Global J. Environ. Sci. Manage. 10(1): 51-68, Winter 2024, Serial #37

Global Journal of Environmental Science and Management
(GJESM)

Homepage: https://www.gjesm.net/

Podcasts

ORIGINAL RESEARCH ARTICLE

Analyzing cellulolytic bacteria diversity in mangrove ecosystem soil using 16 svedberg ribosomal ribonucleic acid gene

I. Dewiyanti[1], D. Darmawi[2,*], Z.A. Muchlisin[3], T.Z. Helmi[4]

[1] *Graduate School of Mathematics and Applied Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia*

[2] *Laboratory of Microbiology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh Aceh 23111, Indonesia*

[3] *Aquaculture Department, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia*

[4] *Laboratory of Biochemistry, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia*

ARTICLE INFO

Article History:
Received 28 February 2023
Revised 05 May 2023
Accepted 14 June 2023

Keywords:
16 svedberg ribosomal ribonucleic acid (16S rRNA)
Bacteria
Cellulase
Mangrove
Molecular biology

DOI: 10.22034/gjesm.2024.01.05

ABSTRACT

BACKGROUND AND OBJECTIVES: Soil is an essential abiotic component serving as a habitat for numerous organisms, including cellulolytic bacteria commonly found in mangrove ecosystems. This bacteria could produce active enzymes needed to improve environmental quality by accelerating the organic matter decomposition. The unique mangrove environment may contain new types of cellulolytic bacteria with new characteristics. Despite several mangrove areas being explored as sources of cellulolytic bacteria, there is currently unexplored data on its diversity in Aceh Province, Indonesia. Accordingly, it is necessary to analyze the molecular biological approach, namely the 16 svedberg ribosomal ribonucleic acid gene, to identify the diversity of cellulolytic bacteria and analyze the phylogenetic relationships between them.
METHODS: Bacteria isolates were collected from mangrove soil at six research locations with three replications. A purposive sampling method was applied to determine the research location. Isolates from soil samples were streaked and purified in carboxymethyl cellulose as selective media for cellulolytic bacteria. Molecular identification adopted 16 svedberg ribosomal ribonucleic acid gene sequencing, and the sequencing data were matched with GenBank data. Phylogenetic analysis and genetic distance between species were evaluated using molecular evolutionary genetics analysis.
FINDINGS: Thirteen isolates were sequenced, and nine species of cellulolytic bacteria dominated by the *Bacillus* genus were identified. These species exhibited an identity value of 97.77-100 percent when compared to data from GenBank, and *B. velezensis* was found to have a close relationship with *B. amyloliquefaciens* at a value of 0.002 percent. Interestingly, the non-rehabilitated mangrove areas had more bacterial species than the rehabilitated ones. Two *Bacillus* genus had different nucleotide bases, proving they were distinct species.
CONCLUSION: Nine cellulolytic bacteria species were identified; the two closely interspecies genetic distance related were *B. velezensis* and *B. amyloliquefaciens*, whereas the farthest were *Bacillus* sp1. and *Bacillus* sp2. Small genetic distances of interspecies indicate a close relationship between species. In comparing the two sampling sites, the non-rehabilitated mangrove contains higher bacterial cellulolytic species than the rehabilitated and *Bacillus-dominated* site. The findings provide valuable insights into the diversity of cellulolytic bacteria in mangrove ecosystems. The abundance of bacterial species could serve as sources of cellulase enzymes with different characteristics, essential in an environmental aquatic management.

NUMBER OF REFERENCES	NUMBER OF FIGURES	NUMBER OF TABLES
76	7	5

*Corresponding Author:
Email: *darmawi@usk.ac.id*
Phone: + 62821 1615 7741
ORCID: 0000-0003-3499-8000

Note: Discussion period for this manuscript open until April 1, 2024 on GJESM website at the "Show Article".

INTRODUCTION

The mangrove ecosystem is a unique marine environment characterized by distinctive features, such as high productivity and providing a habitat for diverse aquatic organisms (Thatoi *et al.,* 2013; Hu *et al*., 2022). Bacteria are the most abundant and diverse microorganisms found in the mangrove ecosystem, and its soil plays a crucial role in providing essential nutrients, such as carbon, nitrogen, and phosphorus, which contributes to high productivity (Becker *et al.,* 2020; Palit *et al.,* 2022; Saneha *et al.,* 2023). However, as a heterotrophic microorganism, bacteria serve as primary decomposers within the ecosystem and are instrumental in decomposing organic matter, mineralization, and nutrient cycling for plant growth (McGuire *et al*., 2012; Liu *et al.,* 2019; Pringgenies *et al.,* 2023). In mangrove ecosystem, fallen vegetation litter contributes to the abundance of cellulose on the soil surface. These are rich in polysaccharides, such as cellulose and hemicellulose, and are major components of plant cell walls and a primary carbon source in the carbon cycle (Furusawa, 2019). Cellulolytic bacteria can digest cellulose and are widespread microorganisms (Watanabe and Tokuda, 2010). Cellulolytic bacteria is one of the essential bacteria types commonly found in mangrove soil; they contribute carbon sources to improve soil fertility. The diversity of bacteria in the ecosystem serves as an indicator of water fertility, and each species has its unique function and role, such as cellulolytic bacteria, which produces cellulase enzyme that accelerates cellulose degradation (Biswa *et al.,* 2020; Nimnoi and Pongsilp, 2022). Microbial enzymes involved in plant cell wall degradation convert these polysaccharides into digestible components (Ejaz *et al.,* 2021). Bacteria participate directly in the nutrient cycle and provide insight into soil environmental quality through decomposition (Hafich *et al.,* 2012). Studying highly active cellulolytic bacteria is essential to understanding microbial cellulose degradation (Talia *et al.*, 2012). Interest in cellulase enzymes has grown in recent years due to its application in bioenergy and biofuel production, alongside other industries, such as beverage, paper, and textile (Srivastava *et al.,* 2015; Ejaz *et al.,* 2021). Safika *et al*. (2018) stated that cellulolytic bacteria has been proposed as a cost-effective method for reducing fibrous feed and increasing its digestibility, as opposed to using commercial cellulase enzymes. In the fisheries sector, cellulose degradation in feed ingredients can enhance fish growth by improving digestibility (Kurniawan *et al.,* 2019). The isolation of cellulolytic bacteria from mangrove organic materials, like sediments and leaf litter, has been reported in several studies (Behera *et al.,* 2014; Yahya *et al.,* 2014; Chantarasiri, 2015; Kurniawan *et al.,* 2018; Ningsih *et al.,* 2014). Cellulolytic bacterial diversity isolated from the mangrove ecosystem using the 16 svedberg ribosomal ribonucleic acid (16S rRNA) gene has been carried out. Pramono *et al.* (2021) reported the species found in mangrove soil, namely *Fictibacillus nanhaiensis*; Kurniawan *et al.* (2019) recorded two species, namely *Vibrio parahaemolyticus* and *Bacillus amyloliquefaciens* from mangrove soil. There are three species of cellulolytic bacteria from mangrove soil in Malaysia, namely *Anoxybacillus* sp., *Bacillus subtilis*, and *Paenibacillus dendritiformis* (Naresh *et al.,* 2019). Bacterial diversity can be assessed using two approaches: cultivation and non-cultivation, with metagenomic analysis being a useful tool to overcome the limitations of cultivation-based methods (Glogauer *et al.,* 2011). In studying bacterial diversity in forest soil enriched with cellulolytic bacteria, the 16S rRNA gene sequencing analysis is commonly employed (Talia *et al.,* 2012). This method is useful for analyzing the diversity of microorganisms (Izquierdo *et al.,* 2010). Diverse studies of cellulolytic bacteria in Indonesia's mangrove ecosystems have been carried out for morphology approach and molecular biology. However, there is no information about the diversity of cellulolytic bacteria in the soil of rehabilitated and non-rehabilitated mangrove areas in Aceh Besar and Banda Aceh, Aceh Province, Indonesia. Those areas have different bacterial populations, productivity, and soil characteristics, including organic carbon (OC) content and soil texture, making those interesting subjects to study. Non-rehabilitated mangrove areas exhibited higher OC content and cellulolytic bacterial populations than rehabilitated ones. Specifically, the OC content and bacterial populations in non-rehabilitated and rehabilitated mangrove areas were 1.21 percent (%) and 0.90%, categorized low and very low OC content (Dewiyanti *et al.,* 2021), and 5.07×10^7 colony form unit/gram (CFU/g) and 3.47×10^7 (CFU/g), respectively. High bacterial diversity can be associated with the stability and fertility of an ecosystem. The suitable environmental management should be applied in mangrove ecosystem to increase

productivity and fertility. One way is to analyze the presence of cellulolytic bacteria and their species richness. Furthermore, the distinct environmental characteristics of mangrove soil harboring cellulolytic bacteria have encouraged several studies to discover new bacterial strains capable of producing cellulase enzymes with unique properties. Therefore, the current study aims to analyze the molecular biological approach, namely the 16S rRNA gene, to identify the diversity of cellulolytic bacteria species and to analyze the phylogenetic relationships between them in the mangrove ecosystems, Banda Aceh and Aceh Besar. This study was carried out in the northern coast of Aceh Province, Indonesia, in 2021–2022.

MATERIALS AND METHODS

Study area

Cellulolytic bacteria isolates were collected from the soil samples in rehabilitated and non-rehabilitated mangrove areas in the northern coast of Banda Aceh and Aceh Besar, Aceh Province, Indonesia. The rehabilitated mangrove is the vegetation planted after the tsunami catastrophe in 2004, while the non-rehabilitated is the ecosystem that was not destroyed by the tsunami. The rehabilitated mangroves were dominated by *Rhizophora* sp., but the three common species in the non-rehabilitated ecosystem included *Rhizophora* sp., *Avicennia marina*, and *Sonneratia alba*. Identifying these isolates was performed through molecular biological techniques, specifically the phylogenetic analysis of the 16S rRNA gene at the Research Laboratory, Faculty of Veterinary Medicine, Universitas Syiah Kuala (USK).

Sample handling

The study involved using bacterial isolates sourced from the soil samples. These isolates were purified and then tested for their ability to produce cellulase enzymes in the rehabilitated and non-rehabilitated mangrove ecosystems. Then, the isolates producing cellulase continued to the molecular biology step. The soil samples were collected from six locations, with three located in the non-rehabilitated area and the remaining in the rehabilitated mangrove region in Aceh Besar and Banda Aceh. Stations one, two, and three were identified in Lambadeuk, Dayah Teungoh, and Gampong Pande (rehabilitated); four, five, and six were in Ruyung, Lamreh, and Gampong Lampanah (non-rehabilitated). The study area's geographic location and soil sampling location are depicted in Fig. 1. Table 1 explains the location and coordinates of the study area.

Production of carboxymethyl cellulose (CMC) liquid media

The preparation of selective media for cellulolytic bacteria was carried out by weighing specific amounts of various ingredients, such as 1 gram (g) of CMC, 0.02 g of magnesium sulfate heptahidrate ($MgSO_4 7H_2O$), 0.05 g of potassium dihydrogen phosphate (KH_2PO_4), 0.075 g of potassium nitrate (KNO_3), 0.002 g of ferrous sulfate ($FeSO_4$), 0.004 g of calcium chloride ($CaCl_2$), 0.2 g of yeast extract, and 0.1 g of glucose, which were then added to 100 mililiter (mL) of distilled water and placed in an Erlenmeyer flask. Subsequently, one bacterial isolate was introduced into the flask and then centrifuged. The Erlenmeyer flask containing the liquid media was covered with aluminum foil and plastic wrap before transporting to the research laboratory at the Faculty of Veterinary Medicine, USK, for bacterial deoxyribonucleic acid (DNA) extraction.

DNA extraction

This process involved using the commercially available gDNA Presto™ Bacteria Mini kit (Geneaid) to extract the DNA separately. The purified total DNA of 50 microliter (µL), ~200 microgram per mititer (µg/mL) was eluted and then used as a template for Polymerase Chain Reaction (PCR) testing (Sari *et al.,* 2017). To begin the extraction process, a pellet was resuspended by vortexing in 200 mL of extraction buffer. Subsequently, 20 mL of proteinase K was added to the mixture, which was then incubated at 37 degree Celcius (°C) for 30 minutes (min), with the sample being inverted every 10 min, during the incubation period. The present study outlines a DNA extraction and purification protocol using a column-based method. Initially, 200 g of Genomic bind (GB) buffer was added to the sample, and the mixture vortexed for 10 seconds (s) before being incubated at 70°C for 10 min. The elution buffer is preheated to 70°C for the subsequent step, where 200 mL of absolute ethanol is added to the mixture and lysed using a shaker. The resulting sample is then transferred to a column tube of 2 mL capacity and centrifuged at 14.000–16.000 revolution per min (rpm) for 2 min. The supernatant is then discarded, and the pellet is transferred to a new 2 mL tube. Next, 400 mL of wash1 buffer is added to the column and centrifuged at

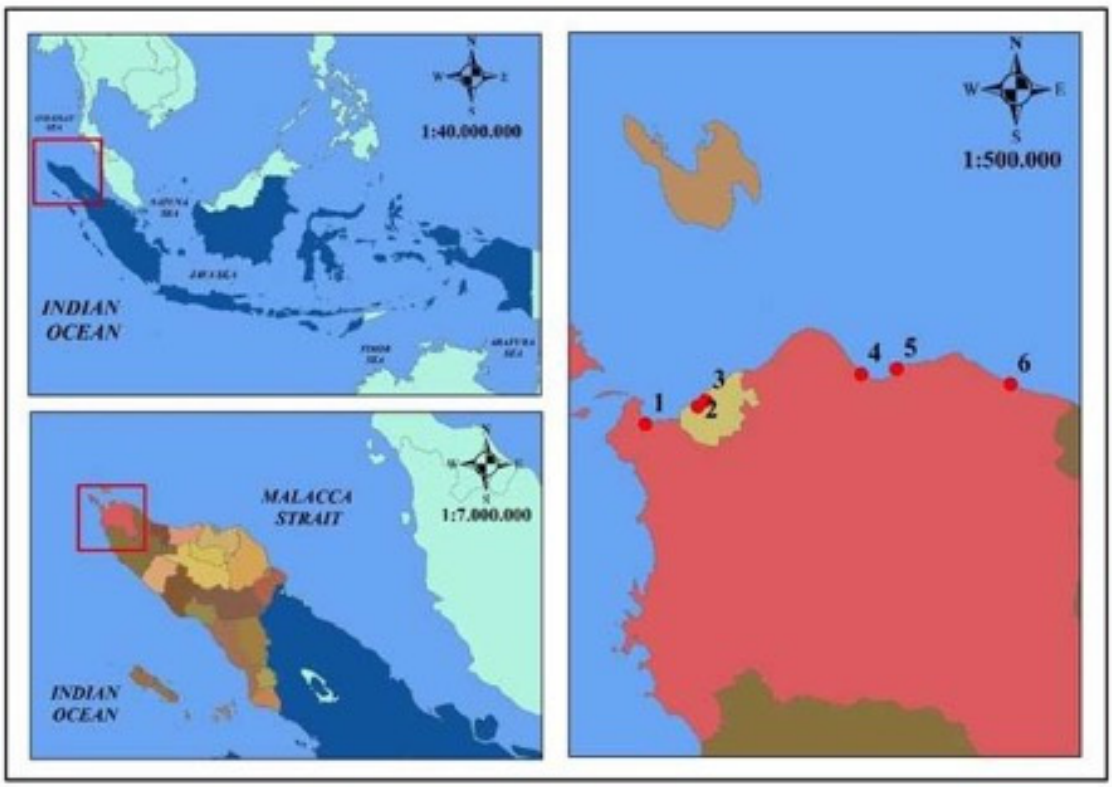


Fig. 1: Geographic location of the study area along with soil sampling locations with red dot in the mangrove ecosystem

Table 1: Location and coordinates of the study area

No.	Location	Coordinate
1	Gampong Lambadeuk	05°32'35.8"(North) N 95°14'30.9"E (East)
2	Gampong Dayan Teungoh	05°33'50.3"N 95°18'13.3"E
3	Gampong Pande	05°34'15.3"N 95°18'46.4"E
4	Gampong Ruyung	05°36'08.2"N 95°29'46.8"E
5	Gampong Lamreh	05°36'30.0"N 95°32'18.1"E
6	Gampong Lampanah	05°35'25.1"N 95°40'20.9"E

14.000–16.000 rpm for 30 s. The resulting supernatant is then discarded, and the process is repeated using 600 mL of wash buffer (containing ethanol). After the final centrifugation at 14.000–16.000 rpm for 30 s, the column is transferred to a 1.5 mL tube. Following this, preheated elution buffer (30–50 mL) is carefully added to the center of the column, and the DNA tube is allowed to incubate at room temperature for 3–5 min before being centrifuged at 14.000–16.000 rpm for 1 min. Finally, the purified DNA tube is stored at −20°C to prevent degradation until it is used in the PCR for DNA amplification.

DNA amplification

A study conducted by Sari *et al.* (2017) involved obtaining approximately 50 µL of pure DNA at a concentration of 200 µg/mL, which was then utilized as a template for PCR testing. The adopted primers were 63Forward (F) 5' (AGA GTT TGA TCM TGG CTC AG) 3' and 1387Reverse (R) 3' (TAC GGY TAC CTT GTT ACG ACT T) 5', thereby amplifying the 16S rRNA gene with a length of approximately 1500 base pair (bp). Meanwhile, 30 nanogram (ng) of DNA was combined with a total of 25 mL mixture containing 10 picomole (pmol) of each primer and 12.5 mL of the main mix

(Kapa Biosystems, Boston, Massachusetts, United States). The PCR amplification process involved 25 cycles and a pre-denaturation temperature of 95°C for 5 min. During each of the 25 cycles, denaturation was performed at 95°C for 1 min, followed by annealing at 50°C for 30 min, and extension at 72°C for 2 min. A final extension step was performed at 72°C for 10 min. In determining the purity and size of the amplification products, 1.2% weight/volume (w/v) agarose gel electrophoresis was used in 1× Tris-Acetate-ethylene diaminete traacetic acid (EDTA) buffer (TAE buffer), potential of hydrogen (pH) = 8.3 (containing 40 miliMolar (mM) Tris-hydrochloric acid (HCl), 40 mM acetate, 1.0 mM EDTA), and the Gel Doc XR+ System (Bio-Rad) was used for analysis. TAE is commonly used as a buffer for nucleic acid electrophoresis.

DNA electrophoresis and sequencing

The Gel Doc XR+ System from Bio-Rad was used for analyzing the gel electrophoresis results. The resulting electrophoresis bands were visualized using an ultra-violet (UV) transilluminator, such as the UVITEC Fire-Reader V10-Plus machine. The presence of a single, clear band with a size of relatively 1500 bp indicates good PCR product visualization. PCR products showing satisfactory electrophoresis results were sent to First BASE Laboratories, in Malaysia for further analysis. Cycle sequencing was performed using the PCR Kapa 2G Fast ReadyMix kit with dye, and the reaction product was sequenced with Dye Terminator (3'-labeled dideoxy nucleotide triphosphate).

Identification and Phylogenetic tree construction

To identify the bacteria species, the 16S rRNA gene based on sequencing techniques commonly employed for detecting and classifiying bacteria (Ntushelo, 2013). The 16S rRNA gene sequence is the most common genetic marker applied for almost all bacteria due its function has not changed over time, suitable for bacterial classification, and has sufficient variation to distinguish between taxa (Ntushelo, 2013; Manjul and Shirkot, 2018). The Clustal W program in the molecular evolutionary genetics analysis (MEGA X) version was used to align the obtained sequencing results alongside the sequencing data from GenBank (Tamura *et al.,* 2013). The edited sequencing alignments were 1386 bp; it is used to construct a phylogenetic tree with 1000× bootstrap support (Gusakov *et al.,* 2011; Naresh *et al.,* 2019; Sari *et al.,* 2017). However, to confirm the species similarity with existing databases, the Basic Local Alignment Search Tool (BLAST) was used to analyze the obtained sequencing results and determine the similarity between DNA sequencing and that of bacteria. Meanwhile, the BLAST-nucleotide, programmed at the National Center for Biotechnology Information (NCBI), was adopted to analyze the homology of 16S rRNA gene sequencing using data in the GenBank database. Using the kimura-2-parameter (K2P) model, genetic distances were estimated, and bacterial relationships were presented in a phylogenetic tree constructed using the Neighboring Joining (NJ) method with 1000× bootstrap and the K2P model. The MEGA X program was used to construct the phylogenetic tree based on the methods that Kumar *et al.* (2018) and Tamura *et al.* (2013) adopted.

RESULTS AND DISCUSSION

Composition of cellulolytic bacteria species in soil on mangrove ecosystems

The 16S rRNA gene was amplified from cellulolytic bacteria using the primer pair 63F and 1387R, yielding a fragment size of 1500 bp upon alignment of the DNA ladder (Fig. 2). After alignment, the 16S rRNA gene fragment size was determined to be 1386 bp. This primer pair is capable of amplifying genes of approximately 1300 bp, resulting in an average size of ±1350 bp for the 16S rRNA gene fragment (Faturrahman, 2005; Kusumaningrum *et al*., 2016). The 16S rRNA is commonly used and selected as a method of bacterial identification because of its distribution in all bacteria species, its function that never changes, and this gene is large enough to reach 1500 bp in size (Safika *et al*., 2018).

The sequencing results were compared to the GenBank database, revealing species similarities ranging from 97.77% to 100%. The specific similarity values for each species are presented in Table 2. This indicates that the values between 97.77% and 100% of the sequencing matched the database. For instance, the isolates BTM121, BTM622, BTM632, and BTM511 were identified as *B. altitudinis* with 100% homology, whereas isolate BTM113 was identified as *Bacillus* sp1. with 97.77% homology, and *Bacillus* sp2. with 99.86%. According to Petti

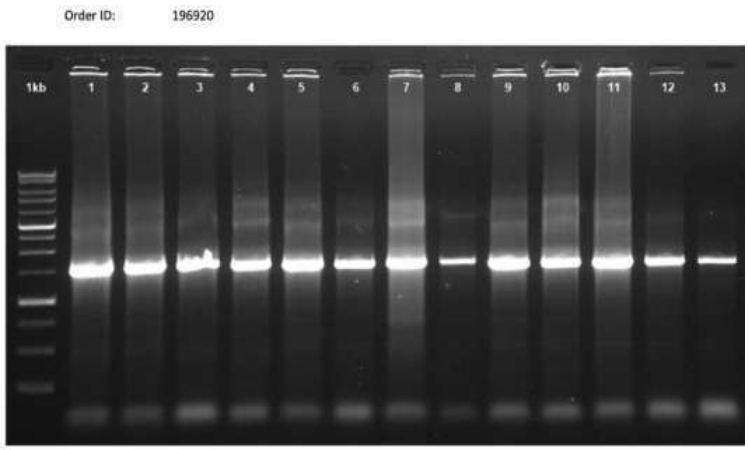


Fig. 2: Visualization of DNA from each cellulolytic bacterial isolate on geldoc *Description: 1 kilobyte (kb) = 250 DNA Ladder (bp)*

Table 2: Species of cellulolytic bacteria identified using 16S rRNA

No. of species	Sample code	Species	BLAST (%)	bp
1	BTM113	*Bacillus* sp1.	97.77	1386
2	BTM512	*Bacillus* sp2.	99.86	1386
3	BTM 121	*B. altitudinis*	100	1386
	BTM 622	*B. altitudinis*	100	1386
	BTM 632	*B. altitudinis*	100	1386
	BTM 511	*B. altitudinis*	100	1386
4	BTM211	*B. amyloliquefaciens*	99.78	1386
5	BTM533	*B. safensis*	100	1386
6	BTM123	*B. subtilis*	99.93	1386
	BTM621	*B. subtilis*	99.93	1386
7	BTM611	*B. velezensis*	99.93	1386
8	BTM431	*Brevibacillus* sp.	99.57	1386
9	BTM321	*P. aeruginosa*	98.85	1386

(2007), a genus is considered similar assuming the similarity score is 97%, and a species is deemed one supposing the similarity obtained is 99%. However, assuming *Bacillus* sp1. from isolate BTM113 does not reach the 99% to 100% homology threshold, it may suspect a new species, and there are nucleotide base differences compared to GenBank. The same sequencing result has not been recorded or registered in GenBank. Additionally, isolate Bakteri tanah mangrove (BTM)432 and BTM321 were identified as *Brevibacillus* sp., and *Pseudomonas aeruginosa*, with a 99.57% and 98.85% similarity to the data in GenBank. The highest individual composition is *B. altitudinis* followed by *B. subtilis*.

Briefly, nine species of cellulolytic bacteria were identified among the 13 sequenced isolates obtained from the mangrove ecosystem soil (Table 2). These include *B. altitudinis, B. amyloliquefaciens, Bacillus* sp1., *Bacillus* sp2., *B. safensis, B. subtilis, B. velezensis, Brevibacillus* sp., and *P. aeruginosa*. However, among these species, *Bacillus* was the most commonly found genus, with four isolates identified as *B. altitudinis*, and two identified as *Bacillus* sp. and *B. subtilis*. This finding is consistent with previous studies, such as Shome *et al*. (1995) who identified 38 bacterial isolates, and reported that *Bacillus* sp. is the most dominant species (>50%) isolated from mangrove sediments in South Andaman. Yahya *et al*. (2014) reported that *Bacillus* sp. is the most abundant bacteria involved in the decomposition process of mangrove litter in the coast of Pasuruan Palace, thereby exhibiting the ability to degrade cellulose. Vásquez and Millones (2023) reported two species of bacteria that produce cellulase enzymes: *B. safensis*

and *B. subtilis*. *Bacillus* is a genus of Firmicutes group; Basak *et al*. (2016) found that Bacteroidetes, Acidobacteria, Firmicutes, Actinobacteria, Nitrospirae, Cyanobacteria, Planctomycetes, and Fusobacteri are groups of microorganisms mostly found in mangrove sediments in Dhulibhashani, India. *Brevibacillus* sp. is a type of cellulolytic bacteria that can produce cellulase enzymes. Liang *et al*. (2009) reported that strain JLX of *Brevibacillus* sp., can break down various unique polymers, including cellulose, and utilizes various carbohydrates, such as cellulose, cellobiose, glucose, and xylose. Several studies have reported that *P. aeruginosa* is another cellulase-producing bacteria found in soil (Gunavathy and Boominathan, 2015; Gunavathy and Boominathan, 2016) and sawdust (Agarwal *et al.*, 2014). Kurniawan *et al*. (2018a) identified *P. aeruginosa* as one of the cellulose-degrading bacteria found in mangrove forest soil in Bangka Island, Indonesia. The diversity of cellulolytic bacteria varies in each area of the mangrove ecosystem; various species can be found in different mangrove ecosystems due to differences in their environmental characteristics, such as soil and water. The higher the species diversity and abundance of bacteria, the faster process of organic matter degradation. The bacterial richness indicates ecosystem fertility because they uniquely functions as a decomposer, such as cellulolytic bacteria, which produces cellulase enzyme that accelerates cellulose degradation (Biswas *et al*., 2020). Several studies have been conducted on mangrove soil's bacteria. For example, Kurniawan *et al*. (2019) found cellulolytic bacteria using molecular biology of the 16S rRNA gene, including TBL1 isolate with 85% similarity to *Vibrio parahaemolyticus*, and strains HY3 and TBL2 with 98% similarity to *B. amyloliquefaciens*. Pramono *et al*. (2021) identified isolate LG2 as the species *Fictibacillus nanhaiensis* strain JSM 082006, which produced the highest cellulase enzyme activity. Naresh *et al.* (2019) successfully identified three species using the 16S rRNA gene: *Anoxybacillus* sp., *B. subtilis*, and *Paenibacillus dendritiformis*, and Biswas *et al.* (2020) discovered strains T2-D2 as *Bacillus* sp. and E1-PT as *Pseudomonas* sp. The composition of cellulolytic bacteria found in mangrove sediments is high, reaching 87.87% (Pramono *et al*., 2021). In the fisheries sector, several cellulase bacteria were found to have potential as probiotics. According to Yanbo and Zirong (2006) and Soltani *et al.* (2019), *Bacillus* sp. is one of the bacteria believed to increase the digestibility of fish because these bacteria are classified as probiotics, so they are often applied to feed to increase feed digestibility. The species of *Bacillus* often applied as a probiotic is *B. subtilis* (El Dakar *et al*., 2007; Olmos *et al*., 2020; Truong *et al*., 2021), and this bacterium was found at the study site. The use of *Bacillus* sp. as a probiotic bacterium in fish feed is believed to enhance their digestive capacity by converting unutilized feed into a digestible form (Putra *et al*., 2021; Widanarni *et al*., 2022). This approach can be an alternative to producing dual-function feed and indirectly improve feed quality.

Genetic distance of cellulolytic bacteria species

Table 3 shows the genetic distance within-species (intraspecies) values, and the values of interspecies distances. Four isolates detected as the *B. altitudinis* species exhibited a species distance of zero, meaning that these isolates are identical. Similarly, two isolates identified as the *B. subtilis* species exhibited a within-species distance of zero. *Bacillus* sp. has a interspecies distance value of 0.084%, indicating differences between the two species (*Bacillus* sp1. and *Bacillus* sp2.). The interspecies genetic distance is 0.069% between *Bacillus* sp2. and *B. amyloliquefaciens*, 0.067% between *Bacillus* sp2. and *B. altitudinis*, and 0.067% between *B. velezensis* and *Bacillus* sp2. The closest intraspecies distances were found to be between *B. velezensis* and *B. amyloliquefaciens* at 0.002%, followed by *B. safensis* and *B. altitudinis* at 0.004%, *B. velezensis* and *B. subtilis* at 0.004%, as well as *B. subtilis* and *B. amyloliquefaciens* at 0.006%. Maduppa *et al*. (2018) stated that a smaller genetic distance implies a closer a relationship between species and vice versa.

In a study by Fan *et al*. (2017), *B. velezensis, B. methylotrophicus,* and *B. amyloliquefaciens* were found to have a high degree of similarity in their nucleotide base sequence, depicting that they belong to the same clade. Furthermore, Hossain *et al*. (2015) reported that a recent phylogenomic study has shown that the genomes of *B. methylotrophicus, B. velezensis, B. oryzicola,* and *B. amyloliquefaciens* are highly similar. A specific strain isolate code of *B. amyloliquefaciens* (DSM 23117T), first identified as *B. amyloliquefaciens* in 2008 (Wang *et al*., 2008), was later revised as *B. amyloliquefaciens* sub-sp. plantarum in 2011 (Borris *et al*., 2011), and finally

Table 3: Within-species and interspecies distance values of cellulolytic bacteria

No.	Species	1	2	3	4	5	6	7	8	9	10	11	12	13
1	*B. altitudinis*	0												
2	*B. altitudinis*	0.000	0											
3	*B. altitudinis*	0.000	0.000	0										
4	*B. altitudinis*	0.000	0.000	0.000	0									
5	*B.amylo liquefaciens*	0.030	0.030	0.030	0.030	0								
6	*Bacillus* sp1.	0.045	0.045	0.045	0.045	0.019	0							
7	*Bacillus* sp2.	0.067	0.067	0.067	0.067	0.069	0.084	0						
8	*B. safensis*	0.004	0.004	0.004	0.004	0.032	0.048	0.067	0					
9	*B. subtilis*	0.029	0.029	0.029	0.029	0.006	0.021	0.066	0.031	0				
10	*B. subtilis*	0.029	0.029	0.029	0.029	0.006	0.021	0.066	0.031	0.000	0			
11	*B. velezensis*	0.028	0.028	0.028	0.028	0.002	0.019	0.067	0.030	0.004	0.004	0		
12	*Brevibacillus* sp.	0.969	0.969	0.969	0.969	0.960	1.006	0.914	0.963	0.957	0.957	0.960	0	
13	*P. aeruginosa*	5.428	5.428	5.428	5.428	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0

reclassified as *B. velezensis* in 2016 based on DNA hybridization, and phenotypic and phylogenetic analyses (Dunlap *et al*., 2016). Although some recent publications and GenBank data sets still refer to this strain as *B. amyloliquefaciens*, it was later confirmed as *B. velezensis* strain using molecular methods (Fan *et al*., 2017; Liu *et al*., 2019; Santiago *et al*., 2021). Despite having similar morphology, physiology, phenotypic properties, and 16S rRNA gene sequences, *B. velezensis* and *B. amyloliquefaciens* can be distinguished based on their fatty acid (FA) cellular composition, as noted by Huynh *et al.* (2022). Both *B. velezensis* and *B. amyloliquefaciens* belong to the *B. subtilis* group, which suggests a close taxonomic relationship (Fan *et al.*, 2017).

Phylogenetic tree of cellulolytic bacteria

Using the NJ method with 1000× bootstrap repetitions, the phylogenetic analysis consistently revealed that *B. velezensis* is closely related to *B. amyloliquefaciens* and *B. subtilis* and showed that *B. subtilis* is closely related to *B. safensis* (Fig. 3). Recently, Vásquez and Millones (2023) reported the close phylogenetic relationship of cellulolytic bacteria of *B. subtilis* and *B. safensis* isolated from solid waste of palm forests, Peru. Constructing the phylogenetic tree from 13 cellulolytic bacteria isolates divided into nine species, and two main clades (groups). Clade one consist of *B. altidudinis, B safensis, B. amyloliquefaciens*, *B. subtilis, Bacillus* sp1. (BTM113), *Bacillus* sp2. (BTM512), *B. velezensis,* and *Brevibacillus* sp. Meanwhile, clade two consists of *P. aeruginosa* at 1000× bootstrap. One main clade was then divided into two subclades, which separated *Brevibacillus* sp. Species of *A. hydrophila* (access code LR991675.1), an outgroup species in the phylogenetic tree (Fig. 3). The *Bacillus* and *Brevibacillus* genera gather in the same class and order, namely the Bacilli class and the order Bacillales, but in different families. *Bacillus* belongs to the Bacillaceae family while *Brevibacillus* belongs to the Paenibacillaceae family. Both families are the phylum Firmicutes. Sulistiyani *et al*. (2021) reported two families and three genera identified using the 16S rRNA gene, namely Bacillaceae (genus *Bacillus* and *Fictibacillus*), and Paenibacillaceae (genus *Brevibacillus*). These families can produce cellulase, xylanase, amylase, and lipase enzymes (Shanti and Roymon, 2018; Powthong and Suntornthiticharoen, 2017).

Comparatively, there are five species of cellulolytic bacteria in the rehabilitated mangrove area: *B. amyloliquefaciens, Bacillus* sp1., *B. subtilis, B. altitudinis,* and *P. aeruginosa*. The non-rehabilitated area has six species of celluloytic bacteria, including *Bacillus* sp2., *B. subtilis, B. altitudinis, B. velezensis, B. safensis*, and *Brevibacillus* sp. Based on the analysis, *Bacillus* sp1., *B. amyloliquefaciens*, and *P. aeruginosa* were not found in non-rehabilitated areas, while *Bacillus* sp2., *B. safensis*, *B. velezensis* and *Brevibacillus* sp. were also not found in the rehabilitated areas. The phylogenetic trees of the rehabilitated and non-rehabilitated mangrove areas, shown in Fig. 4a and Fig. 4b, respectively, consist of two main clades, with one serving as an outgroup. The species used as an outgroup in this phylogenetic tree is *A. hydrophila* (access code LR991675.1).

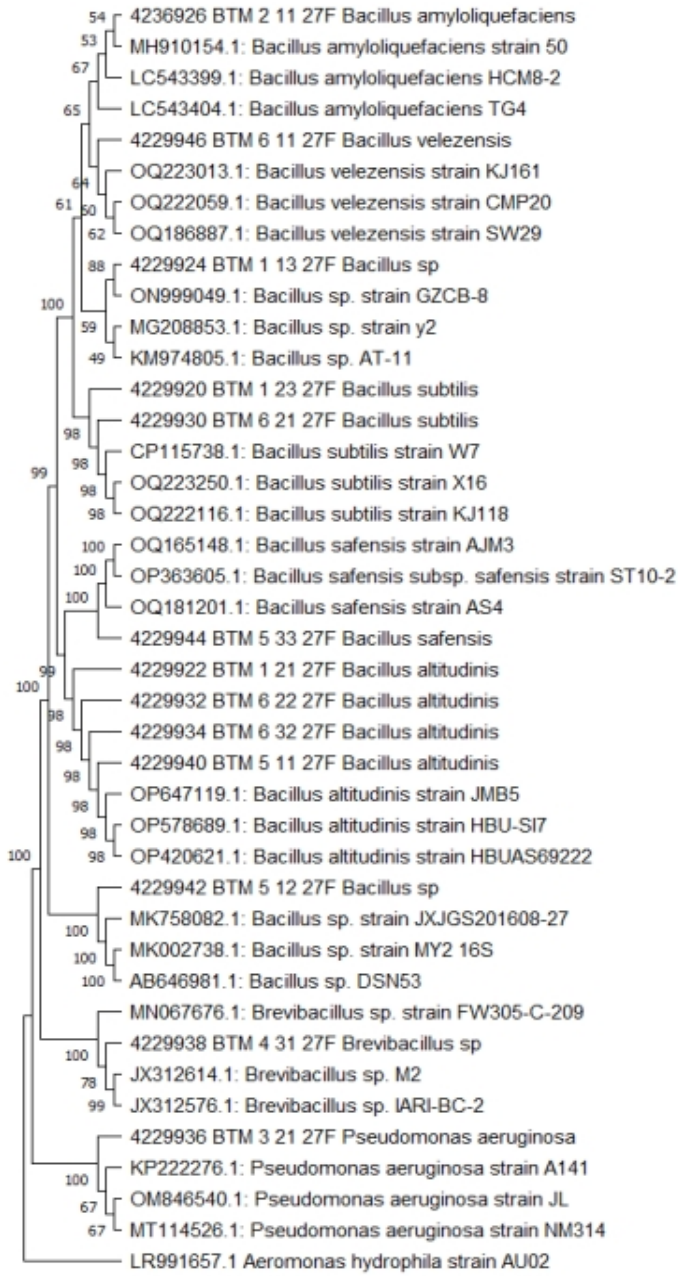
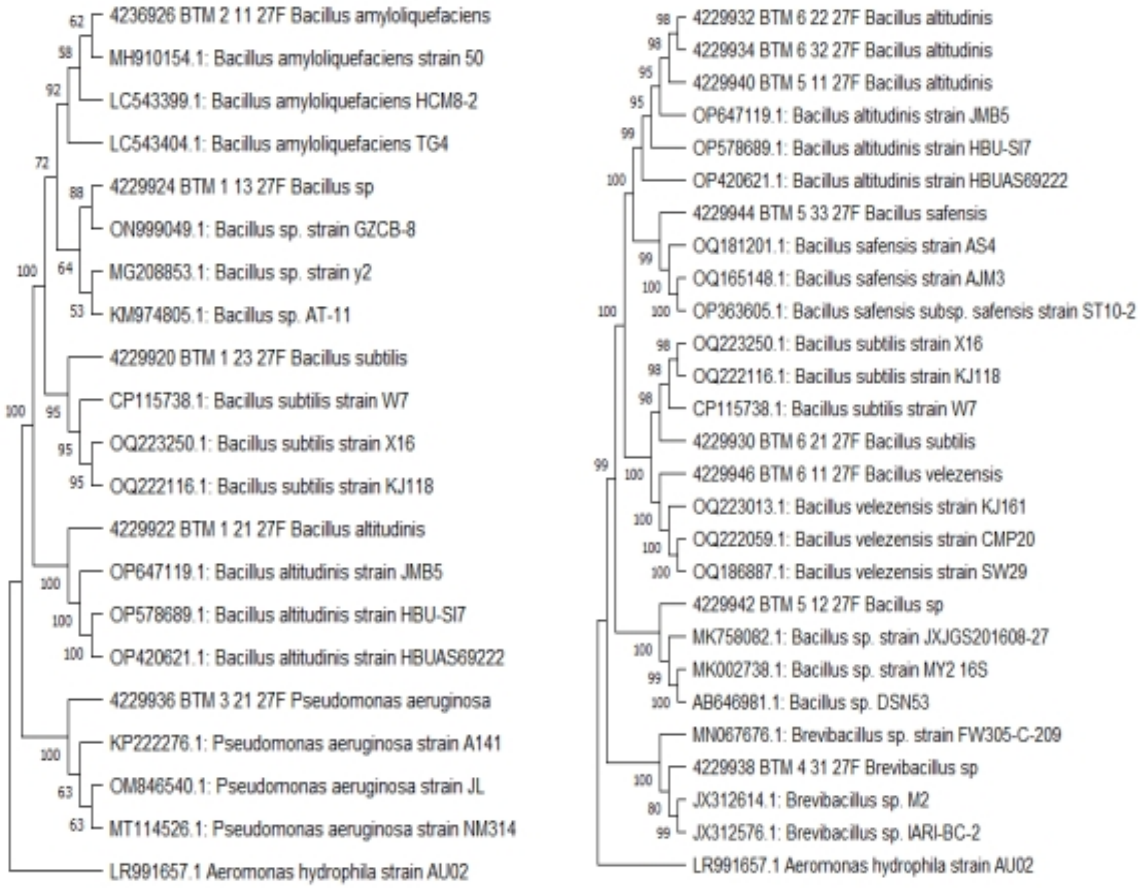


Fig. 3: Phylogenetic tree of 9 cellulolytic bacterial sequences isolated from mangrove soil using NJ method

The present study found that the number of cellulolytic bacteria species was higher in the non-rehabilitated mangrove area than in the rehabilitated one. Preliminary studies suggested that this difference in species richness was due to lower OC in the rehabilitated mangrove soil. The OC in the non-rehabilitated and rehabilitated mangrove were 1.23% and 0.90% (Dewiyanti *et al*., 2021), followed by a higher percentage of silt fraction (fine fraction) in non-rehabilitated mangrove (43%) than in



a b

Fig. 4: Phylogenetic tree of cellulolytic bacteria in soil in mangrove ecosystems (a) rehabilitated mangrove (b) non-rehabilitated mangroves

rehabilitated (31%). Soil organic C content is higher in fine soil with small porosity (silt) than in coarse soil with big porosity (sand). Carney and Matson (2005) mentioned that fine-textured soils support more microbial biomass and species than their coarse-textured. Huang *et al.* (2019) supported this view, stating that bacterial diversity tends to increase with higher organic matter content in the soil. A higher number of mangrove vegetation in non-rehabilitated than in rehabilitated area was assumed to contribute more litter production that can increase OC content. There were 70 individuals/100 square meter (m^2) in non-rehabilitated than 39 individuals/100 m^2 in rehabilitated. Litter production significantly contributes OC into the soil, promoting the diversity of cellulolytic bacteria (Leff *et al.,* 2012). Another factor that could explain the higher number of bacterial species in the non-rehabilitated areas is the more extensive root systems in these ecosystems. Batubara (2021) reported that the rhizosphere, the soil layer where roots grow, is enriched with nutrients from root exudates (materials released from living root cells, such as sugars, amino acids, organic acids, and FA) and other soil organisms. Bacteria tend to dominate in the rhizosphere due to organic matter abundance; many bacteria species form symbiotic relationships with plant roots, as these exudates serve as the primary food source for microorganisms

Table 4: Nucleotide base composition and accession number registered in GenBank of cellulolytic bacteria

No.	Bacterial isolate	Species	Accession number	T (%)	C (%)	A (%)	G (%)	GC (%)
1	BTM121	*B. altitudinis*	OQ152604	20.00	23.78	25.02	31.20	54.98
	BTM632	*B. altitudinis*	OQ152605	20.00	23.78	25.02	31.20	54.98
	BTM511	*B. altitudinis*	OQ152606	20.00	23.78	25.02	31.20	54.98
	BTM622	*B. altitudinis*	OP363153	20.00	23.78	25.02	31.20	54.98
2	BTM211	*B. amyloliquefaciens*	OQ152607	19.93	23.78	24.87	31.42	55.20
3	BTM113	*Bacillus* sp1.	OQ152608	20.65	22.98	24.95	31.42	54.40
4	BTM512	*Bacillus* sp2.	OQ152609	20.87	22.76	25.82	30.55	53.31
5	BTM123	*B. subtilis*	OQ152610	20.07	23.71	24.95	31.27	54.98
	BTM621	*B. subtilis*	OQ152611	20.00	23.64	25.02	31.35	54.98
6	BTM611	*B. velezensis*	OQ152612	20.00	23.64	25.02	31.35	54.98
7	BTM622	*B. safensis*	OP363154	19.93	23.71	24.87	31.49	55.20
8	BTM431	*Brevibacillus* sp.	OQ152613	20.51	23.64	24.80	31.05	54.69
9	BTM321	*P. aeruginosa*	OQ152614	20.36	22.62	25.38	31.64	54.25

Table 5: Average nucleotide composition

Nucleotide	Average	Minimum	Maximum
T %	20.18	19.93	20.87
C %	23.51	22.62	23.78
A %	25.06	24.80	25.82
G %	31.26	30.55	31.64
GC %	54.76	53.31	55.20

and their activities (Raaijmakers *et al*., 2009; Sulistiyani *et al*., 2020). A previous study investigating cellulolytic bacteria: *B. altitudinis* and *B. safensis* showed a higher cellulolytic index (CI) than other bacteria found in mangrove non-rehabilitated areas. Dewiyanti *et al*. (2022) reported that *B. safensis* had CI index of 4.82, followed by *B. altitudinis* with 2.09 CI index, categorized as a high CI index. A high CI index indicates high cellulase production (Naresh *et al.,* 2019). Enzyme produced by microorganism could be good indicators to ecosystem response's to environmental changes (Luo and Gu, 2015).

Composition of nucleotide bases in cellulolytic bacteria

The sequencing of 13 isolate samples resulted in nucleotides with a base length of 1386 bp, and Guanine (G) being the most abundant nucleotides, followed by Adenine (A), Cytosine (C), and Thymine (T). The nucleotide base composition for each cellulolytic bacterial species is presented in Table 4. The nucleotide composition had an average of 20.18% (T), 23.51% (C), 25.06% (A), and 31.26% (G). The GC content of the first, second, and third codon positions increased, with mean GC and AT contents of 27.39%, and 22.62%, respectively. The average nucleotide composition is shown in Table 5.

After conducting a nucleotide alignment, the two *Bacillus* sp. isolates were discovered to be distinct species, as revealed by Multalin (multiple sequence alignment) program (Fig. 5). The interspesies were identified in both isolates, with nucleotide base changes observed at several points, denoted by the following base sequence numbers 9, 11, 12, 16, 17, 28, 29, 74, 75, 118, 119, 120, 121, 122, etc. The nucleotide base of these two species had a variation of 106 base sequence points (7.7%) due to nucleotide base substitution. These nucleotide base variations are highlighted in blue and black (Fig. 5), and were primarily caused by nucleotide base changes, including transition and transversion substitutions. Transitions are changes between A and G bases (purines) or disparities between C and T bases (pyrimidines), while transversions are alterations

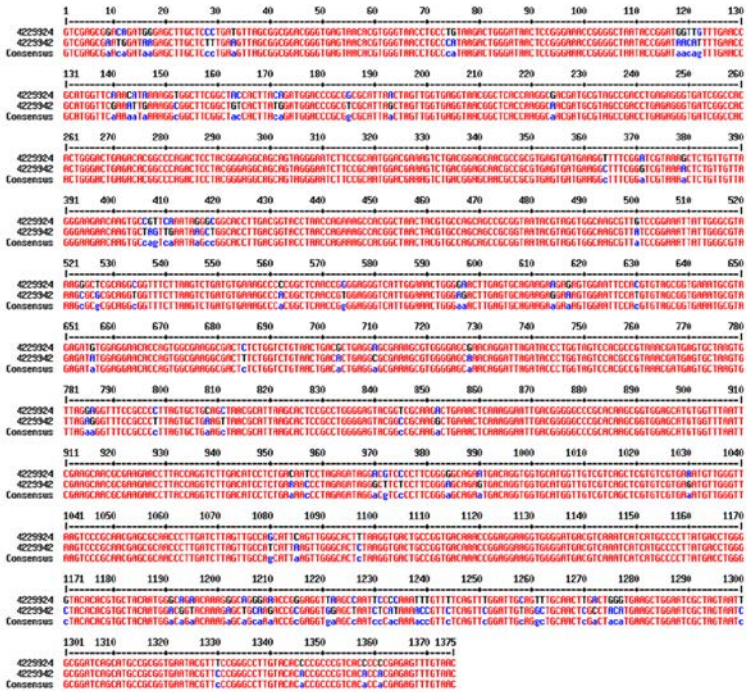


Fig. 5: Sequence alignment of *Bacillus* sp. (BTM113 and BTM512) using Multalin

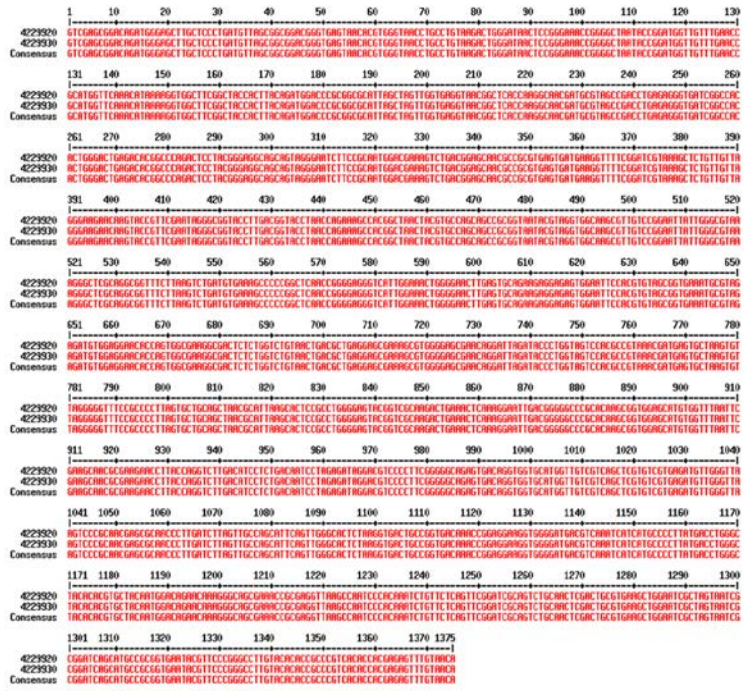


Fig. 6: Sequence alignment of *B. subtilis* (BTM123 and BTM621) using Multalin

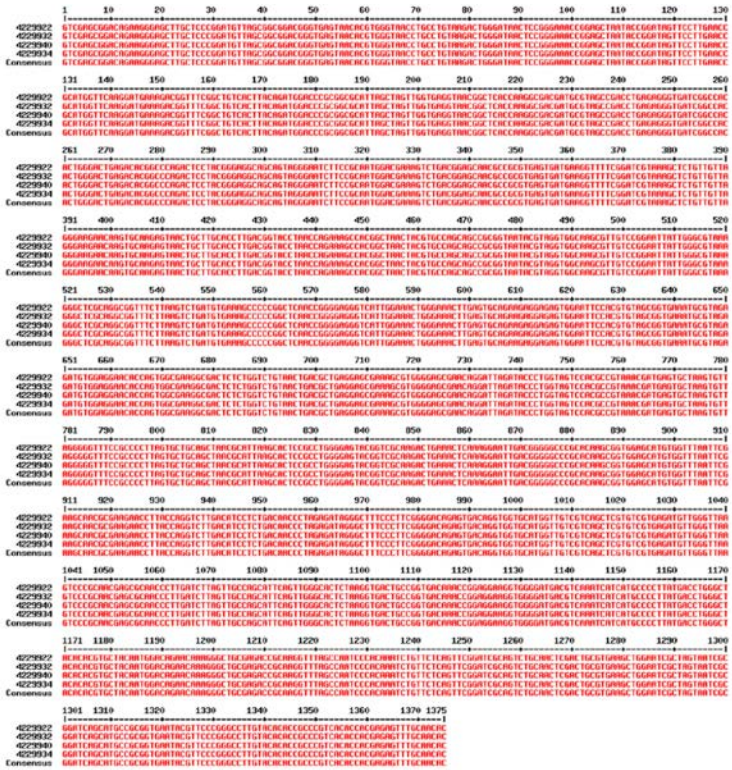


Fig. 7: Sequence alignment of *B. altitudinis* (BTM121, BTM622, BTM632 and BTM511) using Multalin

between a purine and pyrimidine base (Elyvra *et al*., 2009; Nandy, 2009; Ubaidillah and Sutrisno, 2009). Specifically, the nucleotide chain polymorphism in *Bacillus* sp. includes 74 nucleotide transitions and 32 transversions. Among these substitutions, nucleotide transitions were more commonly found than transversions (Kochzius and Nuryanto, 2008). The nucleotide composition of *Bacillus* sp1 (BTM113) was 20.65% (T), 22.98% (C), 24.95% (A), and 31.42% (G), and the highest average was the G+C pair with a value of 27.2% while the lowest is the A+T pair (22.8%), the G+C nucleotide pair composition was also higher in *Bacillus* sp2. (BTM512). Assuming both species have a higher A+T nucleotide base value based on composition, the species will have many similarities due to independent parallel substitutions. Conversely, supposing both species have a higher G+C nucleotide base composition, the species will have fewer similarities (Lam and Morton, 2006). Transition mutations were found to occur more frequently and easily than transversion mutations, which explains why transitions were more commonly found among substitutions (Graur and Li, 2000). Notably, the average content of the G+C nucleotide base was greater than that of the A+T nucleotide base in each identified species of cellulolytic bacteria, with values of 27.39% (GC) and 22.62% (AT), respectively. This formula also occurred in each identified species of cellulolytic bacteria. This finding is consistent with previous research suggesting that G+C nucleotide pairs are more prevalent in bacterial genomes.

Meanwhile, two isolates, identified as *B. subtilis*, and the four isolates, identified as *B. altitudinis,* were confirmed through alignment to be the same species. Figs. 6 and 7 show that the nucleotide base variations in these isolates were identical, indicating that they belonged to the same species.

CONCLUSION

The study results revealed cellulolytic bacteria in mangrove soil for rehabilitated and non-rehabilitated, Banda Aceh and Aceh Besar, Aceh Province, Indonesia.

Based on the alignment using MEGA X and comparing to the BLAST database in GeneBank, nine species were identified from the 13 isolates successfully sequenced using the 16S rRNA gene: *B. altitudinis*, *B. amyloliquefaciens*, *Bacillus* sp1., *Bacillus* sp2., *B. safensis, B. subtilis*, *B. velezensis*, *Brevibacillus* sp., and *P. aeruginosa*. The similarity of data sequencing and GenBank database ranged from 97.77% to 100%. The isolates BTM121, BTM622, BTM632, and BTM511 were identified as *B. altitudinis* with 100% homology, whereas isolate BTM113 was identified as *Bacillus* sp1. with 97.77% homology, and *Bacillus* sp2 with 99.86%. Species of *B. amyloliquefaciens*, *B. safensis*, *B. subtilis, B. velezensis, and P. aeruginosa* have 99.78%, 100%, 99.93%, 99.57%, and 98.85% homology, respectively. The species obtained are considered similar due to similarity achieving 97%, and categorized as same species with database if the similarity obtained 99%. Further analysis revealed that *B. velezensis* is closely related to *B. amyloliquefaciens*, with interpecific genetic distance of 0.002%. The farthest interspecies genetic distance was 0.084% related between *Bacillus* sp1. and *Bacillus* sp2. Small genetic distances of interspecies indicate a close relationship between species. Research studies were located at two kinds of mangrove habitats (rehabilitated and non-rehabilitated mangrove) with different characteristics, for example, substrate fraction, OC, and mangrove species. Previous research showed that non-rehabilitated mangrove contain higher OC, silt fraction, and mangrove vegetation species and abundance. This condition makes the non-rehabilitated mangrove soil acquire more bacterial cellulolytic species than the rehabilitated habitat. The characteristics of a suitable soil environment strongly support the life and diversity of cellulolytic bacteria, so an action is needed to increase the presence of cellulolytic bacteria in mangrove soils by applying cellulolytic species that produce high enzyme cellulase. The bacteria diversity is a critical aspect to the functioning of the ecosystem, managing the ecosystem while maintaining diversity may be one of the approaches to ensure the stability and productivity of the mangrove ecosystem in the study area. Notably, *Bacillus* is a genus commonly found in the study area, and two isolates from the *Bacillus* sp. were identified as different species, as proven by different nucleotide bases that have been aligned. *Bacillus* sp1. could be a novel species due to low homology percentage and the absence of nucleotide bases identical to the aligned data on GenBank. *B. subtilis* found is a cellulolytic bacterium often applied in the industrial, animal husbandry, and fishery sectors.

AUTHOR CONTRIBUTIONS

D. Darmawi, as a corresponding author, developed the study idea and concepts and supervised the manuscript. I. Dewiyanti, as the first author, has contributed to study conceptualization, surveys, data collection and analysis, result interpretation, and manuscript preparation. Z.A. Muchlisin contributed to the research design, result interpretation, and manuscript review. T.Z. Helmi contributed to the result interpretation and manuscript review.

ACKNOWLEDGEMENT

The authors are grateful to the Directorate of Research and Community Service for providing research grants through the Doctoral Dissertation scheme, the contract research number was [154/SP2H/LT/DRPM/2021]. Furthermore, the authors are grateful to Dila, Iko, Nanda, Thariq, Fajar for their fieldwork and to the local people for support and cooperation during the study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been completely observed by the authors.

OPEN ACCESS

PUBLISHER'S NOTE

GJESM Publisher remains neutral with regard to jurisdictional claims in published maps and institutional afflictions.

ABBREVIATIONS

16S rRNA	16 svedberg ribosomal ribonucleic acid
%	Percent
µg	Microgram
µL	Microliter
°C	Degree Celsius
A	Adenine
BTM	Bakteri tanah mangrove (mangrove soil bacteria) as isolate code
BLAST	Basic local alignment search tool
bp	Basepair
C	Cytosine
$CaCl_2$	Calcium chloride
CFU	Colony form unit
CI	Cellulolytic index
CMC	Carboxymethyl cellulose
DNA	Deoxyribonucleic acid
DSM 23117T	Isolate code of *B. amyloliquefaciens*
E1-PT	Isolate code of *Pseudomonas*.
E	East
EDTA	Ethylene diaminete traacetic acid
etc	et cetera
F	Forward
$FeSO_4$	Ferrous sulphate monohydrate
FA	Fatty acid
G	Guanine
g	Gram
GB	Genomic bind
HCL	Hydrogen chloride
HY3	Isolate code of *B. amyloliquefaciens*
JLX	Genus in the *Brevibacillus*
JSM	Strain code
K2P	Kimura-2-parameter
KH_2PO_4	Potassium Dihydrogen Phosphate
KNO_3	Potassium nitrate
LG2	Isolate code of *Fictibacillus nanhaiensis*
LR991675.1	Access code of *A. hydrophila*
m^2	Square meter
MEGA	Molecular evolutionary genetics analysis
$MgSO_4.7H_2O$	Magnesium sulfate heptahydrate
min	Minute
mL	Mililiter
mM	miliMolar
Multalin	Multiple sequence alignment
N	North
NCBI	National Center for Biotechnology Information
ng	Nanogram
NJ	Neighboring joining
No	Number
OC	Organic carbon
PCR	Polymerase Chain Reaction
pH	Potential of hydrogen
pmol	Picomole
R	Reverse
rpm	Revolution per minute
s	Second
T2-D2	Isolate code of *Bacillus* sp.
T	Thymine
TAE buffer	Tris-acetate-EDTA buffer
TBL1	Isolate code of *Vibrio parahaemolyticus*
TBL2	Isolate code of *B. amyloliquefaciens*
USK	Universitas Syiah Kuala
UV	Ultra Violet
w/v	Weight/volume

REFERENCES

Agarwal, T.; Saxena, M.K.; Chandrawat, M.P.S., (2014). Production and optimization of cellulase enzyme by *Pseudomonas aeruginosa* MTCC 4643 using sawdust as a substrate. Int. J. Sci. Res., 4(1): 1-3 **(3 pages).**

Becker, C.C.; Weber, L.; Suca, J.J.; Klopiz, J.K.; Mooney, T.A.; Apprill, A., (2020). Microbial and nutrient dynamics in mangrove, reef, and seagrass waters over tidal, and diurnal time scales. Aquat. Microb. Ecol., 85: 101-119 **(19 pages).**

Basak, P.; Pramanik, A.; Sengupta, S.; Nag, S.; Bhattacharyya, A.; Roy, D.; Pattanayak, R.; Ghosh, A.; Chattopadhyay, D.; Bhattacharyya, M., (2016). Bacterial diversity assessment of pristine mangrove microbial community from Dhulibhashani, Sundarbans using 16S rRNA gene tag sequencing. Genom. Data. 1(7): 76-78 **(3 pages).**

Batubara, U.M.; Suparjo, S.; Maritsa, H.U.; Pujianto, E.; Herlini, M., (2022). Screening and determination of potential cellulolytic bacteria from mangrove ecosystem. J. Perikanan dan Kelautan., 27(2): 264-271 **(8 pages).**

Behera, B.C.; Parida, S.; Dutta, S.K.; Thatoi, H.N., (2014). Isolation and identification of cellulose degrading bacteria from mangrove soil of Mahanadi River Delta and Their Cellulase. Am. J. Microbiol. Res., 2(1): 41-46 **(6 pages).**

Biswas, S.; Al Saber, Md.; Tripty, I.A.; Karim, A.Md.; Islam, A.Md.; Hasan, S.Md.; Rubayet Ul Alam, A.S.M.; Jahid, K.Md.I.; Hasan, N.Md., (2020). Molecular characterization of cellulolytic (endo- and exoglucanase) bacteria from the largest mangrove forest (Sudarbans), Bangladesh. Anal. Microbiol., 7(70): 1-11 **(11 pages).**

Borriss, R.; Chen, X.H.; Rückert, C.; Blom, J.; Becker, A.; Baumgarth, B.; Fan, B.; Pukall R.; Schumann, P.; Spröer, C., (2011). Relationship of *Bacillus amyloliquefaciens* clades associated with strains DSM 7T and FZB42T: A proposal for *Bacillus amyloliquefaciens* subsp. *amyloliquefaciens* subsp. nov. and *Bacillus amyloliquefaciens* subsp. *plantarum* subsp. nov. based on complete genome sequence comparisons. Int. J. Syst. Evol. Microbiol., 61(8): 1786-1801 **(16 pages).**

Carney, K.M.; Matson, P.A., (2005). Plant communities, soil microorganisms, and soil carbon cycling: does altering the world belowground matter to ecosystem functioning?. Ecosystems., 8(8): 928–940 **(13 pages).**

Chantarasiri, A., (2015). Aquatic *Bacillus cereus* JD0404 isolated from the muddy sediments of mangrove swamps in Thailand and characterization of its cellulolytic activity. Egypt. J. Aquat. Res., 41(3): 257–64 **(8 pages).**

Dewiyanti, I.; Darmawi, D.; Muchlisin, Z.A.; Helmi, T.Z.; Imelda, I.; Defira, C.N., (2021). Physical and chemical characteristics of soil in mangrove ecosystem based on differences habitat in Banda Aceh and Aceh Besar. IOP Conference Series. Earth. Environ. Sci., 674: 012092 **(7 pages).**

Dewiyanti, I.; Darmawi, D.; Muchlisin, Z.A.; Helmi, T.Z.; Arisa, I.I.; Rahmiati, R.; Destri, E.; Fanisha, S., (2022). Characteristic and activity of cellulolytic bacteria isolated from mangrove soil in Northern Coast of Aceh Province, Indonesia. Biodivers

Dunlap, C.A.; Jin Kim, S.; Wo Kwon, S.; Rooney, A.P., (2016). *Bacillus velezensis* is not a later heterotypic synonym of *Bacillus amyloliquefaciens*; *Bacillus methylotrophicus*, *Bacillus amyloliquefaciens* subsp. *plantarum* and '*Bacillus oryzicola*' are later heterotypic synonyms of *Bacillus velezensis* based on phylogenomics. Int. J. Syst. Evol. Microbiol., 66(3): 1212-1217 **(6 pages).**

Ejaz, U.; Sohail, M.; Ghanemi, A., (2021). Cellulases: from bioactivity to a variety of industrial applications. Biomimetics., 6(3): 44-54 **(11 pages).**

El Dakar, A.Y.; Shalaby, S.M.; Saoud, I.P., (2007). Assessing the use of a dietary probiotic/prebiotics as an enhancer of spinefoot Rabbitfish *Siganus rivulatus* Survival and Growth. Aquac. Nutr., 13(6): 407-412 **(6 pages).**

Elyvra, R.; Solihin, D.D.; Affandi, R.; Junior, Z.; Yus, Y., (2009). Keanekaragaman genetika dan hubungan kekerabatan *Kryptopterus Limpok* dan *Kryptopterus Apogon* dari Sungai Kampar dan Sungai Indragiri Riau berdasarkan Gen Sitokrom B1. J. Ilmu-ilmu Perairan Indonesia., 16(1): 55-61 **(7 pages).**

Fan, B.; Blom, J.; Klenk, H.P.; Borriss, R., (2017). Bacillus amyloliquefaciens, Bacillus velezensis, and Bacillus siamensis form an "Operational Group B. amyloliquefaciens" within the B. subtilis species complex. Front. Microbiol., 8(22): 22-36 **(15 pages)**

Faturrahman., (2005). Genetic diversity of *Clostridium bifermentans* strains by amplified ribosomal DNA restriction analysis. J. Biol. Tropi**.,** 6(2): 57-64 **(7 pages).**

Furusawa, G., (2019). Biodiversity of plant polysaccharide-degrading bacteria in mangrove ecosystem. Trop. Life. Sci. Res., 30(3): 157–172 **(17 pages).**

Glogauer, A.; Martini, V.P.; Faoro, H.; Couto, G.H.; Santos, M.M.; Monteiro, R.A.; Mitchell, D.A.; de Souza, E.M.; Pedrosa, F.O.; Krieger, N., (2011). Identification and characterization of a new true lipase isolated through metagenomic approach. Microb. Cell. Fact., 10(54): 1-15 **(15 pages).**

Graur, D.; Li, H.W. (2000). Fundamentals of molecular evolution. 2nd edition. Sinauer **(439 pages)**.

Gunavathy, P.; Boominathan, M., (2015). Isolation and molecular characterization of cellulase producing bacteria from soil of Sacred Grove, Puducherry, India. Int. J. Curr. Microbiol. Appl. Sci., 4(12): 584–590 **(7 pages).**

Gunavathy, P.; Boominathan., (2016). Optimization of cellulase production of *Pseudomonas aeruginosa* Sg21 isolated from sacred Groove, Puducherry, India. Int. J. Sci. Res., 6(5): 603-607 **(5 pages).**

Gusakov, A.V.; Kondratyeva, E.G.; Sinitsyn, A.P., (2011). Comparison of two methods for assaying reducing sugars in the determination of carbohydrase activities. Int. J. Anal. Chem., 2011(283658): 1-4 **(4 pages).**

Hafich, K.; Perkins, E.J.; Hauge, J.B.; Barry, D.; Eaton, W.D., (2012). Implications of land management on soil microbial communities and nutrient cycle dynamics in the lowland tropical forest of northern Costa Rica. Trop. Ecol., 53(2): 215–224 **(10 pages).**

Hossain, M.J.; Ran, C.; Liu, K.; Ryu, C.M.; Rasmussen-Ivey, C.R.; Williamsm, M.A., (2015). Deciphering the conserved genetic loci implicated in plant disease control through comparative genomics of *Bacillus amyloliquefaciens* subsp. plantarum strains. Front. Plant. Sci., 17(6)**:** 631-644 **(14 pages).**

Hu, B.; Liao, J.; Zhang, Q.; Ding, S.; He, M.; Qiao, Y.; Zhang, Z.; Shang, C.; Chen, S., (2022). Diversity and vertical distribution of sedimentary bacterial communities and its association with metal bioavailability in three distinct mangrove reserves of South China. Water., 14(6): 1-27 **(27 pages).**

Huang, X.; Zhao, J.; Zhou, X.; Zhang, J.; Cai, Z., (2019). Differential responses of soil bacterial community and functional diversity to reductive soil disinfestation and chemical soil disinfestation. Geoderma., 348(2019): 124-134 **(11 pages).**

Huynh, T.; Vörös, M.; Kedves, O.; Turbat, A.; Sipos, G.; Leitgeb, B.; Kredics, L.; Vágvölgyi, C.; Szekeres, A., (2022). Discrimination between the two closely related species of the operational group *B. amyloliquefaciens* based on whole-cell fatty acid profiling. Microorganisms., 10(2): 418-430 **(13 pages).**

Izquierdo, J.A.; Sizova, M.; Lynd, L.R., (2010). Diversity of bacteria and glycosyl hydrolase family 48 genes in cellulolytic consortia enriched from thermophilic biocompost. Appl. Environ. Microbiol., 76(11): 3545-53 **(9 pages).**

Kochzius, M.; Nuryanto, A., (2008). Strong genetic population structure

in the boring giant clam, *Tridacna crocea,* across the Indo-Malay Archipelago: implications related to evolutionary processes and connectivity. Mol Ecol., 17(17): 3775-3787 **(13 pages).**

Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K., (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol., 35(6): 1547-1549 **(3 pages).**

Kurniawan, A.; Sari, S.P.; Asriani, E.; Kurniawan, A.; Sambah, A.B.; Prihanto, A.W., (2018). Cellulolytic bacteria mangrove leaf litter in Bangka Island. Samakia., 9(2018): 06-11 **(6 pages).**

Kurniawan, A.; Prihanto, A.A.; Sari, S.P.; Febriyanti, D.; Kurniawan, A.; Sambah, A.B.; Asriani, E., (2018a). Isolation and identification of cellulolytic bacteria from mangrove sediment in Bangka Island. IOP Conference Series: Earth. Environ. Sci., 137: 012070 **(7 pages).**

Kurniawan, A.; Suci, P S.; Euis, A.; Andi, K.; Abu Bakar, S.; Asep, A.P., (2019). Molecular identification of cellulolytic bacteria from mangrove sediment at Tin Minning Region In West Bangka. Int. J. Appl. Boil., 3(2019): 7-14 **(14 pages).**

Kusumaningrum, H.D.; Handayani, L.; Novrianti, R., (2016). Partial sequencing of 16S rRNA gene of selected *Staphylococcus aureus* isolates and its antibiotic resistance. Media Peternakan., 39(2016): 67-74 **(8 pages).**

Lam, K.; Morton, B., (2006). Morphological and mitochondrial-DNA analysis of the Indo-West Pacific rock oysters (Ostreidae: Saccostrea species). J. Molluscan. Stud., 72(2006): 235-245 **(11 pages).**

Leff, J.W.; Nemergut, D.R.; Grandy, A.S.; O'Neil, S.P.; Wickings, K.; Townsend, A.R.; Cleveland, C.C., (2012). The effects of soil bacterial community structure on decomposition in a tropical rain forest. Ecosystems., 15(2012): 284–298 **(15 pages).**

Liang, Y.; Yesuf, J.; Schmitt, S.; Bender, K.; Bozzola, J., (2009). Study of cellulases from a newly isolated thermophilic and cellulolytic *Brevibacillus* sp. strain JXL. J. Ind. Microbiol. Biotechnol., 36(2009): 961–970 **(10 pages).**

Liu, M.; Huang, H.; Bao, S.; Tong, Y., (2019). Microbial community structure of soils in Bamenwan mangrove wetland. Sci. Rep., 9(2019): 1-11 **(11 pages).**

Luo, L.; Gu, J.D., (2015). Particle-size fractions-dependent extracellular enzyme activity in sediments and implications for resource allocation in a subtropical mangrove ecosystem. Global J. Environ. Sci. Manage., 1(1): 15-26 **(12 pages).**

Maduppa, H.H.; Timm, J.; Kochzius, M., (2018). Reduced genetic diversity in the clown anemonefish *Amphiprion ocellaris* in exploited reefs of Spermonde Archipelago, Indonesia. Front. Mar. Sci., 5(80): 1-8 **(8 pages).**

Manjul, A.S.; Shirkot, P., (2018). 16S rRNA gene sequencing for bacterial identification of pullulanase synthesizing thermophilic bacteria contributing to big data. Intl. J. Chem. Stud., 6(2): 2769-277 **(5 pages).**

McGuire, K.L.; Fierer, N.; Bateman, C.; Treseder, K.K.; Turner, B.L., (2012). Fungal community composition in neotropical rain forests: the influence of tree diversity and precipitation. Microb. Ecol., 63(4): 804–812 **(9 pages).**

Nandy, A., (2009). Empirical relationship between intra-purine and intrapyrimidine differences in conserved gene sequences. PLoS ONE., 4(8): e6829 **(12 pages).**

Naresh, S.; Balakrishnan, K.; Ahmad, A.N.G.; Yi Peng., (2019). Isolation and partial characterisation of thermophilic cellulolytic bacteria from North Malaysian tropical mangrove soil. Trop. Life Sci. Res., 30(1): 123–147 **(25 pages).**

Nimnoi, P.; Pongsilp, N., (2022). Insights into bacterial communities and diversity of mangrove forest soils along the Upper Gulf of Thailand in response to environmental factors. Biology., 11(2): 1-22 (**22 pages**).

Ningsih, R.L.; Khotimah, S.; Lovadi, I., (2014). Cellulose degradation bacteria from *Avicennia alba* blume leaf litter in the safety mangrove forest in Pontianak Regency. Protobiont., 3(1): 34-40 **(7 pages).**

Ntushelo, K., (2013). Identifying bacteria and studying bacterial diversity using the 16S ribosomal RNA genebased sequencing techniques: a review. Afr. J. Microbiol. Res., 7(49): 5533-5540 **(8 pages).**

Olmos, J.; Acosta, M.; Mendoza, G.; Pitones, V., (2020). *Bacillus subtilis*, an ideal probiotic bacterium to shrimp and fish aquaculture that increase feed digestibility, prevent microbial diseases, and avoid water pollution. Arch. Microbiol., 202(3): 427-435 **(9 pages).**

Palit, K.; Rath, S.; Chatterjee, S.; Das, S., (2022). Microbial diversity and ecological interactions of microorganismsin the mangrove ecosystem: Threats, vulnerability, and adaptations. Environ. Sci. Pollut. Res., 29(2022): 32467–32512 **(45 pages).**

Petti, C.A., (2007). Detection and identification of microorganisms by gene amplification and sequencing. Clin. Infect. Dis., 44(8): 1108-1114 **(7 pages).**

Powthong, P.; Suntornthiticharoen, P., (2017). Antimicrobial and enzyme activity produced by *Bacillus* spp. isolated from soil. Int. J. Pharm.Sci., 9(3): 205 **(6 pages).**

Pramono, H.; Mariana, A.; Ryandini, D.; Sudiana, E., (2021). Short communication: Diversity of cellulolytic bacteria isolated from coastal mangrove sediment in Logending Beach, Kebumen, Indonesia. Biodiversitas., 22(4): 1869-1878 **(10 pages).**

Pringgenies, D.; Setyati, W.A.; Feliatra, F.; Ariyanto, D., (2023). The antibacterial and antifungal potential of marine natural ingredients from the symbiont bacteria of mangrove. Global J. Environ. Sci Manage., 9(4): 819-832 **(14 pages)**.

Putra, A.N.; Mustahal.; Syamsunarno, M.B.; Hermawan, D.; Fatimah, D.G.; Putri, P.B.; Sevia.; Isnaini, R.; Herjayanto, M., (2021). Dietary *Bacillus* NP5 supplement impacts on growth, nutrient digestibility, immune response, and resistance to *Aeromonas hydrophila* infection of African catfish, *Clarias gariepinus*. Biodiversitas., 22(1): 253-261 **(9 pages).**

Raaijmakers, J.M.; Paulitz, T.C.; Steinberg, C.; Alabouvette, C.; Moënne-Loccoz, Y., (2009). The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil., 321(2009): 341–361 **(21 pages).**

Safika.; Sari, W.N.; Darmawi.; Fahrimal, Y.; Santosa, S.F., (2018). Isolation and identification of a cellulilytic *Bacillus* from rumen of Aceh's cattle. Asian Jr. of Microbiol. Biotech. Env. Sc., 20(3): 798-804 **(6 pages).**

Saneha, S.; Pattamapitoon, T.; Bualert, S.; Phewnil, O.; Wararam, W.; Semvimol, N.; Chunkao, K.; Tudsanaton, C.; Srichomphu, M.; Nachaiboon, U.; Wongsrikaew, O.; Wichittrakarn, P.; Chanthasoon, C., (2023). Relationship between bacteria and nitrogen dynamics in wastewater treatment oxidation ponds Global J. Environ. Sci. Manage., 9(4): 707-718 **(12 pages)**.

Santiago, C.M.; De Vicenta, A.; Romero, D., (2021). Bacterial extracellular matrix as a natural source of biotechnologically multivalent materials. Comput. Struct. Biotechnol. J., 19(2021): 2796-2805 **(10 pages).**

Sari, W.N.; Safika, S.; Darmawi, D.; Fahrimal, Y., (2017). Isolation and identification of a cellulolytic Enterobacter from rumen of Aceh cattle. Vet. World., 10(12): 1515-1520 **(6 pages).**

Shanti, V.; Roymon, M.G., (2018). Isolation and screening of alkaline thermostable xylanase producing bacteria from soil in Bhilal durg region of Chhattisgarh, India. Int. J. Curr. Microbiol. Appl. Sci., 3(8): 303-311 **(9 pages).**

Shome, B.R.; Mandal, A.B.; Bandhopadyay, A.K., (1995). Bacteri flora in mangrove of Andaman: part I: isolation, identification and antibiogram studies. Ind. J. Mar. Sci., 24(1995): 97-98 **(2 pages).**

Soltani, M.; Hosenifar, S.H.; Roy, S.; Kumar, V.; Lymbery, A.; Ringoe, E., (2019). Genus *Bacillus*, promising probiotics in aquaculture: aquatic animal origin, bio-active components, bioremediation and efficacy

in fish and shellfish. Rev. Fish., 27 (3): 331-379 **(49 pages).**
Srivastava, N.; Srivastava, M.; Mishra., P. K.; Singh, P.; Ramteke, P. W., (2015). Application of cellulases in biofuels industries: An overview. J. Biofuels. Bioenergy., 1(1): 55-63 **(9 pages).**
Sulistiyani, T.R.; Masrukhin; Purnaningsih; Napitupulu, T.P.; Budiyanto, A.; Kanti, A.; Sudiana, I.M., (2020). Endophytic bacteria diversity from Zingiberaceae and anticandidal characterization produced by *Pseudomonas helmanticensis*. J.Teknologi., 83(1): 7-17 **(11 pages).**
Talia, P.; Sede, S.M.; Campos, E.; Rorig, M.; Principi, D.; Tosto, D.; Hopp, H.E.; Grasso, D.; Cataldi, A., (2012). Biodiversitas characterization of cellulolytic bacteria present on native Chaco soil by comparison of ribosomal RNA genes. Res. Microbiol., 163(3): 221-232 **(12 pages).**
Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S., (2013). MEGA6: Molecular evolutionary genetics analysis version 4.0. Mol. Biol. Evol., 30(12): 2725-2729 **(4 pages).**
Thatoi, H.; Bikash, C.B.; Rashmi, R.M.; Sushil., (2013). Biodiversity and biotechnological potential of microorganisms from mangrove ecosystems: a review. Ann. Microbiol., 63(2013): 1-19 **(19 pages).**
Truong, Q.P.; Phan, T.C.T.; Vu, H.H.; Pham, T.T.N.; Huynh, T.G.; Vu, N.U., (2021). Isolation of potential probiotic *Bacillus subtilis* CM3.1 and its effects on water quality and growth performance of the white leg shrimp *Litopenaeus vannamei* in the Mekong Delta, Vietnam. AACL. Bioflux., 14(6): 3347-3357 **(11 pages).**
Ubaidillah, R.; Sutrisno, H., (2012). Pengantar biosistematika: teori dan praktek. Pusat Penelitian Biologi. Lembaga Ilmu Pengetahuan Indonesia. Bogor, Indonesia: LIPI Press. **(198p).**
Vásquez, E.; Millones, C., (2023). Isolation and identification of bacteria of genus *Bacillus* from composting urban solid waste and palm forest in Northern Peru. Microorganisms., 11(3): 1-16 **(16 pages).**
Wang, L.T.; Lee, F.L.; Tai, C.J.; Kuo, H.P., (2008). *Bacillus velezensis* is a later heterotypic synonym of *Bacillus amyloliquefaciens*. Int. J. Syst. Evol. Microbiol., 58(3): 671–675 **(5 pages).**
Watanabe, H.; Tokuda, G., (2009). Cellulolytic systems in insects. Annu. Rev. Entomol., 55(2009): 609-632 **(23 pages).**
Widananarni.; Sukenda.; Anggraeni, A.A.; Maharani, R.B.A.; Mulyadin, A.; Yuhana, M., (2022). The effects of different dosage applications of *Bacillus* sp. NP5 para-probiotic on the growth performance and resistance of Nile tilapia against *Streptococcus agalactiae* infection. J. Akuakultur Indonesia., 21(2): 186-197 **(12 pages).**
Yahya.; Happy, N.; Yenny, R.; Soemarno., (2014). Characteristic of extracelluler metabolic of coastal ecosystem bacteria producing histidine decarboxylase crude. Int. Food Res. J., 21(4): 1337-1347 **(11 pages).**
Yanbo, W.; Zirong, X., (2006). Effect of probiotics for common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. Anim. Feed. Sci. Tec., 127(3-4): 283-292 **(10 pages).**

AUTHOR (S) BIOSKETCHES

Dewiyanti, I., Ph.D. Candidate, Graduate School of Mathematics and Applied Science, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia.
- Email: *irmadewiyanti@usk.ac.id*
- ORCID: 0000-0002-1361-6280
- Web of Science ResearcherID: E-5877-2017
- Scopus Author ID: 56140589200
- Homepage: https://fsd.usk.ac.id/irmadewiyanti/

Darmawi, D., Ph.D., Professor, Laboratory of Microbiology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Aceh 23111, Indonesia.
- Email: darmawi@usk.ac.id
- ORCID: 000-0003-3499-8000
- Web of Science ResearcherID: NA
- Scopus Author ID: 57190664731
- Homepage: https://fsd.usk.ac.id/darmawi/

Muchlisin, Z.A., Ph.D., Professor, Aquaculture Department, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia.
- Email: muchlisinza@usk.ac.id
- ORCID: 0000-0002-0858-1853
- Web of Science ResearcherID: NA
- Scopus Author ID: 55937322900
- Homepage: https://fsd.usk.ac.id/muchlisinza/

Helmi, T.Z., Ph.D., Assistant Professor, Laboratory of Biochemistry, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia.
- Email: zahrial_fkh@usk.ac.id
- ORCID: 000-0001-8623-2854
- Web of Science ResearcherID: NA
- Scopus Author ID: 57192716683
- Homepage: https://fsd.usk.ac.id/zahrial/

HOW TO CITE THIS ARTICLE

Dewiyanti, I.; Darmawi, D.; Muchlisin, Z.A., Helmi, T.Z., (2024). Diversity of cellulolytic bacteria in mangrove ecosystems soil using the 16S rRNA Gene. Global J. Environ. Sci. Manage., 10(1): 51-68.

DOI: 10.22034/gjesm.2024.01.05

URL: https://www.gjesm.net/article_705424.html