VOLUME 12 NOMOR 1 JUNE 2025 ISSN 2548 – 611X

JURNAL
BIOTEKNOLOGI & BIOSAINS INDONESIA

Homepage Jurnal: http://ejournal.brin.go.id/JBBI/index

THE DIVERSITY AND CHARACTERISATION OF CELLULOLYTIC BACTERIA ISOLATED FROM VARIOUS CONDITIONS OF EMPTY FRUIT BUNCHES IN OIL PALM PLANTATION

Keragaman dan Karakterisasi Bakteri Selulolitik yang Diisolasi dariBerbagai Kondisi Tandan Kosong Kelapa Sawit di Perkebunan Kelapa Sawit

Edo Agam Pamungkas[1], Sylvia Madusari[1*], Halida Adistya Putri[1], Risa Rosita[2]
[1]Production Technology of Plantation Crop, Politeknik Kelapa Sawit Citra Widya Edukasi
Jl. Gapura No.8 Rawa Banteng, Cibuntu, Cibitung, Bekasi, West Java, Indonesia
[2]Science Innovation Technology Department, Southeast Asian Regional Centre for Tropical Biology (SEAMEO BIOTROP) Jl. Km 6 Tajur, Bogor, West Java, Indonesia.
*e-mail: smadusari@cwe.ac,id

ABSTRACT
The utilisation of oil palm empty fruit bunches (EFB) remains underexplored. Harnessing cellulolytic microorganisms for the production of cellulase enzymes offers sustainable approach to addressing waste management challenges while aligning with the principles of the Sustainable Development Goals (SDGs) and address waste management challenges. This study aims to isolate, characterize, identify, and test the potential cellulase activity of cellulolytic bacteria from EFB taken from three different locations: PO code from organic fertilizer plantations (POU1, POU2, POU3), PL code from oil palm plantations (PLU1, PLU2, PLU3), and PK code from Sulung mills (PKU1, PKU2, PKU3). This study used three isolated cultures in its testing. The research process includes sample preparation, bacterial isolation, gram staining, catalase test, hypersensitivity test, DNA amplification, bioinformatics analysis and cellulase activity analysis. The results of the bacterial isolation obtained 28 colonies. The results of the characterisation were all 3 non-pathogenic bacterial isolates, with a positive catalase test. The result of staining Gram-negative with bacilli-shaped bacteria. The amplification results obtained a band size of 1500 bp. The results of the identification obtained the species *Aeromonas enteropelogenes, Nitrosomonas stercoris, and Methylobacillus caricis*. The results of phylogenetic analysis showed low homology. Cellulase activity of six positive isolates with medium ability isolates code POU3 (1.3), PLU2 (1.0), PLU3 (1.0); low isolates POU1 (0.2), POU2 (0.2), PLU (0.8) and 3 negative isolates no enzyme activity PKU1 (-1), PKU2 (-1), and PKU3 (-1).

Keywords: *Cellulase Enzyme, Cellulolytic Bacteria, DNA Barcoding, Molecular Identification, Empty Fruits Bunches (EFB)*

ABSTRAK
Pemanfaatan limbah tandan kosong kelapa sawit (TKKS) masih terbatas, pemanfaatan bakteri selulolitik sebagai sumber penghasil selulase menjadi solusi permasalahan pengelolaan limbah dan bentuk penerapan *Sustainable development goals* (SDGs). Penelitian ini bertujuan untuk mengisolasi, mengkarakterisasi, mengidentifikasi dan menguji potensi aktivitas selulase bakteri selulolitik asal TKKS yang diambil dari tiga lokasi berbeda: kode PO asal kebun pupuk organik (POU1, POU2, POU3), Kode PL asal kebun sawit (PLU1, PLU2, PLU3), dan Kode PK asal pabrik Sulung (PKU1, PKU2, PKU3). Penelitian ini menggunakan tiga kultur isolat dalam pengujiannya. Proses penelitian meliputi preparasi sampel, isolasi bakteri, pewarnaan gram, uji katalase, uji hipersensitivitas, amplifikasi DNA, analisis bioinformatika dan analisis aktivitas selulase. Hasil iisolasi bakteri mendapatkan 28 koloni. Hasil karakterisasi seluruh isolat bakteri

***Received**: 7 March 2025* ***Accepted**: 6 May 2025* ***Published**: 7 June 2025*

nonpatogen, dengan uji katalase positif. Hasil pewarnaan gram negatif dengan bakteri berbentuk basil. Hasil amplifikasi mendapatkan pita ukuran ±1500 bp. Hasil identifikasi mendapatkan spesies *Aeromonas enteropelogenes*, *Nitrosomonas stercoris*, *Methylobacillus caricis*. Hasil analisa filogentik menunjukan homologi yang rendah antara spesies. Aktivitas selulase enam isolat positif memiliki aktivitas selulase dengan kemampuan sedang isolat kode POU3 (1.3), PLU2 (1.0), PLU3 (1.0); rendah isolat POU1 (0.2), POU2 (0.2), PLU1 (0.8) dan 3 isolat negatif tidak memiliki aktivitas enzim PKU1 (-1), PKU2 (-1), dan PKU3(-1).

Keywords: *Enzim Selulase, Bakteri Selulolitik, DNA Barcoding, Identifikasi Molekuler dan Tandan Kosong Kelapa Sawit (TKKS)*

INTRODUCTION

Sustainable development goals (SDGs) are activities to achieve sustainable development. SDGs have 17 pillars (development programs) carried out by all United Nations member countries to attain community welfare and environmental sustainability (Khairina et al., 2020). The pillars of the SDGs related to this study are pillar 9 (industrial infrastructure and innovation) as a form of innovation in the palm oil plantation industry, pillar 12 (responsible consumption and production) as a form of responsibility for palm oil waste production, and pillar 15 (maintaining terrestrial ecosystems) as an action to prevent environmental pollution in maintaining terrestrial ecosystems. Palm oil is an industry with rapid development; the total area of oil palm plantations in 2022 reached 15.34 million/ha, consisting of 40.51% of smallholder oil palm plantations, 3.57% of large state plantations, and 55.92% of large private plantations (BPS, 2023). The high level of consumption of palm oil and palm oil derivative products influences the development of the palm oil plantation industry. It affects the availability of empty oil palm bunches (EFB) in line with the development of the palm oil industry. The total production of EFB waste from processed Fresh Fruit Bunches is around 22-24% of the total weight produced in palm oil mills, currently the utilisation of EFB is still very limited. The use of EFB, which is commonly used as biochar, mulch, or compost, can increase plant growth by 49.2%, and EFB can be used as a soil improvement material to improve soil properties and increase crop yields (Adu et al., 2022)

EFB waste is an abundant biomass and is still rarely utilised. The use of cellulolytic bacteria from empty oil palm bunches as a source of cellulase production is a solution to the problems of waste management, the environment and the implementation of the SDGs pillars. The use of EFB waste is still very limited and less than optimal; the decomposition process takes 6-12 months (Kurniawan et al., 2021). Decomposition is influenced by microorganisms that can decompose organic waste into organic fertiliser. One of the microorganisms that plays an important role in the decomposition process is cellulolytic bacteria. Suryaningrum and Samsudin (2018) stated that cellulolytic bacteria are bacteria that can hydrolyse materials containing cellulose, degrading complex molecules in insoluble substrates using enzymes to break bonds at different locations in the substrate. The enzyme produced is cellulase which plays a role in the hydrolysis of cellulose into simpler products, namely glucose. Cellulolytic bacteria can be found in palm oil industry waste, namely in piles of oil palm leaves, fronds and empty oil palm bunches. Murtiyaningsih and Hazmi (2017); Arifin et al. (2019) reported that these bacteria are found in agricultural land, forests, organic compost, decaying plants, and dry leaf litter. Previous research related to the isolation and characterisation of cellulolytic bacteria from various samples including soil, bagasse, vermicomposting EFB, and cow rumen. These bacteria have a relatively faster growth rate compared to other microbes, which has an impact on good time efficiency when producing cellulase (Yusnia et al., 2019). Cellulase is used in various industrial fields such as food technology, textiles, animal feed, paper, and agriculture. (Seprianto, 2017).

According to Azizah et al, (2013), Screening of cellulolytic bacteria from

vermicomposting can accelerate the composting process in 2-3 months, based on enzyme activity measurements obtained from 21 isolates having cellulolytic activity from 51 isolates of TKKS vermicomposting bacteria. Selpani, (2015) succeeded in identifying bacteria from Situ Gede soil using the 16s rDNA gene to obtain the species *Cellvibrio fibrivorans* and *Cellvibrio gandavensis*, which had a maximum rate of enzyme activity on the seventh day. The genus of bacteria capable of degrading cellulose includes the genera *Nisseria, Micrococcus, Bacillus, Pseudomonas, Flavobacterium, and Actinobacillus* (Fauziah and Ibrahim, 2020). However, exploration of cellulolytic bacteria diversity is still lacking. Thus, this experiment aims to determine the identity of the species and genus of EFB cellulolytic bacteria and to determine the cellulase activity in EFB so that it can be utilised further. These findings are expected to answer the availability of EFB waste.

MATERIALS AND METHODS

This research was conducted in February – November 2024, and it isolated and characterised cellulolytic bacteria at the Sulung Research Station (SRS) -Citra Borneo Indah (CBI GROUP), PT. Sawit Sumbermas Sarana Tbk, Pangkalan Bun, Central Kalimantan. The multiplication, biochemical test, and purification of bacterial isolates were carried out at the Microbiology Laboratory of the Citra Widya Edukasi Palm Oil Polytechnic, Bekasi, West Java. Molecular Identification was carried out at the Biotechnology Laboratory and Silviculture Laboratory of the Southeast Asian Regional Centre for Tropical Biology (SEAMEO BIOTROP), Bogor, West Java.

The tools used in this study were oven, centrifuge, orbital shaker, incubator, autoclave, biosafety cabinet, hot plate, vortex, analytical balance, spatula, petri dish tweezers, pyrex glassware, spirit burner, ose needle, tube rack, vortex, 1.5ml tube, 2ml micro tube, micro pipette with sizes (2; 10; 200; 100 and 1000 µL), micro centrifuge, heating block, freezer, analytical balance, 250 ml Erlenmeyer flask, microwave, chamber, comb, electrophoresis set, UV transilluminator box, thermocycler (PCR Machine). The materials used in this study were samples of EFB from the SRS organic fertiliser plantation (PO); PT. SSMS oil palm plantation (PL); Sulung Factory (PK), bunsen, plastic wrapping, congo red, samples of empty oil palm bunches, Dipotassium phosphate (K_2HPO_4), Monopotassium phosphate (KH_2PO_4), Ammonium sulphate (NH_4SO_4), Magnesium sulfate heptahydrate ($MgSO_4.2H_2O$), Calcium chloride dihydrate ($CaCl_2$), Sodium chloride (NaCl), Yeast extract, Carboxyl methyl cellulose (CMC, Avicel, Guaiacol), aquadest, aquabidest (ddH2O), agar, agarose, primer 27F, primer 1492R, PCR MIX 2x My Tag HS red mix (Bioline, US), TAE Buffer 1x, Florosafe DNA stain. The Stages carried out in this research on Figure 1.

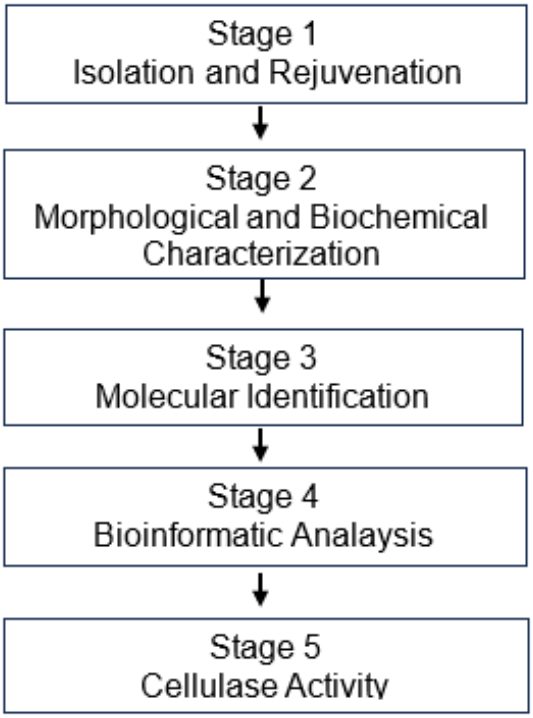

Figure 1. Research Stages

Isolation and Rejuvenation

The cellulolytic bacteria were isolated from three different locations: organic fertiliser plantation (PO), oil palm plantation (PL), and Sulung factory (PK) from SRS. The media used in isolation and rejuvenation was selective media for cellulolytic bacteria Carboxy Methyl Cellulose (CMC). Isolation was carried out by taking 100 µL to be inoculated on the press using the pour plate technique and incubated at a temperature of 31℃ for 48 hours. The three isolates were rejuvenated using solid media for 24 hours,

and then the three isolates of rejuvenation were inoculated into liquid media for 24 hours while stirring using a shaker at 150-200 rpm. The results of the isolation of bacteria that grew on selective media for cellulolytic bacteria with dilution using NaCl were calculated using a Colony Counter.

Morphological and Biochemical Characterisation

Gram Staining

Gram staining was conducted by applying 1 loop of bacterial colonies to a slide and fixing/heating with a Bunsen burner, then dropping gentian violet dye and leaving for 5 minutes. Lugol dye and leave for 2 minutes. Safranin dye and leave for 2 minutes, add 1-2 drops of 95% alcohol (here using 70% alcohol). In each solution before dropping another solution, the sample is rinsed with distilled water. After that, drop a little immersion oil and observe using a microscope with a 100x magnification lens.

Catalase Test

The catalase test was conducted by taking 1 colony using an ose, then flattened on a glass preparation and dripped with 3% H_2O_2. The results of the catalase test are divided into positive and negative. A positive catalase test is indicated by the appearance of bubbles on the isolate that has been dripped as a form of reaction. The hydrolysis process is based on the reaction of the decomposition of H202 into H20 and O2, which characterizes the appearance of bubbles when positive (Chusniasih et al., 2023).

Hypersensitivity Test

The hypersensitivity test is achieved by injecting 500 µL of isolates into liquid culture into tobacco leaves aged ± 3 months. The injection is done using a sterile needle, and the leaves are incubated and observed for 48 hours after the injection.

Molecular Identification

DNA Amplification

Amplification was carried out directly on the pellet without DNA extraction using PCR (thermocycler). In DNA isolation and amplification, pure isolates were cultured in selective liquid media for 24 hours using an orbital shaker with stirring at 150 rpm. A total of 1 mL of bacteria in liquid culture was centrifuged for 3 minutes at a speed of 7,500 rpm. The resulting pellets were used for DNA extraction (Rosita, 2021). The molecular marker used by Sharma et al, (2020) was 16S rRNA, such as 27F (5′- AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′- GGT TAC CTT ACG ACT T-3′). PCR reaction was performed in a total volume of 50 µl, using PCR MIX My Tag HS red mix with 5 µl DNA template, 2 µl forward and reverse primers, 25 µl 2x My Tag HS red mix, and 16 µl nuclease-free water. The PCR program were performed in initial denaturation at 95 °C for 1 minute 30 seconds, further denaturation at 95 °C for 30 seconds, annealing process at 50 °C for 30 seconds, extension process at 72 °C for 1 minute 30 seconds and final extension at 72 °C for 5 minutes, the amplification process was carried out for 30 cycles (Rosita, 2021) The amplification results were visualized using 100V DC electrophoresis technique for 45 minutes.

Bioinformatic Analysis

The PCR product from the amplification that has been obtained is then read through the DNA nucleotide sequence (Sequencing) with the help of PT. Genetika Science. The sample sequence by DNA Sequencing sample qualification by agarose gel. The sequencing data is then subjected to bioinformatics analysis to identify species and reconstruct phylogenetic trees. The analysis to identify the species using the Bioedit and the BLASTN (Basic Local Alignment Search Tool Nucleotide) program on the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and the Mega11 software applications to reconstruct the phylogenetic trees.

Cellulase Activity

The method is used to measure cellulase enzyme activity qualitatively through cellulolytic testing. Measurements are made by measuring the clear zone around the colony on selective media that has been given a Congo red reagent. The testing stages started based on namely by making selective bacterial media using 0.5% CMC (Carboxy Methyl Cellulose) media by inoculating pure isolates in the middle of the media using an ose (Syukri et al., 2021; Chusniasih

et al., 2023). Testing was carried out on each bacterial isolate (PO, PL, PK) with 3 repetitions, with a total of 9 experimental units. The ability of cellulolytic bacteria to degrade cellulose is indicated by a clear zone, to clarify the clear zone, 0.1% Congo red reagent was given, soaked for 15 minutes, then rinsed with 5% NaCl, then left for 15 minutes, and the liquid was discarded. The reaction of giving Congo red will show a clear zone, to clarify the clear zone formed, incubate for 48 hours at a temperature of 30℃. The diameter of the bacterial colony and the diameter of the clear zone formed are measured using a ruler (mm). Qualitatively, the larger the diameter of the enzyme produced by the cellulolytic bacterial colony, the greater the amount of enzyme produced by the bacteria (Alkahfi et al., 2021).

The formula for calculating cellulase activity through the clear zone, colony, and cellulolytic index is as follows:

1. The formula is used to calculate DB (Clear Zone Diameter):

$$DB = \frac{\text{DB vertical+ DB horizontal}}{2}$$

2. The formula is used to calculate DK (Colony diameter):

$$DK = \frac{\text{DK vertical+DK harizontal}}{2}$$

3. The formula is used to calculate IS (Cellulolytic Index):

$$IS = \frac{\text{Clear zone Diameter (DB)-Colony Diameter (DK)}}{\text{Colony Diameter (DK)}}$$

Description:
DB = Clear zone diameter
DK = Colony diameter
IS = Cellulolytic Index

The results of the clear zone measurement can be seen from the amount of enzyme produced through the comparison between the diameter of the clear zone and the diameter of the colony, the further the distance of the edge of the clear zone from the edge of the colony means the enzyme produced by the bacteria is also greater. The classification of cellulose degradation power is based on the cellulolytic index value, with a measurement value of ≤ 1 included in the low category, 1-2 included in the medium category and ≥ 2 included in the high category (Alkahfi et al., 2021).

RESULTS AND DISCUSSION

Isolation, Rejuvenation and Characterisation

The results of cellulolytic bacterial isolation using TKKS samples from 3 different locations showed high microorganism activity, obtaining 300+ colonies of total colony count in 10^{-7} dilution, inoculated with 100µL. Purification was carried out until three isolates were obtained that represented the origin of the isolation location: organic fertiliser plantation (PO), oil palm plantation (PL), and Sulung factory (PK) in SRS. The bacterial isolates were then subjected to a hypersensitivity test, which showed that the three isolates were non-pathogenic and were positive for hydrolysis of H_2O_2 based on the catalase test carried out. The results of the isolation and characterisation of bacterial isolates can be seen in Table 1.

Table 1. Characterisation Results of the three isolates include Colony Description, Morphological and Biochemical characteristics.

Isolate Code	Sampele Origin	Colony Count	Characterization of Three Isolated							
			Colony Description			Morphological			Biochemical	
			Colour	Shape	Elevation	Margin	Shape	Gram	Catalase	Hypersensitivity
PO	Organic fertilezer plantati	300	White	Circular	Raised	Like wool	Bacilli	Negative	Positive	Negative
PL	Oil palm plantation	300	White	Circular	Raised	Like wool	Bacilli	Negative	Positive	Negative
PK	Sulung mills	300	White	Circular	Raised	Like wool	Bacilli	Negative	Positive	Negative

DNA Amplification

The amplification result exhibited that the three samples had a thick and clear single band with a length of 1500 bp (Figure 2), as expected from the selected molecular marker. The amplicons from the three samples had good quality and showed that bacterial DNA amplification without the DNA isolation process using 16S rRNA primers was successful. Bacterial DNA amplification was carried out using the Polymerase Chain Reaction technique with primer pairs 27F and 1492R, then the sequencing process could be carried out.

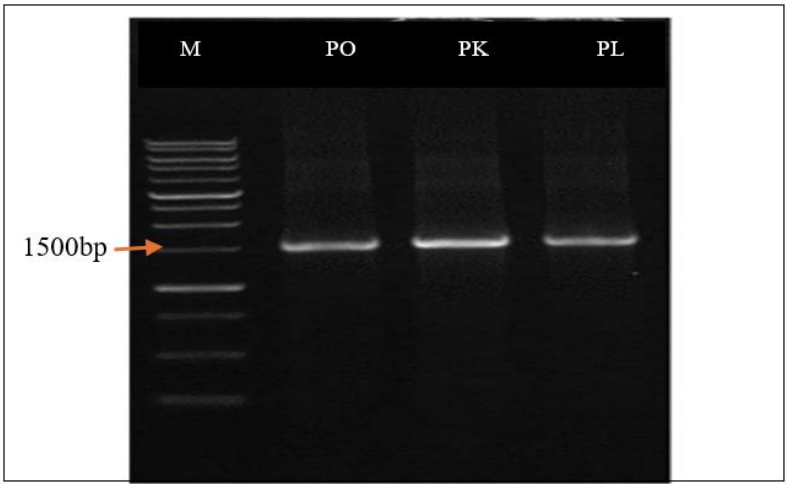

Figure 2. Electrophoregram of the results of DNA isolation of cellulolytic bacteria, without the DNA isolation process, with a single band 1500 bp long.

BIOINFORMATIC ANALYSIS

Species Identification

The sequence data was then matched with the database using the BLASTn program to determine the level of homology. The BLAST results in Table 2 show that the 3 cellulolytic bacterial isolates have varying levels of homology, and the three isolates identified <97% have similarities with the bacterial genus on the NCBI website. The PK code isolate has a maximum identity value of 80.43%, included in the Aeromonas enteropelogenes, code PL genus Nitrosomonas stercoris with a maximum identity value of 72.14%, and the PO isolate is identified as Methylobacillus caricis with a maximum identity value of 68.82%. Table 2. Results of the identification of Cellulolytic Bacterial Species.

Table 2. Results of the identification of cellulolytic bacteria species.

Code	Type of Samples	BLAST Results			
		Top Species	*Query Cover* (%)	*Perc. Identity*	*Accession*
PO	Organic fertilizer plantation	*Methylobacillus caricis*	83%	68.82	NR_179144.1
PL	Oil palm plantation	*Nitrosomonas stercoris*	41%	72,14	NR_146824.1
PK	Sulung factory	*Aeromonas enteropelogenes*	21%	80,43	NR_044846.1

The species *Methylobacillus caricis* belongs to the genus *Methylobacillus. Methylobacillus* is a genus and species of obligate methylotrophic bacteria, with gram-negative rods (Yordy and Weaver, 1977). This bacterium was found from isolation results in the area around the roots of nutsedge (Agafonova et al., 2017). The *Nitrosomonas stercoris* species was found by Nakagawa and Takahashi (2015), who isolated it from composted cow dung as a gram-negative bacterium capable of oxidizing *Chemoautotrophic ammonia*, which is tolerant to high ammonium. *Aeromonas enteropelogenes* is a species of gram-negative bacteria found and first identified in human faeces in India (Schubert et al., 1990). Faeces are livestock waste containing complex organic materials, which can be processed as compost through a decomposition process influenced by microorganisms (Hidayatulloh et al., 2022). Mahendra (2022) identified cellulolytic bacteria from horse faeces (Equus caballus) using Bergey's Manual of Systematic Bacteriology, the 9th edition obtained the genus Fibrobacter and Bacillus. Based on the results of the molecular identification carried out, these species had similarities to cellulolytic bacteria from TKKS <97%, which means there is potential to obtain new species of cellulolytic bacteria. Species with <97% similarity are said to be different species (novel species) but are said to be the same species if they are 99% similar (Stackbrant and Gobel, 1994; Petti, 2007). The limited studies related to cellulolytic bacteria from TKKS have an impact on the low percentage of bacteria identified and the absence of data sequences in the NCBI gene bank.

Phylogenetic Tree

The phylogenetic tree was reconstructed based on the identified cellulolytic bacterial isolates by comparing the identified bacterial sequences in the NCBI database. The phylogenetic tree is shown in Figure 3. The results of the phylogenetic tree showed that the bacterial species *Nitrosomonas stercoris NCBI* and *Aeromonas enteropelogenes NCBI* have 96% homology with *Methylobacillus caricis NCBI. Methylobacillus caricis NCBI* has 94% similarity with the species *Methylobacillus caricis PO*, with 20% similarity to the species *Aeromonas enteropelogenes PK,* and 13% similarity to the species *Nitrosomonas stercoris PL.* The result indicated that it has the potential to get new species of cellulolytic bacteria from EFB; however, further related studies are required to explore cellulolytic bacteria from EFB.

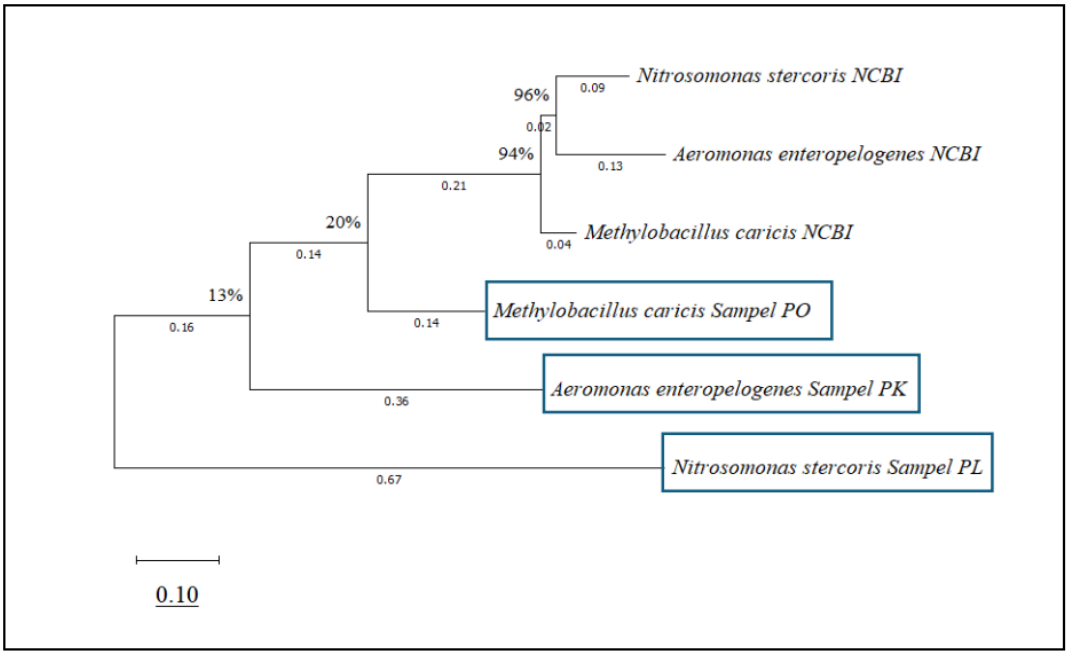

Figure 3. Phylogenetic tree construction. Bacterial isolates with NCBI gene bank data.

Cellulase Activity

The results of the clear zone measurement were carried out by calculating the cellulolytic index and classification based on Alkahfi et al., (2021) the greater the enzyme produced, the more the clear zone produced, the classification of cellulose degradation power values using the cellulolytic index <1 is included in the low category, 1-2 is included in the medium category, and >2 is included in the high category. The result of cellulase activity measurements (Table 3) with cellulolytic tests based on quantitative descriptions showed 6 positive isolates (POU3, PLU1, PLU2, POU1, POU2, PLU1), and 3 negative isolates (PKU1, PKU2, PKU3). Based on measurements (IS), the cellulolytic index, three isolates (POU3, PLU1, PLU2) showed moderate values 1-2, three isolates (POU1, POU2, PLU1) showed low values <1, and 3 (PKU1, PKU2, PKU3) isolates did not have cellulase activity. Measurement of the clear zone in each isolate using the cellulolytic index aims to determine the ability of the isolate to produce cellulase. The results of the cellulase activity test, with 0.1% Congo red reactant, are marked with a clear zone, which can be seen in Figure 4.

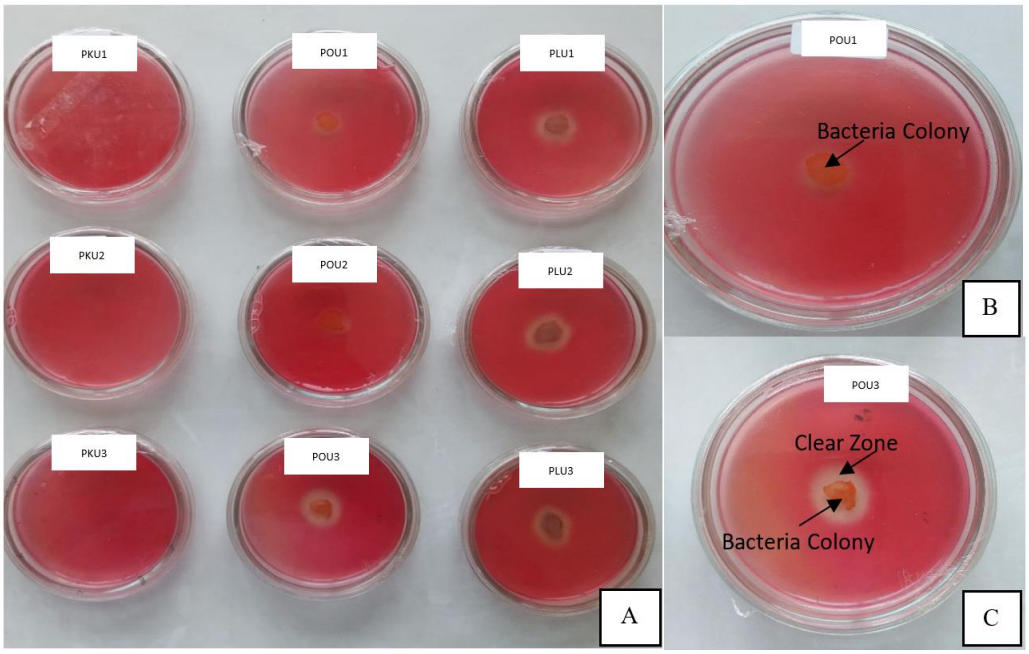

Figure 4. Cellulase Activity Test. 9 experimental units (PLU1, PLU2, PLU3, POU1, POU2, POU3, PKU1, PKU2, and PKU3) (A), Isolate without cellulase activity (B), Isolate with cellulase activity (C).

Table 3. The result of Cellulase Activity Analysis

Result of Cellulase Activity Analysis				
Sample Code	Qualitative	Quantitative		
		Cellulolytic Index	Power Classification	Value Description
POU1	+	0.2	<1	Low
POU2	+	0.2	<1	Low
POU3	**+**	**1.3**	**1-2**	**Medium**
PLU1	+	0.8	<1	Low
PLU2	**+**	**1.0**	**1-2**	**Medium**
PLU3	**+**	**1.0**	**1-2**	**Medium**
PKU1	-	-1.0	<1	None
PKU2	-	-1.0	<1	None
PKU3	-	-1.0	<2	None

CONCLUSION

Characterization results showed that the three isolates have white colony physiology, round shape, raised elevation with wool-like edges, and are non-pathogenic bacteria, gram-negative and bacillus-shaped, and positive for H_2O_2 catalase. The results of molecular identification of the three cellulolytic bacterial isolates are as follows: the PK sample is the *Aeromonas enteropelogenes* species, the PL sample is the *Nitrosomonas stercoris* species, and the PO sample is *Methylobacillus caricis*. Isolates were identified at <97%, so further studies are needed to explore new species of cellulolytic bacteria. The results of the analysis of cellulase activity using the cellulolytic test method showed activity in 6 positive isolates with moderate ability (1-2) in isolate codes POU3 (1.3), PLU2 (1.0), PLU3 (1.0); low in isolates POU1 (0.2), POU2 (0.2), PLU (0.8) and three negative isolates did not have PKU1 (-1), PKU2 (-1), and PKU3(-1) enzyme activity. Based on the research findings, the cellulolytic bacteria derived from TKKS waste has the potential to be used in cellulose production. However, further studies are needed regarding production optimisation, enzyme characterisation, and others.

ACKNOWLEDGEMENT

The authors would like to thank Badan Pengelola Dana Perkebunan Kelapa Sawit (BPDPKS), the institution that provided full scholarship during the study and research period. Sulung Research Station, PT. Sawit Sumbermas Sarana tbk. Citra Borneo Indah (CBI Group) and Southeast Asian Regional Centre for Tropical Biology (SEAMEO BIOTROP) have facilitated and helped during the research process. Gratitude is also given to the Politeknik Kelapa Sawit Citra Widya Edukasi, which has facilitated the facilities and infrastructure so that the author can complete this research well.

AUTHOR CONTRIBUTIONS

Pamungkas EA. Methodology; Investigation Formal analysis; Writing – original draft; Madusari S: Conceptualisation; Data curation; Project administration; Supervision; Writing – review & editing. Putri HA: Conceptualisation; Data curation; Supervision; Writing – review & editing. Rosita R: Conceptualization; Data curation Supervision; Writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Adu MO, Atia K, Arthur E, Asare PA, Obour PB, Danso EO, Frimpong KA, Sanleri KA, Larbi SA, Adjel R, Mensah G, Andersen MN. 2022. The use of oil palm empty fruit bunches as a soil amendment to improve the growth and yield of crops. A meta-analysis. Agronomy for Sustainable Development. 42:13

Agafonova NV, Kaparullina EN, Doronina NV, Trotsenko YA. 2017. Methylobacillus caricis sp. nov., an Obligate Methylotroph Isolated from Roots of Sedge (Carex sp.). *Mikrobiologiya,* 86(6), 720–728.

Alkahfi F, Adiartayasa W, dan Wirawan IGP. 2021. Isolasi dan Identifikasi Bakteri Selulolitik pada Sampah Organik di TPA Suwung Denpasar. *Jurnal Agroekoteknologi Tropika* ISSN, 2301, 6515.

Arifin Z, Gunam IBW, Antara NS, Setiyo Y. (2019). Isolasi bakteri selulolitik pendegradasi selulosa dari kompos. *Jurnal Rekayasa dan Manajemen Agroindustri* ISSN, 2503, 488

Azizah SN, 2013. Skrining Bakteri Selulolitik asal Vermicomposting Tandan Kosong Kelapa Sawit [Skripsi]. Jember: Universitas Negeri Jember.

BPS (Badan Pusat Statistik). 2023.Statistik *Kelapa Sawit Indonesia 2022*. Direktorat Statistik Tanaman Pangan, Hortikultura, dan Perkebunan, editor. Jakarta (ID): Badan Pusat Statistik/BPS–Statistics Indonesia

Chaudhary N, Qazi JI, Irfan M. 2015. Isolation and Identification of Cellulolytic and Ethanologenic Bacteria from Soil. Iranian Journal of Science and

Technology Articles in Press. DOI 10.1007 / s40995- 017-02821

Chusniasih D, Suryanti E, Safitri E. 2023. Isolasi dan Uji Aktivitas Selulolitik Bakteri Asal Limbah Bagas (Isolation and Cellulolytic Activity Assay of Bacteria from Bagasse). Jurnal Ilmu Pertanian Indonesia (JIPI). 28(3); 386 - 395. DOI: 10.18343/jipi.28.3.386.

Fauziah SI, Ibrahim M. 2020. Isolasi dan Karakterisasi Bakteri Selulolitik Pada Tanah Gambut di Desa Tagagiri Tama Jaya Kecamatan Pelangiran Kabupaten Inhil, Riau. *Lentera Bio*. 9(3): 194-203.

Gaol MRLL, Sitorus R., Yanthi S, Surya I, dan Manurung R 2013. Pembuatan Selulosa Asetat dari α-Selulosa Tandan Kosong Kelapa Sawit. Jurnal Teknik Kimia USU, 2(3), 33-39.

Hidayatulloh A, Yahdiyani N, Nurhayati LS. 2022. Isolasi dan seleksi bakteri kandidat selulolitik dari proses pembuatan pupuk organik pada pengolahan limbah peternakan.Jurnal Teknologi Hasil Peternakan. 3(2);64-72.

Khairina E, Purnomo EP, Malawi AD. 2020. Sustainable development goals: Kebijakan Berwawasan Lingkungan Guna Menjaga Ketahanan Lingkungan Di Kabupaten Bantul Daerah Istimewa Yogyakarta. *Jurnal Ketahanan Pangan*. 26(2);155-181.

Kurniawan CA, dan Gusmawartati. 2021. Uji Isolat Bakteri Selulolitik Sebagai Dekomposer Pada Dekomposisi Tandan Kosong Kelapa Sawit. *Jurnal Agrotek*. 5(1).

Mahendra MI. 2022. Isolasi dan karakterisasi bakteri selulolitik asal feses kuda (Equus caballus) [Skripsi]. Bogor:Institute Pertanian Bogor.

Murtiyaningsih M, Hazmi M. 2017. Isolasi dan uji aktivitas enzim selulase pada bakteri selulolitik asal tanah sampah. *Journal Agritrop*. 15(2): 293-308.

Nakagawa T, dan Takahashi R. 2015. Nitrosomonas stercoris sp. nov., a Chemoautotrophic Ammonia-Oxidising Bacterium Tolerant of High Ammonium Isolated from Composted Cattle Manure. *Microbes Environ.* 30(3), 221-227.

Rahmasita ME, Farid M, Ardhyananta H. 2017. Analisa Morfologi Serat Tandan Kosong Kelapa Sawit Sebagai Bahan Penguat Komposit Absotpsi Suara. Jurnal Teknik ITS. 6(2): 23337-2520.

Ramadhan ML, Buwono IDE, Mulyani Y. 2012. Analisis Potensi dan Karakterisasi Molekuler Gen 16S rRNA Bakteri Selulolitik yang Diisolasi dari Makroalga Eucheuma sp. dan Sargassum sp. Sebagai Penghasil Enzim Selulase. *Jurnal Perikanan dan Kelautan*. 3(3):61-67.

Rosita, R. (2021). Pertumbuhan dan Kemampuan Fitoremediasi Brachiaria decumbens Stapf. Yang Diperkaya Claroideoglomus etunicatum dan Bacillus sp. pada Tanah Bekas Tambang Batu Bara (Doctoral dissertation, IPB University).

Rosita, R., Apriana, E., Hazra, F., & Eris, D. D. (2023). Characterisation of Phosphate-Solubilising Bacteria from Three Types of Soil Rhizosphere and Their Potency to Increase Growth of Corn Plants (Zea mays). Jurnal Ilmiah Biologi Eksperimen dan Keanekaragaman Hayati (J-BEKH), 10(1), 30-39.

Schubert RHW, Hegazi M. Wahlig W. 1990. Aeromonas enteropelogenes species nova. *Hyg Med* 15;471-472.

Selpani NB. 2015. Identifikasi Bakteri Selulolitik Asal Tanah Situ Gede Dengan Teknik Genetika Molekuler. [Skripsi]. Bogor: Institut Pertanian Bogor. selulolitik dari rumput laut *Turbinaria sp.* dan *Sargassum sp.* sebagai kandidat pendegradasi serat kasar pakan ikan. *J Ris Akua*. 10(1): 2015.

Seprianto, 2017. Isolasi dan Penapisan Bakteri Selulolitik dari Berbagai Jenis Tanah Sebagai Penghasil Enzim Selulase. *Indonesian Journal of Biotechnology B*. 5(1).

Sharma S, Kumar S, Khajuria A, Ohri P, Kaur R. 2020. Biocontrol potential of chitinases produced by newly isolated *Chitinophaga* sp. S167. *World J. Microbiol. Biotechnol.*36:90. Doi: 10.1007/s11274-020-02864-9.

Soesetyaningsih E, Azizah. 2020. Akurasi Perhitungan Bakteri pada Daging Sapi Menggunakan Metode Hitung Cawan. *BERKALA SAINSTEK 2020*, 8(3): 75-79

Suryaningrum LH dan Samsudin R.2018. Potensi enzim selulase dalam mendegregasi material lignoselulosa sebagai bahan pakan ikan. Prosiding seminar nasional hasil riset pengolahan produk dan bioteknologi kelautan dan perikanan 2018 Pertemuan ilmiah ke10 kongres MPHI; 2018 okt 16-17; Jakarta, Indonesia. Balai riset perikanan budidaya air tawar dan penyuluhan perikanan. hlm 71-76.

Syukri N, Kasprijo P, Tjahja H, Syakuri H, Listiowati E. 2021. Penapisan bakteri selulolitik pada saluran pencernaan ikan kerapu cantang yang dibudidayakan di Desa Babakan, Kecamatan Pangandaran, Kabupaten Pangandaran. *Jurnal Ruaya*. 9(2): 1–10. https://doi.org/10.29406 /jr.v9i2.3000.

Yordy JR, Dan Weaver TL. 1977. *Methylobacillus*: a New Genus of Obligately Methylotrophic Bacteria. *Journal of Systematic Bacteriology*. 247-255

Yusnia ED, Gunam IBW, Antara NS. 2019. Isolasi dan Skrining Bakteri Selulolitik dari Beberapa Tanah Hutan di Bali. Jurnal Rekayasa dan Manajemen Agroindustri.7(1):11-20.