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Characterization of fermentation liquid from mangrove leaves *Avicennia marina* and its inhibitory potential for bacterium causing ice-ice disease

Karakterisasi cairan fermentasi daun mangrove *Avicennia marina* dan daya hambatnya terhadap bakteri penyebab penyakit *ice-ice*

Samsu Adi Rahman[1,2], Sukenda[2*], Widanarni[2], Alimuddin[2], Julie Ekasari[2]

[1]Study Program of Aquaculture, Faculty of Fisheries, University of Muhammadiyah Luwuk, Luwuk, Indonesia
[2]Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Bogor, Indonesia.
*Corresponding author: sukenda@ipb.ac.id

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ABSTRACT

Fermentation liquid from mangrove leaves *Avicennia marina* contains microorganisms, nutrients, and secondary metabolites. This study aimed to identify bacteria and the compounds in fermentation liquid of mangrove leaves *A. marina* and measured their inhibitory capacity against pathogenic bacteria *Stenotrophomonas maltophilia* which causes ice-ice disease in seaweed. Molecular analysis which aimed the 16S rRNA gene showed that the bacteria in fermentation liquid consisted of eight types of *Bacillus*, *Bacillus subtilis* MSAR-01, *Bacillus megaterium* MSAR-02, *Bacillus firmus* MSAR-03, *Bacillus thuringiensis* MSAR-04, *Bacillus subterranerus* MSAR-05, *Bacillus vietnamensis* MSAR-06, *Bacillus* sp. MSAR-07, *Bacillus circulans* MSAR-08, with the best inhibitory power indicated by *B. subtilis* MSAR-01, *B. vietnamensis* MSAR-06, and *Bacillus* sp. MSAR-07. The administration of lactic acid, bacteriocin, total fermentation liquid, and supernatant as much as 15 µL produce inhibition to *S. maltophilia* indicated better result than using one or a combination of several types of bacterial isolates. The inhibition of single bacterial enriched fermentation and supernatant liquids was better than bacterial combination enrichment.

Keywords: *Avicennia marina*, fermentation, ice-ice, mangrove

ABSTRAK

Cairan fermentasi daun *mangrove Avicennia marina* mengandung mikroorganisme, nutrient, dan metabolit sekunder. Penelitian ini bertujuan untuk mengidentifikasi bakteri dan senyawa dalam cairan fermentasi daun *mangrove A. marina* dan mengukur daya hambatnya terhadap bakteri patogen *Stenotrophomonas maltophilia* penyebab penyakit *ice-ice* pada rumput laut. Hasil analisis molekuler dengan target gen 16S rRNA menunjukkan bahwa bakteri dalam cairan fermentasi terdiri atas delapan jenis *Bacillus*, yaitu *Bacillus subtilis* MSAR-01, *Bacillus megaterium* MSAR-02, *Bacillus firmus* MSAR-03, *Bacillus thuringiensis* MSAR-04, *Bacillus subterranerus* MSAR-05, *Bacillus vietnamensis* MSAR-06, *Bacillus* sp. MSAR-07, *Bacillus circulans* MSAR-08, dengan daya hambat terbaik ditunjukkan oleh *B. subtilis* MSAR-01, *B. vietnamensis* MSAR-06, dan *Bacillus* sp. MSAR-07. Pemberian asam laktat, bakteriosin, cairan fermentasi total, dan supernatan sebanyak 15 µL menghasilkan daya hambat terhadap bakteri *S. maltophilia* lebih baik daripada menggunakan salah satu atau kombinasi beberapa jenis bakteri isolat. Daya hambat cairan fermentasi dan supernatan yang diperkaya bakteri tunggal lebih baik daripada pengayaan kombinasi bakteri.

Kata kunci: *Avicennia marina*, fermentasi, *ice-ice*, *mangrove*

INTRODUCTION

Seaweed *Kappaphycus alvarezii* is fisheries main commodity. Seaweed production in 2015 reached 14.7 million tons and Indonesia's seaweed production contributed 39% of world total seaweed production (FAO, 2018). One of the obstacle in seaweed production is disease. The infectious disease in seaweed usually is ice-ice disease. Ice-ice is most common disease in seaweed culture since it can be very harmful and could lowered seaweed production up to 100% (Vairappan, 2006). Ice-ice disease caused by opportunistic bacteria (Egan *et al.,* 2017; Kumar *et al.,* 2016), is *Stenotrophomonas maltophilia* (Achmad *et al.,* 2016).

Various efforts to control ice-ice disease has done by introduction to gene encoding lisozyme enzyme (Handayani *et al.,* 2014), dismutase superoxide enzyme (Triana *et al.,* 2016), inhibition test for mangrove leave *Sonnerati alba* extract (Syafitri *et al.,* 2017), inhibition test for endophytic bacteria of mangrove leave *Avicennia marina.* Mangrove leaves *A. marina* used as *indigenous microorganisms* (IMO) source, is group of innate microbial consortium that inhabits the soil and the surface of all living things inside and outside (Umi & Saria, 2006), and easily available in that environment (Kumar & Gopal, 2015). IMO has potentiality in nitrogen fixation, increase soil fertility, phosphates solvent, and increase plant growth (Umi & Saria, 2006), bioremediation (Kao *et al.,* 2016; Sarkar *et al.,* 2016; Kumar & Gopal, 2015), and protect host from pathogenic microorganism (Kumar & Gopal, 2015), biactivator (Mirwandono *et al.,* 2018), increase the availability of nutrients for host (Sakimin *et al.*, 2017; Suyanto & Irianto, 2016), and as fermentation agent (Anyanwu *et al.,* 2015).

Mangrove leaves contains endophytic microorganism (IMO) (Rahman *et al.,* 2019) that has potentially used as antibacteria (Sanchez *et al.,* 2018). The use of fermenting liquid from various source of IMO has been done to increase plant growth and soil fertility (Kumar *et al.,* 2015), and to control pathogen in plant (Kumar & Gopal, 2015). Fermentation liquid is commonly used to inhibit pathogenic bacteria, anti-fungal, anti-mycotoxin (Waters *et al.,* 2015), and to increase growth (Sakimin *et al.*, 2017). Fermentation liquid contains primer and secondary metabolite produced by lactic acid bacteria (lactic acid and bacteriocin). Lactic acid is antimicrobial substance produced by lactic acid bacteris to inhibit the growth of pathogenic microbes (Wang *et al.,* 2015). Bacteriocin is peptide substance that synthesized by bacteria in ribosome (Hegarty *et al.,* 2016). This two has bactericidal and bacteriostatic activity against pathogen. Therefore, this study aimed to identify bacteria and fermentation liquid compound of mangrove leaves *Avicennia marina,* and to test the inhibitionagainst ice-ice disease caused by *S. maltophilia* bacteria.

MATERIALS AND METHOD

Pathogenic bacteria culture

Pathogenic bacteria as causative agent of ice-ice disease *S. maltophilia* with highest pathogenicity obtained from previous study of Achmad *et al*. (2016). Koch's Postulates used for pathogenicity test. Bacteria isolate was cultured in *sea water complex* (SWC) agar medium (0.5 g of bacto pepton, 0.1 g of yeast extract, 0.3 mL of glycerol, 1.5 g of bacto agar, 75 mL of sea water, dan 25 mL of aquades) and was incubated in 28°C for 24 hours. Then, it was vcultured into SWC liquid media and was homogenizedby shaker in 140 rpm for 24 hours.

Fermentation of mangrove leaves *Avicennia marina* and bacteria identification

Fermentation procedure was according to method of Budiyani *et al.* (2016) and Valli *et al.* (2016). Old and wet mangrove leaves was minced and added some palm sugar, sterile sea water with ratio of 1:1/4:2. The mangrove leaves were fermented in closed container without any exposure for two weeks. The fermentation liquid filtered with cheese cloth, then it put in glass bottle in -20°C.

Filtered fermentation liquid was isolated, then the bacteria was identified, test the inhibitionagainst disease and pythochemical compound was analyzed. As much as 0.1 mL of fermentation liquid was put and placed in a tube for serial dilution (10^{-5}) by using sterile seawater. Afterward, 0.1 mL of every isolate was cultured in SWC agar medium in 28°C for 24 hours to obtain a pure isolate.Bacteria identification has done by molecular technology with gene target was 16S rRNA. PCR amplification used primer 63F (5'-CAG GCC TAA CAC ATG CAA GTC-3') and 1387R (5'-GGG CGG WGT GTA CAA

GGC-3') (Marchesi *et al*., 1998) with gene target of 1,300 base pair. DNA extraction was according to Presto™ mini gDNA bacteria kit (Geneaid manufacture's Taiwan) protocol. Sequence analysis has done by sending the amplification product to 1st BASE DNA Sequencing, Malaysia. DNA sequencing result was analyzed by using Bio Edit Software. Then, the sequence was macthed against 16S rRNA sequence database in GeneBank (http://www.ncbi.gov/ BLAST) by using basic local allignment search tool-nucleotide (BLAST-N) program.

Inhibition test against lactic acid, bacteriocin, total fermentation liquid, and supernatant of bacteria caused ice-ice disease

Lactic acid compound in fermentation liquid was measured by using high performance liquid chromatography (HPLC). As much as 5 mL of supernatant put in a 25 mL measuring cup, some aquades was added into it, then the mixture was homogenized by using a sentrifuge in 7500 rpm for three minutes. The supernatant was filtered by using 0.45 µm filtration membrane in a vial then it was injected into HPLC. Afterward, the lactic acid compound can be measured (Gezginc *et al.,* 2015). Bacteriocin raw extract compound was measured from total fermentation liquid. The supernatant was extracted twice by using 10% ammonium sulfate solvent. The extract was steamed in an evaporator to obtain thick extract then it was stored in desiccator (Badi & Bhat, 2017). Lactic acid and bacteriocin inhibition test was done by using paper disc with diameter of 0.5 cm (Whatman no.4). Paper disc was put in SWC agar medium with 10^6 cells/mL of *S. maltophilia* inoculation. Every paper disc was dropped with lactic acid (5, 10, and 15 µL) and bacteriocin (5, 10, and 15 µL) then it was incubated in 28 °C for 24 hours. The dosage was according to Biswas *et al*. (2017). After incubation, the inhibition zone was measured and analyzed.

The total fermentation liquid was sentrifuged in 10,000 rpm for 10 minutes and the supernatant was filtered by using 0.22 µm filtration paper. The paper disc with diameter of 0.5 cm (Whatman no.04) was put in SWC agar medium with 10^6 cells/mL of *S. maltophilia* inoculation. Then, the paper disc was dropped with the total fermentation liquid and the supernatant (5, 10, dan 15 µL), it was incubated in 28 °C for 24 hours. After incubation, the inhibition zone was measured and analyzed.

Inhibition test of bacteria isolate of fermentation liquid

Inhibitation test of bacteria isolate from fermentation liquid (endophyte) toward *S. maltophilia* was done by *in vitro test* of Kirby-Bauer method as in line with Achmad *et al.* (2016), then it was dropped by 10^5 cell/mL of endophytic bacteria. The inhibition test result was selected on best three and it was combined with Kirby-Bauer method against *S. maltophilia.* Then, the fermentation liquid and supernatant was enriched with three endophytic bacteria with best inhibition and was enriched with the combination of three endophytic bacteria. The density of endophytic bacteria was 10^5 cells/ mL of each. This density is considered able to suppress pathogen. As much as 100 µL of bacteria isolate was put into 10 mL of fermentation liquid and supernatant to test the resistibility towards the combination of three endophytic bacteria. Then, the challenge test was done by dropped the liquid as much as 5 µL above paper disc in *S. maltophilia* isolation. It was incubated in 28°C for 24 hours. The diameter was measured and analyzed.

Phytochemical analysis

Phytochemical analysis of fermentation liquid was done by qualitative method. Phytochemical compounds that was analyzed were alkaloid, saponin, tannin, phenolic, flavonoid, triterpenoid, steroid, and glycoside. It was analyzed by standard procedure.

Data analysis

Molecular and phytochemical identification data used descriptive analaysis, while inhibition of lactic acid, bacteriocin, fermentation liquid, supernatant, and inhibition of bacteria was analyzed by using ANOVA test of SPSS 20 version. In case, if it would significantly different, the test further used Tukey test.

RESULTS

Isolat bakteri dari cairan fermentasi bacteria isolate of fermentation liquid

The moleculer identification of bacteria isolate of fermentation liquid was obtained eight strain of *Bacillus* (Table 1). The result of BLAST showed the similarity between 16S rRNA sequences from bacteria isolate with BLAST database was 99–100%. This similarity showed degree of accuracy in moleculer identification of certain strain bacteria. The result showed that the sequence was in species level.

Table 1. Bacteria isolate from fermentation liquid of mangrove *A. marina* leaves according to the percentage of similarity of 16S rRNA gene nucleotide sequences.

Isolate	Bacteria	Similarity (%)	Subject	Access number
MSAR-01	*Bacillus subtilis* strain BR4	100	1256/1256 (100%)	KU052617.1
MSAR-02	*Bacillus megaterium* YC4-R4	100	1247/1247 (100%)	CP026740.1
MSAR-03	*Bacillus firmu*s strain PGRP4	99	1098/1099 (99%)	MG229068.1
MSAR-04	*Bacillus thuringiensis* strain VKK-SL-2	100	1116/1116 (100%)	KT714055.1
MSAR-05	*Bacillus subterranerus* strain FJAT-47744	99	1250/1252 (99%)	MG651149.1
MSAR-06	*Bacillus vietnamensis* strain FJAT-46928	100	1086/1086 (100%)	MG651539.1
MSAR-07	*Bacillus* sp. strain FJAT 47851	100	995/995 (100%)	MG651253.1
MSAR-08	*Bacillus circulans* strain MD1	100	1253/1253 (100%)	KT757520.1

Table 2. Inhibition activity of lactic acid and bacteriocin against *S. maltophilia*

Doses of compound	Inhibition diameter (mm) (X ± SD)	
	Lactic acid	Bacteriocin
Control (aquades 10 µL)	00.00 ± 0.00^{a}	00.00 ± 0.00^{a}
5 µL	8.95 ± 0.54^{b}	9.05 ± 0.11^{bc}
10 µL	10.00 ± 0.50^{cd}	10.85 ± 0.49^{d}
15 µL	13.30 ± 0.84^{e}	12.25 ± 0.59^{f}

Note: different superscript in same column and bar showed significantly different result (P<0.05).

Table 3. Inhibition activity of fermentation liquid and supernatant against *S. maltophilia*

Doses of product	Inhibition diameter (mm) (X ± SD)	
	Fermentation liquid	Supernatant
Control (aquades 10 µL)	00.00 ± 0.00^{a}	00.00 ± 0.00^{a}
5 µL	9.55 ± 0.33^{b}	9.35 ± 0.34^{b}
10 µL	11.45 ± 0.37^{c}	11.25 ± 0.59^{c}
15 µL	15.25 ± 1.00^{d}	14.15 ± 1.08^{d}

Note: different superscript in same column and bar showed significantly different result (P<0.05).

Table 4. Inhibition activity of single bacteria and bacteria combination against *S. maltophilia*

Bacteria	Inhibition diameter(mm) (X ± SD)
B. subtilis MSAR-01	9.50 ± 0.11^{a}
B. megaterium MSAR-02	8.43 ± 0.08^{e}
B. firmus MSAR-03	8.02 ± 0.24^{f}
B. thuringiensis MSAR-04	7.76 ± 0.12^{f}
B. subterranerus MSAR-05	8.70 ± 0.20^{cd}
B. vietnamensis MSAR-06	9.11 ± 0.11^{b}
Bacillus sp. MSAR-07	9.41 ± 0.14^{a}
B. circulans MSAR-08	8.90 ± 0.14^{bc}
B. subtilis MSAR-01+ *B. vietnamensis* MSAR-06	8.00 ± 0.00^{f}
B. subtilis MSAR-01+*Bacillus* sp. MSAR-07	8.50 ± 0.00^{de}
B. vietnamensis MSAR-06+ *Bacillus* sp. MSAR-07	8.00 ± 0.00^{f}
B. subtilis MSAR-01+ *B. vietnamensis* MSAR-06+ *Bacillus* sp. MSAR-07	8.00 ± 0.00^{f}

Note: Different superscript in same column showed significantly different result (P<0.05)

Table 5. Inhibition activity of fermentation liquid and fermentation enriched with single bacteria and bacteria combination against *S. maltophilia*

Enrichment	Inhibition diameter (mm) (X ± SD)	
	Liquid fermentation	Supernatant
B. subtilis MSAR-01	16.60 ± 0.55^{a}	13.10 ± 0.14^{b}
B. vietnamensis MSAR-06	15.00 ± 0.94^{a}	11.55 ± 1.04^{bcd}
Bacillus sp. MSAR-07	15.55 ± 0.45^{a}	12.60 ± 0.55^{bc}
B. subtilis MSAR-01+*B. vietnamensis* MSAR-06	12.20 ± 0.27^{bc}	12.65 ± 1.14^{bc}
B. subtilis MSAR-01+ *Bacillus* sp. MSAR-07	12.00 ± 1.00^{bcd}	11.70 ± 0.84^{bcd}
B. vietnamensis MSAR-06+*Bacillus* sp. MSAR-07	12.50 ± 1.00^{bc}	11.00 ± 1.00^{cd}
B. subtilis MSAR-01+*B. vietnamensis* MSAR-06+ *Bacillus* sp. MSAR-07	12.80 ± 0.76^{b}	10.40 ± 0.89^{d}

Note: Different superscript in same column showed significantly different result (P<0.05)

Table 6. Phytochemical compound of fermentation liquid and supernatant

Compound	Qualitative results	
	Fermentation liquid	Supernatant
Alkaloid	+	+
Saponin	+	-
Tannin	+	+
Phenolic	+	+
Flavonoid	+	+
Triterpenoids	+	-
Steroids	-	-
Glycoside	+	+

Keterangan: (+): ada, (-): tidak ada Note : (+) Existed, (-) Not existed

Inhibition of lactic acid, bacteriocin, fermentation liquid, and supernatant

By using HPLC, it was known that lactic acid content in fermentation liquid amounted 19,117.78 mg/L, whereas, crude extract of bacteriocin was 2.5%. Lactic acid and bacteriocin inhibition of different dose against ice-ice disease showed in Table 2.

According to inhibition test against *S. maltophilia,* highest lactic acid inhibition (P<0.05) showed at dose of 15 µL (13.30 mm), followed by dose of 10 µL (10.00 mm), 5 µL (8.95 mm), and control (0.00 mm). Likewise, highest bacteriocin inhibition showed at dose of 15 µL (12.25 mm), followed by 10 µL (10.85 mm), 5 µL (9.05 mm), and control (0.00 mm). This results showed that as the increase of treatment dose of lactic acid and bacteriocin, the inhibition activity increased. The treatment of lactic acid that showed significantly different rather than bacteriocin was dose of 15 µL (P<0.05).

Inhibition activity of fermentation liquid and supernatant against *S. maltophilia* showed various value within dosage. The inhibition activity increased as the treatment of fermentation liquid and supernatant increased (Table 3).

The study results showed that fermentation liquid and supernatant assigned significantly different result (P<0.05) of inhibition activity against *S. maltophilia*. The respons between fermentation liquid and supernatant treatment with various doses was significantly different. Highest inhibition activity of fermentation liquid showed at dose of 15 µL (15.25 mm), followed by 10 µL (11.45 mm), 5 µL (9.55 mm), and control (0.00 mm). Likewise, highest inhibiton activity of supernatant showed at dose of 15 µL (14.15 mm), followed by 10 µL (11.25 mm), 5 µL (9.35 mm), and control (0.00 mm). Inhibition activity of fermentation liquid in all treatment was not significantly different (P>0.05) compare to inhibition activity of supernatant.

Bacteria inhibition activity

Single cell bacteria inhibition from fermentation liquid and combination of two or three kind of bacteria against *S. maltophilia* showed in Table 4, meanwhile the inhibition of fermentation liquid and supernatant enriched with bacteria showed in Table 5. The combination of bacteria by using single cell bacteria were *B.*

subtilis MSAR-01 isolate, *Bacillus* sp. MSAR-07 isolate, and *B. vietnamensis* MSAR-06 isolate, which all of them has best inhibition activity against disease (Table 4). Then, the combination of isolate was expected to generate better inhibition activity. Yet, the result showed that inhibition activity among the combination of two or three bacteria was lower than single bacteria. Highest inhibition activity has showed by *B. subtilis* MSAR-01 isolate, followed by *Bacillus* sp. MSAR-07, and *B. vietnamensis* MSAR-06 isolate.

The results of inhibition activity from fermentation liquid and supernatant enriched with selected potential bacteria showed that every treatment could generate significantly different inhibition activity ($P<0.05$) against *S. maltophilia*, showed in Table 5.

Inhibition activity of fermentation liquid that enriched with single bacteria was better than supernatant that enriched with single bacteria (Table 5). Then, fermentation liquid enriched with single cell bacteria had higher inhibition activity than fermentation liquid and supernatant enriched with combination of two or three bacteria. Inhibition activity of fermentation liquid that combined with single bacteria did not significantly different with supernatant enriched with single bacteria. Meanwhile, supernatant combined with two kind of bacteria did not significantly different with supernatant combined with single bacteria, otherwise it did significantly with supernatant combined with three kind of bacteria, whereas the treatment of *B. subtilis* MSAR-01+*B. vietnamensis* MSAR-06+ *Bacillus* sp. MSAR-07 combined with supernatant has lower inhibition activity than it combined with fermentation liquid.

Phytochemical compound

Phytochemical screening was done to obtain bioactive compound in fermentation liquid. The result of phytochemical test indicated that fermentation liquid contains alkaloid, saponin, tannin, phenolic, flavonoid, triterpenoids, and glycoside that allegedly as antibacterial (Table 6).

DISCUSSION

Molecular identification showed that there were eight isolates from *Bacillus* sp species. Eight bacteria isolates were *Firmicutes* that are very common found in marine water. This bacteria was isolated from mangrove *Avicennia marina* leaves and (Rahman *et al.,* 2019) and found abundantly in green algae *Ulva* and *Gracilaria* (Singh *et al.,* 2015). Commonly, *Bacillus* sp. is lactic acid bacteria (BAL) produced several antimicrobial compound like lactic acid, alcohol, carbon dioxide, diacetyl, hydrogen peroxide, bacteriocinand other metabolic compounds (Gaggia *et al*., 2010). This bacteria is used as probiotic and biocontrol agent to suppress the growth of pathogenic bacteria through various mechanism, one of them is produced metabolic compound (Widanarni *et al.,* 2015). Bacteriocin that produced by this bacteria can increase health (Woraprayote *et al.,* 2016), has high specific target, and is effective to control the pathogen (Ansari *et al.,* 2018).

Inhibition activity towards lactic acid and bacteriocin from fermentation liquid against *S. maltophilia* bacteria was significantly different in dosage of 15 µL. This difference due to different inhibition spectrum, was suspected cause by organism sensitivity in higher dosage. The ability to inhibit lactic acid and bacteriocin showed bactericidal and bacteriostatic characteristic, as higher the lactic acid and bacteriocin concentration, the inhibition ability gets higher (Table 2). This results showed that lactic acid and bacteriocin produced by fermentation liquid has extensive inhibition ability against *S. maltophilia*. It was in line with Smid and Gorris (2007) that stated organic acid produced by lactic acid bacteria has extensive inhibition spectrum against microorganism. The mechanism started with attack cell wall, cell membrane, enzyme metabolism, interfere both protein synthesis system and genetic. Identified bacteria of fermentation liquid was Gram positive bacteria with stronger inhibition ability. Then, Sandi and Salasia (2016) explained that Gram positive bacteria has smaller bacteriocin with more extensive antimicrobe spectrum than Gram negative bacteria.

Fermentation liquid showed better inhibition activity than the supernatant. Antimocrobe compound of fermentation liquid worked optimally than the supernatant (Tabel 3). This was happen due to the loss of some compound through particles sediment when sentrifuged and cell separated from its supernatant by using filter of 0.22 µm. Antimicrobes compound in fermentation liquid was whole antimicrobe with other particles compund therefore it worked more specific against *S. maltophilia.* Inhibition activity of fermentation liquid and supernatant of every treatment has different ability according its concentration level. The amount of inhibiton

activity is according to its concentration level. As higher the concentration, the higher its inhibition activity. Some compounds that worked for *S. maltophilia* inhibition were lactic acid, bacteriocin, and other identified active compound in phytochemist test (alkaloid, saponin, tannin, phenolic, flavonoid, and glycoside) (Table 6). Sandi and Salasia (2016) stated that lactic acid bacteria produced some bioactive compounds with wider inhibition activity and spectrum against pathogen. Then, Cebrian *et al.* (2018) stated that bacteriocin is antimicrobe molecule with selective ability to inhibit certain bacteria species that produced bacteriocin (Ahmad *et al.,* 2016).

Bacteria and fermentation liquid had different inhibition activity against *S. maltophilia* (Table 4). Three species of *Bacillus* sp. with best inhibition activity was *B. subtilis* MSAR-01, *Bacillus* sp. MSAR-07, and *B. vietnamensis* MSAR-06. This three species was potential bacteria candidate to control ice-ice disease in seaweed. This study results showed that the combination of this three potential bacteria had lower inhibition activity than single potential bacteria and enrichment of single bacteria was better that bacteria combination (Table 5). This bacteria combination was not effective yet to suppress the pathogen since every single bacteria had its own mechanism to inhibit the pathogen and their effectivity was become lower if it was combined with other species. This was in line with enrichment both of single bacteria and bacteria combination due to both bacteria synergies and antimicrobes compound in fermentation liquid and supernatant before bacteria enrichment. Faust (2018) stated that to optimize consortium performance is by right design or microorganism combination through some experiments. Cookson (1995) explained that the different inhibition activity is depend on microbes preference during biodegradation process, so did the biochemist process and specific enzymes in it.

Phytochemist compound (Table 6) from qualitative fermentation liquid contained alkaloid, saponin, tannin, phenolicm flavonoid, and glycoside, meanwhile, the supernatant had no saponin, triterpenoid, and steroid. This was caused by the compounds was degraded through particles sediment while centrifugation process and cell separation from its supernatant by using filter of 0.22 µm, therefore, it made lower inhibition ability of supernatant than fermentation liquid against *S. maltophilia*. Some compounds had antibacterial activity. Alkaloid explored the inhibition of bacteria quorum sensing (Rabin *et al.,* 2015). Flavonoid, phenolic, and tannin played an important role as antioxidant source (Hamli *et al.,* 2017). Glycoside engaged to destroy microbe cell wall by catalytic or anabolic (Rijai, 2016).

CONCLUSION

Some bacteria in fermentation liquid were eight species of *Bacillus.* Fermentation liquid of mangrove leaves was very potential to control ice-ice disease in seaweed. The enrichment of single bacteria in fermentation liquid and supernatant was worked better than single bacteria, bacteria combination, and enrichment of bacteria combination. The best enrichment bacteria of fermentation liquid was *B. subtilis* MSAR-0.

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