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Cellulolytic bacteria from *Coptotermes curvignathus* termite gut isolated from coastal area of Bengkulu, Indonesia

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Abstract. *Oktiarni D, Lusiana, Marsuganda A. 2025. Cellulolytic bacteria from* Coptotermes curvignathus *termite gut isolated from coastal area of Bengkulu, Indonesia. Biodiversitas 26: 3252-3261.* The conversion of lignocellulosic biomass into bioethanol addresses both renewable energy production and waste management challenges on a global scale. Utilizing diverse types of biomass is particularly advantageous, as lignocellulosic biomass is not a food source for humans and offers an economically viable alternative for waste processing while also enhancing bioethanol production. As highly social insects, termites degrade cellulosic biomass into glucose with the aid of cellulolytic enzymes produced by bacteria residing in their guts. This research aimed to isolate cellulolytic bacteria suitable for hydrolyzing cellulose biomass into glucose for bioethanol production. Cellulolytic bacteria were isolated from the guts of *Coptotermes curvignathus* (Holmgren, 1913) termites collected in the coastal area of Bengkulu, a region known for its abundant termite populations. The isolated bacteria were characterized and analyzed using biomolecular assays. The identification of cellulolytic bacterial species was performed through biomolecular techniques, by using the PCR method. Eight isolates of cellulolytic gut bacteria were obtained, and showed cellulolytic activity. The PCR results revealed that the cellulolytic bacteria belonged to the species *Bacillus cereus* and *Bacillus thuringiensis*. Eight isolates of cellulolytic gut bacteria that obtained, have potential as a source of microbial cellulases for use in hydrolyzing cellulosic biomass into ethanol production.

Keywords: Bioethanol, biomass, cellulolytic bacteria, cellulolytic enzyme, termite

INTRODUCTION

The production of second-generation bioethanol as a renewable energy source has advanced quickly and is now considered a viable alternative energy solution. Cellulose, found in wood, organic litter, and agricultural residues, serves as a viable biomass source for bioethanol production. Several countries have developed technology to hydrolyze cellulosic biomass, which uses fermentation technology to produce bioethanol (Oktiarni et al. 2021).

The conversion of lignocellulosic biomass into bioethanol is more complex than converting starch into ethanol. The intricate composition of lignocellulose requires delignification to reduce lignin content as a pretreatment step in the bioethanol production process (Mosier et al. 2005; Zabed et al. 2016). The pretreatment process is essential for removing lignin, reducing crystallinity, and increasing material porosity (Karimi et al. 2006). Among the various pretreatment methods, using an acid solution is one of the simplest. After pretreatment, the saccharification and fermentation processes convert lignocellulose into bioethanol. When hydrolysis is performed enzymatically, the conversion process can either be Simultaneous Sacharification and Fermentation (SSF) or Separate Hydrolysis and Fermentation (SHF) (Hou et al. 2019).

The role of cellulase enzymes and cellulolytic bacteria in hydrolyzing lignocellulosic biomass is vital. One organism with this capability is termites, which can be found in the coastal areas of Bengkulu. The gut of termites harbors cellulolytic bacteria capable of hydrolyzing cellulose into glucose. Isolating these bacteria requires selective media to obtain single bacterial colonies (Oktiarni et al. 2021).

The enzymatic hydrolysis of cellulose is a sustainable and efficient approach that avoids generating harmful chemical residues. Some organisms, like termites, rely on cellulose as a source of nutrition. The bacteria in termite guts produce enzymes that hydrolyze cellulose into glucose. Termites are classified into two types based on their gut symbionts: lower termites, (which host fungi and bacteria), and higher termites (which contain only bacteria) (Ni and Tokuda 2013). The termite digestive system accommodates a variety of bacteria and fungi with cellulolytic enzyme activity that breaks down cellulose into simple sugars.

The potential and ability of cellulases can be developed for sustainable purposes, such as energy production, bioremediation, pest-control, antimicrobial production, vitamins, amino acids, and lactic acid. The availability of cellulase enzymes with high activity will impact enzyme production results and can be used to meet industrial needs for enzyme availability (Bhardwaj et al. 2021).

Bacteria in termite guts can naturally break down cellulose, a potential biomass. Most lower termites consume wood (Hongoh 2011), while higher termites feed on lignocellulosic biomass such as wood, plant litter, grass litter, and soil. The study of termites has gained significant interest due to their capacity to break down lignocellulose through symbiotic bacteria in their guts, making them specialized agents for hydrolyzing cellulose biomass. Termites play a critical role in ecosystem services but are sensitive to habitat disturbances, which can lead to biodiversity loss. Termite diversity decreases with increasing land disturbance (Neoh et al. 2017; Jalaludin et al. 2018; Handayani and Winara 2020), including in coastal areas.

Indonesia is rich in biodiversity, both in terms of its flora and fauna; and its location on the equator gives it tropical rainforests and coastal areas which are spread across various provinces. Indonesia, which has a long coastline, one of which is in Bengkulu Province, has multiple organisms that live in this area, including termites. Bengkulu is affected by weather factors such as tides, wind, and others (Syukhriani et al. 2017). Tides can disrupt the coastline, damage coastal plants, and ecological damage, including termite ecosystems. The number of organisms in this location is very low because sandy soil conditions inhibit the ability of organisms to grow. High temperatures and the ability to retain water in sandy soil are very low (Mutmainah et al. 2018). Termites must be able to adapt to these conditions. Termites that live in colonies are fauna whose primary food source is cellulose, which comes from trees, including wood, stems, roots, and leaves (Hongoh 2011; Brune 2014).

Correspondingly, the present study isolated and identified termite and bacterial species from termite guts. The discovered cellulolytic bacterial species are anticipated to shed light on the bacterial diversity within termite guts in Bengkulu's coastal regions. These cellulolytic bacteria can serve as biocatalysts for hydrolyzing biomass into glucose, a raw material for bioethanol production.

MATERIALS AND METHODS

Selection and identification of termites

Termite samples were collected from the coastal area of Bengkulu, specifically in the Muara Bangkahulu area, Bengkulu City, Bengkulu Province, Indonesia (Figure 1). The termites were placed in labeled plastic containers and taken to the laboratory for analysis. The samples were identified by observing their shape, color, and size and comparing them with existing literature.

The sampling technique involved the direct collection, targeting termite colonies that had infested tree roots (Figure 2). The samples included soldier and worker termites, stored in sealed plastic containers and transported to the laboratory. To maintain humidity, they were placed in plastic containers lined with moist tissue (Arif et al. 2019; Oktiarni et al. 2021).

Isolation of bacteria

Six worker termite samples were sterilized in 70% alcohol for 30 seconds. The termite guts were dissected and soaked in 0.9% NaCl solution in a petri dish. The samples were rinsed with sterile distilled water and allowed to dry for one minute. The termite guts were ground in 0.9% NaCl to obtain a suspension. The suspension was subsequently centrifuged at 3000 rpm to separate the supernatant from the pellet. Next, 0.1 mL of the suspension was evenly spread onto NA media and incubated at 37°C for 24 hours. A series of dilutions were performed, ranging from 10^{-1} to 10^{-5} (Ferbiyanto et al. 2015; Boontanom and Chantarasiri 2021; Fathollahi et al. 2021; Hidayat 2021; Oktiarni et al. 2021). The CMC agar medium used contained the following components: 0.18 g K_2HPO_4, 0.1 g $NaNO_3$, 0.05 g KCl, 0.09 g $MgSO_4.7H_2O$, 0.2 g yeast extract, 1 g peptone, 1 g CMC, and 2 g agar, and was adjusted to pH 5 (w/v).

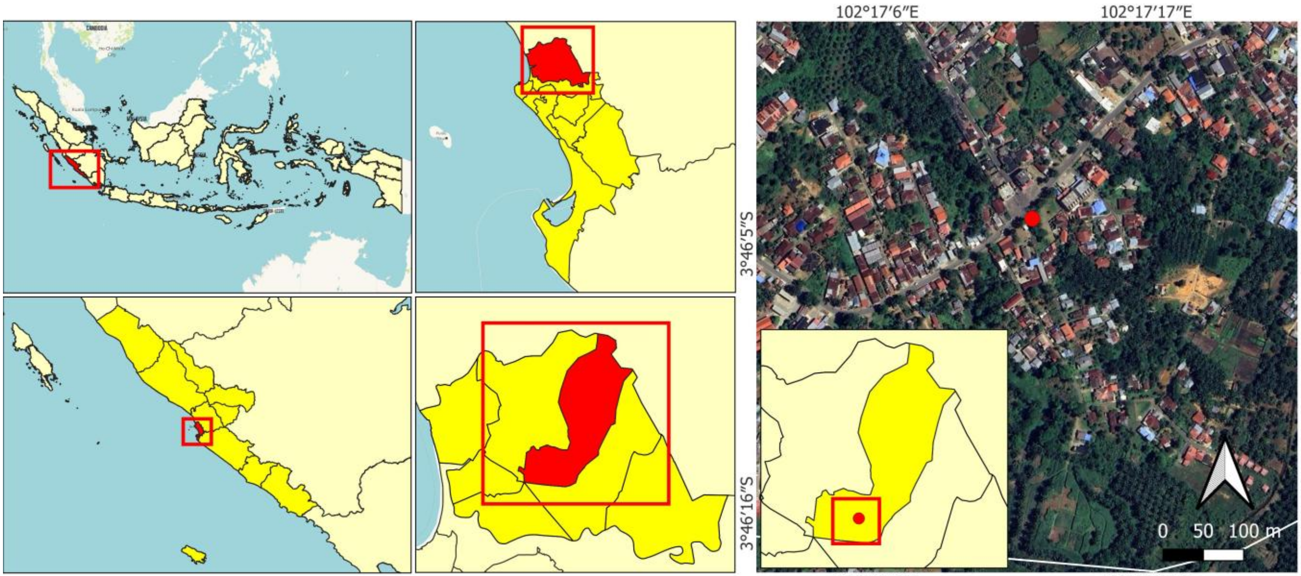


Figure 1. The location of termite sample (3°46'3.78"S, 102°17'12.23"E), Muara Bangkahulu, Bengkulu, Indonesia

Screening of cellulolytic bacteria

The suspension was spread on Carboxymethyl Cellulose (CMC) agar media, a selective media used to obtain cellulolytic bacteria. Bacterial colonies that grew on CMC agar media were transferred to new CMC agar media to obtain single bacterial isolates. The single bacterial isolates obtained were then determined by iodine solution for their hydrolysis ability. Single bacterial isolates with high clear zones were subjected to morphological, physiological, biochemical, and biomolecular tests (Fathollahi et al. 2021; Hidayat 2021). The cellulose index can be calculated using the following formula (Ferbiyanto et al. 2015; Peristiwati et al. 2018; Oktiarni et al. 2021):

$$\text{Cellulolytic index} = \frac{\text{diameter of clear zone} - \text{diameter of bacterial colonies}}{\text{diameter of bacterial colonies}}$$

Morphology and biochemical assay

Morphological and physiological tests were carried out by doing macroscopic test, for texture, colour, shape, colony size, and elevation, and also microscopic test, for Gram staining and colony cell. Biochemical assay was carried out on bacterial isolates, followed by indole test, motility test, Triple Sugar Iron (TSI) test, urease test, catalase test, citrate test, Methyl Red (MR) test, and Voges Proskauer (VP) test (Sharma et al. 2015; Ferbiyanto et al. 2015; Peristiwati et al. 2018; Oktiarni et al. 2021).

DNA isolation and purification

Cellulolytic bacterial isolates were identified using a biomolecular approach. The 16S rRNA genes from eight bacterial isolates were extracted and purified utilizing the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research) according to the manufacturer’s protocol.

DNA quantification using a spectrophotometer nanodrop

A 1 µL DNA sample from each bacterial isolate was placed on a drop plate and analyzed for optical density ratios (260/280 for DNA and 260/230 for RNA) using a NanoDrop™ spectrophotometer (Thermo Scientific™). Electrophoresis was conducted on 1 µL of PCR products using 0.8% TBE agarose gel, and DNA bands were visualized with Cyber Green staining under a UV transilluminator.

PCR amplication of 16S RNA genes

PCR amplification of the 16S RNA genes from the eight purified isolates was performed using (2x) MyTaq HS Red Mix (Bioline, BIO-25048) with universal primers 27F (5'-`AGAGTTTGATCMTGGCTCAG`-3') and 1492R (5'-`TACGGYTACCTTGTTACGACTT`-3'). The thermo-cycling conditions consisted of an initial denaturation at 94°C for 4 minutes, followed by 35 cycles of 94°C for 40 seconds for denaturation, 55°C for 1 minute for annealing, and 72°C for 1 minute and 10 seconds for extension, concluding with a final extension at 72°C for 10 minutes. Amplification and quantification were conducted using an ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems).

16S RNA gene sequence

The DNA extracts were sequenced utilizing the BigDye® Terminator v3.1 Cycle Sequencing Kit. The PCR products were analyzed utilizing bidirectional DNA sequencing. The obtained 16S rRNA gene sequences were analyzed by blasting them with the GenBank database using the BLAST-N program provided by the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

Phylogenetic tree analysis of amplified 16S RNA genes

The 16S rRNA gene sequences were utilized to construct a phylogenetic tree using MEGA 5.05 software. The tree was created using the neighbor-joining method with bootstrap analysis performed on 1000 replicates.

RESULTS AND DISCUSSION

Selection and identification of termites

Termite samples (Figure 1) were collected in the Pematang Gubernur area, Muara Bangkahulu, Bengkulu City. These samples were taken from the roots of trees infested by termite colonies. The termites identified belonged to the species *Coptotermes curvignathus* (Holmgren, 1913) and were classified as lower termites. This species is commonly found in dead trees and roots (Arsyad et al. 2018; Arif et al. 2019). The major soldiers measured 6 mm in length, while the minor soldiers ranged from 3 to 3.2 mm. The head capsules of the major soldiers were less than 2 mm long and exhibited a brownish-yellow color. Previous research indicates that termites in rubber plantations are typically from the *Microtermes* species and *Macrotermes gilvus* (Hagen, 1858), whereas those in oil palm plantations are often *Odontotermes* species. Conversely, termites found in drywood structures are commonly associated with the *Cryptotermes* species (Arif et al. 2019).

Screening, isolation, purification and characterization of bacteria present in the gut of termites

Screening of cellulolytic bacteria

Figure 3 shows bacterial colonies obtained on CMC agar media in a 10^{-4} dilution series. Eight bacterial isolates were obtained and labeled as BRP 1, BRP 2, BRP 3, BRP 4, BRP 5, BRP 6, BRP 7, and BRP 8.

Figure 2. A. Termite habitat. B. *Coptotermes culvignathus* termite

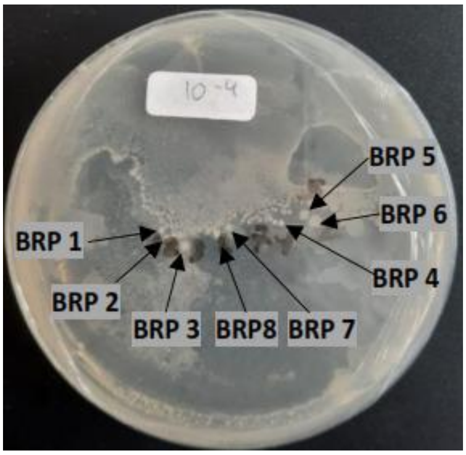


Figure 3. Bacterial colonies found in the termites gut (10^{-4} dilution series)

Isolation of cellulolytic bacteria

The eight bacterial isolates were further isolated and purified to obtain single colonies from each isolate (Figure 4). Morphological and physiological tests of cellulolytic bacterial isolates include macroscopic and microscopic tests. Morphological tests are related to cell shape and cell arrangement (microscopic); and macroscopic tests are tests on bacterial isolates that include color, shape, size of bacteria, and margins. In contrast, physiological tests include the biochemical properties of bacterial isolates (Ferbiyanto et al. 2015; Fathollahi et al. 2021; Hidayat 2021; Oktiarni et al. 2021). Macroscopic and microscopic data and biochemical tests of each bacterial isolate obtained were compared with the data in Bergey's Manual of Determinative Bacteriology 9th Edition.

Morphological and physiological (macroscopic) characteristics observed in the isolates revealed that all had smooth textures, flat elevations, and a round colony shape. The colonies were white, and the size of the isolates ranged from 0.5 to 5 mm (Table 1). However, the bacteria did not grow on the BRP3 isolate, so the isolate was not further measured.

Microscopic observation was carried out by performing Gram staining on bacterial isolates. Seven bacterial isolates showed a Gram positive bacterial with bacilli shape (see Figure 5). The seven bacterial isolates showed the ability to hydrolyze cellulose with the emergence of a clear zone around the bacterial isolates (Figure 6). Almost all bacterial isolates had cellulolytic activity, as seen by iodine staining on CMC growth plates, resulting in clear zones from cellulose degradation. The cellulolytic activity index of each bacterial isolate showed differences from each other (Table 2).

Biomolecular assay

Preparation of bacterial isolates

Isolation using a biomolecular assay was performed on five bacterial isolates: BRP 1, BRP 2, BRP 4, BRP 7, and BRP 8. Since BRP 5 isolate was similar to BRP 7 isolate, while BRP 6 isolat was similar to BRP 1 isolate (Figure 3), however, the isolates of BRP 5 and BRP 6 were not futher measured. The DNA of these isolates was measured for concentration using a nanodrop spectrophotometer.

Isolation of bacterial isolate DNA and amplification of 16S rRNA gene

The DNA concentration of the bacterial isolates ranged from 13.0 to 16.5 ng/μL (Table 3). The 260/280 nm ratios of the isolates ranged from 1.78 to 2.35, indicating high DNA purity. The 16S rRNA gene sequencing method was employed to identify, classify, and quantify microbial samples. The 16S rRNA gene is a highly conserved element of the transcriptional machinery present in all DNA-based cells. It is an excellent target for sequencing microbial DNA in samples with diverse species. The 16S rRNA gene contains both conserved and variable regions. The conserved regions enable universal amplification, while sequencing the variable regions allows for distinguishing specific microorganisms, such as bacteria.

The amplification of the 16S rRNA gene was performed using PCR, and the resulting gene fragments were analyzed through gel electrophoresis to evaluate the amplification outcomes. A total of five 16S rRNA gene fragments from bacterial isolates each displayed a single band of approximately 1400 to 1500 base pairs (Figure 7).

The purity of the amplified 16S rRNA gene fragments was confirmed by the presence of a single, thick band on the electrophoresis gel. Subsequently, the 16S rRNA gene fragments were sequenced, and their nucleotide sequences were analyzed to identify the species of each bacterial isolate. This analysis enabled the construction of a phylogenetic tree for each of the bacterial isolates. BLAST-N data for the 16S rRNA genes of isolates BRP 1, BRP 2, BRP 4, BRP 7, and BRP 8 (Table 4) were used in conjunction with the phylogenetic trees of these isolates (Figure 8).

Table 2. Cellulolytic index of cellulolytic bacteria isolated from termite gut

Isolate	Diameter of colony (mm)	Diameter of cellulolytic zone (mm)	Cellulolytic index
BRP 1	2.0 ± 0.0	5.5 ± 0.0	1.8 ± 0.0
BRP 2	1.8 ± 0.0	6.2 ± 0.0	2.4 ± 0.0
BRP 3	1.0 ± 0.0	2.0 ± 0.0	1.0 ± 0.0
BRP 4	2.3 ± 0.0	5.0 ± 0.0	1.2 ± 0.0
BRP 5	1.5 ± 0.0	2.0 ± 0.0	0.3 ± 0.0
BRP 6	2.0 ± 0.0	5.0 ± 0.0	1.5 ± 0.0
BRP 7	1.0 ± 0.0	2.0 ± 0.0	1.0 ± 0.0
BRP 8	2.2 ± 0.0	8.5 ± 0.0	2.9 ± 0.0

Table 1. Colony morphology and biochemical tests of cellulolytic bacteria isolated from termite gut

Isolate	Texture	Color	Shape	Elevation	Gram staining	Indole	Motility	Triple Sugar Iron			Urease	Catalase	Citrate	MR	VP
								Ferm	H_2S	Gas					
BRP 1	Smooth	White	Round	Flat	+ Bacilli	-	+	+	-	+	-	+	+	-	+
BRP 2	Smooth	White	Round	Flat	+ Bacilli	-	+	+	-	+	-	+	+	-	+
BRP 4	Smooth	White	Round	Flat	+ Bacilli	-	+	+	-	+	-	+	+	-	+
BRP 5	Smooth	White	Round	Flat	+ Bacilli	-	+	+	-	+	-	+	+	-	+
BRP 6	Smooth	White	Round	Flat	+ Bacilli	-	+	+	-	+	-	+	+	-	+
BRP 7	Smooth	White	Round	Flat	+ Bacilli	-	+	+	-	+	-	+	+	-	+
BRP 8	Smooth	White	Round	Flat	+ Bacilli	-	+	+	-	+	-	+	+	-	+

Note: (+): positive result; (-): negative result; Ferm: Fermentation result; MR: Methyl Red test; VP: Voger Proskauer test

Figure 4. Subculture of single isolates of bacteria on CMC agar media

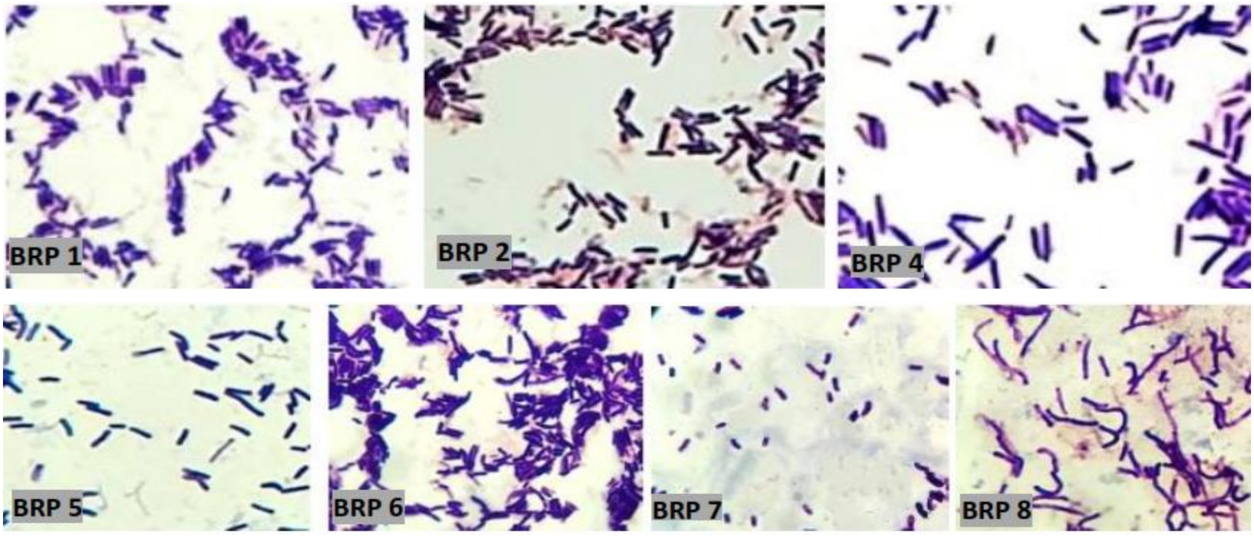


Figure 5. Bacterial isolates after Gram staining under microscope (100 ×)

The BLAST-N data revealed that isolates BRP 1 and BRP 4 were closely related to *Bacillus cereus* strain SA1 (MH0326051), with similarities of 99.86% and 100%, respectively. Isolate BRP 2 showed a 99.08% similarity to *Bacillus thuringiensis* strain LDC 507 (KF779471.1). Meanwhile, isolate BRP 7 exhibited almost 100% similarity to *Bacillus cereus* strain GT-5 (MT492023.1), and isolate BRP 8 was nearly identical to *Bacillus thuringiensis* strain JC-3 (MT492011.1) with 99.93% similarity.

These results indicate that the DNA sequences of isolates BRP 1, BRP 2, BRP 4, BRP 7, and BRP 8 were highly similar to sequences in the database (see Table 4). The isolates BRP 1, BRP 4, and BRP 7 were similar to *Bacillus cereus*, while the isolates BRP 2 and BRP 8 were similar to *Bacillus thuringiensis*. These bacterial species have also been reported in the gut of the termite in Egypt (Ali et al. 2019), in Nepal (Sharma et al. 2015; Tsegaye et al. 2019), in Iran (Javaheri-Kermani and Asoodeh 2019), in Thailand (Boontanom and Chantarasiri 2021), as well as in Malaysia (Ayeronfe et al. 2019), and West Kalimantan, Indonesia (Hidayat et al. 2021).

Discussion

The eight bacterial isolates showed the ability to hydrolyze cellulose with the emergence of a clear zone around the bacterial isolates. The clear zone formed is the ability of cellulase released by bacterial cells to break down carbohydrate macromolecules into oligosaccharides. The difference in cellulolytic activity is likely due to the cellulase excreted by each bacterial isolate having different potentials to decompose substrates in the growth medium. The larger the clear zone in the bacterial isolate, the greater the cellulolytic activity produced (Oktiarni et al. 2021).

The results of microscopic testing of bacterial isolates showed that all isolates had flat outer edges, were white in colour, and had round colonies with flat elevation. The diameter of the bacterial colonies varied from 0.1 mm to 5 mm. Microscopic tests included Gram staining tests on each bacterial isolate. The bacteria in this study were Gram-positive, with the shape of the colonies in the form of bacilli.

Gram staining aims to characterize the morphology of bacterial cells by grouping them into groups of Gram-positive bacteria, characterized by the appearance of purple bacterial cells that remain purple even though they have been given a bleaching solution. In contrast, Gram-negative bacteria are characterized by the appearance of red cells due to the fading of the purple colour of crystal violet after being given a bleaching solution so that it absorbs the second dye, namely safranin, which is red (Irawati et al. 2022).

Table 3. DNA concentration of bacterial isolates using a nanodrop spectrophotometer

Isolate	Concentration (ng/µL)	A $_{260/280}$	A $_{260/230}$
BPP 1	13.3	2.35	0.68
BRP 2	16.5	2.23	0.83
BRP 4	13.0	2.27	0.54
BRP 7	13.5	2.10	0.67
BRP 8	14.6	1.78	0.56

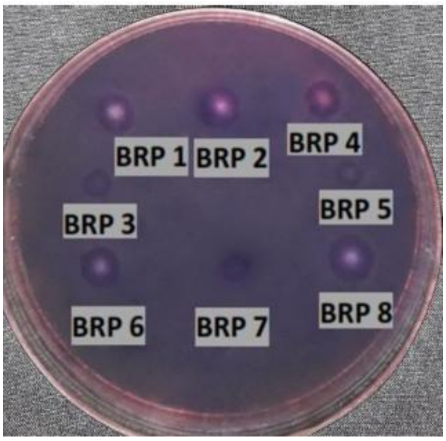
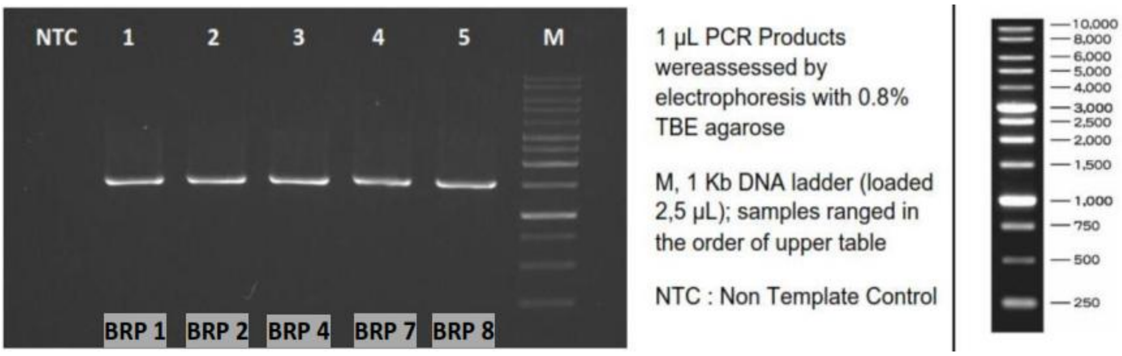


Figure 6. Cellulolytic activity of bacterial isolates on CMC media (stained with iodine)



1 µL PCR Products wereassessed by electrophoresis with 0.8% TBE agarose

M, 1 Kb DNA ladder (loaded 2,5 µL); samples ranged in the order of upper table

NTC : Non Template Control

Figure 7. PCR product results of 16S rDNA gene fragments on gel electrophoresis

Table 4. BLAST-N data of 16S rRNA gene of isolates BRP 1, BRP 2, BRP 4, BRP 7, and BRP 8

Isolate	Accession number	Related species	Max score	Total score	Query coverage	E value	Percent. identity
BRP 1	MH032605.1	*Bacillus cereus* strain *SA1*	2627	2627	100%	0	99.86%
	MK600526.1	*Bacillus* sp. strain *LSB7*	2617	2617	100%	0	99.86%
	MH569447.1	*Bacterium* strain *Glm17*	2617	2617	100%	0	99.86%
	MH569425.1	*Bacillus* sp. strain *Glm17*	2617	2617	100%	0	99.86%
	MG706137.1	*Bacillus anthracis* strain *SAK4*	2617	2617	100%	0	99.86%
BRP 2	KF779471.1	*Bacillus thuringiensis* strain *LDC 507*	2549	2549	99%	0	99.08%
	LK392517.1	*Bacillus cereus*	2542	2542	99%	0	99.01%
	KX343996.1	*Bacillus cereus* strain *G37*	2536	2536	100%	0	98.87%
	JF937058.1	*Bacillus* sp. *JSG1*	2529	2529	100%	0	98.73%
	LC483989.1	*Bacillus cereus MSA1*	2525	2525	100%	0	98.73%
BRP 4	MH032605.1	*Bacillus cereus* strain *SA1*	2628	2628	100%	0	99.93%
	MK600526.1	*Bacillus* sp. strain *LSB7*	2623	2623	100%	0	99.93%
	MH569447.1	*Bacterium* strain *Glm17*	2623	2623	100%	0	99.93%
	MH569425.1	*Bacillus* sp. *GLM17*	2623	2623	100%	0	99.93%
	MG706137.1	*Bacillus anthracis* strain *SAK4*	2623	2623	100%	0	99.93%
BRP 7	MT492023.1	*Bacillus cereus* strain *GT-5*	2638	2638	100%	0	100%
	MN543842.1	*Bacillus cereus* strain *ATCC 14579T.52*	2628	2628	100%	0	100%
	MT312782.1	*Bacillus cereus* strain *YP20170200*	2628	2628	100%	0	100%
	MT158659.1	*Bacillus cereus* strain *IIWM-OMS-06N*	2628	2628	100%	0	100%
	MN786930.1	*Bacillus cereus* strain *LXJ91*	2628	2628	100%	0	100%
BRP 8	MT492011.1	*Bacillus thuringiensis* strain *JC-3*	2629	2629	100%	0	99.93%
	MK088304.1	*Bacillus cereus* strain *F5*	2623	2623	100%	0	99.93%
	KY910254.1	*Bacillus thuringiensis* strain *BH49*	2623	2623	100%	0	99.93%
	KY910255.1	*Bacillus thuringiensis* strain *BH39*	2623	2623	100%	0	99.93%
	KY910252.1	*Bacillus thuringiensis* strain *EBCH18*	2623	2623	100%	0	99.93%

The difference in Gram groups is the result of differences in the structure of the cell wall components, where Gram-positive bacteria have a very thick peptidoglycan layer so that they are able to retain crystal violet dye during the Gram staining procedure, while the peptidoglycan layer in Gram-negative bacteria is thin and close to the cytoplast so that they are unable to retain crystal violet dye during the decolourization process using alcohol (Aqlinia et al. 2020).

The catalase test of all bacterial isolates was positive, indicating that all bacterial isolates produce the catalase enzyme through the degradation of hydrogen peroxide present in the bacteria so that the bacteria can live in an aerobic environment. The Simmon's citrate test was also positive, indicating that each bacterial isolate can use citrate as the sole source of carbon and energy (Ayitso and Onyango 2016), causing the pH to increase and the bacterial isolates to be able to live in an acidic environment. A Methyl Red-Voges Proskauer (MR-VP) test on bacterial isolates was conducted to determine the ability of bacteria to oxidize glucose to produce acid (Fallo and Sine 2016). In the MR test, five bacterial isolates showed negative results. The VP test showed all the bacteria-positive results.

The Triple Sugar Iron biochemical test aims to distinguish various Enterobacteriaceae genera that can ferment glucose by producing acid (Gram-negative bacteria). In addition, it can also determine the genus Enterobacteriaceae from other genera of *Bacillus* (Gram-negative bacteria). Bacteria from the termite digestive tract consist of bacterial species belonging to the Enterobacteriaceae, Bacteroidales and Lactobacillales groups (Husseneder et al. 2005). Termites that obtain nutrients from coastal area are closely related to symbiotic microorganisms in the termite digestive tract.

Biomolecular tests, supported by biochemical test data, macroscopic tests and microscopic tests, showed that two species of bacteria were obtained from the gut of coastal termites in Bengkulu. The results of macroscopic and microscopic tests show these bacteria are Gram-positive bacteria in the form of bacilli, do not form spores, are facultative anaerobes, and the bacterial colonies are white. The results of the biochemical tests gave negative results for the MR test, positive results for the VP test, and negative results for the indole test. These bacteria can use citrate as a carbon source. These bacteria are usually found in the digestive tract of humans, soil, animals, and plants and are also usually found in food (Kus 2014).

Based on the observation results, the five bacterial isolates are close to the genus Bacillus, namely bacterial isolates BRP 1, BRP 2, BRP 4, BRP 7, and BRP 8. Two species of cellulolytic bacteria were identified from the gut of *Coptotermes curvignathus* and classified as *Bacillus cereus* and *Bacillus thuringiensis*. In the biochemical tests that have been carried out, these five bacterial isolates can produce the enzyme catalase, which plays a role in breaking down hydrogen peroxide into water and oxygen, are motile, are citrate, cannot hydrolyze urea, which cannot break down urea into ammonia. The five bacterial isolates are included in Gram-positive with a bacilli cell shape.

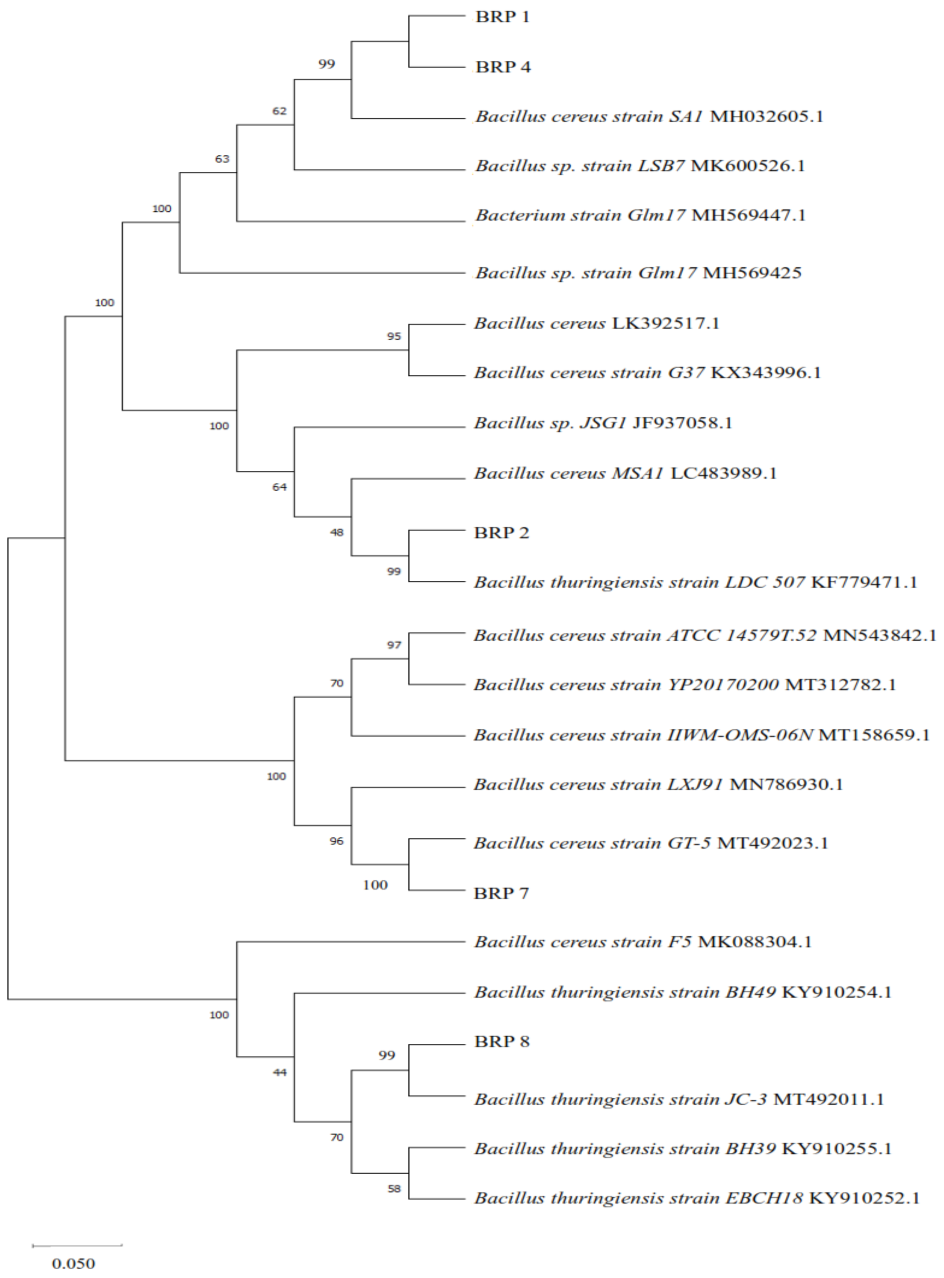


Figure 8. Dendogram of bacterial isolate BRP 1, BRP 2, BPR 4, BRP 7, and BRP 8

Wright and Jones (2006) reported that termites play a crucial role in ecosystems by decomposing organic matter into nutrients, chemically and physically modifying soil, maintaining the stability of nitrogen and carbon cycles, and enhancing microbial activity. Of the 3,106 known termite species, 10% are found in Indonesia, and 5% of these pose a serious threat to plantations as pests. Tropical and subtropical regions serve as habitats for a large number of termites (Arif et al. 2019). According to Sharma et al. (2015), in tropical regions, termites consume 50-100% of the biomass from dead plants, of which 74-99% is cellulose and 65-87% is hemicellulose.

Xylanase activity has been observed in *Bacillus subtilis*, which was isolated from the lower-level termite *Reticulitermes santonensis* (Tarayre et al. 2015). Meanwhile, *B. licheniformis* HI-08 was isolated from another lower-level termite. Cellulase, a cellulolytic enzyme, is produced by *Heterotermes indocola* (Afzal et al. 2019). Furthermore, Ayeronfe et al. (2019) identified three bacteria from lower-level termites: *Lysinibacillus* sp., *Bacillus* sp., and *Acinetobacter* sp., all of which produce ligninolytic enzymes (manganese peroxidase, laccase, and lignin peroxidase). The lower-level termite *Psammotermes hypostoma* hosts five bacteria that exhibit Carboxymethyl Cellulase (CMCase) activity: *Paenibacillus lactis*, *Lysinibacillus macroides*, *Stenotrophomonas maltophilia*, *Lysinibacillus fusiformis*, and *Bacillus cereus* (Ali et al. 2019). The β-1,4-glucanase activity of *Bacillus* sp. CF96 was isolated from *Anacanthotermes* sp., another lower-level termite (Javaheri-Kermani and Asoodeh 2019). Similarly, *Bacillus* sp.

BMP03 was found in the lower-level termite *Cryptotermes brevis*, displaying xylanase and carboxymethyl cellulase activity (Tsegaye et al. 2019).

High-level termite bacteria, including *Paenibacillus macerans* IIPSP3 and *Pseudocitrobacter anthropic* MP-4 from *Microtermes pakistanicus*, demonstrated ligninolytic enzyme activity (Li et al. 2019). Members of the *Termitidae* family produce thermostable xylanase (Dheeran et al. 2012). According to Kamsani et al. (2016), three bacteria isolated from high-level termites of the genus *Bulbitermes* (*Bacillus* sp. B1, *Bacillus* sp. B2, and *Brevibacillus* sp. Br3) contain lignocellulolytic enzymes.

Termites hydrolyze cellulose in their digestive tracts through symbiotic bacteria. For instance, *Bacillus megaterium* and *Paracoccus yeei*, isolated from the gut of the high-level termite *Macrotermes gilvus* in Bogor, exhibited cellulolytic activity under both aerobic and anaerobic conditions (Ferbiyanto et al. 2015). Sharma et al. (2015) also observed cellulolytic activity in *Enterobacter*, *Bacillus*, and *Cellulomonas* species. In the gut of lower-level termites such as *Coptotermes* sp., bacteria like *Pseudomonas alcaligenes*, *Brevibacillus parabrevis*, and *Brevibacillus* sp. produced amylolytic activity (Mulyani et al. 2018). Cellulolytic bacteria, including *Clostridium* sp., members of the *Mycobacteriaceae* and *Lactobacillaceae* families, and *Proteus* species, were isolated from the gut of the termite *Cryptotermes* sp. (Peristiwati et al. 2018). Additionally, *Shigella flexneri*, *Shigella* sp., and *Salmonella paratyphi* were identified in the gut of high-level termites *Macrotermes michaelseni* and found to exhibit cellulolytic activity (Ayitso and Onyango 2016).

Termites break down cellulose into glucose through bacterial symbiosis in their digestive tracts. Glucose, a monosaccharide, is the repeating unit of cellulose polymers. During the hydrolysis of cellulose or hemicellulose, glucose is produced. In recent years, the industrial sector has shown significant interest in the hydrolysis of cellulose into glucose using cellulases of microbial origin (Peristiwati et al. 2018). Cellulase enzymes are essential in this process, facilitating the fermentation of glucose into ethanol. Through enzymatic methods, glucose acts as a key raw material for boosting bioethanol production. Glucose can be hydrolyzed from lignocellulose biomass, including wood and plant litter, using cellulolytic enzymes sourced from termite guts (Keshk 2016).

In conclusion, this study isolated two cellulolytic gut bacteria from the gut of *Coptotermes curvignathus*: *Bacillus cereus* and *Bacillus thuringiensis*. Their elevated cellulolytic index on CMC agar media indicates their capability to hydrolyze cellulose. These bacteria use nitrate as their sole source of carbon and energy, thriving in both aerobic and acidic environments. Further research is needed to optimize the cellulolytic gut bacteria for enzyme production and prospecting for cellulolytic genes for possible biotechnological use. Investigating termites' unique cellulolytic enzyme properties is also essential to fully understand their ability to degrade cellulose.

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REFERENCES

Afzal M, Qureshi MZ, Khan S, Khan MI, Ikram H, Ashraf A, Iqbal A, Qureshi NA. 2019. Production, purification and optimization of cellulase by *Bacillus licheniformis* HI-08 isolated from the hindgut of wood-feeding termite. Intl J Agric Biol 21: 125-134. DOI: 10.17957/ijab/15.0872.

Ali HRK, Hemeda NF, Abdelaliem YF. 2019. Symbiotic cellulolytic bacteria from the gut of the subterranean termite *Psammotermes hypostoma* Desneux and their role in cellulose digestion. AMB Expr 9: 111. DOI: 10.1186/s13568-019-0830-5.

Aqlinia M, Pujiyanto S, Wijanarka W. 2020. Isolasi bakteri (*Zingiber cassumunar* Roxb) dan uji antibakteri supernatan crude metabolit sekunder isolat potensial terhadap *Staphylococcus aureus*. Jurnal Akademika Biologi 9 (1): 23-31. [Indonesian]

Arif A, Muin M, Larekeng SH, Lestari PI. 2019. Survey and morphological identification of termites (Insecta: Isoptera) in Teaching Forest of Hasanuddin University, Indonesia. IOP Conf Ser: Earth Environ Sci 270: 012001. DOI: 10.1088/1755-1315/270/1/012001.

Arsyad S, Wiyono S, Herliyana EN. 2018. Screening of cellulolytic bacteria origined from termite gut for decomposing rubber tree stump. Jurnal Silvikultur Tropika 9 (3): 217-222. DOI: 10.29244/j-siltrop.9.3.217-222. [Indonesian]

Ayeronfe F, Kassim A, Hung P, Ishak N, Syarifah S, Aripin A. 2019. Production of ligninolytic enzymes by *Coptotermes curvignathus* gut bacteria. Environ Clim Technol 23 (1): 111-121. DOI: 10.2478/rtuect-2019-0008.

Ayitso AS, Onyango DM. 2016. Isolation and identification by morphological and biochemical methods of antibiotic producing microorganism from the gut of *Macrotermes michaelseni* in Maseno, Kanya. J Appl Biol Biotechnol 4 (1): 027-033. DOI: 10.7324/jabb.2016.40105.

Bhardwaj N, Kumar B, Agrawal K, Verma P. 2021. Current perspective on production and applications of microbial cellulases: A review. Bioresour Bioprocess 8 (1): 95. DOI: 10.1186/s40643-021-00447-6.

Boontanom P, Chantarasiri A. 2021. Diversity and cellulolytic activity of culturable bacteria isolated from the gut of higher termites (*Odontotermes* sp.) in eastern Thailand. Biodiversitas 22 (8): 3349-3357. DOI: 10.13057/biodiv/d220831.

Brune A. 2014. Symbiotic digestion of lignocellulose in termite guts. Nat Rev Microbiol 12 (3): 168-180. DOI: 10.1038/nrmicro3182.

Dheeran P, Nandhagopal N, Kumar S, Jaiswal YK, Adhikari DK. 2012. A novel thermostable xylanase of *Paenibacillus macerans* IIPSP3 isolated from the termite gut. J Ind Microbiol Biotechnol 39 (6): 851-860. DOI: 10.1007/s10295-012-1093-1.

Fallo G, Sine Y. 2016. Isolasi dan uji biokimia bakteri selulolitik asal saluran pencernaan rayap pekerja (*Macrotermes* spp.). Bio-Edu: J Pendidikan Biologi 1: 27-29. [Indonesian]

Fathollahi Z, Habibpour B, Imani S, Hassanzadeh N, Abdigoudarzi M. 2021. Identification of cellulolytic bacteria from guts of *Microcerotermes diversus* Silvestri (Isoptera: Termitidae) in Southern Iran. Curr Microbiol 78 (4): 1305-1316. DOI: 10.1007/s00284-021-02369-0.

Ferbiyanto A, Rusmana I, Rafiudin R. 2015. Characterization and identification of cellulolytic bacteria from gut of worker *Macrotermes gilvus*. Hayati J Biosci 22: 197-200. DOI: 10.1016/j.hjb.2015.07.001.

Handayani W, Winara A. 2020. Keanekaragaman makrofauna tanah pada beberapa penggunaan lahan gambut. Jurnal Agroforestri Indonesia 3 (2): 77-88. DOI: 10.20886/JAI.2020.3.2.77-88. [Indonesian]

Hidayat MR. 2021. Isolation and identification of cellulolytic bacteria symbiont from various termites on different nest type in Bukit Baka Bukit Raya National Park, West Kalimantan, Indonesia. Walailak J Sci Technol 18 (14): 12708. DOI: 10.48048/wjst.2021.12708.

Hongoh Y. 2011. Toward the functional analysis of uncultivable, symbiotic microorganisms in the termite gut. Cell Mol Life Sci 68 (8): 1311-1325. DOI: 10.1007/s00018-011-0648-z.

Hou R, Hu J, Wang Y, Wei H, Gao M-T. 2019. Simultaneous production of cellulase and ferulic acid esterase by *Penicillium decumbens* with rice straw as the sole carbon source. J Biosci Bioeng 129 (3): 276-283. DOI: 10.1016/j.jbiosc.2019.09.013.

Husseneder C, Wise BR, Higashiguchi DT. 2005. Microbial diversity in the termite gut: A complementary approach combining culture and culture-independent techniques. In: Lee C-Y, Robinson WH (eds). Proceeding of the fifth International Conference On Urban Pests. Perniagaan Ph'ng @ P&Y Design Network, Malaysia.

Irawati W, Ambarita PP, Sihombing DL, Advenita VESR, Marvella EB. 2022. Isolation and characterization of indigenous copper resistant bacteria from Yogyakarta tannery factory waste. Jurnal Biologi Tropis 22 (3): 795-802. DOI: 10.29303/jbt.v22i3.3621.

Jalaludin N-A, Rahim F, Yaakop S. 2018. Termite associated to oil palm stands in three types of soils in Ladang Endau Rompin, Pahang, Malaysia. Sains Malays 47 (9): 1961-1967. DOI: 10.17576/jsm-2018-4709-03.

Javaheri-Kermani M, Asoodeh A. 2019. A novel beta-1,4 glucanase produced by symbiotic *Bacillus* sp. CF96 isolated from termite (*Anacanthotermes*). Intl J Biol Macromol 131: 752-759. DOI: 10.1016/j.ijbiomac.2019.03.124.

Kamsani N, Salleh MM, Yahya A, Chong CS. 2016. Production of lignocellulolytic enzymes by microorganisms isolated from *Bulbitermes* sp. termite gut in solid-state fermentation. Waste Biomass Valor 7: 357-371. DOI: 10.1007/s12649-015-9453-5.

Karimi K, Taherzadeh MJ. 2016. A critical review on analysis in pretreatment of lignocelluloses: Degree of polymerization, adsorption/desorption, and accessibility. Bioresource Technol 203: 348-356. DOI: 10.106/j.biotech.2015.12.035.

Keshk SMAS. 2016. Cellulase application in enzymatic hydrolysis of biomass. In: Gupta VK (eds). New and Future Developments in Microbial Biotechnology and Bioengineering. Microbial Cellulase System Properties and Applications. Elsevier Inc., Amsterdam, Netherlands. DOI: 10.1016/B978-0-444-63507-5.00016-2.

Kus JV. 2014. Infections due to *Citrobacter* and *Enterobacter*. In: Reference Module in Biomedical Sciences. Elsevier Inc., Amsterdam, Netherlands. DOI: 10.1016/b978-0-12-801238-3.05089-3.

Li F, Xie R, Liang N, Sun J, Zhu D. 2019. Biodegradation of lignin *via Pseudocitrobacter anthropi* MP-4 isolated from the gut of wood-feeding termite *Microtermes pakistanicus* (Isoptera: termitidae). BioRes 14 (1): 1992-2012.

Mosier N, Wyman C, Dale B, Elander R, Lee YY, Holtzapple M, Ladisch M. 2005. Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 96 (6): 673-686. DOI: 10.1016/j.biortech.2004.06.025.

Mulyani PD, Hamid RM, Janatunaim RZ, Purwestri YA. 2018. Amylolytic ability of bacteria isolated from termite (*Coptotermes* sp.) gut. Indones J Biotechnol 23: 14-20. DOI: 10.22146/ijbiotech.32445.

Mutmainah E, Feriady A, Rani FNA. 2023. Karakteristik usahatani pertanian di pesisir pantai Kota Bengkulu. Jurnal Agribisnis 16 (2): 2235-2243. DOI: 10.36085/agribis.v16i2.5578. [Indonesian]

Neoh K-B, Bong L-J, Muhammad A, Itoh M, Kozan O, Takematsu Y, Yoshimura T. 2017. The effect of remnant forest on insect successional response in tropical fire-impacted peatland: A bi-taxa comparison. PLoS One 12 (3): e0174388. DOI: 10.1371/journal.pone.0174388.

Ni J, Tokuda G. 2013. Lignocellulose-degrading enzymes from termites and thei symbiotic microbiota. Biotech Adv 31: 838-850. DOI: 10.1016/j.biotechadv.2013.04.005.

Oktiarni D, Hermansyah, Hasanudin, Miksusanti, Nofyan E, Kasmiarti G. 2021. Isolation and identification cellulolytic bacteria from termite gut obtained from Indralaya peatland area. IOP Conf Ser: Earth Environ Sci 926: 012024. DOI: 10.1088/1755-1315/926/1/012024.

Peristiwati, Natamihardja YS, Herlini H. 2018. Isolation and identification of cellulolytic bacteria from termite gut (*Cryptotermes* sp.). J Phys: Conf Ser 1013: 012173. DOI: 10.1088/1742-6596/1013/1/012173.

Sharma D, Joshi B, Bhatt MR, Joshi J, Malla R, Bhattarai T, Sreerama L. 2015. Isolation of cellulolytic organisms from the gut contents of termites native to Nepal and their utility in saccharification and fermentation of lignocellulosic biomass. J Biomass Biofuel 2: 11-20. DOI: 10.11159/jbb.2015.002.

Syukhriani S, Nofridiansyah E, Sulistyo B. 2017. Analisis data citra landsat pemantauan perubahan garis pantai Kota Bengkulu. Jurnal Enggano 2: 90-100. DOI: 10.31186/jenggano.2.1.90-100. [Indonesian]

Tarayre C, Bauwens J, Brasseur C, Mattéotti C, Millet C, Guiot PA, Destain J, Vandenbol M, Portetelle D, De Pauw E, Haubruge E, Francis F, Thonart P. 2015. Isolation and cultivation of xylanolytic and cellulolytic *Sarocladium kiliense* and *Trichoderma virens* from the gut of the termite *Reticulitermes santonensis*. Environ Sci Pollut Res Intl 22 (6): 4369-4382. DOI: 10.1007/s11356-014-3681-2.

Tsegaye B, Balomajumder C, Roy P. 2019. Isolation and characterization of novel lignolytic, cellulolytic, and hemicellulolytic bacteria from wood-feeding termite *Cryptotermes brevis*. Intl J Microbiol 22 (1): 29-39. DOI: 10.1007/s10123-018-0024-z.

Wright JP, Jones CG. 2006. The concept of organisms as ecosystem engineers ten years on: Progress, limitations, and challenges. BioScience 56 (3): 203-209. DOI: 10.1641/0006-3568(2006)056[0203:TCOOAE]2.0.CO;2.

Zabed H, Sahu JN, Boyce AN, Faruq G. 2016. Fuel ethanol production from lignocellulosic biomass: An overview on feedstocks and technological approaches. Renew Sustain Energy Rev 66: 751-774. DOI: 10.1016/j.rser.2016.08.038.