 123-130)

Sugarcane (*Saccharum sp*.) is a vital commodity for global sugar production and biomass generation,with sucrose being the primary sugar accumulated predominantly in the stem. The sucrose transporter protein is essential in facilitating sucrose transportacross cells and over longdistances within plants, from source to sinktissues. This study focused on the cloning and expression analysis of the SoSUT4 gene in the Bululawang sugarcane variety. A partial coding sequence of SoSUT4, comprising 802 nucleotides and encoding a 267-amino acid protein, was successfully cloned and sequenced. Sequence analysis revealed that the SoSUT4 protein shares high similarity with other SUT4 proteins in monocotyledonous plants, particularly with *Saccharum spontaneum* and Saccharum hybrid. Bioinformatics predictions indicated that the SoSUT4 protein is localized to the plasma membrane and contains six transmembrane helices. Gene expression analysis further demonstrated that SoSUT4 expression was significantly higher in the middle internodes of the stem compared to the youngest midsection of the leaves. This expression pattern correlates with higher sucrose accumulation in the stem, as reflected by elevated Brix levels in the stem (19.61%) compared to the leaves (19.48%). This finding suggests that SoSUT4is essential for sucrose translocation to the stem, which serves as the primary storage site forsugar.The study provides valuable insights into the SoSUT gene family in sugarcane, particularly highlighting the role of SoSUT4 in sugar transport and accumulation. Future research should further investigate the underlying mechanisms of SoSUT4 and related genes to enhance our understanding of their impact on sugarcane yield, with potential applications for genetic engineering aimed at improving crop productivity.

[Keywords: brix, relative expression, SoSUT4]

Masna Maya Sinta, Rizka Tamania Saptari, Imron Riyadi, & Sumaryono

Callus induction and regeneration of date palm (*Phoenix dactylifera* L.) cv. Zambli through somatic embryogenesis from four layers of young leaves explant*e* (page 131-140)

The Zambli variety of date palm shows potential for cultivation in tropical regions, as its fruits are edible during the Rutab stage. However, large-scale production of Zambli seedlings presents a significant challenge. In vitro propagation offers a solution for producing large quantities of clonal planting material. This study focuses on inducing callus formation from the four-layered shoot tips of young leaves and regenerating these calli into plantlets through somatic embryogenesis. Explants were cultured on a modified MS medium with 10, 50, or 100 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), combined with 1 or 3 mg L^{-1} N6-(2-isopentenyl)adenine (2-iP). Embryo maturation was performed on the same medium without 2,4-D, while a hormone-free medium was used for plantlet regeneration. The results indicated that the highest callus induction occurred from the younger leaf layer (layer 1) in the medium containing 100 mg L^{-1} 2,4-D and 1 mg L^{-1} 2-iP, achieving a callus formation rate of 82.3%. Successful callus induction was achieved from the first, second, and third layers of young leaves. Somatic embryo maturation and plantlet regeneration were also completed, producing vigorous, well-rooted plantlets. Additionally, the development of date palm cv. Zambli in vitro culture through somatic embryogenesis was confirmed through histological analysis.

[Keywords: 2,4-D, embryogenic callus, in vitro propagation]

Firda Dimawarnita, Khairy Yunda Maharani, Yora Faramitha, Donny Nugroho Kalbuadi, Haryo Tejo Prakoso, Indah Puspita Sari, Dedy Prasetyo, Sutanto Sutanto & Didiek Hadjar Goenadi

Optimization of fulvic acids production from oil palm empty fruit bunches using microwave extractor (page 141-152)

Fulvic acid (FA) derives from a non-renewable source, Shilajit, known as highly commercial values for its benefit for human health. Fulvic acid can also be extracted from materials such as coal, lignite, and peat. Extraction methods of FA generally use solid acids and bases, ion exchange chromatography, and their combinations. However, these methods cause corrosion, low purity, and environmental pollution. The FA extraction using organic solvents is common, but low yielded, and many organic solvents are toxic. Therefore, an effective way to separate organic solvents from FA must be determined. This research aims to extract the FA from renewable biomass, namely oil palm empty fruit bunches (OPEFB), using a microwave extractor combined with hydrogen peroxide. The advantage of using a microwave is its quick and efficient extraction process. Hydrogen peroxide is an environmentally friendly solvent that can be converted into water and oxygen. Fulvic acid extraction was optimized using expert design with the Response Surface Methodology method with optimization of four 4 factors (H_2O_2 concentration and volume, reaction time, and microwave power). The extracted FA was then characterized using FTIR, H-NMR, and Fluorescennce spectroscopy. The highest FA concentration namely 24.716%, was obtained using H_2O_2 at a concentration of 30.46% with a volume of 137.4139 mL, reaction time of 9.384 minutes, and microwave power of 351.39 W. *Fourier-Transform Infrared Spectroscopy* peaks at 3213 cm^{-1}, 2935.47 cm^{-1}, and 2825.13 cm^{-1} in the OPEFB-FA sample indicate existence of FA. The fluorescent emission intensity ratio between 450/500 nm wavelengths of OPEFB-FA was 0.719.

[Keywords: FTIR, hydrogen peroxide, H-NMR, humic substance, spectrofluorosence]

Yuli Setiawati, Imron Riyadi, Dini Astika Sari, Rizka Tamania Saptari, Masna Maya Sinta, Hayati Minarsih, Turhadi & Riza Arief Putranto

Characterization and morphological development of oil palm transformed-callus on modified culture media (page 153-161)

Genome editing through cisgenesis develops into scientific breakthroughs in accelerating oil palm breeding programs. However, one remaining problem is the low success of transformed-calli regeneration, while its scientific explanation is still underexplored. This study aimed to characterize and regenerate transformed-calli using various amino acids and antioxidants. Transformed callus that did not regenerate (un-regenerated transformed callus or UTC) after the transformation process was taken, then T-DNA integration was detected using the *NPTII* gene. Furthermore, the UTC was divided into four types based on morphological characteristics. The four types of UTCs were regenerated on media enriched with glutamine (for Type-1 callus), cysteine and putrescine (for Type-2 callus), and a combination of cysteine and ascorbic acid (for Type-3 and Type-4 callus). The research results obtained *NPTII* successfully amplified with a band size of 700bp. The results showed that on Type-1 callus, enrichment media with 10 mg L^{-1} L-glutamine could induce the formation of new nodular structures on UTC Type-1. On Type-2, media enriched with 5 mg L^{-1} L-cysteine + 20 mgL^{-1} putrescine increased the density of callus structures. Media enriched with 25 mg L^{-1} ascorbic acid + 25 mg L^{-1} L-cysteine could prevent the spread of brown callus on Type-3 callus, while Type-4 callus did not show any response and became dry. Our new findings will facilitate the basic research and unregenerated transformed callus and morphological callus development behavior in oil palm.

[Keywords: oil palm, plant development, transformed callus]

Irma Kresnawaty, Farhan Palgunadi, Yora Faramitha, Kenny Lischer, Ayu Rahayu Saraswati, Fauziatul Fitruyah, & Djoko Santoso

Extraction of recombinant fatty acid photodecarboxylase-*E.Coli* and its use for biohydrocarbon synthesis (page162-171)

The use of fossil fuels still becomes a problem of unsustainable and environmental issues, so it is crucial to use renewable energy. Biohydrocarbons as renewable energy, could be generated from biomass that contains fatty acids and produce compounds such as alkanes and alkenes. *Chlorella variabilis* Fatty Acid Photodecarboxylase (*Cv*FAP) enzyme from *E. coli* recombinant is a remarkable recent technique for producing bio-hydrocarbons. According to extensive studies, this enzyme can change free fatty acids when induced by blue light and accompanied by the addition of substrates. This research aims to synthesize bio-hydrocarbons that focus on the enzyme activation process with variations in protein concentration, activation time, and the type of substrate CPO compared with palmitic acid regarding bio-hydrocarbon concentration produced. The research focuses on cloning the synthetic gene of *Cv*FAP in *E. coli* and the *Cv*FAP enzyme produced was used to convert palmitic acid to pentadecane, which was measured using Gas Chromatography. This study has confirmed the potency of producing bio-hydrocarbons in the form of pentadecane with a yield of 16.44%. The synthesis of pentadecane can run optimally using the light activation method with several substantial factors needed in the activation process, namely the optimum growth medium for TB, protein volume of 1777.5 μL, activation time of 3 hours, and a substrate preference of 50% CPO.

[Keywords: biocatalyst, cap, light-driven enzyme, recombinant protein, renewable energy]

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