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Short Communication: Bacterial diversity of mangrove ecosystem in Klawalu Sorong, West Papua, Indonesia

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Abstract. *Sukmawati S, Rosalina F, Sipriyadi, Dewi NK, Yunita M, Sarhan ART, Rahayu Y, Kusumawati E. 2022. Short Communication: Bacterial diversity of mangrove ecosystem in Klawalu Sorong, West Papua, Indonesia. Biodiversitas 23: 1427-1432.* The mangrove ecosystem is a producer of detritus and a source of nutrients and organic matter. Some of the ecological functions of mangrove forests are coastline protector, preventing seawater intrusion, as a habitat for various living creatures, a microclimate regulator, a nursery ground, spawning ground, as well as a feeding ground for various aquatic biota. The mangrove forest ecosystem cannot be separated from the role of microbes in helping the process of soil biochemical cycles. In the biochemical cycle, microbes are able to maintain the availability of macronutrients in the soil. The objective of this study was to identify the diversity of bacteria in the mangrove ecosystem in Klawalu, Sorong City. The research method descriptively described the diversity of bacterial species found in the mangrove ecosystem in Klawalu, Sorong City, West Papua Province, Indonesia. The results indicated that the DNA fragments of the four isolates obtained from this study were around 1300 bp. Meanwhile, the bacterial species obtained were isolated SA3, identified as *Bacillus safensis* strain C251, isolate SA8 identified as *Bacillus amyloliquefaciens* strain NO10, isolate SL8 was identified as *Clostridium* sp. JC336, and isolate SL1 was identified as *Bacillus australimaris* strain IIHR GAPB01.

Keywords: Bacterial identification, mangrove ecosystem, Sorong City

INTRODUCTION

Mangrove forest is a coastal ecosystem and generally has a tropical coastal vegetation community that is dominated by mangrove plants, Avicennia pants, and others. Mangrove forests are found in coastal areas, inundated by seawater, influenced by tides yet not affected by climate (Putriningtias et al. 2014). Some of the ecological functions of mangrove forests are coastline protector, preventing seawater intrusion, as a habitat for various organisms, a microclimate regulator, a nursery ground, spawning ground, as well as a feeding ground for various aquatic biota. In addition, mangrove sediments recorded exclusively higher abundance of ecologically important bacterial classes including *Gamma-proteobacteria*, *Alphaproteobacteria*, *Deltaproteobacteria*, and *Bacilli* (Haldar and Nazareth 2018).

The mangrove ecosystem is a producer of detritus and a source of nutrients and organic matter. Therefore, the mangrove ecosystem has a very important role, particularly as a source of fish, shrimp, and other biodiversity (Feller et al. 2010; Ramli 2012; Wicaksono and Muhdi 2015). Mangrove forests provide a variety of needs for all organisms (Kurniadi and Koeslulati 2020). The mangrove ecosystem is a source of various kinds of microbes that are able to produce enzymes and molecules that are beneficial for human life, both in agriculture, fisheries, industry, and as bioremediation (Thatoi et al. 2013; Subagiyo et al. 2017; Behera et al. 2014; Kurniawan et al. 2019). For example, the combination of probiotic bacteria from mangroves and ponds was able to significantly control the organic matter content ($P<0.05$) (Atmomarsono et al. 2009).

Mangroves are unique ecosystems under a combination of marine and terrestrial influence. This diversity in their habitat, leads them to produce variable root exudates,

which support the growth of different types of microorganisms (Shakia et al. 2016). The complex mangrove ecosystem with acidic soil conditions requires microbes to produce extracellular enzymes that are able to work in complex and extreme environments.

It is known that the mangrove forest ecosystem cannot be separated from the role of microbes in helping the process of soil biochemical cycles. In the biochemical cycle, microbes are able to maintain the availability of macronutrients in the soil. Macronutrients, especially nitrogen (N), are very important for plants. Basically, nitrogen in N_2 is freely available in the atmosphere, yet cannot be directly absorbed by Phanerogamae plants. Generally, plants absorb nitrogen from their environment in the form of ammonium compounds (NH_4^+). Ammonium can be obtained from the soil with the help of microorganisms, such as nitrogen-fixing bacteria. Organic nitrogen originating from within cells, such as proteins and nucleic acids, will be released into the environment and dead bacterial cells can be utilized by other organisms, particularly plants, after going through the mineralization process (Singh et al. 2005; Yanti et al. 2020).

Bacteria, being an important component of the mangrove environment, not only play a very critical role in creating and maintaining this biosphere but also serve as a source of biotechnologically valuable and important products (Thatoi et al. 2013). The results of studies on nitrogen-fixing bacteria are generally applied in various fields, including the environment, industry, and agriculture. Some bacteria live freely on roots and in plant tissues, particularly for the genus of *Pseudomonas*, *Azotobacter*, *Azospirillum*, *Enterobacteria*, and *Bacillus*. These genus have been shown to be able to fix nitrogen (Huergo et al. 2018). In addition, non-symbiotic nitrogen-fixing bacteria had also been found in the Wonorejo mangrove forest area with a total of 4 genus, namely *Azotobacter*, *Azospirillum*, *Streptomycetes*, *Pseudomonas* (Kathiresan and Selvam 2006).

Three genus of nitrogen-fixing bacteria had been found in the soil of the mangrove forest of Sungai Peniti, Mempawah Regency including *Azotobacter*, *Azospirillum*, and *Pseudomonas* (Santoso and Rahmawati 2019). Subsequently, two genus of nitrogen-fixing bacteria were found from the Rhizosphere Mangrove in Kuala Singkawang, namely *Nitrosococcus* and *Bacillus* (Saputri et al. 2021). Previously, a study was conducted on screening probiotic bacteria candidates in the Klawalu subdistrict, Sorong City, West Papua Province. The results of the study indicated that 9 isolates had potential as lactic acid bacteria (Sukmawati and Badaruddin 2019). However, exploration of nitrogen-fixing bacteria at the genetic level in mangrove ecosystems has not been conducted. The objective of this study was to identify the diversity of bacteria in the mangrove ecosystem in Klawalu, Sorong City, West Papua, Indonesia.

MATERIALS AND METHODS

This research is a descriptive study that describes the results of identifying bacterial species found in the mangrove ecosystem in Klawalu, Sorong City, West Papua, Indonesia. The research was conducted in May-December 2021. The research was conducted at the Laboratory of the Faculty of Fisheries, Muhammadiyah University of Sorong, West Papua, Indonesia, and the Biology Laboratory of Bengkulu University, Bengkulu, Indonesia.

The samples tested included water and mud samples, each taken at a depth of ± 20 cm. Samples were taken randomly and then put into sterile bottles. Water samples were coded SA3 and SA8, while mud samples were coded SL8 and sample SL1. The samples were then grown on deMan, Rogosa and Sharpe (MRS) media. Furthermore, a molecular identification stage was carried out to determine the diversity of bacterial species obtained.

Bacterial genomic DNA isolation (modified from Sambrook and Russell 2001)

A total of 1.5 mL bacterial culture was added to 1.5 ml eppendorf and then centrifuged at 8000 rpm for 10 minutes. Furthermore, the supernatant was discarded and the pellet formed was washed using STE buffer and then centrifuged at 8000 rpm for 10 minutes. Pellets were washed 3 times repeatedly. Subsequently, the supernatant was removed and 200 L of STE buffer and 45 L of lysozyme were added to the pellet obtained, the pellet mixture was slowly inverted and then incubated at 55ºCfor 1 hour to form protoplasts. A total of 20 L proteinase-K was added to the mixture and incubated at 55ºC for 60 minutes. After that, 400 L 10% CTAB was added in a solution of 0.7 M NaCl and then incubated at a temperature of 65ºC for 30 minutes. Then, 1 volume of phenol: chloroform (25:24) was added and centrifuged at 12000 rpm for 10 minutes into the solution. The clear phase was transferred to a new tube and added 0.6 times the volume of isopropanol and 20 L sodium acetate and incubated at a temperature of -20ºC for one night. Furthermore, the solution mixture was centrifuged at 12000 rpm for 10 minutes. The supernatant was discarded, while the pellet was washed using 1 mL of 70% alcohol. The DNA was dried for 1 hour to remove the alcohol and then dissolved in 50 L sterile ddH_2O, then the DNA isolation was stored at 4ºC or -20ºC.

16S rRNA gene amplification of bacterial isolates

The 16S rRNA gene from genomic DNA was amplified by a Polymerase Chain Reaction (PCR) machine and prokaryotic-specific primers (Marchesi et al. 1998), namely primer 63f (5′-CAG GCC TAA CAC ATG CAA GTC-3′) and primer 1387r (5′-GGG CGG WGT GTA CAA GGC-3′). The PCR conditions used were as follows: pre-denaturation (94ºC, 4 minutes), denaturation (94ºC, 45 seconds), annealing (55ºC, 1 minute), elongation (72ºC, 1 minute 10 seconds), and post PCR (72ºC, 7 minutes) with a total of 30 cycles. DNA separation of PCR product was carried out on a mini-gel electrophoresis machine using 1% agarose at 75 volts for 45 minutes. DNA visualization was carried out on a UV transilluminator using Ethidium Bromide (EtBr) dye.

The raw data from the sequencing were then trimmed and assembled using the ChromasPro version 1.5 program. The assembled data were then performed by BLAST with genomic data that had been registered by NCBI/ National

Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/). Furthermore, the data were further analyzed by aligning the sequences using the MEGA 5.0 program (Tamura et al. 2011), and a phylogenetic tree construction was carried out to show the degree of relationship between isolatesXyl_22 with actinomycetes and other non-actinomyceteses using thNeighbor-Joiningng Tree method with 1000 bootstrap replications (Felsenstein 1985).

RESULTS AND DISCUSSION

Whole Genome amplification of the samples by PCR method using primer 63f and primer 1387r resulted in a DNA fragment of around 1300 bp (Figure 1). The results of the identification of four isolates showed that the bacterial species found in the mangrove ecosystem in Klawalu, Sorong City were quite diverse. Isolate SA3 was identified as *Bacillus safensis* strain C251, isolate SA8 was identified as *Bacillus amyloliquefaciens* strain NO10, isolate SL8 was *Clostridium* sp. JC336, and isolate SL1 was identified as *Bacillus australimaris* strain IIHR GAPB01 (Table 1; Figure 2). The size of the DNA fragments from the four isolates was about 1300 bp (Figure 1).

First, *B. safensis* are Gram-positive bacteria that can form spores, have aerobic chemoheterotroph properties, and are tolerant of salt and ultraviolet radiation (Kothari 2013). This is evidenced by its ability to grow in mangrove areas, where the area is an environment that has a high salt content. Moreover, *B. safensis* plays an important role in plant growth because these bacteria are able to produce hormones that function to strengthen plants. In addition, these bacteria are also capable of producing plant growth hormones such as auxins and cytokinins (Castro et al. 2018).

Primastoeti's research (2020) stated the same thing that *B. safensis* bacteria have the ability to produce the hormone IAA, can dissolve phosphate, are able to secrete ammonium, and have the ability to be antagonistic to *F. Oxysporum*. Apart from that, these bacteria are able to produce xylanase which can be used to produce bagasse, through bioconversion efforts. So that it is able to produce several derivative products with high economic value, one of which is xylooligosaccharides (XOS) (Lateef 2015). No less important, *B. safensis* can be applied to the bioremediation process (Rajesh et al. 2014).

Second, *B. amyloliquefaciens* is a species of bacteria that belongs to the genus *Bacillus*. These bacteria have potential as probiotics, because of their ability to grow in a salt environment and are able to grow on deMan, Rogosa and Sharpe (MRS) media (AlGburi 2016). *B. amyloliquefaciens* is a potential microbe in the synthesis of bioactive compound including exopolysaccharides and peptides. It can synthesize antimicrobial compounds. Which makes its novelty in the food sector greater. It imparts and improves the functional, sensory, and shelf life of the end products. The hydrolysis of complex compounds including insoluble proteins, carbohydrates, fibers, lignans, and hemicellulose also shows that *B. amyloliquefaciens* is a multifunctional and potential microbe which can be applied in the food industry and in functional food processing (Woldemariam et al. 2010).

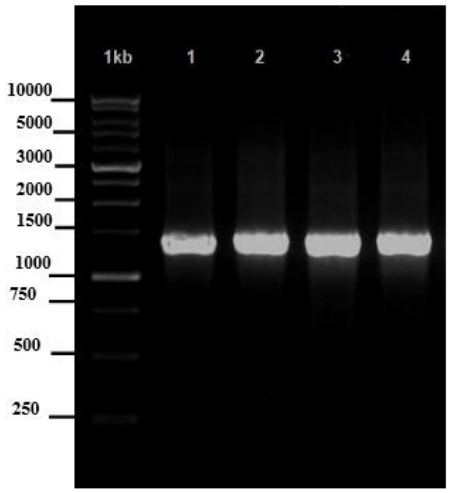

Figure 1. PCR amplification of the 16s rRNA gene using primer 63f and primer 1387r; M = marker 1 Kb ladder; (1) Isolate SA3, (2) Isolate SA8, (3) Isolate SL8, (4) isolate SL1

Table 1. The16S rRNA gene sequence similarity of bacterial isolates with data in the genebank accessed through the NCBI and BlastN sites (Basic Local Alignment Search Tools)

Specimen	No access	Description	Similarity (%)	E-value
Isolate	KC519441.1	*Bacillus safensis* strain C251 16S ribosomal RNA gene, partial sequence	99.67	0.00
SA3	LC034564.1	*Bacillus pumilus* gene for 16S ribosomal RNA, partial sequence, strain: NKCM3202	99.67	0.00
	OL477458.1	*Bacillus safensis* strain C251 16S ribosomal RNA gene, partial sequence	99.67	0.00
Isolate	MT377854.1	*Bacillus amyloliquefaciens* strain NO10 16S ribosomal RNA gene, partial sequence	100.0	0.00
SA8	MT124532.1	*Bacillus amyloliquefaciens* strain ER7 16S ribosomal RNA gene, partial sequence	100.0	0.00
	MN240443.1	*Bacillus velezensis* strain z21 16S ribosomal RNA gene, partial sequence	100.0	0.00
Isolate	LN846811.1	*Clostridium* sp. JC336 partial 16S rRNA gene, strain JC336, isolate GJ11	99.92	0.00
SL8	DQ978211.1	*Clostridium bifermentans* strain HT2 16S ribosomal RNA gene, partial sequence	99.83	0.00
	LC515632.1	*Paraclostridiumbenzoelyticum* H001 gene for 16S ribosomal RNA, partial sequence	99.83	0.00
IsolateSL1	OL477458.1	*Bacillus australimaris* strain IIHR_GAPB0116SrRNA 16S ribosomal RNA gene, partial sequence	100.0	0.00
	OK415424.1	*Bacillus safensis* strain Q en 16S ribosomal RNA gene, partial sequence	100.0	0.00
	OK336467.1	*Bacillus safensis* strain AYE_4 16S ribosomal RNA gene, partial sequence	100.0	0.00

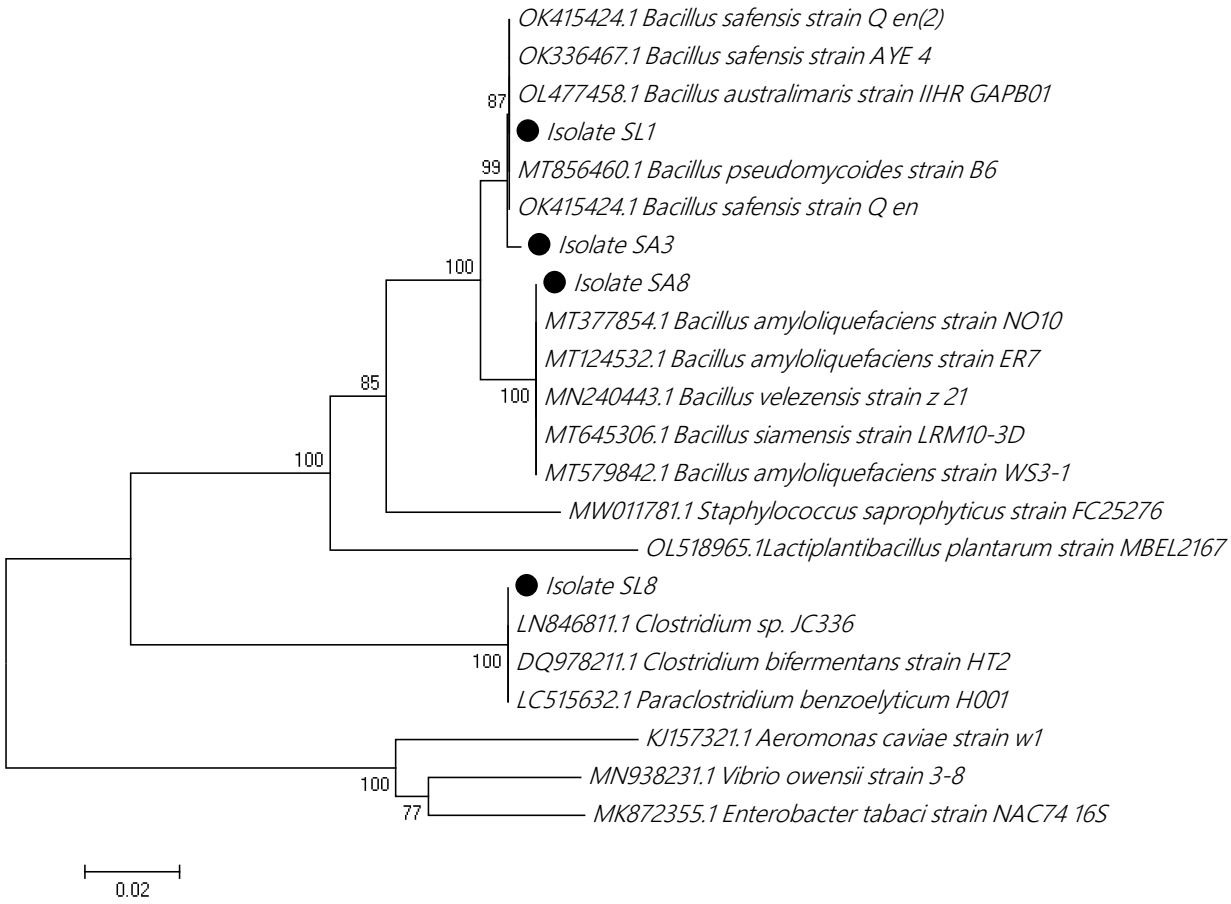

Figure 2. Phylogenetic tree that describes the genetic distance of the isolates obtained to other bacteria either in one clade or in another clade (outer group). Construction was based on the Neighbor-Joining Tree method with a bootstrap value of 1000x replications

Bacillus amyloliquefaciens SC06 (Ba)-induced autophagy and its antibacterial activity against *Escherichia coli* in murine macrophage cells (Wu et al. 2017). The mixed probiotics of *B. amyloliquefaciens* 54A and *B. pumilus* 47B were isolated from striped catfish (*Pangasianodon hypophthalmus*) intestine to stimulate growth performance and innate immunity, stress tolerance of striped catfish (Thy 2017). *Bacillus amyloliquefaciens* are capable of producing the restriction enzyme BamHI. In addition, these bacteria are also capable of producing barnase enzymes, ribonuclease enzymes that act as intracellular inhibitors against *Bacillus anthracis* (Chowdhury et al. 2015). *Bacillus amyloliquefaciens* can be applied in agriculture such as cultivation and hydroponics because these bacteria are able to inhibit root pathogens such as *Ralstonia solanacearum, Rhizoctonia solani, Alternaria tenuissima, Pythium,* and *Fusarium.* It is also known that *B. Amyloliquefaciens* is considered rhizobacteria that can promote plant growth and is tolerant of salt stress (Chen et al. 2017). According to the conditions of the sampling site and the growth medium of bacteria during the isolation process, it is appropriate that the environmental conditions have high salt content.

Third, *Clostridium* spp. are obligate anaerobic bacteria, Gram positive, motile, capable of forming spores under extreme environmental conditions, capable of producing botulin toxin and tetanus toxin, and are generally found in soil and mangrove areas (Shanmugam et al. 2018). This is in accordance with the results of this study in which the isolate SL8 was identified as *Clostridium* sp. JC336 was isolated from mud with a depth of about ± 20 cm from the mangrove surface. It is known that *Clostridium* sp. is pathogenic bacteria that cause botulism. These bacteria are found in meat products, sausage products, seafood, and canned food. Spores produced by these bacteria can be found in soil, intestines of mammals, poultry, and fish (Poharkar et al. 2017).

Fourth, *B. australimaris* are Gram-positive bacteria, circular and rod-shaped, motile, and obligate aerobe. This bacteria was first isolated from sediments at a depth of 756 m in the South China Sea (Liu et al. 2016). However, the samples studied in this study came from mud with a depth of ± 20 cm from the surface of the mangrove. These bacteria are known to be able to produce indole and arginine. *Bacillus australimaris* is also classified as denitrifying bacteria and are able to accelerate plant growth (Qian et al. 2020). These bacteria can also ferment glucose, sucrose, and arabinose (Dutta et al. 2020). These bacteria can grow on MRS media, as has been conducted in this study. Furthermore, *B. australimaris* can be used as a

biocontrol, because it is able to inhibit the growth of the pathogen M. oryzae (Chen et al. 2019). These bacteria also include lactic acid bacteria that can be used as probiotics and found in fermented food products (Cissé et al. 2019).

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