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Research Article

Exploration of Antimicrobial Potency of Mangrove Symbiont against Multi-Drug Resistant Bacteria

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Abstract

Antimicrobial property of mangrove symbiont have the ability to fight Multi Drug Resistant bacteria which were *Staphylococcus aureus*, *Escherichia coli*, and *Vibrio haryeyi*. This study aimed to determine the potential of symbiont microbes from the root of *Rhizophora mucronata* and *Acanthus ilicifolius* as antimicrobial agents against multi-drug resistant (MDR) pathogenic microbes. This research was conducted during July to November 2020. The MDR bacteria were *S. aureus*, *E. coli*, and *V. harveyi* MDR test microbes. The symbiont microbes were identified through molecular analyses (PCR 16S rDNA). Isolation of symbiont microbes from *R. mucronata* resulted in 16 isolates, while isolation from *A. ilicifolius* resulted in 14 isolates. Based on the antimicrobial qualitative test against *S. aureus*, 8 out of 16 microbial isolates from *R. mucronata* were found to show antimicrobial properties. The testing of *A. ilicifolius* symbiont microbes against *S. aureus* showed 8 out of 14 isolates with antimicrobial properties. The test against *E. coli* resulted in 2 out of 16 microbial isolates from *R. mucronata* and 5 out of 14 isolates from *A. ilicifolius* with antimicrobial properties. The test against *V. harveyi* resulted in two out of 16 microbial isolates from *R. mucronata* and 4 out of 14 isolates from *A. ilicifolius* with antimicrobial properties. The quantitative test found 2 isolates from *R. mucronata*, namely isolates RM10 and RM12, with antimicrobial properties against MDR strain *E. coli*, with the best isolate being RM10, which produced 11.22 mm of inhibition zone diameter. Furthermore, the selection of isolates was based on the size of the inhibition zone, the clearness of the inhibition zone and the potential for antibacterial activity. Based on their overall anti-microbial potential against the test microbes, four isolates were selected. Molecular analyses of RM12 isolate showed 95% homology with *Bacillus subtilis*, of RM 10 isolate showed 97% homology with *Bacillus oceanisediminis*, of AC isolate showed 96% homology with *Paracoccus caeni*, and of AC 5 isolate showed 89% homology with *Bacillus circulans*. The study found four isolates with antimicrobial potency against MDR pathogenic microbes. The symbiont microbes taken from *R. mucronata* and *A. ilicifolius* were determined to be of the genus *Bacillus* and *Paracoccus*.

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1. Introduction

Mangrove is a common species spread throughout the Indo-Pacific region. In Indonesia, mangrove can be found in shallow waters along the Indonesian coastal regions (Ariyanto *et al.*, 2018a; Ariyanto *et al.*, 2019a; 2019b). After the 2004 Aceh tsunami which devastated the region and its immediate vicinity, there has been a rising awareness of the importance of mangrove ecosystems among the community in the affected areas. Mangrove green belt cannot stop an extreme tidal wave in its entirety, however, it can mitigate the destructive impact from such natural disaster (Osti *et al.*, 2009). After this incident, Indonesian people together with the Government planted mangroves to improve the mangrove ecosystem on the Indonesian coast. Moreover, mangrove can be used as a source nutrition and habitat for marine organism (Ariyanto *et al.*, 2020; Ningsih *et al.*, 2020). The high level of nutrients in the mangrove area was caused by the mangrove waste, which turned into mangrove litter and was decomposed by bacteria, making it useful as a nutrient. Mangrove extract has proven potential as a multi-drug resistant anti-bacterial (Pringgenies *et al.*, 2020). Mangrove symbiont bacteria were thought to have the potential to be anti-bacterial because the active compounds in the symbiont were suspected to be similar to the host.

Various species of mangroves has been identified. Two species are particularly abundant along the coastal area of Jepara, namely *R. mucronata* and *A. ilicifolius*. *R. mucronata* is a true mangrove species (Quadros and Zimmer, 2017), meaning that its natural habitat are areas which are affected by oceanic tides. On the other hand, *A. ilicifolius* is a pseudo-mangrove species in that it is only associated to mangrove plants (Ragavan *et al.*, 2015). True mangrove species generally have special adaptive mechanisms which help them thrive in their natural habitats. Pseudo-mangrove species do not have these special adaptive mechanisms, instead they have high environmental tolerance which allow them to live in the environment typically classified as mangrove ecosystem (Yang *et al.*, 2015).

Dead mangrove leaves decomposes on the ground and turn into source of nutrition which contributes to their immediate vicinity (Ariyanto *et al.*, 2018b). The decomposition process is enabled by microbes which are found in the mangrove ecosystem. Symbiont microbes of marine life can act as antimicrobial agents, even against Multi-Drug Resistant (MDR) strains. The MDR microbes are pathogenic strains that have mutated and become immune to a range of antibiotics.

Studies found that the symbiont microbe in gastropod *Conus miles* has the potential to be used as anti MDR microbe agents (Pringgenies *et al.*, 2019). The symbiont microbe in gastropod *Pleuroploca trapezium* has potential to be used as anti MDR microbe agents, of which the microbe is identified to be closely related to *Paracoccus* sp. MBIC4019 with 95% homology (Pringgenies and Renta, 2014).

There are four significant factors to make microbes resistant to antibiotics, namely permeability, inactivation process by enzymes, change of the target's receptor cells, and the increase in the synthesis of antagonistic metabolites (Ventola, 2015). Moreover, resistant microbes, which are initially sensitive to antibiotics, may happen through the mutation in their chromosomes or the exchange of genetic materials between microbes. Exchange of chromosome material is rarely found and genetic material exchange is commonly the case. Based on the findings above, this study aimed to determine the potential of symbiont microbes associated with *R. mucronata* and *A. ilicifolius* as the source of anti MDR microbial agent and to identify the symbiont microbes which are active against those MDR strains.

2. Materials and Methods

2.1. Sampling

This research was conducted from July to November 2020. Symbiont microbes from one root of *R. mucronata* and *A. ilicifolius* were collected from Jepara waters and its immediate areas. The samples were then placed inside polyethylene bags (Whir-pak, Nasco, USA) and stored in a cooling container.

2.2. Media Preparation

Zobell agar is a non-selective agar medium made by mixing 0.25 grams of peptone, 0.05 grams of yeast, and 1.5 grams of bactoagar with 100 ml of distilled water in an Erlenmeyer flask. The medium is homogenized on a hotplate and stirred with a stirring rod. After the medium became homogeneous, it is sterilized using an autoclave for 15 minutes at 121°C. Liquid Zobell medium is made using the same steps, except it does not use bactoagar as a solidifier.

2.3 Isolation of Microbes and Purification of Microbial Isolates

A total of 10 g mangrove sample was immersed in 90 ml of sterile saline water and then diluted until 10^{-1} , 10^{-2} , and 10^{-3} dilutions. Using pipettes, 100 μ l of

each dilution was dripped into the prepared Zobell 2216E in Petri dishes. The preparation was then spread and incubated for 2x24 hours at room temperature. The purification of microbial isolate was performed using streak method. Streak method is used in obtaining purified microbial isolates. Microbial colonies are sorted out based on their morphological characteristics and then purified using streak plates (Badieyan *et al.*, 2018)

2.4 Qualitative and Quantitative Test of Antimicrobial Properties

The test of *S. aureus*, *E. coli*, and *V. harveyi* were obtained from the repository of Doctor Kariadi, General Hospital Semarang. The qualitative test was performed using overlay method according to Jiang *et al.* (2013). The overlay method used two agar plates which was placed on top of one another. A total of 10 microbial isolates were inoculated on petri dish and incubated for 4x24 hours. Each preparation was then added with soft agar media, inoculated with test microbe, and incubated for 24 hours. The positive control test was performed using the antibiotic Streptomycin which was tested against the test bacteria with a concentration of 80 g/ disk. The interaction between the isolates and microbes test might form transparent zone on the periphery of the isolate. The results of this test were used to determine the potency of symbiont microbe as MDR antimicrobial agents. Agar diffusion method is used in the quantitative antimicrobial properties test (Balouiri *et al.*, 2016). In this method, the zone of inhibition resulted from the interaction between the isolate and test microbes is measured using calipers (Altarugio *et al.*, 2018).

2.5 Identification of *R. mucronata* and *A. ilicifolius* Symbiont Microbes

Screened microbial isolates were identified using molecular DNA method. In order to determine the microbes, the microbial isolates were put through DNA extraction process. The resulting DNA genomes were added with 200µl of instagene matrix. The mixture was then put into a heat block at 56°C for 15 minutes and into centrifuge at 2,000 rpm for 3 minutes. The resulting supernatants are used in 16S rDNA PCR amplification. The PCR amplification is performed by using universal primers (Sune *et al.*, 2020).

The amplified DNA is put through electrophoresis to determine DNA bands. The

observation of the DNA band using UV. The parts of DNA band formed are then purified and amplified using 765R and 1141R primers in 30 cycles. Afterwards, the sequencing is performed using an automatic DNA sequencer device. The result of the PCR sequencing were then sent to PT. Genetics Science Indonesia, which is in Jakarta for the sequencing of the nitrogen bases.

2.6 Data Analysis

The data obtained were analyzed using descriptive method. The homology of DNA sequence was determined using BLAST. The BLAST analysis results were then compiled into a phylogenetic tree using Clustal X Version 1.60.

3. Results and Discussion

3.1 Isolation of Mangrove Symbiont Microbe

Isolation of symbiont microbes from *R. mucronata* and *A. ilicifolius* resulted in 16 isolates and 14 isolates respectively. The microbial isolates are categorized based on their color, shape, texture, and margin. There were seven isolates of microbes from *R. mucronata* which were predominantly white: isolates RM6, RM9, RM10, RM11, RM12, RM13, and RM16 as well as four isolates from *A. ilicifolius*, which are: isolates AC7, AC10, AC11, and AC14. Out of all isolates from *R. mucronata* were predominantly circular, with seven of them observed to be in this form, namely isolates RM3, RM4, RM5, RM9, RM10, RM13 and RM14, and 10 of them were convex in texture, namely isolates RM1, RM3, RM6, RM7, RM8, RM10, RM12, RM13, RM 14, and RM16. The most predominant shape for *A. ilicifolius* isolates were circular, with six isolates, namely AC1, AC3, AC5, AC6, AC10 and AC13 showed this shape. The most prevalent texture for *A. ilicifolius* isolates were convex, with 10 isolates showing this texture, while the other four, isolates AC5, AC6, AC9 and AC11, showed crateriform. Most isolates from both mangrove species were observed to have even margins.

3.2 Antimicrobial Activity Screening

The qualitative test results against MDR strain of *S. aureus*, *E. coli*, and *V. harveyi* indicated that eight isolates from each mangrove species possess antimicrobial activity against MDR strain *S. aureus*. Qualitative test results of Symbiont Microbe from *R. mucronata* and *A. ilicifolius* against *E. coli* indicated that two microbial isolates from *R. mucronata* were active against

MDR strain *E. coli* and five isolates from *A. ilicifolius* were active against MDR strain *E. coli*. Qualitative test results of Symbiont Microbe from *R. mucronata* and *A. ilicifolius* against *V. harveyi* indicated that two microbial isolates from *R. mucronata* were active against MDR strain *V. harveyi* and four isolates from *A. ilicifolius* were active against MDR strain *V. harveyi* (Table 1).

The qualitative test results of microbial isolates from *R. mucronata* indicated that 8 of 16 isolates showed potential as antimicrobial agents against MDR strain *S. aureus*, of which isolates codes are RM1, RM2, RM5, RM9, RM10, RM11, RM12, and RM15. The remaining 8, namely isolates RM3, RM4, RM6, RM7, RM8, RM13, RM14, and RM16 did not show any antimicrobial properties against MDR strain *S. aureus*. The qualitative test results of microbial isolates from the mangrove species *A. ilicifolius* indicated that eight isolates showed antimicrobial properties, while the remaining six did not show antimicrobial activity against MDR strain *S. aureus*. The isolates indicating antimicrobial properties were AC1, AC2, AC4, AC5, AC9, AC10, AC12, and AC14. The isolates that did not indicate antimicrobial properties were AC3, AC6, AC7, AC8, AC11, and AC13.

Out of 16 microbial isolates from *R. mucronata* showed antimicrobial properties against MDR strain *E. coli*, namely isolates RM10 and RM12. The testing of *ilicifolius* symbiont microbes against MDR strain *E. coli* showed five out of 14 isolates with antimicrobial properties: AC5, AC6, AC7, AC8, and AC9. Out of 16 microbial isolates from *R. mucronata* showed antimicrobial properties against MDR strain *V. harveyi*, namely isolates RM10 and RM14. And for microbial isolates from *A. ilicifolius*, four out of 14 microbial isolates, namely AC2, AC4, AC5 and AC12, showed antimicrobial properties against MDR strain *V. harveyi* (Table 1).

3.3 Quantitative Antimicrobial Activity Test against MDR Strains

The quantitative antimicrobial test was performed using agar diffusion method in accordance to Kirby-Bauer principles, using 8 mm paper disks. The quantitative test found eight isolates from *R. mucronata*, namely isolates RM1, RM2, RM3, RM5, RM7, RM10, RM11, and RM12, with antimicrobial properties against MDR strain *S. aureus* (Table 2). The test also found that 6 microbial isolates from *A. ilicifolius* with potential to be used as antimicrobial agents, namely isolates AC1, AC2, AC5, AC10, AC12, and AC14.

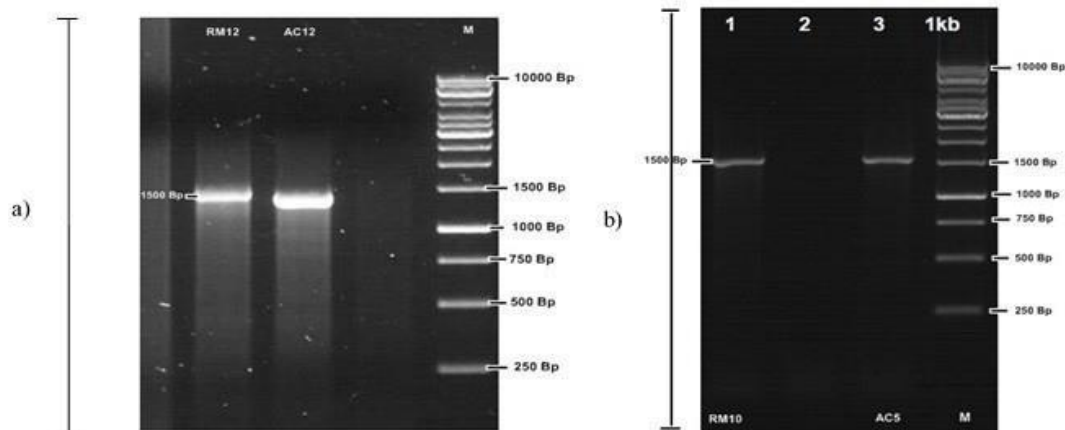


Figure 1. a) 16S rDNA Amplification of RM12 and AC 12 (M : DNA Marker), b) 16S rDNA Amplification of RM10 and AC 5. (M : DNA Marker)

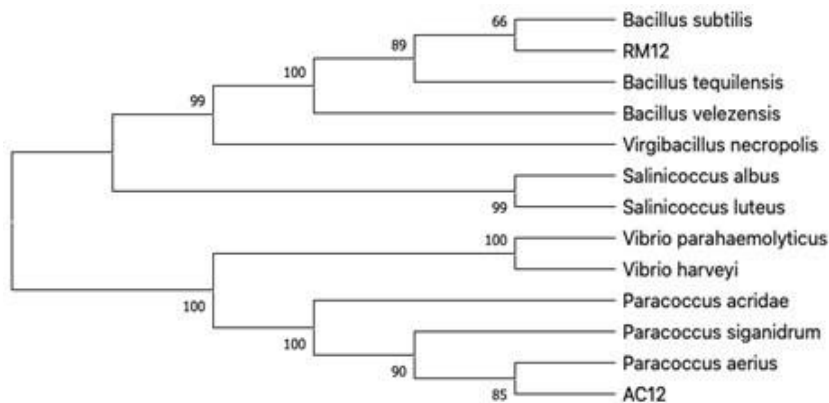


Figure 2. The phylogenetic tree describes the match between isolate RM12 and *B. subtilis*, and the match between isolate AC12 and *Paracoccus aerius*, using the interface of the program Tree View.

Table 1. Antimicrobial activity qualitative test of mangrove symbiont microbe against *S. aureus*, *E. coli* and *V. Harveyi*

Isolates	Test microbes			Isolates	Test microbes		
	<i>S. aureus</i>	<i>E.coli</i>	<i>V.harveyi</i>		<i>S. aureus</i>	<i>E.coli</i>	<i>V.harveyi</i>
RM.10.1.1	+	-	-	AC.10.1.1	+	-	-
RM.10.1.2	+	-	-	AC.10.1.2	+	-	+
RM.10.1.3	-	-	-	AC.10.1.3	-	-	-
RM.10.1.4	-	-	-	AC.10.1.4	+	-	+
RM.10.1.5	+	-	-	AC.10.1.5	+	+	+
RM.10.2.6	-	-	-	AC.10.1.6	-	+	-
RM.10.2.7	-	-	-	AC.10.1.7	-	+	-
RM.10.3.1.8	-	-	-	AC.10.2.8	-	+	-
RM.10.3.2.9	+	-	-	AC.10.3.1.9	+	+	-
RM.10.3.2.10	+	+	+	AC.10.3.2.10	+	-	-
RM.10.4.1.11	+	-	-	AC.10.4.1.11	-	-	-
RM.10.4.1.12	+	+	-	AC.10.4.2.12	+	-	+
RM.10.4.2.13	-	-	-	AC.10.5.1.13	-	-	-
RM.10.5.1.14	-	-	+	AC.10.5.2.14	+	-	-
RM.10.5.1.15	+	-	-				
RM.10.5.2.16	-	-	-				

Table 2. Antimicrobial activity quantitative test of mangrove symbiont microbe against *S. aureus*

Parameters	Isolates	Zone of Inhibition Diameter (mm)	Isolates	Zone of Inhibition Diameter (mm)
<i>S. aureus</i>	RM.10.1.1	9.08	AC.10.1.1	9.6
	RM.10.1.2	10.30	AC.10.1.2	9.28
	RM.10.1.3	8.52	AC.10.1.5	10.16
	RM.10.1.5	11.21	AC.10.3.2.10	10.03
	RM.10.2.7	9.07	AC.10.4.2.12	12.32
	RM.10.3.2.10	12.20	AC.10.5.2.14	10.20
	RM.10.4.1.11	10.22		
	RM.10.4.1.12	12.38		
<i>E. Coli</i>	RM.10.3.2.10	11.22	AC.10.1.5	10.76
	RM.10.4.1.12	10.56	AC.10.1.6	10.42
			AC.10.1.7	10.33
			AC.10.1.8	10.23
			AC.10.3.1.9	10.62
<i>V. harveyi</i>	RM.10.3.2.10	10.80	AC.10.1.2	10.47
	RM.10.4.1.14	10.13	AC.10.1.4	10.53
			AC.10.1.5	10.46
			AC.10.4.2.12	10.58

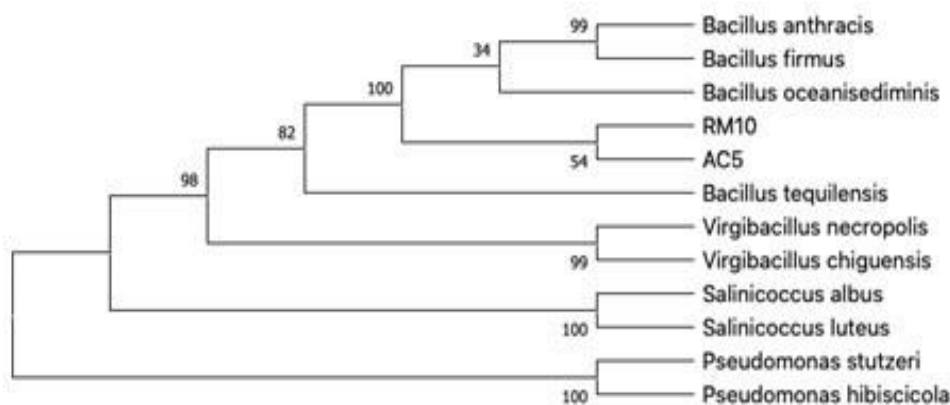


Figure 3. Phylogenetic tree showing the relative match of isolate RM10 and isolate AC5 using the program Tree View

Table 3. BLAST homology of mangrove symbiont microbe

Isolates	Relative Match	Homology (%)	Access No.
RM12	<i>Bacillus subtilis</i>	97%	MN696502.1
AC12	<i>Paracoccus aerius</i>	99%	NR_157753.1
RM10	<i>Bacillus oceanisediminis</i>	94%	MH283842.1
AC5	<i>Bacillus firmus</i>	76%	EU418717.1

Based on the ability to inhibit the growth of pathogenic microbe by the diameter of inhibition zone, one isolate, RM 12 with 12.38 mm diameter of inhibition zone, from *R. mucronata* was selected, meanwhile from *A. ilicifolius*, isolate AC 12 was with 12.32 mm of inhibition zone diameter was chosen. The quantitative test found two isolates from *R. mucronata*, namely isolates RM10 and RM12, with antimicrobial properties against MDR strain *E. coli*, with the best isolate being RM10, which produced 11.22 mm of inhibition zone diameter. The test found five isolates from *A. ilicifolius* with promising antimicrobial properties, namely isolates AC5, AC6, AC7, AC8, and AC9. The best *A. ilicifolius* microbial isolate, AC5, produced 10.76 mm of inhibition zone diameter against MDR strain *E. coli*.

The test found that against MDR strain *V. harveyi*, two isolates from *R. mucronata*, namely isolates RM10 and RM14, with antimicrobial properties, with the best isolate being RM10, which produced 10.80 mm of inhibition zone diameter. The same test found four isolates from *A. ilicifolius* with promising antimicrobial properties, namely isolates AC2, AC4, AC5, AC8, and AC12. The best *A. ilicifolius* isolate, AC12, produced 10.58 mm of inhibition zone diameter against MDR strain *V. harveyi*.

3.4 DNA Amplification

A total of two isolates, namely isolate RM12 and AC12, of which each has 1500 base pairs according to comparison using DNA marker was shown with single band (Figure 1a). Meanwhile, two isolates, namely isolate RM10 and AC5, of which each has approximately 1500 base pairs according to comparison using DNA marker was also shown with single band (Figure 1b).

3.5 Molecular Phylogenetic Analysis

Homology tracing of 16S rDNA sequences of isolates RM12, AC12, RM10, and AC5 with DNA sequences of the GeneBank database using the BLAST system resulted in the homology of each bacterial isolate (Table 3). The match value between isolate RM12 with the sequence in the database was approximately 97%, isolate AC12 was approximately 99%, RM10 was approximately 94%, and for isolate AC5 was approximately 76%. The findings indicated that the molecular identification of the symbiont microbes represented match up to the genus level. The phylogenetic tree describes the match between isolate RM12 and *B. subtilis*, and the match between isolate AC12 and *P. aerius* (Figure 2). The relative matching of isolates RM10 and AC5 using Tree View indicated that the isolates were most identical to *B. oceanisediminis* and *B. firmus* respectively. Isolate RM10 and isolate AC5 have the relative match in Phylogenetic tree (Figure 3)

Secondary metabolites are compounds that are synthesized by an organism not to meet its basic needs, but to preserve its existence in interaction with the ecosystem (Mitri and Foster, 2013). Secondary metabolism plays a role in the survival of a species in the struggle against other organisms (Lopanik, 2014). Secondary metabolites can be used as self-defence mechanism against predators as sex attractor and pheromones (De-main and Fang, 2000). Chemical compounds produced by microbial symbionts that can block these undesirable microbial organisms are categorized as antibiotics. The term antibiotic comes from anthibios, which means substances produced by a microorganism in small amounts that can inhibit the development of or kill other organisms (Kelsic et al., 2015)

The results of this study showed that the microbial symbionts of *R. mucronata* and *A. ilicifolius*

have anti-microbial activity against MDR strain bacteria from *S. aureus*, *V. harveyi*, and *E. coli*. The findings indicated that microbial symbiont of mangrove produced secondary metabolites which acted as antimicrobial agents. Antimicrobial agents are compounds which kill or inhibit the reproduction of microbes. Therefore, this medicine group is useful in treating bacterial infection (Li *et al.*, 2017). The performance of antimicrobial agents are affected by several factors, among which are the concentration of the compound, the number of microbial species, temperature, the existence of other organic compounds, and acidity levels (Gómez-García *et al.*, 2019; Peh *et al.*, 2020). The results of research on mangroves show that mangroves contain very strong tannins (Pringgienies *et al.*, 2017). Tannin compounds have known potential as antibacterial. Tannins are plant poly phenolic compounds which bind to proteins, amino acids, alkaloids and precipitate them. They are known antimicrobial biomolecules (Kurhekar, 2016). Thus, the results of the study showing that symbiotic bacteria have the potential as antibacterial in MDR bacteria are suspected because they have compounds that are similar to their host. The results showed that there were four microbial isolates which had potential as MDR antimicrobial agents, namely RM12 isolate which showed homology (97%) with *B. subtilis*, RM10 isolate which showed homology (94%) with *B. oceanisediminis*, AC12 isolate which showed homology (99%) with *P. aereus*, and AC5 isolate showing homology (76%) with *A. firmus*. This finding indicated that among the symbiotic microbes of mangrove with antimicrobial MDR potential are the genus *Bacillus* and *Paracoccus*.

The microbe of the genus *Bacillus* is a gram-positive rod-shaped bacterium and a member of the phylum *Firmicutes*, can be aerobic and obligate aerobes (dependent on oxygen), or facultative anaerobes (have the ability to be aerobic or anaerobic). Microbes from this genus will be tested positive for catalase enzymes when there is oxygen used or present (Kaushal *et al.*, 2018). *Bacillus* can be found in a variety of environments and includes free-living species (non-parasitic) and parasitic pathogens (Celandroni *et al.*, 2016). Under stressful environmental conditions, *Bacillus* can produce oval endospores, which are not true 'spores',

with which they can reduce themselves and remain dormant for long periods of time. This characteristic at first defined the genus, but not all of these species are closely related, and many have been transferred to other genera from *Firmicutes* (Hashmi *et al.*, 2020).

The genus *Paracoccus* is one of the most distantly related of the Proteobacteria to *E. coli* as judged by 16S rRNA sequence. For many years, the sole representative of the genus was *P. denitrificans*. The original selection of this species was based on its ability to convert nitrate into molecular nitrogen. The genus *Paracoccus* has been known to produce antimicrobial compounds (Lee *et al.*, 2012a). The microbe *Paracoccus* sp. MBIC4019 is also found in *Pleuroploca trapesium* from Ternate waters, and has been indicated to have antimicrobial properties (Pringgienies and Renta, 2014).

Symbiotic bacteria of the genus *Bacillus* are known to be found in several hosts that live in the sea, namely in mangroves (Pringgienies *et al.*, 2015), sea cucumber (Pringgienies *et al.*, 2019; Santosa *et al.*, 2020), molluscs (Bahry and Pringgienies, 2016), sediment (Ariyanto, 2019), and fish (Pringgienies *et al.*, 2021). This indicates that *Bacillus* has natural affinity in marine environment, and that they may be categorized as pathogenic or non-pathogenic. Several non-pathogenic species that have potential as antimicrobial MDR agents are *B. subtilis*, *B. firmus*, and *B. oceanisediminis*. The microbial consortium of *B. subtilis* is known to break down waste into compost (Pringgienies *et al.*, 2018), and research results show that compost produced has high concentrations of Nitrogen and Phosphorus (Pringgienies *et al.*, 2016).

B. circulans are named so because the inside of the colony flows in a circular pattern. This microbial species is classified into Bacillaceae family and is gram-positive, rod-shaped, and motile with peritrichous flagella. *B. firmus* also produces endospores (Alebouyeh *et al.*, 2011). This species is commonly found in soil, wastewater, food, and baby bile. This microbe can be isolated from the intestines of bee larvae. *B. circulans* is a pathogen that is known to cause fatal sepsis in immunocompromised patients (Alebouyeh *et al.*, 2011). The finding is very interesting because the results of this study found that *B. circulans* in symbiosis

with mangrove host has the potential as antimicrobial agent against MDR strain pathogens. Studies of DNA linkages and G C (The guanine-plus-cytosine) analysis showed that most of the strains analyzed were incorrectly classified as *B. firmus* (Susić *et al.*, 2020). This results in the phenotypic heterogeneity of this species, which is not due to its inherent nature of genetically related strain variability, but because of the inclusion of genetically unrelated organisms. This means that *B. circulans* which was found and was shown to have MDR antimicrobial activity has a different strain.

The microbe *B. oceanisediminis* was found to have antimicrobial activity against MDR pathogens. This corroborates the findings of (Lee *et al.*, 2012b) who stated that *B. oceanisediminis* 2691 is an aerobic, gram-positive, spore-forming, and quite halophilic bacterium isolated from marine sediments of the Yellow Sea coast, South Korea. It is also known that the design of the genome sequence of *B. oceanisediminis* 2691 may have an important role in bioremediation of marinesediments.

4. Conclusion

The results of this study found that symbiont microbe of mangrove *R. mucronata* and *A. ilicifolius* have the potential as antimicrobial agents against MDR strain pathogens. Of all the isolates obtained from the mangrove samples, four isolates showed significant antimicrobial potential. *R. mucronata* and *A. ilicifolius* are shown to have the largest inhibition diameter zone against *S. aureus* with the diameter of 12.38 mm and 12.32 mm respectively. Molecular analyses of RM12 isolate showed 97% homology with *Bacillus subtilis*, of RM10 isolate showed 94% homology with *B. ocean-isediminis*, of AC12 isolate showed 99% homology with *P. aeri*, and of AC5 isolate showed 76% homology with *B. firmus*.

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Authors' Contributions

All authors have contributed to the final manuscript. The contribution of each author as follows, Delianis pringgenies; contributed ideas, data generation, data analysis, funding, revised and perfected sentences and vocabulary in the publication manuscript, and a project coordinator. Wilis Ari Setyati, Ali Djenaedi, and Rini Pramesti; devised the main conceptual ideas and critical revision of the article. Meanwhile Siti Rudiyan-ti, and Dafit Ariyanto; contributed manuscript preparation and submission. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The authors declare that have no conflict of interests.

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