BIODIVERSITAS
Volume 25, Number 7, July 2024
Pages: 3035-3042

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250725

Antibacterial and biosurfactant activity of endophytic bacteria isolated from mangrove plant in Lamongan, Indonesia

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Manuscript received: 21 February 2024. Revision accepted: 12 July 2024.

Abstract. *Fatimah, Rahayuningtyas ND, Nastiti A, Alawiyah DD, Ramadhan E, Geraldi A, Junairiah. 2024. Antibacterial and biosurfactant activity of endophytic bacteria isolated from mangrove plant in Lamongan, Indonesia. Biodiversitas 25: 3035-3042.* This study aims to determine the antibacterial and biosurfactant activity of 61 endophytic bacteria from mangrove plants in Kutang Lamongan Beach. Antibacterial screening of supernatant of endophytic bacteria was performed using the disc diffusion method against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. The most potent isolate was identified using 16s rRNA. The chemical compounds of the most potent isolate were identified using GC-MS analysis. The data were analyzed descriptively. Biosurfactant activity was also performed on the most potential isolate. All the isolates showed inhibitory zones against *E. coli* and *S. aureus*. Isolate LMG-2 produced the highest antimicrobial activity, averaging 9.57±0.4 mm against *E. coli* and 8.87±0.36 mm against *S. aureus*. The biosurfactant activity of LMG-2 isolate produced a surface tension of 39.09+0.49 mN/m and emulsification activity of 65.03+0.03% against kerosene, 47.63+0.10% against crude oil and 46.53+0.27 to diesel. The isolate LMG-2 had 98,41% similarity with the *Bacillus amyloliquefaciens* strain NBRC 15535 with a query cover of 100%. The biosurfactant extract contains 9-octadecenoic acid methyl ester, (E)-, 9,12-octadecadienoic acid (Z, Z) methyl ester, and 9,12,15-octadecadienoic acid methyl ester. These compounds, known as Fatty Acid Methyl Esters (FAMEs), were integral components of biosurfactants, surface-active agents produced by microorganisms.

Keywords: Antibacterial agent, *Bacillus amyloliquefaciens* LMG-2, endophytic bacteria, Lamongan mangrove

INTRODUCTION

Antibiotics are common drugs used to treat microbial infections. These microbial infections can become major health problems in several countries, especially developing ones. Antibiotics are chemical substances produced by microorganisms that can kill or inhibit the growth of pathogenic microbes (Kasanah and Hamann 2004). At the same time, in humans, the toxic effect is relatively low (Baran et al. 2023). New problems relate to multi-drug-resistant bacteria (Salam et al. 2023). The improper use of antibiotics, low doses, and prolonged use caused the pathogenic bacteria to resist antibiotics. Around 40-62% of antibiotics are misused, especially for diseases that do not require antibiotics. Studies in several hospitals showed that 30-80% of the use of antibiotics was not based on indications. This problem is worsening, especially in developing countries, due to the lack of trained doctors and health workers to diagnose diseases (Chokshi et al. 2019). So far, antibiotic resistance worldwide has cost $20 billion and is predicted to increase by $100 trillion in 2050, with the deaths reaching 10 million per year (Morehead and Scarbrough 2018). Exploring microbes with the potential to kill pathogenic microbes can be a solution to overcome the problem. These antimicrobial-producing microorganisms can be isolated from various habitats, such as mangroves.

Mangroves are ecosystems where various communities of antimicrobial-producing microorganisms have enzymatic activities (Bibi et al. 2017). Mangroves have dynamic environmental conditions, so organisms have a defense system to survive and adapt to these habitats (Tan et al. 2018). Several factors, including salinity, pH, climate, vegetation, nutrition, and location, influence the presence and diversity of bacteria in the mangrove ecosystem (Li et al. 2022). This unique ecosystem can be a natural resource in finding qualified antibiotic-producer candidates, especially those from endophytic bacteria.

Endophytic bacteria are microorganisms that partially or entirely live in the host plant tissue without causing disease to the host (Purnawati and Nirwanto 2021). Endophytic bacteria can produce primary and secondary metabolites. Primary metabolites are produced during the growth period for survival, while secondary metabolites are by-products of organisms. Those groups of bacteria can increase the growth of host plants by reducing phytopathogens due to the production of antibiotic compounds that protect host plants from pathogens (Afzal et al. 2019). Endophytic bacteria can produce metabolites similar to compounds produced by host plants (Pandey et al. 2022). *Rhizopora apiculata* is a mangrove plant known to produce several compounds with antibiotic, anticancer, and antioxidant effects, such as flavonoids, alkaloids, terpenoids, aliphatic alcohols, and phenol derivatives (Dat et al. 2022).

Irawan et al. (2019) successfully isolated endophytic bacteria from mangrove plants in Kutang Beach, Brondong-Lamongan. There has been no research regarding the potential of bacterial endophytes from mangrove plants in Kutang Beach as antimicrobial. So, this study determines the potency of endophytic bacterial isolates from the Kutang Beach mangroves in producing antimicrobial compounds. These isolates are expected to be developed as antibiotics to overcome pathogenic microbial resistance. The antimicrobial activity of endophytic bacteria from mangrove plants will be tested against two pathogenic bacteria, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 strains.

MATERIALS AND METHODS

Procedures

This study was conducted in the Microbiology Laboratory, Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia. Samples were taken from Kutang Beach, Lamongan, Indonesia.

Bacterial isolates

Sixty-one endophytic bacteria were isolated from four mangrove plant species, identified as *R. apiculata*, *B. silindris*, *S. desandra*, and *E. agallocha.* The endophytic bacteria was isolated from leaves, roots, and stems. The antimicrobial potential tests were pathogenic microbes, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923.

Screening of antimicrobial activity

Antimicrobial screenings in this study aimed to obtain data on isolates that produced the highest antimicrobial activity characterized by the highest clear zone diameter among all isolates. The cell density of the pathogenic bacteria was measured using a spectrophotometer (λ = 600 nm) to obtain an Optical Density (OD) of 0.1. Microbe suspension of 100 µL was swabbed on the surface of the MHA medium (Mueller Hinton Agar). Then, a paper disc filled with 30 µL endophytic bacterial culture was placed on the surface of the MHA medium. The Petri dish with MHA medium was incubated for 48 hours at room temperature, and the clear zone diameter around the paper disc was measured.

Antibacterial activity test of potential isolates

The 3 endophytic isolates with the highest antimicrobial activity were re-screened using the same method with several modifications. MHA inoculated with the pathogenic bacteria. The disc containing 0.1 µL of endophytic bacterial culture was placed on MHA and incubated for 48 hours. Every 24 hours, the isolates were observed for the formation of a clear zone around the disc.

Identification of bacteria using the 16s rRNA gene

Molecular identification was performed on the most potential isolate. DNA isolation followed the Promega Genomic Purification Kit Wizard's protocol. The results of genomic DNA observations were observed using agarose gel electrophoresis in the form of bands. DNA purity was measured quantitatively using a spectrophotometer at 260 and 280 nm wavelengths. Primers used to amplify the 16s rRNA gene consisted of universal 27F and 1492R. The PCR result sequences were blasted using Bioedit and then compared with the data in Genebank.

Potential isolate biosurfactants activity test

Isolate with the highest inhibitory zone, *B. amyloliquefaciens*, was tested for biosurfactant production. The supernatant of the culture was measured for surface tension and emulsification activity against hydrocarbon compounds. Surface tension was measured using a Du-Nouy tensiometer. The emulsification activity was done by adding 2 mL of the supernatant mixed with 2 mL of oil and then homogenizing for 2 minutes. The emulsion was measured in 1 hour and 24 hours of incubation. The emulsification index (E24) is the percentage of the height of the emulsion layer (cm) divided by the total height of the solution.

Fermentation and extraction of biosurfactant

A 10% suspension of *B. amyloliquefaciens* in NB medium was added to 350 mL fermentation medium and homogenized for ±3 days (El-Banna and Qaddoumi 2016; Sun et al. 2019). The culture was centrifuged for 15 minutes at 10,000 rpm. The supernatant was mixed with 6N HCL solution until it reached a pH of 2 and then stored in the refrigerator overnight. The supernatant was centrifuged again for 15 minutes at 10,000 rpm. The precipitate was extracted using ethyl acetate with acetone in a ratio of 2:1. The mixed solution was evaporated using a rotary evaporator at 50°C (Uche-Okereafor et al. 2019).

Profiling of antimicrobial compounds by GC-MS

One µL of ethyl acetate extract was injected into the GC-MS and Agilent 5975 GC/MSD systems. The column temperature was initially set at 35°C for 1 minute before being programmed to 200°C at a rate of 25°C per minute with a holding time of 5 minutes. Unit mass conditions were done using an ion source of 230°C with an ionization energy of 70 eV and an electron current of 1435 mA. Each peak produced on the chromatogram is compared with the mass spectrum in the WILEY library to determine the appropriate organic compound (Adams et al. 1995; Lee et al. 2017).

RESULTS AND DISCUSSION

Antimicrobial activity screening

Sixty-one of bacterial endophytes have been isolated from mangrove plants in Kutang Beach. All isolates produced antimicrobial activity against *E. coli* and *S. aureus*. The diameter of the clear zone of Endophytic isolates was presented in 24 hours of incubation (Table 1).

Table 1. Diameter of the inhibitory zone of endophytic bacteria from mangrove plants against *E. coli* and *S. aureus* (24 hours incubation)

Code of isolates	Inhibitory zone diameter (mm)	
	E. coli	***S. aureus***
LMG-1	7.90±1.33	8.50±1.33
LMG-2	8.68±0.53	9.58±0.44
LMG-3	6.62±0.33	7.28±0.23
LMG-4	7.27±0.23	8.09±0.54
LMG-5	6.54±0.54	7.38±0.38
LMG-6	6.80±0.33	7.98±0.58
LMG-7	6.64±0.62	7.60±0.52
LMG-8	6.68±0.98	8.11±0.28
LMG-9	6.52±0.81	7.47±0.78
LMG-10	6.43±0.51	4.67±4.00
LMG-11	6.92±1.54	7.93±1.11
LMG-12	7.17±0.21	8.32±0.13
LMG-13	7.43±0.67	8.07±0.68
LMG-14	7.24±0.23	7.90±0.18
LMG-15	6.83±0.72	6.95±0.61
LMG-16	7.93±0.67	8.13±0.65
LMG-17	6.97±0.25	6.98±0.58
LMG-18	6.72±0.63	7.28±0.64
LMG-19	7.52±1.50	8.13±0.67
LMG-20	8.03±0.50	7.90±0.88
LMG-21	7.52±2.63	9.13±0.84
LMG-22	6.26±0.45	7.33±0.23
LMG-23	6.95±1.04	7.58±0.36
LMG-24	7.31±0.93	7.37±0.03
LMG-25	6.90±0.70	7.10±0.87
LMG-26	8.22±1.81	8.37±0.56
LMG-27	7.10±0.40	7.87±0.75
LMG-28	7.53±0.41	8.03±0.13
LMG-29	6.89±1.54	7.53±0.18
LMG-30	7.31±0.89	6.78±1.11
LMG-31	7.93±0.79	6.62±1.07
LMG-32	8.73±0.58	7.00±1.08
LMG-33	7.45±0.13	6.07±0.12
LMG-34	7.33±0.21	7.43±0.12
LMG-35	6.77±0.80	7.20±0.28
LMG-36	6.30±0.52	6.10±0.17
LMG-37	6.18±0.32	6.20±0.35
LMG-38	6.75±1.29	6.00±0.00
LMG-39	6.97±1.67	7.90±0.18
LMG-40	6.85±1.47	7.47±0.28
LMG-41	6.00±0.00	8.13±0.72
LMG-42	6.33±0.58	7.50±0.36
LMG-43	6.13±0.23	6.12±0.20
LMG-45	6.55±0.95	4.00±3.46
LMG-47	6.00±0.00	6.25±0.35
LMG-48	6.40±0.69	6.27±0.25
LMG-49	6.63±1.09	6.00±0.00
LMG-50	6.03±0.06	6.03±0.06
LMG-51	6.27±0.46	4.22±3.65
LMG-52	6.00±0.00	4.17±3.62
LMG-53	6.20±0.35	4.53±3.94
LMG-54	6.93±1.62	7.43±1.11
LMG-55	6.17±0.21	4.63±4.04
LMG-56	6.00±0.00	4.77±4.13
LMG-57	6.20±0.20	6.65±0.54
LMG-58	6.62±0.55	8.20±0.79
LMG-59	6.87±1.24	8.20±0.99
LMG-60	7.13±1.27	7.43±0.06
LMG-61	6.83±0.76	4.03±3.49
LMG-62	6.70±0.70	6.27±0.38
LMG-63	6.87±0.75	4.10±3.55
Ciprofloxacin (positive control)	26.2±0.40	29.6±0.34
NB medium (negative control)	0.00±0.00	0.00±0.00

Table 1 shows that all bacterial isolates inhibit pathogenic bacteria' growth. It indicated that the extract of the supernatant contains antibacterial compounds. LMG-2 was the most potential bacterial isolate with an inhibitory zone of 8.68±0.53 against *E. coli* and 9.58±0.44 against *S. aureus* at 24 hours of observation. The inhibitory zone of *B. amyloliquefaciens* LMG-2 was lower than the positive control ciprofloxacin, producing an inhibitory zone of 26.20±0.40 mm against *E. coli* and 29.60±0.34 mm against *S. aureus.* The negative control, NB medium, had no inhibitory zone (0.00±0.00 mm) against *E. coli* and *S. aureus*. According to the Clinical and Laboratory Standards Institute 2013 (CLSI 2013), the ability of the supernatant from the *B. amyloliquefaciens* LMG-2 culture to inhibit the test bacteria was categorized as low (<14 mm). CLSI states that the category of inhibition zones for an antimicrobial compound is as follows: A diameter ≥20 mm indicates strong inhibition, a diameter of 15-19 mm indicates moderate inhibition, and a diameter <14 mm indicates weak inhibition. However, production optimization and appropriate fractionation methods are expected to enhance product yield and promote antibacterial activity at different concentrations to provide data on the antibacterial efficacy against *B. amyloliquefaciens* LMG-2 and other isolates. Figure 1 shows the inhibitory zone of ethyl acetate extract of culture supernatant of *B. amyloliquefaciens* LMG-2 against *E. coli* and *S. aureus.*

B. amyloliquefaciens LMG-2 had the highest inhibitory zone diameter against *E. coli* and *S. aureus* among the 61 endophytic bacteria isolated from mangrove plants.

Potency Bacillus amyloliquefaciens *LMG-2 for biosurfactant production*

Figure 2 shows the activity of the biosurfactant production by *B. amyloliquefaciens*. Supernatant *B. amyloliquefaciens* can emulsify several oils (diesel, kerosene, and crude oil). The emulsifier activity indicates the presence of surfactant in a culture and its ability to reduce the surface tension of liquid culture.

Table 2 shows the *B. amyloliquefaciens* supernatant can emulsify diesel oil and kerosene test oils up to 67.51% and 65.97% of the total liquid, respectively. It indicates that *B. amyloliquefaciens* is potent in producing biosurfactants. A previous study by Panjiar et al. (2015) showed that *Bacillus velezensis* had emulsification activities in the range of 49.89% to 72.9%, indicating strong emulsifying properties. The culture supernatant of *B. amyloliquefaciens* LMG-2 isolate reduced the surface tension by 39.09±0.49 mN/m (Table 3). The decrease of surface tension of distilled water and growth media (nutrient broth) were 32.91 mN/m and 18.08 mN/m, respectively. A reduction in surface tension of more than 10 dyne/cm indicates a high probability that the bacteria produce biosurfactants (Francy et al. 1991).

Profilling of biosurfactant extract from Bacillus amyloliquefaciens *using GC-MS*

Biosurfactant extracts of *B. amyloliquefaciens* LMG-2 were characterized using Gas Chromatography-Mass Spectrometry (GC-MS). Table 4 shows the complete compound profile of biosurfactant extract from *B.*

amyloliquefaciens. Figure 3 shows that the highest compound in biosurfactant extract was 9,12-Octadecadienoic acid (Z, Z) methyl ester with a retention time of 10.982 minutes at an area of 0.87%, followed by 9, 12, 15-Octadecatrienoic acid, methyl ester which has a retention time of 11.083 and an area of 0.75% and 9-Octadecenoid acid, methyl ester, (E)- which has a retention time of 11.083 and an area of 0.50%. 9,12-Octadecadienoic acid (Z, Z) methyl ester possesses an antioxidant, anti-microorganism (Mazumder et al. 2020), and antidiabetic activity (Singh and Chaturvedi 2019; Agada et al. 2020). In addition, Belakhdar et al. (2015) also stated that 9,12-Octadecadienoic acid (Z, Z) methyl ester possesses anticancer activity.

Table 2. Emulsification activity of the *Bacillus amyloliquefaciens* supernatant against hydrocarbons

Test hydrocarbons	Emulsification activity (%) after vortexing		
	Supernatant		Bacterial growth medium (negative control)
	1 h	24 h	24 h
Diesel oil	67.51+0.01	47.63+0.10	0.00±0.00
Crude oil	47.27+0.01	46.53+0.27	0.00±0.00
Kerosene	65.97+0.01	65.03+0.03	0.00±0.00

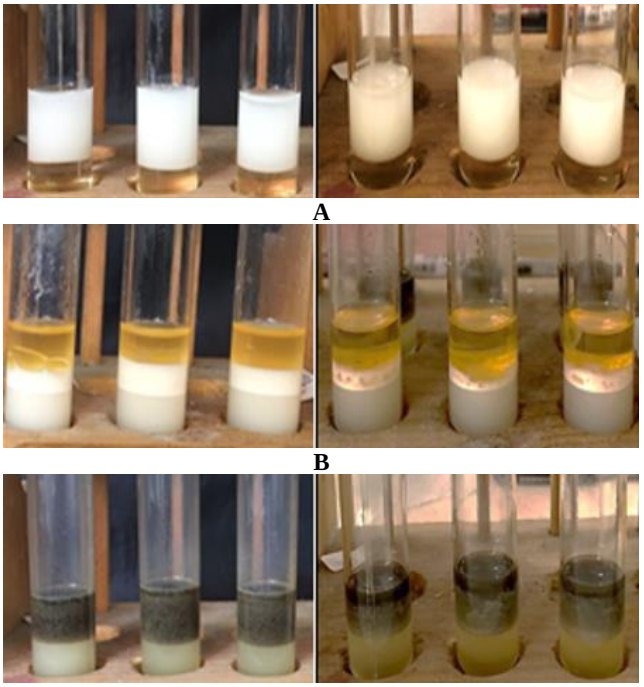

Figure 2. Emulsification activity of *Bacillus amyloliquefaciens* supernatant against: A. Kerosene; B. Diesel oil; C. Crude oil

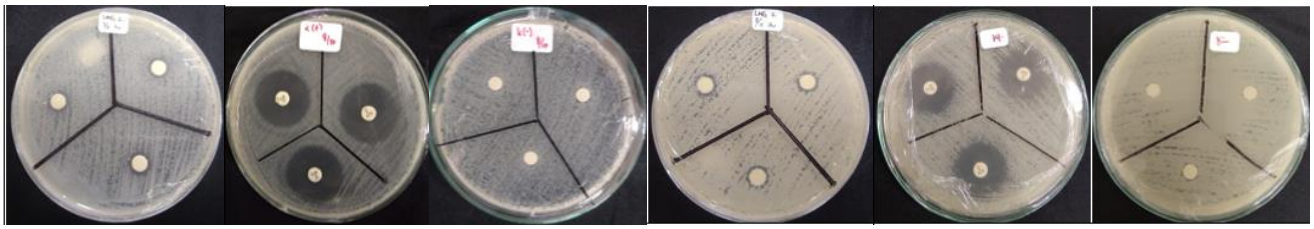

Figure 1. Disc diffusion test of antibacterial activity of the endophytic bacterial of: A. *Bacillus amyloliquefaciens* LMG-2 extract against *E. coli*; B. Positive control of the antibiotic (Ciprofloxacin) against *E. coli*; C. Negative control (NB medium) against *E. coli*; D. *B. amyloliquefaciens* LMG-2 extract against *S. aureus*; E. Positive control of antibiotic (Ciprofloxacin) against *S. aureus*; F. Negative control (water) against *S. aureus*

Table 3. The surface tension of supernatant culture of *Bacillus amyloliquefaciens*, growth medium, and distilled water

Measurement of	The surface tension of liquid (mN/m)	Decrease in surface tension of supernatant culture of *B. amyloliquefaciens* (mN/m) compared to
Supernatant culture of *B. amyloliquefaciens*	39.09+0.49	0
Growth medium (NB)	53.92+0.13	14.83
Aquades (Distilled water)	72.00+0.17	32.91

Table 4. Analysis of *Bacillus amyloliquefaciens* LMG-2 biosurfactant extract using GC-MS

Compound	RT	Area (%)	Formula	Qual (%)
9-Octadecenoid acid, methyl ester, (E)-	11.083	0.50	$C_{19}H_{36}O_2$	99
9,12-Octadecadienoic acid (Z, Z) methyl ester	10.982	0.87	$C_{18}H_{32}O_2$	99
9, 12, 15-Octadecatrienoic acid, methyl ester	11.172	0.75	$C_{19}H_{32}O_2$	99
1, 4-Hexadecanoic acid, methyl ester	9.104	0.09	$C_{18}H_{36}O_2$	90

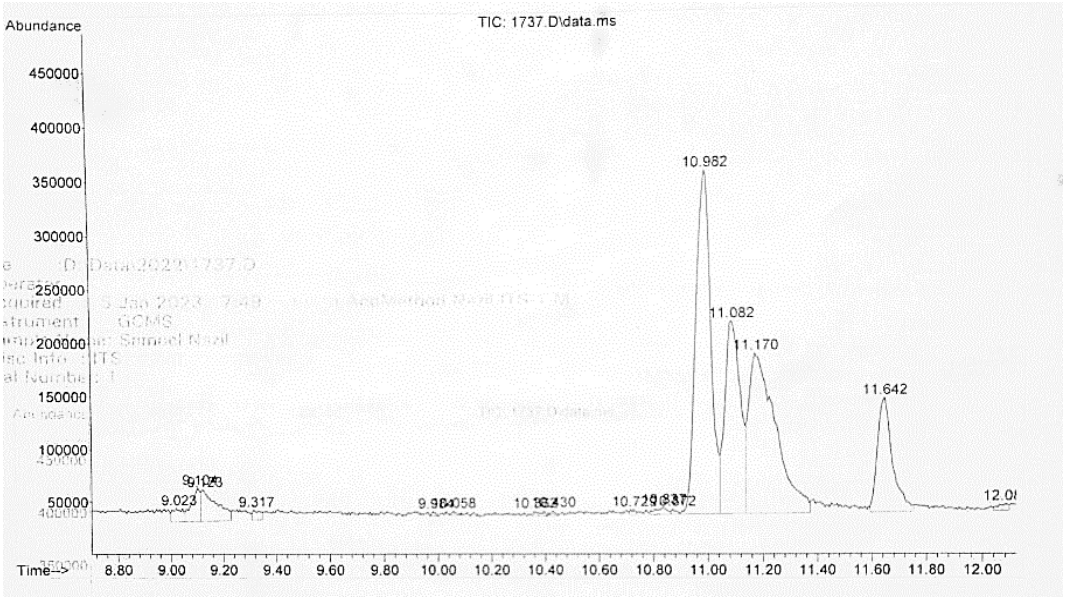

Figure 3. Chromatogram of biosurfactant extract from *Bacillus amyloliquefaciens* LMG-2 culture

Discussion

Endophytic bacteria are bacteria that colonize and grow inside plant tissues. These bacteria do not negatively affect plant growth. Otherwise, they can be neutral or in symbiosis with their host plants (Kandel et al. 2017), which induces gene transfer between plants and endophytic bacteria. Gene transfer occurs under certain environmental conditions, making endophytic bacteria produce compounds similar to their host (Chaudhry et al. 2017). Endophytic bacteria can produce secondary metabolites that are beneficial as antibacterial, antifungal, anticancer, and other potential to reduce surface tension (Kolli et al. 2021).

Naturally, endophytic bacteria can be isolated from mangroves and other plants. Mangroves are plants often found in the intertidal zone in both the tropics and the subtropics. These plants grow in different environments with unique characteristics, such as high permeability, waterlogged areas, and high temperatures. These conditions stimulate endophytic bacteria inside mangrove tissue to develop survival adaptation mechanisms. Therefore, many endophytic bacteria possess a series of putative genes that are known to cope with extreme environmental stresses (Chen et al. 2021).

In this study, 61 isolates of endophytic bacteria were isolated from mangrove plants. All the endophytic bacteria were screened for antimicrobial activity by observing the clear zone formed. Based on the results, isolates of LMG-2 produced compounds with antimicrobial properties indicated by forming an inhibitory zone around the test bacteria. An average inhibitory zone against *E. coli* was 8.68±0.53 mm, and against *S. aureus* was 9.58±0.44 mm at 24 hours of incubation. LMG-2 has been identified as *Bacillus amyloliquefaciens* LMG-2 through molecular identification and had an inhibitory zone wider than other bacterial endophytes. This isolate has a similarity of 98.41% with *Bacillus amyloliquefaciens* strain NBRC 15535; the query covers 99% (acc number NR_041455.1) (data not presented).

Bacillus has been reported to produce antibiotics in the late log phase or early stationary phase (Chalasani et al. 2015). Some *Bacillus* spp. have been known to produce active compounds. Approximately 8.5% of the genome of *Bacillus amyloliquefaciens* FZB42 involves the synthesis of secondary metabolites (Boottanun et al. 2017). These secondary metabolites consist of lipopeptides (Surfactin, bacillomycin D, and fengicin), polyketides (macrolactin, bacillaene, and difficidin), and siderophore (bacillibactin) (Gu et al. 2017).

Endophytic bacteria certainly have natural compounds that can inhibit the growth of pathogenic microbes (Simons et al. 2020). *Bacillus amyloliquefaciens* is a member of the *Bacillus*, which acts as an intracellular inhibitor by generating antibiotics (Lv et al. 2020). *Bacillus* are known to produce many enzymes and antimicrobial compounds. *Bacillus amyloliquefaciens* is a source of natural antibiotics, such as barnase (ribonuclease), alpha-amylase (for starch hydrolysis), Protease subtisilin (for combination with detergents), and restriction enzyme BamH1 (for DNA research) (Shleeva et al. 2023). A study by Ndlovu et al. (2017) reported that *Bacillus amyloliquefaciens* has antibacterial activity against pathogenic bacteria *E. coli* and *S. aureus.*

This study showed that metabolites produced by *Bacillus amyloliquefaciens* could also act as biosurfactants. Generally, biosurfactants are compounds with antimicrobial activity (Ndlovu et al. 2017). Antimicrobial compounds produced by *B. amyloliquefaciens* damage and prevent cell wall synthesis, which will cause the cells to be sensitive to osmotic pressure, which causes bacterial lysis (Lv et al. 2020).

According to Lv et al. (2020), the supernatant of *B. amyloliquefaciens* contains a mixture of lipopeptides, consisting of surfactin and fengycin, which have a strong

inhibitory effect on the growth and viability of *Clostridium difficile*. The mixture of lipopeptides, i.e., surfactin and fengycin, is a biosurfactant with antimicrobial properties (Ndlovu et al. 2017). Studies related to antibacterial mechanisms have shown that lipopeptide C-1 damages the integrity and permeability barrier of cell walls and cell membranes, then causes cell death of *Clostridium difficile*. *Bacillus* synthesizes a mixture of lipopeptides via nonribosomal peptide synthetase, which includes the surfactin, iturin, lichenysin, and fengycin families, with broad-spectrum biological activity (Dimkić et al. 2017; Farias et al. 2018).

Biosurfactants have been widely applied for bioremediation, industrial emulsification, and increasing oil recovery (Banat et al. 2014; Ndlovu et al. 2017). Apart from emulsifying oil and lowering surface tension, biosurfactants also have the potential to be used as biomedical and multipurpose therapeutics, which have applications in antiadhesion, anticarcinogenic, and antimicrobial (Mulligan et al. 2014; Ndlovu et al. 2017). In this study, *B. amyloliquefaciens* LMG-2 has shown the ability to reduce surface tension and emulsify oil. In addition, there are several types of biosurfactant-producing microorganisms, including *Lactobacillus, Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Bacillus licheniformis,* and *Streptococcus thermophilus* (Sari et al. 2015; Kumar et al. 2021).

Biosurfactants produced by *B. amyloliquefaciens* emulsify several oils (diesel, kerosene, and crude oil). The emulsification activity and the decrease in surface tension are indications of the presence of surfactants in liquid bacterial cultures. The supernatant of *B. amyloliquefaciens* produced emulsification activity in diesel and kerosene of 67.51% and 65.97% of the total liquid, respectively. The surface tension of the *B. amyloliquefaciens* supernatant was reduced by 39.09±0.49 mN/m, and the surface tension of distilled water and growth media was 32.91 mN/m, and compared to the growth medium (NB), a decrease of 14.83 mN/m. A reduction in surface tension of more than 10 dyne/cm indicates a high probability that the bacteria produce biosurfactants (Francy et al. 1991).

The results of compound identification using GC-MS showed that the most abundant compound was 9,12-Octadecadienoic acid (Z, Z) methyl ester and 9, 12, 15-Octadecatrienoic acid methyl esters. Both of these compounds belong to the class of linoleic acid compounds. Linoleic acid is an unsaturated omega-6 fatty acid with two double bonds. It is one of the two essential fatty acids with a long carbon chain compound that can undergo denaturation to become bioactive compounds, such as ɣ-linolenic acid and arachidonic acid. Furthermore, arachidonic acid can be converted into bioactive compounds like eicosanoids, such as leukotrienes and prostaglandins. Eicosanoids are essential compounds in cell and tissue metabolism, but if produced continuously, they can cause several chronic diseases, such as inflammation and cancer (Johnson and Fritsche 2012). However, previous research stated that the compound 9,12-Octadecadienoic acid (Z, Z) methyl ester (linoleic acid) functions as an antioxidant and anti-microorganism (Mazumder et al. 2020) and antidiabetic properties (Singh and Chaturvedi 2019; Agada et al. 2020).

Parthipan et al. (2017) and Deshmukh et al. (2012) showed that lipopeptides are compounds extracted from biosurfactants from *Bacillus* sp. Most of the compounds are fatty acids in nature, such as hexadecanoic acid, methyl esters (Parthipan et al. 2017), 9, 12-octadecadienoic acid (Z, Z)-, methyl esters (Cruz et al. 2020), 9-octadecenoic acid, 12-hydroxy-, methyl ester (Akintunde et al. 2015). Parthipan et al. (2017) reported that the biosurfactant isolated from *B. subtilis* A1 revealed the presence of fatty acids and peptides.

The GC-MS analysis showed several metabolites, i.e., that biosurfactant in this study contained 9-Octadecenoic acid, methyl ester, (E)-; 9,12-Octadecadienoic acid (Z, Z) methyl ester; 9, 12, 15-Octadecatrienoic acid, methyl esters and 1,4-Hexadecanoic acid, methyl ester are components of biosurfactants. Ibrahim et al. (2013) also reported that biosurfactant compounds contain fatty acid lipopeptides such as octadecanoic acid and 9-octadecenoic acid as the main components. Hexadecanoic acid is another fatty acid compound also detected in biosurfactants (Mo et al. 2022).

The compound 9,12-Octadecadienoic acid (Z, Z) methyl ester is an unsaturated fatty acid. The three Fatty Acid Methyl Ester (FAMEs) compounds in LMG2 have different chemical structures, melting points, and structural bonds but have almost the same function as biosurfactants. Low melting properties are associated with high reactivity, significantly affecting the membrane's fluidity. The fluidity of fats increases according to the degree of unsaturation of the residual fatty acids (He and Ding 2020). This property is required in the EOR process, where the Surfactant Enhancing Oil Recovery (EOR) is used. Unsaturated fatty acids have great potential as antimicrobials with their potential to emulsify oil and surface tension (Saxena et al. 2019). Hexadecanoic acid, methyl ester, or methyl hexadecanoic acid, also known as methyl palmitate or hexadecanoic methyl ester, belongs to the class of organic compounds known as FAMEs. FAMEs contain esterified fatty acids with a methyl group. Fatty Acid Methyl Esters (FAMEs) are integral components of biosurfactants, surface-active agents that microorganisms produce. These biosurfactants encompass various molecules, including glycolipids, lipopeptides, and phospholipids, with FAMEs frequently identified in the composition of glycolipids and phospholipids. *Klebsiella pneumoniae* contains different fatty acids with biosurfactant properties (Nwaguma et al. 2016). Similarly, the characterization of a biosurfactant activity of *Pseudomonas otitidis* from coal mines identified fatty acid components, demonstrating their significant role in the biosurfactant structure (Singh and Tiwary 2016).

Biosurfactant compounds can also act as antimicrobials through several mechanisms. One of the antibacterial mechanisms is in biosurfactant compounds, showing that lipopeptide C-1 damages the integrity and permeability barrier of cell walls and cell membranes, then causes cell death of pathogenic bacteria (Zhou et al. 2022).

However, it's essential to note that the antimicrobial activity of endophytic bacteria may be limited against certain strains of bacteria or in specific environments.

Therefore, considering other factors, such as the antimicrobial compound's dosage and mode of action, is crucial.

The biosurfactant activity of endophytic bacteria may have positive applications, such as enhancing oil recovery. However, it can also have negative environmental impacts. It disrupts natural oil-water interfaces or alters aquatic organisms' behavior. Antimicrobial-producing microorganisms may contribute to the development of antibiotic-resistant strains of bacteria. Therefore, it is necessary to carefully consider the risks and benefits of using microorganisms. One possible solution is to use microorganisms in combination with other antibiotics.

In conclusion, *B. amyloliquefaciens* exhibits antibacterial activity against *S. aureus* and *E. coli* and can also produce biosurfactants, indicated by the emulsification value and reduced surface tension. The crude extract of the culture supernatant of *B. amyloliquefaciens* contains Fatty Acid Methyl Esters (FAMEs), which are components of biosurfactants. So, the antimicrobial activity in the supernatant of *B. amyloliquefaciens* culture is a component of the biosurfactant-forming compound.

ACKNOWLEDGEMENTS

The authors would like to thank Universitas Airlangga, Surabaya, Indonesia, for funding the research through the *Penelitian Unggulan Fakultas* (PUF) 2022 scheme with contract number 1476/UN3.1.8 /PT/2022.

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