ANTIMICROBIAL ACTIVITY AND 16S rRNA GENE PROFILES OF POTENTIAL ANTIMICROBIAL-PRODUCING ENDOPHYTIC BACTERIA ISOLATED FROM MANGROVES *Rhizophora stylosa* Griff. AND *Sonneratia alba* Sm. FROM ENGGANO ISLAND

Risky Hadi Wibowo[*], Sipriyadi[*], Riziq Ilham Nur Fahmi[**], Denny Kurniawan[***], Putri Hezekiel Claracia Simanjuntak[***]

[*] *Department of Biology, Faculty Mathematics and Natural Sciences, University of Bengkulu, Bengkulu, Indonesia, rhwibowo@unib.ac.id, sipriyadi@unib.ac.id*

[**] *Science Laboratory Study Program, Faculty of Mathematics and Natural Sciences, University of Bengkulu, Bengkulu, Indonesia, rilham.fahmi@unib.ac.id*

[***] *Undergraduate Student, Biology Department, Faculty of Mathematics and Natural Sciences, University of Bengkulu, Bengkulu, Indonesia, dennykrnw@gmail.com, putrihcsimanjuntak@gmail.com*

Email Correspondence : rhwibowo@unib.ac.id

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Abstract: Mangroves *Rhizophora stylosa* Griff. and *Sonneratia alba* Sm. produce secondary metabolite compounds that are useful as potential sources of new antimicrobes because they inhibit the growth of pathogenic microbes. Endophytic bacteria are bacteria that live within plant tissues and are capable of producing metabolite compounds with antimicrobial properties. This study aimed to determine the antimicrobial activity and molecular profile of the 16S rRNA gene from endophytic bacterial isolates of *R. stylosa* Griff. and *S. alba* Sm. from Enggano Island. The isolates were subcultured on Nutrient Agar (NA) medium. The results showed that five isolates (DRSE 16, DRSE 17, ASAE 3, DSAE 17, and DSAE 19) out of 40 endophytic bacterial isolates exhibited potential activity against the testing microbes (*Salmonella typhi*, *Enterococcus faecalis*, and *Candida albicans*). The results of 16S rRNA identification showed that the bacterial isolate DRSE 16 had similarities with the species *Stenotrophomonas nitritireducens* strain BR-49, isolate DRSE 17 with the species *Pseudomonas putida* strain XT214, isolate ASAE 3 with the species *Pseudomonas aeruginosa* strain D3, isolate DSAE 17 with the species *Bacillus subtilis* strain C-2-3-2, and isolate DSAE 19 with the species *Pseudomonas otitidis* strain RR017.

Keywords: Antimicrobe; Endophytic Bacteria; 16S rRNA; Mangroves

Abstrak: Bakau *Rhizophora stylosa* Griff. dan *Sonneratia alba* Sm. menghasilkan senyawa metabolit sekunder yang berguna untuk dijadikan sumber antibiotik baru karena dapat menghambat pertumbuhan mikroba patogen. Bakteri endofit merupakan bakteri yang hidup pada jaringan tumbuhan dan dapat menghasilkan senyawa metabolit yang dapat berfungsi sebagai antimikroba. Penelitian ini bertujuan untuk mengetahui aktivitas antimikroba dan profil molekuler gen 16S rRNA dari isolat bakteri endofit bakau *R. stylosa* Griff. dan *S. alba* Sm. asal Pulau Enggano. Isolat diremajakan pada media Nutrient Agar (NA), kemudian digunakan untuk menguji aktivitas senyawa antimikroba hasil kultur sel bakteri, supernatan, dan pelet. Hasil penelitian didapatkan bahwa lima isolat potensial (DRSE 16, DRSE 17, ASAE 3, DSAE 17 dan DSAE 19) dari 41 isolat bakteri endofit terhadap mikroba uji (*Salmonella typhi*, *Enterococcus faecalis* dan *Candida*

albicans). Hasil identifikasi 16S rRNA menunjukkan bahwa isolat bakteri DRSE 16 memiliki kemiripan dengan spesies *Stenotrophomonas nitritireducens* galur BR-49, isolat DRSE 17 dengan spesies *Pseudomonas putida* galur XT214, isolat ASAE 3 dengan spesies *Pseudomonas aeruginosa* galur D3, isolat DSAE 17 dengan spesies *Bacillus subtilis* galur C-2-3-2, dan isolat DSAE 19 dengan spesies *Pseudomonas otitidis* strain RR017.

Kata Kunci : Antimikroba; Bakteri Endofit; 16S rRNA; Mangroves

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Introduction

Enggano Island is one of Indonesia's outermost islands, with a coastline of 126.71 kilometers and a land area of 39,586.74 hectares. Administratively, Enggano Island is part of North Bengkulu Regency, comprising one sub-district and six villages (Wilopo et al., 2023). Previous study reported that Enggano Island had several types of natural ecosystems, one of which is the mangrove ecosystem. Mangroves provide numerous ecological functions and are a major producer for marine fisheries. They can also support the social and economic development of local coastal communities. The mangrove ecosystem on Enggano Island is still relatively pristine because it is a frontier island that is rarely visited. The condition of its coastal areas is also very well-preserved, making the island highly potential for development, especially in the functional areas of its mangrove ecosystem (Komarudin et al., 2016).

Mangroves are known to be rich in microbial biodiversity, with endophytic bacteria being one key component. The endophytic bacteria found in mangroves are very specific because these plants possess unique characteristics that differ from other plants. Several studies on mangroves have shown that they are a natural source of bioactive compounds such as saponins, tannins, flavonoids, diterpenoids, and octacosyl alcohol, all of which exhibit antimicrobial activity. Mangroves are plants rich in bioactive compounds, but the compounds found within them do not always originate from the plant itself. Instead, they can come from other organisms that synthesize these compounds inside the mangrove. This suggests that endophytic fungi or bacteria living within the mangrove plant tissues could be indicated as the producers of these bioactive compounds. The mangrove species *R. stylosa Griff.* and *S. alba Sm.* have been identified as capable of producing secondary metabolites like flavonoids, saponins, tannins, and alkaloids, which can serve as a source of new antibiotics by inhibiting the growth of pathogenic bacteria (Nia et al., 2017).

Endophytic bacteria are microbes that live inside plant tissues without causing damage to their host plant. These bacteria live inside plant tissues (xylem and phloem), roots, stems, leaves, and fruits. They have a mutualistic symbiotic relationship with their host, as the bacteria get nutrients from the plant's metabolism, and the plant benefits by receiving necessary nutrient derivatives from the bacteria. Endophytes also help the plant protect itself against herbivores, insects, and pathogenic bacteria. Each plant can contain one or more endophytic microbes, which consist of bacteria and fungi. These endophytic bacteria can produce metabolite compounds that act as antibiotics, antivirals, anticancer agents, antidiabetics, antimalarials, antioxidants, anti-insect agents, growth regulators, and enzymes. The secondary metabolites from mangrove plant endophytes are structurally diverse and pharmacologically active. These compounds, known from bicarbonates, include terpenes, chromones, coumarins, polyketides, alkaloids, and peptides. These compounds show a broad spectrum of biological properties, such as antioxidant, α-glucosidase, acetylcholinesterase, cytotoxic, and antimicrobial activity (Ancheeva et al., 2018).

Antimicrobials are substances that can inhibit the growth rate and kill bacteria by disrupting their metabolic processes. There are two types of antibacterial agents: bacteriostatics, which inhibit microbial growth, and bactericides, which kill microbes. Antimicrobial compounds consist of antibacterial and antifungal compounds. Antibacterial compounds are substances that can inhibit the growth and kill the vegetative forms of a microbe. Based on their activity, antibacterials are divided into bacteriostatic and bactericidal. Bacteriostatics are compounds that inhibit bacterial growth, while bactericides are compounds that kill bacteria. The mechanism of action of antibacterial compounds includes inhibition of cell wall synthesis, disruption of the integrity of bacterial cell membrane permeability, inhibition of bacterial metabolism, and inhibition of nucleic acid and protein synthesis (Yan et al., 2021).

In 2023, the isolation of endophytic bacteria from the mangrove plants *R. stylosa Griff.* and *S. alba Sm.* from Enggano Island was successfully carried out (unpublished data). This research successfully isolated 40 endophytic bacteria, which have been characterized for their morphology, Gram staining, and physiological characters. However, the antimicrobial activity of the obtained isolates is not yet known. There has been no previous research on the potential of endophytic bacteria from *R. stylosa Griff.* and *S. alba Sm.* from Enggano Island as producers of antimicrobial compounds. Therefore, this research is needed to investigate the antimicrobial activity of these bacterial isolates against pathogenic microbes and to detect the molecular profiles of the potential endophytic bacteria using the 16S rRNA gene.

Material and Methods

Materials

Five selected isolates (DRSE 16, DRSE 17, ASAE 3, DSAE 17, and DSAE 19) from 40 endophytic bacterial isolates from mangrove plants *R. stylosa Griff.* and *S. alba Sm.* from Enggano Island (unpublished collection from the microbiology laboratory). Nutrient Agar (NA), Nutrient Broth (NB), Trypticase Soy Broth (TSB), Trypticase Soy Agar (TSA), Sabouraud Dextrose Agar (SDA), *Salmonella typhi*, *Enterococcus faecalis*, and *Candida albicans*. Chloramphenicol antibiotic**,** Ethyl acetate**,** Presto™ Mini gDNA Bacteria kit (Geneaid)**,** Lysozyme buffer**,** Proteinase K**,** GB buffer**,** Absolute ethanol**,** 16S rRNA Primers 63F/1387R**,** GoTaq Green Mastermix PCR kit**,** Nuclease-Free Water (NFW)**,** Agarose**,** Ethidium bromide (EtBr) stain**,** Crystal violet**,** Lugol's iodine**,** Safranin**,** 96% alcohol, 70% alcohol, 6 mm paper discs (Whatman)**,** Microtips (pipette tips)**.**

Methods

Subculturing of Potential Endophytic Bacterial Isolates.

Subculturing of the potential endophytic bacterial isolates was performed using the quadrant streak method on Petri dishes containing sterile Nutrient Agar (NA) medium. The plates were then incubated at 30 ℃ for 12–24 hours. The bacterial colonies that grew on the medium was observed based on their morphology (shape, color, surface, elevation, and colony margin) (Ritaqwin et al., 2025). The isolates were then purified using the same medium and the quadrant streak method.

Antimicrobial Activity Assay using Culture, Pellet, and Supernatant.

Two approaches were used for the antimicrobial test: cell culture and disc diffusion using pellets and supernatant (Nassar et al., 2019). In the cell culture test, the isolates were inoculated into a medium containing the pathogen. In contrast, for the antimicrobial test using supernatant and pellets, the culture was centrifuged at 10,000 rpm for 5 minutes in an Eppendorf tube before the pellets and supernatant were separated for the antimicrobial activity test. A total of 8 µl of the pellet and supernatant were dropped onto paper discs and then incubated at 37 ℃ for 2x24 hours. Each treatment was replicated twice. A positive result was indicated by the formation of a clear zone around the paper disc. The clear zone was measured using a caliper, and the inhibition zone index was calculated.

Crude Extraction from Potential Isolates Using the Ethyl Acetate Fraction .

The bacterial isolates were cultured in 500 ml of NB (Nutrient Broth) medium. The culture was then incubated on a rotary shaker (100 rpm, 30 ºC) for three days. Following this, 500 ml of ethyl acetate was added to the culture, stirred for 3 hours, and then evaporated. The crude extract was stored at 5 ºC for subsequent use (Muller et al., 2004).

Crude Extract Assay Against Pathogenic Microbes.

The bacterial crude extract was measured using the agar diffusion method. The extract was dissolved in ethyl acetate, which was then dropped onto 6 mm

paper discs (Whatman). The paper discs were placed in an oven at 40 ℃. Subsequently, the discs were sterilized under UV light for two hours and placed onto agar plates previously inoculated with 1% (v/v) of the testing microbes (*E. faecalis*, *S. typhi*, and *C. albicans*) at a concentration of $1x10^6$ CFU/mL, OD 620 0.45. The plates were incubated for three hours to allow for optimal diffusion of the crude extract into the medium. The diameter of the inhibition zone was measured after 24 hours of incubation at 30 ℃, and the Minimum Inhibitory Concentration (MIC) value was calculated. Negative control discs were soaked in ethyl acetate solvent, while positive control discs were soaked in a chloramphenicol solution and prepared in the same method.

Identification of the 16S rRNA Gene from Potential Isolates.

The 16S rRNA gene was amplified using a PCR (Polymerase Chain Reaction) machine with a forward primer 63F (5'-CAG GCC TAA CAC ATG CAA GTC-3') and a reverse primer 1387R (5'-GGG CGG WGT GTA CAA GGC-3'), following Marchesi et al. (1998), with a target fragment of approximately 1300 bp. The total volume of the PCR reaction was 40 µL, consisting of 20 µL of GoTaq Green Mastermix 2x PCR kit, 2 µL of each primer, 4 µL of genomic DNA (~100 ng), and 12 µL of nuclease-free water (NFW). The PCR conditions were Pre-denaturation: 94 ℃ for 5 minutes, 30 cycles of: Denaturation 94 ℃ for 45 seconds, Annealing 55 ℃ for 1 minute, Extension 72 ℃ for 1 minute 10 seconds, Post-extension 72 ℃ for 7 minutes, Cooling 15 ℃ for 15 minutes. The PCR amplification products were then visualized using 1% agarose gel electrophoresis at 85 Volts for 40 minutes. The gel was stained with ethidium bromide for 10 minutes before being visualized under UV light. The amplified 16S rRNA gene was expected to appear as a band of approximately ±1300 bp. Finally, the amplification products were sequenced through PT. Genetika Science Indonesia.

Data Analysis

Data analysis was performed in two parts: an analysis of the inhibition zone calculation results from the antimicrobial activity test and bioinformatics analysis. The inhibition zone categories were determined by following the inhibition index according to Ouchari et al. (2019), based on the inhibition zone diameter measurements taken with a digital caliper, as shown in Table 1. The bioinformatics analysis was carried out using the Bioedit and BLAST programs from the National Center for Biotechnology Information (NCBI). Phylogenetic tree construction based on the 16S rRNA gene was performed using the Neighbor-Joining method with a bootstrap value of 1000 replicates and the software MEGA 11.

Table 1. Classification of antimicrobial activity based on inhibition zone diameter

Inhibition Zone	Description
> 20 mm	Very Strong
10-20 mm	Strong
5-10 mm	Moderate
<5 mm	Weak

Results and Discussions

Antimicrobial Activity Test

In this research, 40 endophytic bacterial isolates (unpublished) were successfully obtained by re-culturing them from the Microbiology Laboratory's collection. Five bacterial isolates (DRSE 16, DRSE 17, ASAE 3, DSAE 17, and DSAE 19) with the best antimicrobial activity based on culture assay were selected for further testing of antimicrobial activity using pellets, supernatants, and crude extracts (Figure 1).

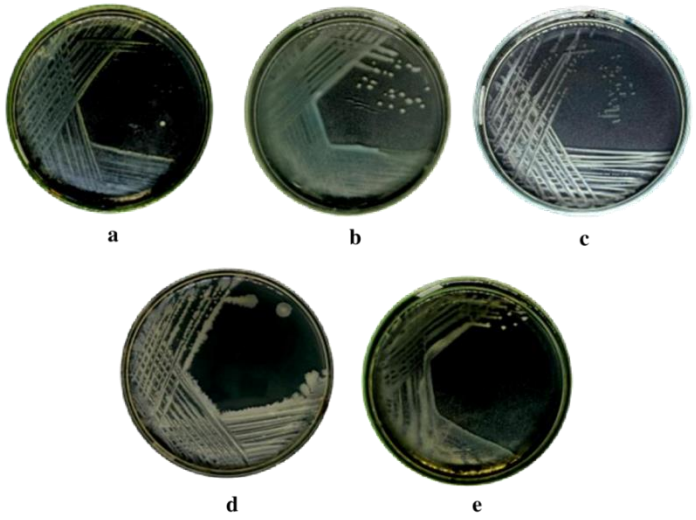


Figure 1. Morphological colonies of five potential mangrove endophytic bacterial isolates (a) DRSE 16, (b) DRSE 17, (c) ASAE 3, (d) DSAE 17, and (e) DSAE 19

The five selected bacterial isolates showed to have inhibitory activity against the three pathogenic microbes: *E. faecalis*, *S. typhi*, and *C. albicans*. The highest inhibitory activity was demonstrated by bacterial isolate DRSE 16 against the pathogenic bacterium *S. typhi*, with a diameter of inhibition zone of 25.45 mm. The results of the supernatant assay also showed that the five bacterial isolates exhibited inhibitory activity against the three pathogenic microbes. The best inhibitory activity was demonstrated by bacterial isolate DSAE 17 against the pathogenic bacterium *S. typhi*, with an inhibition zone diameter of 17.97 mm. The

results of the antimicrobial activity using crude extracts showed that only the ASAE 3 bacterial isolate was able to inhibit the three pathogenic microbes. Crude extracts from the DRSE 16 and DRSE 17 bacterial isolates did not show any inhibitory activity. The crude extract of the DSAE 17 bacterial isolate was only able to inhibit *E. faecalis* bacteria. Meanwhile, the crude extract of the DSAE 19 bacterial isolate was able to inhibit the pathogenic bacteria *S. typhi* and *E. faecalis*. The crude extract of ASAE 3 bacterial isolate had the best inhibitory activity against the pathogenic bacteria *S. typhi* with an inhibition zone diameter of 15.28 mm. The ASAE 3 bacterial isolate was consistently inhibiting the three tested pathogenic microbes, using isolate cultures, pellets, supernatants, and crude extracts (Table 2 and Figure 2).

Table 2. Results of the antimicrobial activity test using culture, pellet, supernatant, and bioactive crude extract from mangrove plants *R. stylosa Griff.* and *S. alba Sm.* from Enggano.

Isolates code	Paper Disc Diameter (mm).	Microbes	Diameter of Inhibition Zone Activity (mm) ± SD			
			Culture.	Pellets.	Supernatant.	Crude Extract.
DRSE 16	6,00	*S. typhi*	+	25,45 ± 0,85	7,17 ± 0,57	0,00 ± 0,00
		E. faecalis	+	19,66 ± 0,68	16,27 ± 0,57	0,00 ± 0,00
		C. albicans	+	3,70 ± 0,20	1,7 ± 0,95	0,00 ± 0,00
DRSE 17		*S. typhi*	+	22,3 ± 0,60	7,77 ± 0,37	0,00 ± 0,00
		E. faecalis	+	8,25 ± 0,10	9,05 ± 0,80	0,00 ± 0,00
		C. albicans	+	6,80 ± 0,30	2,32 ± 0,12	0,00 ± 0,00
ASAE 3		*S. typhi*	+	15,92 ± 0,62	11,15 ± 0,75	15,28 ± 0,28
		E. faecalis	+	20,95 ± 0,95	15,77 ± 0,72	13,03 ± 3,63
		C. albicans	+	8,02 ± 0,72	1,00 ± 0,15	8,30 ± 0,10
DSAE 17		*S. typhi*	+	15,90 ± 4,80	17,97 ± 0,92	0,00 ± 0,00
		E. faecalis	+	14,83 ± 0,96	8,25 ± 0,90	3,55 ± 0,55
		C. albicans	+	7,27 ± 0,42	2,90 ± 0,40	0,00 ± 0,00
DSAE 19		*S. typhi*	+	18,97 ± 0,47	2,90 ± 0,80	6,55 ± 1,05
		E. faecalis	+	10,45 ± 0,85	8,00 ± 0,20	8,85 ± 0,70
		C. albicans	+	7,70 ± 0,90	2,65 ± 0,35	0,00 ± 0,00

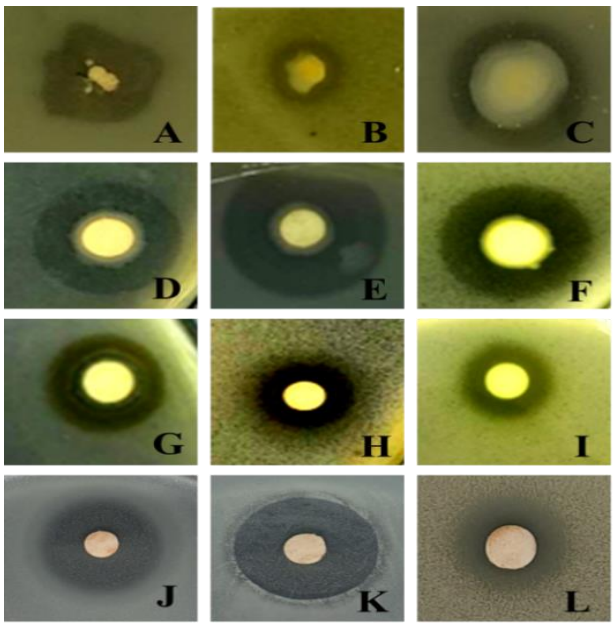


Figure 2. The inhibition zone formed by the of ASAE 3 bacterial isolate against the three testing pathogenic microbes. Inhibition zone using bacterial culture (A, B, C), pellets (D, E, F), supernatant (G, H, I), and crude extract (J, K, L). The tests were conducted against the following testing microbes *E. faecalis* (A, D, G, J), *S. typhi* (B, E, H, K), and *C. albicans* (C, F, I, L), respectively.

The five selected potential endophytic bacterial isolates were extracted using ethyl acetate to obtain crude extract. This extract was re-tested using paper discs to observe the resulting inhibition zones. Srivastava and Kayastha (2014) showed that sometimes crude extracts are more active than pure compounds, and this is due to the synergistic effects of other constituents within the extract. The inhibition activity of the five selected potential endophytic bacterial isolates from *R. stylosa Griff.* and *S. alba Sm.* from Enggano Island against the pathogenic microbes was observed after 24 hours of incubation at 30°C. In general, 30°C is the optimal temperature for the growth of environmental bacteria. While the optimal temperature for many human pathogens is generally 37°C, some environmental bacteria and fungi groups approach or reach peak growth at 30°C. The test results showed differences in inhibition zone activity against the pathogenic microbes. The pathogenic microbes, particularly the bacteria used, differ in their cell wall structure. Khotimah *et al.* 2021, stated that differences in cell wall structure in bacteria is going to lead to differences in inhibition activity. Gram-negative bacteria have a more complex cell wall structure consisting of three layers, while Gram-positive bacteria have a simpler cell wall structure.

Pathogenic microbes generally consist of pathogenic bacteria and/or fungi that are harmful and detrimental to their host. Some of these pathogenic microbes

include *E. faecalis*, *S. typhi*, and *C. albicans*. These three pathogenic microbes were tested due to their high level of resistance to antibiotics. *S. typhi* is a pathogenic bacterium representing Gram-negative bacteria, *E. faecalis* represents Gram-positive bacteria, and *C. albicans* represents the group of fungi that often infect humans. *E. faecalis* is an opportunistic microbe, primarily causing infections in patients in Intensive Care Units, those with severe underlying diseases, or those with compromised immune systems (Ramos et al., 2020). *S. typhi* is the cause of typhoid fever, which is typically contracted by ingesting food or water contaminated with human feces carrying the organism (Ashurst et al., 2022). Candidiasis is a fungal disease that affects the skin, hair, nails, mucous membranes, and internal organs, and is caused by *C. albicans*. The ability of the antimicrobial compounds produced by the isolates to inhibit the growth of the test pathogenic microorganisms is indicated by the formation of a clear zone around the isolates. Bacterial growth rates can vary between species, influenced by their metabolic processes, such as primary metabolites and intracellular molecules. These processes enable cell growth and proliferation, while secondary metabolites and extracellular molecules play a role in the microbe's interactions with its environment (Seyedsayamdost MR, 2019).

Isolates DRSE 16, DRSE 17, ASAE 3, DSAE 17, and DSAE 19 were able to inhibit the wide range of pathogenic microbes: *E. faecalis*, *S. typhi*, and *C. albicans*. This is because antimicrobials are substances that can inhibit the growth rate and kill bacteria by disrupting their metabolic processes (Baran et al., 2023). The production of bioactive compounds plays an important role in the survival of endophytic bacteria (Singh et al., 2017). These bioactive compounds are produced during the stationary phase, where the bacteria have passed the adaptation phase and successfully grown in a new medium containing the pathogens. In the isolate cultures, one of these accumulated end products is the antimicrobial compound that inhibits the growth of pathogenic microbes around the colony.

Identification of the 16S rRNA Gene from Potential Isolates.

DNA amplification of the potential isolates was done using the PCR technique with primers of 63F and 1387R. The PCR products were visualized using electrophoresis on a 1% agarose gel. All five selected isolates were successfully amplified and showed DNA fragments approximately 1300 bp in size, as shown in Figure 3.

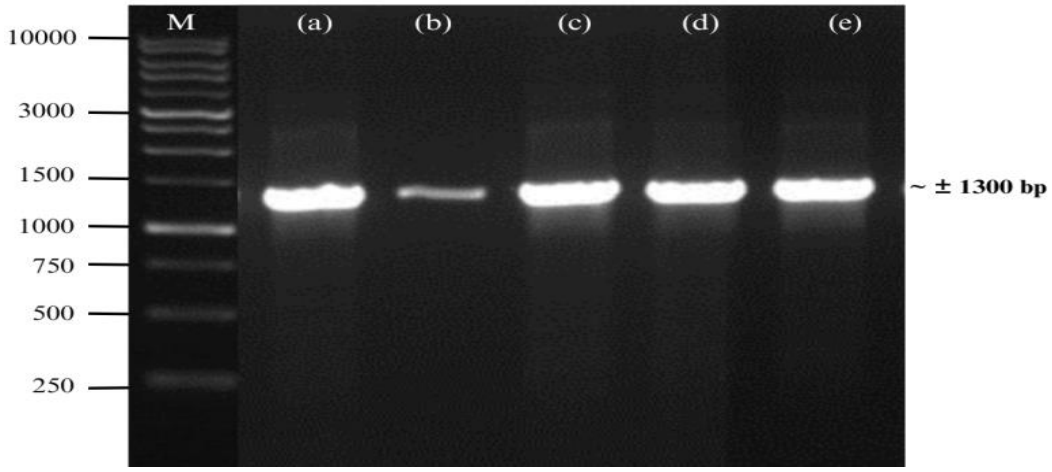


Figure 3. 16S rRNA gene electrophoresis results with a target DNA band size of ~1300 bp.(1kb) 10,000 bp, (M) 1 kb DNA Ladder (Fermentas); (a) DRSE 16, (b) DRSE 17, (c) ASAE 3, (d) DSAE 17, (e) DSAE 19.

The sequencing results of the five potential bacterial isolates were aligned with reference strains from GenBank, showing the highest similarity compared to other control strains (Table 3). All five bacterial isolates have a 99% similarity to the reference strains. Phylogenetic tree construction, based on the 16S rRNA coding gene, was performed using the Neighbor-Joining method with a bootstrap value of 1000 replicates (Figure 4).

Table 3. Identification results of potential mangrove endophytic bacterial isolates based on BLASTn from the site NCBI

Isolat Isolates	Homologi Homology	Query Cover (%)	EE-Value	Similarity	Access Number
DRSE 16	*Stenotrophomonas nitritireducens* strain BR-49	99%	0.0	99.37%	KX082882
	Stenotrophomonas nitritireducens strain MN63	99%	0.0	99.37%	LC106037
	Stenotrophomonas nitritireducens strain KUDC1825	99%	0.0	99.37%	KC355332
DRSE 17	*Pseudomonas putida* strain XT214	99%	0.0	99.51%	MT424797.1
	Pseudomonas putida strain XT215	99%	0.0	99.51%	MT424798.1
	Pseudomonas putida strain XT911	99%	0.0	99.51%	MT424786.1
ASAE 3	*Pseudomonas aeruginosa* strain D3	100%	0.0	99.05%	ON795956
	Pseudomonas aeruginosa strain PaSa1910	99%	0.0	99.05%	OR261093
	Pseudomonas aeruginosa strain PaSa197	99%	0.0	99.05%	OR261061

Isolat Isolates	Homologi Homology	Query Cover (%)	EE-Value	Similarity	Access Number
DSAE 17	*Bacillus subtilis* strain C-2-3-2	100%	0.0	99.76%	KM114001.1
	Bacillus subtilis strain JK0516S	100%	0.0	99.76%	KF135457.1
	Bacillus subtilis strain SSR17	99%	0.0	99.76%	MH889071.1
DSAE 19	*Pseudomonas otitidis* strain RR017	99%	0.0	99.68%	MF426249.1
	Pseudomonas otitidis strain HR-2	99%	0.0	99.68%	MT645611.1
	Pseudomonas otitidis strain RR006	99%	0.0	99.68%	MF426243.1

Isolate DRSE 16 has a 99.37% similarity to the species *Stenotrophomonas nitritireducens* strains BR-49, MN63, and KUDC1825 (Table 3). Isolate DRSE 17 has a 99.51% similarity to the species *Pseudomonas putida* strains XT214, XT215, and XT911. Isolate ASAE 3 has a 99.05% similarity to the species *Pseudomonas aeruginosa* strains D3, PaSa1910, and PaSa197. Isolate DSAE 17 has a similarity level of 99.76% with the species *Bacillus subtilis* strains C-2-3-2, JK0516S, and SSR17. Isolate DSAE 19, has a 99.68% similarity to the species *Pseudomonas otitidis* strains RR017, HR-2, and RR006. According to Newell et al. (2013), the percent identification (Per. ident) on the NCBI site indicated the percentage of nucleotide similarity between the sample and the reference sequence. The results of the phylogenetic tree construction show that the five potential endophytic bacterial isolates from mangrove plants formed three clades, as seen in Figure 4.

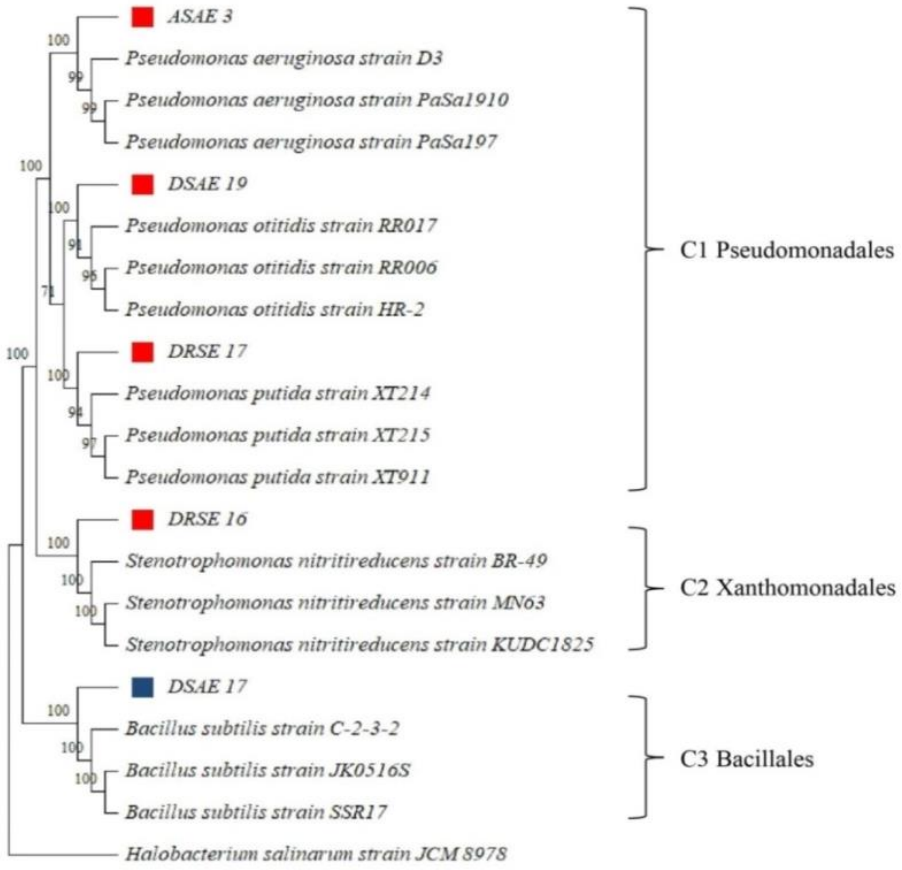


Figure 4: Construction of the phylogenetic tree for five potential mangrove endophytic bacterial isolates (DRSE 16, DRSE 17, ASAE 3, DSAE 17, DSAE 19). The tree was built using the neighbor-joining method based on 16S rRNA gene sequences, with 1000 bootstrap replications.

Clade 1 (C1) is a group of Gram-negative bacteria from the order Pseudomonadales, family Pseudomonadaceae, and genus *Pseudomonas*. Clade 2 (C2) comes from a group of Gram-negative bacteria from the order Xanthomonadales, family Xanthomonadaceae, and genus *Stenotrophomonas*. Meanwhile, Clade 3 (C3) is from the order Bacillales, family Bacillaceae, and genus *Bacillus*. *Halobacterium salinarum* strain JCM 8978 from *Halobacterium* was used as an outgroup to determine the accuracy and suitability of the 16S rRNA gene phylogenetic tree construction. Isolates ASAE 3, DSAE 19, and DRSE 17 belong to the genus *Pseudomonas*. These bacteria can produce phytohormones and secrete organic acids such as formic, acetic, propionic, lactic, glycolic, glyoxylic, fumaric, tartaric, ketobutyric, succinic, and citric acids (Rao, 2007). Isolate DRSE 16 was identified as belonging to the genus *Stenotrophomonas*, which has the ability to reduce nitrite in the soil, convert saturated fatty acids into hydroxylated fatty acids, produce siderophores, dissolve phosphate, and form phytohormones. It also acts as a biocontrol agent against pathogenic fungi, pathogenic bacteria, and pests that can harm plants (Kumar et al., 2023). Isolate DSAE 17 belongs to the genus *Bacillus*, which is capable of

degrading natural substrates and contributing to nutrient turnover, inhibiting the growth of pathogenic microbes, and acting as PGPR (Plant Growth-Promoting Rhizobacteria). The genera Pseudomonas, Stenotrophomonas, and Bacillus are widely recognized as common endophytic bacteria (Chatterjee and Abraham, 2020; Ulrich et al., 2021; Wibowo et al., 2023; Fatimatuzzahra et al., 2024). These microorganisms have been frequently isolated from various plant species and are known for their beneficial roles, including promoting plant growth, enhancing stress tolerance, and exhibiting antagonistic activity against phytopathogens. Their presence as endophytes reflects their adaptability to internal plant environments and their potential applications in sustainable agriculture and biotechnology.

Conclusion

Five potential selected isolates (DRSE 16, DRSE 17, ASAE 3, DSAE 17, DSAE 19) showed the potential to inhibit pathogenic microbes such as *Enterococcus faecalis*, *Salmonella typhi*, and *Candida albicans*. The results of the 16S rRNA gene sequence analysis of five bacterial isolates, namely DRSE 16, DRSE 17, ASAE 3, DSAE 17, and DSAE 19, each had the highest similarity to the species *Stenotrophomonas nitritireducens* strain BR-49, *Pseudomonas putida* strain XT214, *Pseudomonas aeruginosa* strain D3, *Bacillus subtilis* strain C-2-3-2, and *Pseudomonas otitidis* strain RR017. This study concludes that bacterial isolates from mangrove plants may be considered as potential candidates for the development of new antimicrobial agents that play a role as an alternative to antibiotics.

Conflict of interest

The authors declare that they have no conflicts of interest.

Author Contributions

Risky Hadi Wibowo (corresponding author): Conceptualization, methodology, and editing; Sipriyadi: Methodology, data curation, checking, reviewing, evaluating, and journal submitting; Riziq Ilham Nur Fahmi, Denny Kurniawan, and Putri Hezekiel Claracia Simanjuntak: Validation, evaluating, writing, and editing.

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