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Bioactive Crude Extracts from Several *Psychrobacter alimentarius* Isolated from Indonesian Coastal Sediment

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Abstract: In previous study, 141 isolates of marine bacteria were successfully isolated from Indonesian coastal sediment. This study was aimed to investigate whether crude extracts of these selected isolates exerted potential antimicrobial and antioxidant activities *in vitro*. Crude extract was prepared by solvent extraction using 1-butanol. Antimicrobial assay of crude extracts (1-500 µg/mL) against *Bacillus cereus* and *Staphylococcus aureus* was determined by using Minimum Inhibitory Concentration (MIC). Antioxidant assay of crude extracts (5-500 µg/mL) was done by employing 3 assays, including 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferrous chelating activity (FCA), and anti-lipid peroxidation. Our results demonstrated that crude extracts of isolates exerted moderate antimicrobial activities against growth of *B. cereus* and *S. aureus* with MIC values > 500 µg/mL. Some isolates showed potential antioxidant activity in a independent-dose manner. Among 3 antioxidant systems, crude extracts of isolates at 500 µg/mL exerted antioxidant activity < 45% using DPPH assay, < 35% using FCA assay, and < 75% using anti-lipid peroxidation assay. Based on molecular identification using 16S rRNA gene sequence, these isolates with antimicrobial and antioxidant properties were identified as *Psychrobacter alimentarius*, *Bacillus* sp. and *B. niabensis*. These results suggest that crude extracts of *P. alimentarius* may potentially be an alternative antimicrobial and antioxidant agents.
Keywords: antimicrobial activity, antioxidant activity, *Psychrobacter alimentarius*, marine bacteria, Indonesian coastal sediment

INTRODUCTION

One of the countries with the largest coastal region is Indonesia, an archipelago which has sea covering up two-thirds of its entire region. Moreover, sea has greater biodiversity than land, thus, the type of microorganisms obtained from the coastal region will also be more varied compared to soil microorganisms [1]. More than 90% of marine bacteria are psychrophilic Gram-negative bacteria. Usually, marine bacteria require sodium and potassium ions for growth and maintain osmotic balance in the cytoplasm. Requirement of sodium ions is related to the production of indole from tryptophan, transportation of substrate into the cell, oxidation of L-arabinose, mannitol, and lactose. However, the potential of marine bacteria producing antimicrobial and antioxidant compounds has not been explored [2, 3].

Nowadays, the over dosage applications of antibiotics lead to resistance of microorganisms so that they become ineffective. This triggers people to continue to renew the mechanism of existing antibiotics or to seek and obtain new antibiotics [1]. In our previous study, a total of 141 isolates consisting of seven *Streptomyces* sp. isolates and 134 isolates of marine bacteria, were obtained from Indonesian coastal sediment. Those isolates were screened using agar well diffusion method and nine isolates were obtained that inhibit *Bacillus cereus* and *Staphylococcus aureus* [4]. To exclude the solubility problem of the crude extracts in agar, serial dilution assay was performed and the minimum inhibitory concentration (MIC) value was deduced in this study. MIC is the lowest concentration of antimicrobial compounds that can inhibit the growth of bacteria. MIC is used to determine the susceptibility of microorganisms to antimicrobials. MIC is also used to judge the performance of other methods of susceptibility testing, for example MIC gives a definitive answer when a borderline result is obtained by other methods of testing or when disc diffusion method are not appropriate [5].

In addition, degenerative diseases, such as cancer and atherosclerosis, were also increasing. This is caused by free radicals that contain one or more unpaired electrons. It is highly reactive and cause

oxidative damage to important functional groups in biomolecules. These free radicals can be inhibited by antioxidant compounds which donate an electron to free radicals. In general, antioxidants are divided into two groups, namely enzymatic and non-enzymatic antioxidant. Non-enzymatic antioxidant are also called secondary or exogenous antioxidants, such as tocopherol, carotenoids, ascorbic acid, and uric acid. This antioxidant can inhibit the oxidation rate in several ways, such as the abolition of the substrate. Meanwhile, the enzymatic antioxidant also called primary or endogenous antioxidant, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase. As antioxidants, these enzymes inhibit free radical formation by inhibiting the initiation stage or propagation stage, and then turn it into a more stable product [6].

Nowadays, synthetic antioxidant compounds have been widely used, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). However, synthetic antioxidants have risks or side effects. Therefore, natural antioxidant compounds are more preferable than synthetic antioxidants [7]. The objective of this research was to investigate antimicrobial and antioxidant activities of crude extracts from selected Indonesian coastal marine bacteria. In this study, nine isolates that have antimicrobial activity were tested quantitatively using MIC assay. Meanwhile, all isolates from the previous study were screened using three different antioxidant assays. Isolates that have antimicrobial and antioxidant activities will be identified and characterized using molecular identification.

MATERIALS AND METHODS

Bacterial growth and preparation of crude extract

Pure colonies were cultivated in Marine Agar (MA) and incubated at 28°C for 7 days for *Streptomyces* sp. and 5 days for marine bacteria. Preparation of crude extracts from isolates were done for determining antimicrobial and antioxidant properties. For antimicrobial assay, nine selected isolates that have antimicrobial activity were prepared for crude extract production. Meanwhile, for antioxidant assays, 141 isolates were employed and prepared for crude extract production. Isolates were inoculated in 10 mL Brain Heart Infusion (BHI) and incubated at 28°C with 120 rpm agitation for 6 days for *Streptomyces* sp. and 3 days for marine bacteria [4]. After incubation, the medium were centrifuged at 6000 ×g for 15 min. The supernatant was collected and transferred to a new tube. Each 10 mL supernatant was extracted with 10 mL 1-butanol. The organic phase was collected, air-dried overnight, dissolved in Phosphate Buffered Saline (PBS) up to 100 mg/mL and diluted by two-fold serial dilution at a concentration of 0-500 µg/mL [8].

Antimicrobial assay of crude extracts from isolates

Antimicrobial assay of crude extracts from 9 isolates was done by using MIC assay in accordance to Clinical and Laboratory Standards Institute protocol [9]. The test bacteria of *B. cereus* (ATCC 14579) and *S. aureus* (ATCC 25923) were grown at 37°C for 24 h in BHI and standardized by 0.5 McFarland standard. MIC value was the lowest concentration which performed > 90% inhibition. As much as 20 µL of test bacteria were mixed with 180 µL of crude extract of each testing concentration on 96 wells microplates. For positive control was used 20 µL test bacteria and 180 µL BHI, whereas for negative control was used 200 µL BHI. The microplates were incubated at 37 °C overnight. After that, absorbances (A) were measured with a microplate reader at 595 nm. Tetracycline as reference agent was prepared in a similar manner. MIC value was the lowest concentration which performed > 90% inhibition. The inhibition activity was calculated using the equation:

$$\text{Inhibition (\%)} = [(A_{\text{sample}} - A_{\text{negative control}})/(A_{\text{positive control}} - A_{\text{negative control}})] \times 100\%$$

Antioxidant assay of crude extracts from isolates

Crude extracts from 141 isolates were tested for their antioxidant activities using the 1,1-diphenyl-2-picryl hydrazyl (DPPH), ferrous chelating activity (FCA), and anti-lipid peroxidation assays. For DPPH assay, a 125 µL crude extract of each testing concentration were mixed with 50 µL 0.3 mM DPPH on 96-wells microplates [7]. The microplates were incubated at dark room temperature for 30 minutes. Subsequently, absorbances were measured with a microplate reader at 515 nm. Ethanol as control and ascorbic acid as reference agent were prepared in a similar manner. Experiments were done in triplicate. The antioxidant activity was calculated using the equation:

$$\text{Activity (\%)} = [(A_{\text{control}} - A_{\text{sample}})/(A_{\text{control}})] \times 100\%$$

For FCA assay, a one mL crude extract of each testing concentration was mixed with 3.7 mL aquadest and 100 µL $FeCl_2$ 2 mM [7]. After three minutes, 200 µL ferrozine 5 mM were added and incubated at room temperature for 10 minutes. Absorbances were measured with a spectrophotometer at 562 nm. Ethanol as control and EDTA as reference agent were prepared in a similar manner. The antioxidant activity was calculated using the equation:

$$\text{Activity (\%)} = [(A_{\text{control}} - A_{\text{sample}})/(A_{\text{control}})] \times 100\%$$

For anti-lipid peroxidation assay, a 100 µL crude extract of each testing concentration was mixed with 400 µL aquadest, 500 µL egg yolk solution 10% (v/v), and 70 µL $FeSO_4$ 10 mM, and incubated at room temperature for 30 minutes [10]. After incubation, a 1.5

mL thiobarbituric acid 0.8% (w/v) was added into the solution and heated at 95°C for 1 hour. The solution was centrifuged at 3500 ×g for 10 minutes after reaching room temperature. Absorbances were measured with a spectrophotometer at 532 nm. Ethanol as control and ascorbic acid as reference agent were prepared in a similar manner. The antioxidant activity was calculated using the equation:

$$\text{Activity (\%)} = [(A_{control} - A_{sample})/(A_{control})] \times 100\%$$

Molecular identification of isolates

Ten selected isolates that have antimicrobial and antioxidant activities were identified using 16S rRNA gene sequence. The 16S rRNA gene were extracted and amplified using colony polymerase chain reaction (PCR). The PCR conditions were pre-denaturation (94 °C, 5 min.); denaturation (94°C, 30 sec.); annealing (55°C, 30 sec.); extension (72°C, 60 sec.); post-extension (72°C; 10 min.); hold (4°C) with 30 cycles. The primer used were universal primer 63f (5'-CAG GCC TAA CAC ATG CAA GTC-3') and 1387r (5'-GGG CGG WGT GTA CAA GGC-3') [11]. Master mix contains MyTaq® Red Master Mix, forward and reverse primer, nuclease free water, and DNA template. The PCR results were visualized using electrophoresis (80 V, 400 A, 70 minutes) in 1% (w/v) agarose gel. Samples were sequenced for 16S rRNA genes were done by 1st BASE Sequencing INT. The sequences obtained were compared to sequences within NCBI database (http://blast.ncbi.nlm.nih.gov/) using BLASTN 2.2.29+ program. BLAST was carried out on March 2015.

Statistical analysis

All experiments were performed in triplicate with three repeats. All data collected were analyzed statistically using mean ± standard deviation. Statistical analysis of untreatment and marine algae treatment was performed by analysis of variance (SPSS 11.0 for Windows).

RESULTS

Antimicrobial activity of crude extracts from isolates

Crude extracts of nine isolates from Indonesian coastal sediment at concentration of 1 µg/mL had antimicrobial activity 30-70% against *B. cereus* (Table 1) and *S. aureus* (Table 2). Interestingly, F3.S1 isolate showed highest inhibition (~73%) on growth of *B. cereus*. MIC values of all isolates against both *B. cereus* and *S. aureus* were found to be >500 µg/mL, respectively. Meanwhile, MIC value of tetracycline as a reference agent was 8 µg/mL against *B. cereus* and 64 µg/mL against *S. aureus*.

Table 1: MIC values of isolates against *B. cereus*

Isolate	Slight[1]		Moderate[2]		High[3]		MIC[4] (µg/mL)
	[Sample] (µg/mL)	Inhibition (%)	[Sample] (µg/mL)	Inhibition (%)	[Sample] (µg/mL)	Inhibition (%)	
F3.1	-	-	1	44 ± 6	-	-	> 500
F3.5	1	33 ± 5	500	48 ± 2	-	-	> 500
F3.6	1	38 ± 9	2	41 ± 4	-	-	> 500
F3.7	-	-	1	42 ± 3	-	-	> 500
F3.8	-	-	1	48 ± 3	-	-	> 500
F3.S1	-	-	-	-	1	73 ± 0.2	> 500
F3.S2	1	37 ± 2	32	41 ± 8	-	-	> 500
F3.S3	-	-	1	49 ± 6	-	-	> 500
F3.S6	-	-	1	61 ± 3	-	-	> 500
Tetracycline	-	-	-	-	1	85 ± 2	8

[1]Slight Inhibition (20-40%), [2]Moderate Inhibition (40-70%), [3]High Inhibition (70-90%), [4]MIC (>90%).

Table 2: MIC values of isolates against *S. aureus*

Isolate	Slight[1]		Moderate[2]		High[3]		MIC[4] (µg/mL)
	[Sample] (µg/mL)	Inhibition (%)	[Sample] (µg/mL)	Inhibition (%)	[Sample] (µg/mL)	Inhibition (%)	
F3.1	-	-	1	43 ± 2	-	-	> 500
F3.5	-	-	1	49 ± 2	-	-	> 500
F3.6	1	34 ± 4	2	40 ± 2	-	-	> 500
F3.7	-	-	1	47 ± 3	-	-	> 500
F3.8	-	-	1	50 ± 1	-	-	> 500
F3.S1	1	34 ± 3	4	45 ± 3	-	-	> 500
F3.S2	1	27 ± 2	64	40 ± 3	-	-	> 500
F3.S3	-	-	1	62 ± 4	-	-	> 500
F3.S6	-	-	1	62 ± 6	-	-	> 500
Tetracycline	-	-	-	-	1	73 ± 3	64

[1]Slight Inhibition (20-40%), [2]Moderate Inhibition (40-70%), [3]High Inhibition (70-90%), [4]MIC (>90%).

Antioxidant activity of crude extracts from isolates

Among 3 antioxidant systems (DPPH, FCA, and lipid peroxidation), some isolates exerted potential antioxidant activity in a independent-dose manner. However, the relative potency of the isolates in all antioxidant systems was not consistent. For radical scavenging using DPPH assay, the crude extracts of eight isolates at 500 µg/mL exerted antioxidant activity (25-45%) (Table 3). However, antioxidant activity of ascorbic acid standard is higher (70%) than that of the isolates. For FCA system, our results demonstrated that metal-chelating or ferrous-chelating activity of the isolates at highest concentration was found to be low with activity up to 35%, while EDTA standard possesed a significant FCA (99%) than that of isolates (Table 4).

Table 3: Antioxidant activity of ten isolates using DPPH assay

Isolate	Antioxidant Activity (%)						
	Concentration (µg/mL)						
	5	10	25	50	100	250	500
A1.6	10 ± 0.8	14 ± 4	14 ± 4	16 ± 3	20 ± 6	25 ± 0.1	37 ± 3
A9.2	0 ± 0.4	0.4 ± 2	2 ± 1	4 ± 2	5 ± 0.5	14 ± 3	29 ± 2
A11.7	4 ± 3	8 ± 2	8 ± 2	10 ± 2	10 ± 3	14 ± 2	24 ± 3
B1.2	20 ± 3	16 ± 7	20 ± 6	27 ± 3	26 ± 7	31 ± 4	39 ± 4
B2.3	2 ± 1	2 ± 0.7	3 ± 0.3	10 ± 1	15 ± 3	17 ± 0.4	28 ± 6
B3.4	0 ± 7	2 ± 4	3 ± 4	7 ± 9	11 ± 5	15 ± 5	28 ± 4
C1.8	28 ± 1	0.9 ± 1	3 ± 1	3 ± 0.3	4 ± 0.2	7 ± 0.8	12 ± 2
F1.10	5 ± 0.2	4 ± 0.2	10 ± 0.4	10 ± 0.2	13 ± 0.5	17 ± 0.4	39 ± 0.2
F3.6	5 ± 2	3 ± 6	9 ± 9	18 ± 2	17 ± 0.2	22 ± 0.8	31 ± 2
F5.9	14 ± 0.6	9 ± 0.4	8 ± 0.8	11 ± 1	16 ± 3	29 ± 1	46 ± 0.3
Ascorbic acid	62 ± 2	68 ± 1	70 ± 0.2	71 ± 0.7	71 ± 2	70 ± 1	72 ± 0.3

Results are mean ± standard deviation (n=3).

Table 4: Antioxidant activity of ten isolates using FCA assay

Isolate	Antioxidant Activity (%)						
	Concentration (µg/mL)						
	5	10	25	50	100	250	500
A1.6	32 ± 2	32 ± 2	27 ± 3	29 ± 2	30 ± 2	29 ± 2	34 ± 2
A9.2	25 ± 3	23 ± 3	24 ± 2	24 ± 4	20 ± 3	20 ± 0.6	27 ± 3
A11.7	28 ± 0.9	29 ± 0.8	30 ± 0.9	27 ± 0.9	27 ± 0.5	20 ± 1.5	28 ± 0.8
B1.2	25 ± 3	21 ± 3	19 ± 3	20 ± 4	22 ± 3	20 ± 0.5	19 ± 1
B2.3	20 ± 2	26 ± 2	24 ± 2	18 ± 2	20 ± 2	30 ± 2	30 ± 2
B3.4	31 ± 4	32 ± 3	33 ± 3	25 ± 8	31 ± 3	32 ± 3	35 ± 3
C1.8	4 ± 0.2	9 ± 0.2	15 ± 2	6 ± 0.2	17 ± 0.6	4 ± 0.2	11 ± 0.4
F1.10	9 ± 0.3	7 ± 0.1	20 ± 3	12 ± 0.7	21 ± 2	14 ± 0.2	21 ± 2
F3.6	17 ± 2	17 ± 2	20 ± 3	18 ± 2	19 ± 2	19 ± 1	29 ± 3
F5.9	21 ± 2	21 ± 4	20 ± 2	24 ± 4	22 ± 3	23 ± 2	36 ± 4
EDTA	35 ± 6	50 ± 2	75 ± 4	89 ± 2	98 ± 0.5	99 ± 1	99 ± 2

Results are mean ± standard deviation (n=3)

For lipid peroxidation, B2.3 isolate at lowest dose (5 µg/mL) inhibited up to 65% lipid peroxidation (Table 5). For other isolates, only at highest concentration (500 µg/mL), most isolates exerted >50% of inhibition on lipid peroxidation. EDTA standard at 250 µg/mL had up to 50% inhibition of lipid peroxidation.

Table 5: Antioxidant activity of ten isolates using anti-lipid peroxidation assay

Isolate	Antioxidant Activity (%) Concentration (µg/mL)						
	5	10	25	50	100	250	500
A1.6	11 ± 1	70 ± 2	79 ± 2	63 ± 2	43 ± 3	62 ± 2	49 ± 1
A9.2	83 ± 0.3	86 ± 0.2	86 ± 0.2	33 ± 0.4	26 ± 2	18 ± 1	64 ± 2
A11.7	0 ± 1	0 ± 0	0 ± 1	0 ± 0	8 ± 2	26 ± 0.9	48 ± 0.4
B1.2	0 ± 0	0 ± 0	22 ± 3	15 ± 1	8 ± 7	67 ± 3	77 ± 2
B2.3	65 ± 3	74 ± 2	78 ± 5	2 ± 0.3	75 ± 0.8	76 ± 2	65 ± 4
B3.4	0 ± 0	0 ± 15	0 ± 0	3 ± 0.2	0 ± 0	0 ± 0	22 ± 1
C1.8	0 ± 0	0 ± 30	36 ± 2	0 ± 0	15 ± 0.6	0 ± 0	46 ± 4
F1.10	17 ± 0.5	36 ± 5	25 ± 2	34 ± 4	19 ± 2	35 ± 3	35 ± 4
F3.6	44 ± 4	0 ± 9	39 ± 4	6 ± 0.2	15 ± 1	33 ± 3	71 ± 0.8
F5.9	0 ± 0	0 ± 47	16 ± 0.6	46 ± 3	2 ± 0.4	59 ± 3	66 ± 3
Ascorbic acid	0 ± 0	2 ± 0.3	1 ± 0.2	20 ± 1	25 ± 0.2	46 ± 2	100 ± 3

Results are mean ± standard deviation (n=3)

Molecular identification of isolates

Molecular identification of isolates was done by sequencing. The results showed that eight isolates were identified as *Psychrobacter alimentarius* and two isolates were identified as *Bacillus* sp. and *B. niabensis* (Table 6).

Table 6: Molecular identification of isolates from Indonesian coastal sediment

Isolate	Origin of Location	Predicted Genus / Species	Identity(%)	Accession Number
A1.6	Carita Beach	*Psychrobacter alimentarius*	95	KP980554
A9.2	Kongsi Island	*Psychrobacter alimentarius*	97	KP980555
A11.7	Pasir Perawan Beach	*Psychrobacter alimentarius*	96	KP980556
B1.2	Tanjung Pendam Beach	*Psychrobacter alimentarius*	97	KP980557
B2.3	Pasir Padi Beach	*Psychrobacter alimentarius*	98	KP980558
B3.4	Mabay Beach	*Psychrobacter alimentarius*	97	KP980559
C1.8	Akarena Beach	*Psychrobacter alimentarius*	97	KP980560
F1.10	Bali Beach	*Bacillus sp.*	90	KJ670159
F3.6	Sindhu Sanur Beach	*Psychrobacter alimentarius*	97	KP980561
F5.9	Tuban Beach	*Bacillus niabensis*	94	KP980562

DISCUSSION

The microorganisms living and growing in the marine environment are metabolically and physiologically diverse from terrestrial microorganisms. However, the potential of marine microorganisms has not been explored, especially marine microorganisms in Indonesian marine environment. The most studied marine microorganisms are *Actinomycetes*, while other marine bacteria have not yet been extensively studied. From a previous study, marine *Actinomycetes* with antimicrobial activity were isolated from three beaches in Indonesia, namely Cirebon Desa Gebang Beach, Anyer Beach, and Kukup Gunung Kidul Yogyakarta Beach [1]. In this study, marine *Actinomycetes* and marine bacteria from different locations in Indonesia were examined for antimicrobial and antioxidant activities.

The results of antimicrobial activity showed that the isolates were active against Gram-positive bacteria (*B. cereus* and *S. aureus*) compared to Gram-negative bacteria (Table 1 and Table 2). It is caused by the structural differences between these microorganisms. Gram-negative bacteria have an outer polysaccharide membrane that makes the cell wall impermeable to lipophilic solutes, while Gram-positive bacteria only have a peptidoglycan layer which is not an effective permeability barrier [12]. The crude extracts of nine isolates were less active against Gram positive test bacteria in agar diffusion test, while Gram negative test bacteria were resistant to the applied substances.

To exclude the solubility problem of the crude extracts in agar, serial dilution assay was performed and the MIC value was deduced. Results of serial dilution assay showed that crude extract of nine isolates at concentration of 1 µg/mL had antimicrobial activity (30-70%) against *B. cereus* and *S. aureus*. The results obtained in this test coincided with the agar diffusion test, where the extracts were weakly active against the susceptible Gram positive test bacteria with MIC value above 500 µg/mL. Similar results were also obtained by Al-Zereini reporting that marine bacteria from Red Sea sediments had poor inhibition against *S. aureus* and *B. subtilis* with MIC value between 500-1000 µg/mL [8].

Furthermore, we also tested antioxidant activity from crude extracts of isolates by using various antioxidant assays including DPPH, FCA, and lipid peroxidaton. Our results showed that crude extracts had lower antioxidant activity than ascorbic acid (Table 3). In line with our study, marine bacteria from Red Sea sediment had also been reported to have low antioxidant activity (30%) measured with DPPH [8]. It is known that DPPH is a stable free radical and very popular for the study of natural antioxidants. In spite of the wide use of DPPH, this test gives incorrect results in some cases. Some complications may be caused by partial ionization of the tested compounds, which affects the rate of their reaction with DPPH [13].

Through the Fenton reactions, hydroxyl radicals generated by transition metals could stimulate lipid peroxidation. By stabilising transition metals, chelating agents might impair the production of free radicals [14]. The results of FCA assay also showed the crude extracts had lower antioxidant activity than EDTA (Table 4). However, Abubakr *et al.* reported that lactic acid bacteria isolated from kefir had high antioxidant activity (>90%) and almost similar to EDTA activity. In this study, the results of FCA assay is almost similar to the results of DPPH assay. This is different from the results obtained by Abubakr *et al.* that the results of FCA assay is higher than the results of DPPH assay [7]. It is caused by milk fraction in kefir possessing a greater number of phosphoseryl serine groups that have greater affinity for iron. In addition, milk fraction also contain amino acid asparagine and glutamine that can bind iron on carboxyl terminus.

On the other hand, the level of MDA was taken as an indicator of lipid peroxidation. A lower concentration of MDA reflects a higher inhibitory potential [14]. The results of anti-lipid peroxidation assay (Table 5) showed that crude extracts had high antioxidant activity. It is known that this method has low sensitivity and specificity. If thiobarbituric acid reacts with a variety of compounds, such as sugar, amino acids, and bilirubin, it will produce interference in colorimetric MDA measurement [15]. Marine bacteria isolated from coastal sediment in this study had similar antioxidant activity compared to other marine bacteria isolated from different locations. *Bacillus* sp. associated with sponge *Tedania anhelans* showed enhanced free radical scavenging activity ranging from 40 to 46% activity [16]. On the other hand, *Bacillus* sp. associated with seaweed *Acanthophora dendroides* and *Sargassum sabrebandum* showed free radical scavenging activity ranging from 46 to 49% activity [17].

In this study, the compounds that act as an antioxidant agent are still unclear. However, in other studies, the compounds as antioxidant agents have been determined. Chemical identification of crude extract in marine bacteria associated with sponge *Jaspis* sp.possess bioactive properties were identified as flavonoids, alkaloids, and terpenoids [18]. Meanwhile, *Bacillus* sp. associated with sponge *Tedania anhelans* consist of pyrrolo[1,2-a]pyrazine-1,4-dione that might be a major compound for antioxidant activity [16]. Other compounds isolated from marine bacteria *Edwardsiella tarda* by ion-exchange and size-exclusion chromatographies were mannan-water soluble extracellular polysaccharides [19].

Marine bacteria isolated from Indonesian coastal sediment were represented by two bacterial phyla, namely *Proteobacteria* and *Firmicutes* (Table 6). Another report also showed *Proteobacteria* and *Firmicutes* were the predominant phyla in marine sediment [20]. *P. alimentarius* are cocci, 1.4-2.0 µm in diameter, smooth colonies, and cream (yellowish-white) in colour. Members of the genus *Psychrobacter* are widely distributed in various habitats, including fish, food, clinical specimens, Antarctic ornithogenic soils and sea water [21]. Six strains of *Psychrobacter* spp. isolated from Spitsbergen island carried plasmids that contained a gene encoding a putative subunit C of alkyl hydroperoxide reductase (AhpC), an antioxidant enzyme and major scavenger of reactive oxygen species [22]. *B. niabensis* are rod-shaped bacteria, 2-3 mm in diameter, yellowish-white in colour, and can tolerate up to 80 g/L of NaCl (halophilic) [23]. Study by Nithya and Pandian reported *B. niabensis* and other *Bacillus* sp. isolated from Palk Bay sediment had antibacterial activity against *S. aureus, S. typhi, P. aeruginosa, E. coli*, and *Shigella boydii* [20]. However, there is no study that showed *B. niabensis* has antioxidant activity. Therefore, *B. niabensis* with antioxidant activity in this study is a novel finding.

CONCLUSION

Our results suggest that *P. alimentarius* and *Streptomyces* sp. isolated from Indonesian coastal sediment may have potential as an antimicrobial agent against growth of *B. cereus* and *S. aureus* and antioxidant agents through chelating mechanism *in vitro*. Further research is needed to determine the exact bioactive compounds in *P. alimentarius* and other bacteria which act as antimicrobial and antioxidant agents.

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