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Screening of Antibiofilm Activity from Marine Bacteria against Pathogenic Bacteria

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Bacterial biofilms produced by pathogenic bacteria have become a serious issue in several chronic diseases such as atherosclerosis, cystic fibrosis, endocarditis, inner ear infections, and kidney stones. Thus, inhibition and destruction of bacterial biofilm from pathogenic bacteria is needed. The purpose of this study is to analyze biofilm inhibition and destruction activities of marine bacteria associated with hard and soft corals isolated from several oceanic regions in Indonesia. Fifteen marine isolates collected from several regions in Indonesia such as Bali Province, South East Sulawesi Province, East Java Province, Lampung Province, and Banten Province were tested using static biofilm assay against several pathogenic bacteria. Biofilm of the pathogenic bacteria tested were stained using 0.4% crystal violet. Several isolates were sequenced using 16S rRNA PCR method. Most of marine isolates presented higher inhibition and or destruction activity at 10% crude concentration. Few isolates were further identified using 16S rRNA and proven to have antibiofilm activity against several pathogenic bacteria. In conclusion, marine bacteria have broad applications in medical and pharmaceutical industries and the oceanic regions of Indonesia are promising sources for the discovery of novel bacteria with antibiofilm activity.

Key words: antibiofilm compounds, biofilm, chronic diseases, marine isolates, pathogenic bacteria

Biofilm bakteri yang diproduksi oleh bakteri patogen saat ini sudah menjadi masalah serius penyebab penyakit kronis seperti aterosklerosis, sistik fibrosis, endocarditis, infeksi telinga bagian dalam, dan batu ginjal. Oleh karena itu, inhibisi dan destruksi biofilm bakteri patogen sangat diperlukan. Tujuan dari penelitian ini adalah menganalisis aktivitas inhibisi dan destruksi bakteri laut yang berasal dari *hard coral* dan *soft coral* di beberapa perairan Indonesia. Sebanyak lima belas isolat bakteri laut yang dikoleksi dari beberapa perairan Indonesia seperti Provinsi Bali, Sulawesi Tenggara, Jawa Timur, Lampung, dan Banten diuji terhadap biofilm bakteri patogen menggunakan metode biofilm statis. Biofilm bakteri patogen yang digunakan diwarnai menggunakan Kristal violet dengan konsentrasi 0.4%. Beberapa isolat kemudian di-sekuens dengan metode 16S rRNA PCR. Sebagian besar isolate bakteri laut menunjukan aktivitas inhibisi dan destruksi yang lebih tinggi pada konsentrasi *crude* 10%. Beberapa isolate kemudian diidentifikasi lebih lanjut menggunakan metode 16S rRNA dan terbukti memiliki aktivitas antibiofilm terhadap beberapa bakteri patogen. Bakteri laut memiliki aplikasi yang luas dalam bidang medis dan industri obat-obatan, dan wilayah lautan Indonesia merupakan sumber yang menjanjikan dalam penemuan bakteri dengan aktivitas antibiofilm.

Kata kunci: bakteri pathogen, biofilm, isolate bakteri laut, penyakit kronis, senyawa antibiofilm

Biofilms produced by pathogenic bacteria are important issues in a number of chronic infections and diseases such as atherosclerosis, chronic sinusitis, chronic wounds, cystic fibrosis, endocarditis, inner ear infections, kidney stones, leptospirosis, osteomyelitis, osteonecrosis, osteomyelitis of the jaw, periodontal disease, prosthetic joints and heart valves, urinary tract infections, and also veterinary diseases (Skogman 2012). These naturally existing biofilms are major threats to humans as they are far more resistant to antibiotics and can particularly escape from host immune system (Skogman 2012). Bacterial attachment and biofilm formation are important steps in the establishment of chronic infections, thus, making them causative agents of some diseases (Skogman 2012). Biofilms can also appear in drinking water systems and serve as a significant environmental reservoir for pathogenic microorganisms (Skogman 2012).

Biofilm is defined as a complex structure of microbial cells aggregate covered by self-synthesized matrix consist of extracellular polysaccharides, DNA, and proteins. These cells aggregate then permanently attached into biotic or abiotic surfaces. Biofilm formation is associated with the virulence factor of pathogenic microbes and cell protection of the

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pathogens which result in antibiotic resistance (Dheilly *et al.* 2010). Therefore, inhibiting or destroying pathogenic bacterial biofilms could reduce the resistance, and research efforts are focused on developing novel antibioflm compounds.

The marine environment hosts a large biodiversity of many organisms which are important for source of food-related resources, energy, or medicine (Parraga *et al*. 2011). Despite this importance, thousands of marine microorganisms and their roles in the ecosystem still remain undiscovered (Parraga *et al.* 2011). During the last decades, marine organisms have provided huge advantages to human life, by producing antioxidant and antimicrobial substances that help prevent the spread of serious diseases in human body (Ngo *et al.* 2012). Marine organisms are believed to be a potential source not only for biologically active substances for the development of pharmaceuticals, but also essential substances for the inhibition of biofilm formation by pathogenic bacteria (Ngo *et al.* 2012). The bioactive compounds produced by marine bacteria are usually secondary metabolite compounds generated in response of external pressure such as competition for nutrient or space for example demonstrated antibiofilm activity from coral-associated bacteria againsts *Streptococcus pyogenes* biofilm formation (Nithya *et al.* 2010). The aim of this study was therefore to assess marine-associated bacteria from Indonesian waters as potential sources for novel antibiofilm compounds.

MATERIALS AND METHODS

Marine Isolates Preparation. Fifteen out of fifty six marine isolates from previous study (Nataprawira 2014; Putra 2014) which did not have antimicrobial activity were chosen to test their antibiofilm activity. Marine isolates from several Indonesian regions as shown in Table 1 were inoculated using loop or sterile tooth pick onto marine agar (MA) (Difco) and incubated at 28 °C for 2 days to produce single, well-isolated colonies. Pure colony were then refreshed into new MA medium and incubated at 28 °C for 2 d to obtain sufficient amount of isolates. Refreshed isolates in MA medium were stored at 4 °C.

Pathogenic Bacteria Preparation. Seven pathogenic bacteria (*Staphylococcus haemolyticus, Streptococcus pneumonia* ATCC 49619*, Staphylococcus aureus* ATCC 29213*, Pseudomonas aeruginosa* ATCC 27853*, Vibrio cholera* C43*, Enterotoxigenic Escherichia coli* (ETEC)*, Enteropathogenic Escherichia coli* (EPEC)) were streaked onto Brain Heart Infusion Agar (BHIA; Oxoid) to obtain single pure colonies. Pure colonies from BHIA were picked and cultured in new sterile culture tubes containing 5-8 mL of Brain Heart Infusion Broth (BHIB; Oxoid) and incubated at 37 °C overnight. Pathogenic bacteria being used were also adjusted to 0.5 McFarland (600 nm = 0.132) with dilution methods using BHIB. Each diluted pathogen was placed in a sterile conical tube for further use.

Crude Preparation. Pure colonies from MA were picked and inoculated in sterile culture tubes containing 5-8 mL Brain Heart Infusion Broth (BHIB) (Oxoid) supplemented with 1% D-glucose and incubated aerobically using waterbath shaker (Lab Companion, BS-21) for 3 d at 28 °C. The optical density of isolates was measured using spectrophotometer (Genesys 20, Thermo Spectronic). 1 mL of broth with marine bacteria was adjusted to 0.5 McFarland (600 nm = 0.132) with dilution methods using BHIB. Each diluted marine isolate was placed in sterile conical tube which then be centrifuged at 8000 rpm for 10 min using Sorvall Legend RT centrifuge. Supernatants were transferred to new sterile tubes and then centrifuged again at 8000 rpm for 10 min. Supernatants were separated from pellets to smaller conical tubes and were then ready to use (Kwasny and Opperman 2010). Supernatants obtained from the process above were used as crudes for antibiofilm test.

Antibiofilm Activity Assays of Marine Isolates. The methods were divided into two, to see whether the isolates inhibit the formation of biofilm from pathogenic bacteria (inhibition activity), or whether the isolates destruct the biofilm once it is formed (destruction activity). The assays were all under taken using 96-well polystyrene assay plates (round bottom; Iwaki) with lids under sterile conditions inside a laminar hood. Each isolate was used in two concentrations, 5% and 10%. For the inhibition activity, marine crudes (10 µL and 20 µL) were put first into the sterile 96-well assay plate. Then, 200 µL of pathogens were transferred to 96-well microplate. Pathogens alone were used as positive controls. BHIB medium without pathogens was used as negative control. Microplates were incubated at 37 °C overnight.

For the destruction activity, 200 µL of pathogens were transferred to the plate first, and then incubated at 37 °C overnight. After that, 10 µL and 20 µL for 5% and 10% of marine crudes respectively were placed into the plates and incubated at 37 °C for 30 min. All the assays were done under sterile condition inside a laminar airflow. Once finished, each plate was covered with the

lid, which increased biofilm formation by creating an environment with reduced oxygen tension. Biofilms of the bacteria usually appears at the bottom of each assay well (Moscoso *et al.* 2006).

Biofilm Staining. After incubation, planktonic cells and spent media were discarded from microplates and adherent cells were gently rinsed with deionized water (dH2O) twice and allowed to air dry before being stained. The biofilms were stained by 200 µL crystal violet solution (0.4% w/v; Merck) for 30 minutes. After that, crystal violet was removed from the plates and the wells were rinsed with deionized water 3-5 times. Rinsed plates were dried before the addition of 200 µL 96% analytical grade ethanol (Merck). After addition of ethanol, microplates were incubated for 30 min at room temperature. Ethanol solubilized the remaining crystal violet in the microplates. After the incubation period, ethanol from each well was transferred to new microplates and optical density measured at 595 nm using Biorad 680 Microplate reader (Moscoso *et al.* 2006). Absorbance attained was then used to calculate the antibiofilm activity using the following formula (Sun *et al*. 2005):

$$\% \text{ Activity} = \frac{\text{Positive absorbance - sample absorbance}}{\text{Positive absorbance - negative absorbance}} \times 100\%$$

DNA Sequencing. Six out of fifteen isolates were further sequenced to identify the bacteria. The amplification of DNA was performed using 16S rRNA PCR (Hoa *et al.* 2000). DNA extraction was done using CTAB and NaCl (Aris *et al*. 2013) which further amplified by PCR (100™ termocycler,Biorad) using forward primer 63f (5'- CAG GCC TAA CAC ATG CAA GTC-3') and reverse primer 1387r (5'- GGG CGG WGT GTA CAA GGC-3') (Aris *et al*. 2013). The PCR conditions were as followed: 94 °C, 2 min of pre-denaturation, 92 °C 30 s of denaturation, followed by 55 °C 30 s of annealing, continued with elongation at 72 °C 1 min (30 cycles). Next was post PCR at 75 °C, 20 min and stopped at 4 °C (Aris *et al*. 2013). The PCR products were run in electrophoresis using 0.8% agarose gel. Then samples were sent for further sequencing to Gentika Science and the results were aligned using Bioedit and BLAST program from NCBI. Sequences obtained were submitted to GenBank to obtain accession numbers. The accession numbers obtained from GenBank were then used to construct phylogenetic tree using Mega 7 program.

RESULTS

Marine isolates were analyzed for their potential to inhibit and destruct biofilm formation of several pathogenic bacteria. For the inhibition activity, it can be seen from Table 2 that most samples showed an increased inhibition activity when using crudes with concentrations of 10%. These results indicated that the more crudes are added, the higher the inhibition activity of marine crudes against pathogenic biofilms. However, in some samples like BC 11-1 against *Staphylococcus aureus* and BF 08-4 against *Pseudomonas aeruginosa*, though not significant according to the t-test value, the inhibition activity diminished as the crude concentration increased. Some crudes such as BC 11-2, BC 11-3, and BF 14-1 showed no inhibition activity to several pathogens like *Staphylococcus haemolyticus* and *Vibrio cholera* at 5% nor 10%. This phenomenon might show up when higher crude concentration was needed to reach optimum inhibition activity or else they do not have activity at all. Overall, results showed that all marine isolates had different inhibition activity against several pathogens with the most positive results against *Pseudomonas aeruginosa*, and the least against *Vibrio cholera.*

Beside inhibition activity, marine crudes are thought to possess destruction activity against mature pathogenic biofilms (Amador *et al.* 2003). From Table 3, most samples showed an increased destruction activity when using crudes with concentration of 10%. Most samples showed good destruction activity against several pathogenic bacteria with the most positive destruction activity against *Staphylococcus aureus* and least activity against *Vibrio cholera.* The results showed some marine samples performed inhibition activity only such as BF 13-5 against *Staphylococcus haemolyticus* and BB 07-7 against *Streptococcus pneumonia* while others such as BF 09-7 and BC 11-3 performed destruction activity only against *Staphylococcus haemolyticus* and *Streptococcus pneumonia*, respectively.

Based on the results of inhibition and destruction activity, six out of fifteen marine isolates with the most stable inhibition or destruction activity were chosen for sequencing. The six isolates were BC 11-1, BF 04-1, BF 13-2, BF 13-5, BC 12-3, and BF 14-2. After the sequences were obtained, nucleotide BLAST program from NCBI was used to find out what bacteria are those compared to the highest former sequenced bacteria. The results are presented in Table 3. Nucleotide BLAST results showed that marine isolate BC 11-1 has 99% similarity with *Erythrobacter* sp. Marine isolate BF 04-1 has 98% similarity with *Enterobacter cloacae,*

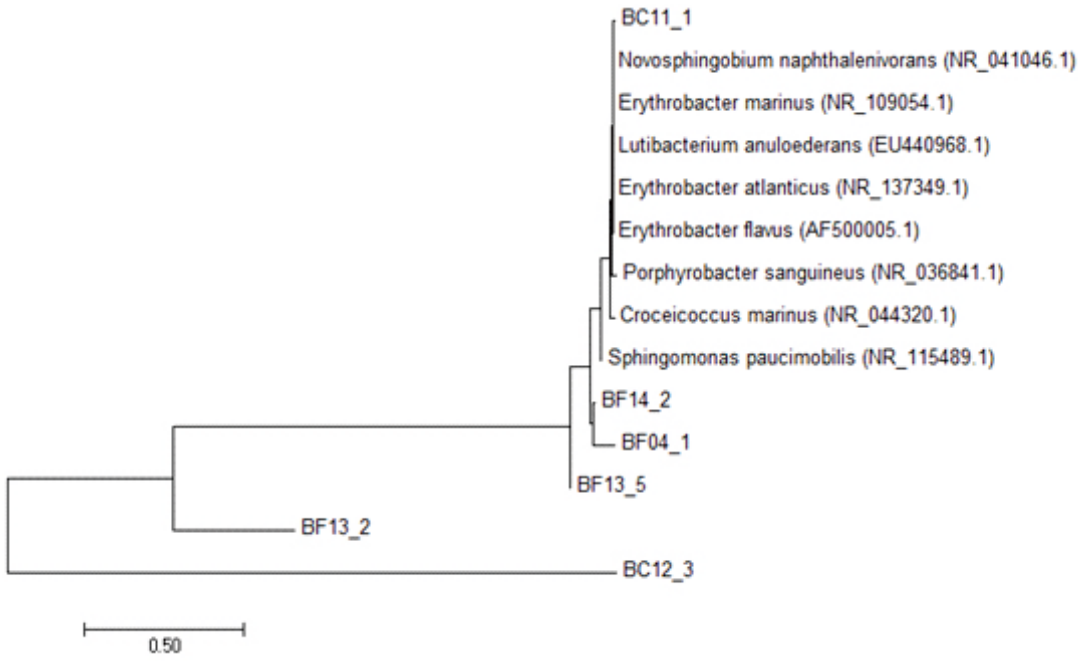

Fig 1 Phylogenetic tree of marine isolates based on 16S rRNA gene sequences compared to existing marine bacteria.

Table 1 Colony and cell morphology of endospore-forming rhizobacteria from rhizosphere of cabbage

Isolates	Sample	Sample type	Origin of Sample
BC 11-1	*Acropora desalwi*	Hard Coral	Bali North, Bali Province
BC 11-2	*Acropora desalwi*	Hard Coral	Bali North, Bali Province
BC 11-3	*Acropora desalwi*	Hard Coral	Bali North, Bali Province
BB 08-3	*Lobophytum* sp.	Soft Coral	Bali Province
BF 08-4	*Scleronephthya* sp.	Soft Coral	Kapuran, East Java Province
BF 09-7	*Heteroxenia*	Soft Coral	Bali Province
BF 13-2	*Studeriotes* sp.	Soft Coral	Kapuran, East Java Province
BF 08-1	*Scleronephthya* sp.	Soft Coral	Kapuran, East Java Province
BF 13-5	*Studeriotes* sp.	Soft Coral	Kapuran, East Java Province
BF 14-1	*Nephthyigorgia* sp.	Soft Coral	Kapuran, East Java Province
BF 14-2	*Nephthyigorgia* sp.	Soft Coral	Kapuran, East Java Province
BC 12-3	*Acropora echinata*	Hard Coral	Kendari, South East Sulawesi Province
BF 04-1	*Scleronephthya* sp.	Soft Coral	Lampung Province
BB 07-4	*Tubastrea micrantha*	Hard Coral	Cilegon, Banten Province
BB 07-7	*Tubastrea micrantha*	Hard Coral	Cilegon, Banten Province

BF 13-2 has 99% similarity with *Bacillus jeotgali*, BF 13-5 has 99% similarity with *Paracoccus marcusii*, BC 12-3 has 96% similarity with *Micrococcus luteus*, and BF 14-2 has 97% similarity with *Cobetia marina*. Those marine isolates had already given the accession number from Genbank (NCBI), KP763639, KP 763640, KP763641, KP763642, KP763643, and KP7763644 respectively.

Marine sequences from the nucleotide BLAST were then used to analyze genetic relationship of each individual sample using Mega7 program. The phylogenetic tree was constructed using neighbor-joining algorithm. From the phylogenetic tree, it can be seen that 4 samples (BC 11-1, BF 04-1, BF13-5, and BF

Table 2 Inhibition activity of marine isolates (%)

Crude	Crude Concentration	Pathogens						
		SH	SP	SA	PA	VC	ETEC	EPEC
BC 11-1	5%	27.6	-	45.1	58.9	-	-	-
	10%	50.6	27.4	39.7	67.0	-	130.4	-
BC 11-2	5%	57.6	38.6	29.0	60.2	-	-	-
	10%	40.2	59.3	39.5	66.1	-	-	-
BC 12-3	5%	-	12.8	-	55.1	-	-	-
	10%	21.5	22.0	32.3	45.5	-	61.0	162.7
BF 13-2	5%	70.8	32.7	5.4	53.5	-	59.9	1.1
	10%	37.1	42.1	11.5	57.8	-	72.9	-
BF 14-2	5%	6.0	28.0	13.4	55.7	-	-	-
	10%	-	20.9	31.9	61.1	-	-	-
BF 04-1	5%	-	5.1	14.5	5.4	-	-	-
	10%	30.1	18.8	8.4	37.9	-	61.0	-
BF 08-1	5%	15.4	12.4	10.0	30.3	-	-	-
	10%	33.4	14.9	8.7	16.9	-	120	7.0
BF 09-7	5%	-	63.3	-	14.5	-	1.9	8.7
	10%	-	43.7	45.0	37.5	-	108.7	-
BF 14-1	5%	18.5	-	39.0	39.5	-	66.7	77.3
	10%	16.2	-	47.6	49.6	-	48.0	-
BF 13-5	5%	46.1	36.1	32.8	53.9	-	1.9	0.4
	10%	49.9	22.1	22.5	47.1	-	-	-
BB 07-7	5%	27.7	25.2	2.7	28.9	-	-	-
	10%	33.8	17.8	0.6	13.0	-	-	13.4
BB 07-4	5%	-	-	-	28.2	-	117.4	8.7
	10%	-	-	-	15.4	-	8.7	-
BB 08-3	5%	32.1	4.5	-	-	7.9	83.6	-
	10%	33.1	-	30.4	28.3	19.7	67.5	84.5
BF 08-4	5%	4.5	-	-	55.7	-	-	32.2
	10%	4.5	-	-	38.9	36.7	-	6.7
BC 11-3	5%	35.2	-	9.3	-	27.1	39.3	10.2
	10%	14.0	-	42.1	-	41.1	49.0	-

SH: *Staphylococcus haemolyticus;* SP: *Streptococcus pneumonia;* SA: *Staphylococcus aureus;* PA: *Pseudomonas aeruginosa;* VC: *Vibrio cholerae;* ETEC: Enterotoxigenic *Escherichia coli;* EPEC: Enteropathogenic *Escherichia coli*

14-2) showed close relationship with existing marine bacteria from GenBank, while the other two samples (BC 12-3 and BF 13-2) showed distant relationship with marine bacteria (Fig 1).

DISCUSSION

From the inhibition and destruction activity, most of the results showed higher activity when concentrations of 10% were used (Table 1 and Table 2). This indicates that 10% crudes concentration might be the optimum concentration for several crudes against many pathogenic bacterial biofilms. Some other isolates showed no activity at both concentrations, 5% and 10%. This could happen if higher concentration of crudes were needed. Another possibility was that some crude were not suitable for pathogens biofilm inhibition or were specific to certain pathogens only (Kalpana *et al*. 2011).

Mechanisms between inhibition and destruction were different. Biofilm control can be achieved in several ways, such as, reduction of the planktonic

Table 3 Destruction activity of marine isolates (%)

Crude	Crude Concentration	Pathogens						
		SH	SP	SA	PA	VC	ETEC	EPEC
BC 11-1	5%	-	37.9	13.3	-	15	71.4	53.9
	10%	15.7	58.4	23.6	16.4	56.3	95.7	35.2
BC 11-2	5%	-	17.8	43.1	30.4	-	10.9	20.6
	10%	-	30.3	24.2	20.7	-	0.3	5.1
BC 12-3	5%	-	16.4	-	11.4	4.8	56	-
	10%	8.1	4.7	28.2	23.8	15.8	23.1	10.4
BF 13-2	5%	-	-	18.8	48.6	-	-	-
	10%	-	12.7	37.2	15.6	-	-	-
BF 14-2	5%	16.9	-	14.1	-	-	64.2	-
	10%	33.2	-	14.2	-	-	49.6	84.2
BF 04-1	5%	-	-	2.9	80.1	-	28.1	28.7
	10%	-	-	19.5	82.1	3.2	69.4	56.8
BF 08-1	5%	43.8	12.8	15.2	-	25.2	36.6	12
	10%	35.6	37.4	34.4	3.7	42.5	134	-
BF 09-7	5%	26.6	-	16.3	3.4	-	-	35.9
	10%	12.3	-	-	6.2	-	3.6	9.7
BF 14-1	5%	-	32.8	9.4	18.2	-	-	18.4
	10%	-	35.5	1	3.4	-	-	14
BF 13-5	5%	-	3.6	0.8	67.7	11.2	-	0.4
	10%	-	0.7	35	43.5	-	-	27
BB 07-7	5%	81.9	-	-	75.3	-	78.3	55.5
	10%	-	-	12.8	81.3	-	26.9	69.6
BB 07-4	5%	26.4	27.7	19.3	16.2	11.8	7.6	28.1
	10%	36	9.6	18	3.4	-	53.4	17.3
BB 08-3	5%	-	11.6	8.5	45	-	-	4.6
	10%	22	10.4	1.8	21.3	-	-	0.3
BF 08-4	5%	-	-	18.5	75.2	-	55.2	20.4
	10%	-	11.6	4.2	81.7	1.1	17.9	-
BC 11-3	5%	-	35.5	28	27.3	-	5.2	-
	10%	-	48.7	30.9	12.9	-	13.3	-

SH: *Staphylococcus haemolyticus;* SP: *Streptococcus pneumonia;* SA: *Staphylococcus aureus;* PA: *Pseudomonas aeruginosa;* VC: *Vibrio cholerae;* ETEC: Enterotoxigenic *Escherichia coli;* EPEC: Enteropathogenic *Escherichia coli*

population, prevention of the initial adhesion of cells to the surface, and removal of the established biofilm (Jorge *et al.* 2012). In inhibition activity; marine crudes would inhibit the surface attachment so that biofilms could not be formed. Whereas, in destruction activity, marine crudes may exhibit bioactive compounds, mainly from extra polymeric polysaccharides or proteins which can destroy and remove mature biofilms from the surface (Jorge *et al.* 2012).

One of the components assembling biofilm formation is the production of an extracellular matrix composed of 90% water and 10% extracellular polymeric substances (EPS) (Flemming and Wingender 2010). The EPS mediates cell to cell and cell to surface interaction that are needed for biofilm formation and stabilization. The EPS matrix consists of cell-surface proteins, proteinaceous pili, DNA, RNA, lipids, and polysaccharides (Flemming and Wingender 2010). Many different biofilm matrix polysaccharides have been characterized, for instance Pel and Psl produced by *Pseudomonas aeruginosa*, poly-N-acetylglucosamine (PNAG) produced by *Escherichia coli*, and glucans produced by *Streptococcus mutans* (Rendueles and Kaplan 2012). This building agent of

biofilm formation is important to maintain the biofilm integrity.

However, some bacterial exopolysaccharides can perform functions that inhibit or destabilize the biofilm (Kostakioti *et al*. 2013). This confirms the polysaccharide nature of the antibiofilm activity. Culture supernatants derived from several marine bacteria have been shown to exhibit antibiofilm activity (Gopal *et al.* 2013).The biofilm inhibition activity can be due to a high molecular weight polysaccharide, for example the SP1 EPS from *Bacillus licheniformis* which can inhibit biofilm formation by several other bacteria such as *E.coli, Acinetobacter sp., S.aureus, B.cereus, Listeria monocytogenes, Salmonella typhimurium, Shigella sonnei, Bacillus amyloliquefaciens, Bacillus pumilus* and *Bacillus subtilis* without inhibiting growth (Sayem *et al.* 2011).

Other study said that there are several mode of actions for antibiofilm activity which likely to be mediated by mechanisms other than growth inhibition (Kostakioti *et al.* 2013). First mode is that antibiofilm polysaccharides act as molecules that modify the physical characteristics of bacterial cells and abiotic surfaces (Kostakioti *et al.* 2013). Second, some studies have indicated that polysaccharides might act as signaling molecules that modulate gene expression of recipient bacteria (Kim *et al.* 2009). Another possibility is the competitive inhibition of multivalent carbohydrate-protein-interactions (Wittschier *et al.* 2009).

Antibiofilm activity of bacteria can also be isolated from biofilms which exhibit an antagonist effect over competing microorganisms (Rendueles *et al.* 2012). Beside the exopolysaccharide, lipopolysaccharides from Gram negative bacteria can alter biofilm structure and reduced adhesion, hence inhibit biofilm formation of competing strains (Lau *et al.* 2009). For example LPS from *Vibrio cholerae* is able to partially inhibit *in vitro* adhesion on colonic cell lines HT29-18N2 (Benitez *et al.* 1997).

Based on the sequencing results and phylogenetic tree, samples BC11-1 (*Erythrobacter* sp.), BF04-1 (*Enterobacter cloacae)*, BF13-5 (*Paracoccus marcusii)*, and BF14-2 (*Cobetia marina*) showed close relationship to existing marine bacteria (Figure 1). A previous study on antibiofilm activity of *Cobetia marina* conducted by Trentin *et al.* 2011 showed that *Cobetia marina* reduced the number of adherent bacteria in sterile 96-well microtiter plates containing *S.epidermidis* compared to the untreated biofilm (control). The study also suggested that the size of aggregates were also reduced to small cluster or even single cells and very little EPS appeared to have been formed (Trentin *et al*. 2011). The results corroborate with the inhibition observed in the biofilm assay. Though the mechanism was still unclear, due to a connection between cell density and the EPS production which is quorum sensing (QS)-regulated, *Cobetia marina* was thought to modulate the QS of pathogenic bacteria and thereby prevent EPS production, blocking the irreversible adhesion to a surface, and consequently, the formation of biofilm (Trentin *et al*. 2011). Meanwhile, there have not been any findings in scientific research on how the other 3 marine isolates perform antibiofilm activity.

The other two samples; BC 12-3 (*Micrococcus luteus*) and BF 13-2 (*Bacillus jeotgali*), however, showed distant relationship to existing marine bacteria. Further studies should be conducted in order to analyze the genetic relationship between those two samples and to observe their antibiofilm activity mechanisms against pathogenic bacteria.

Most marine isolates tested have antibiofilm activity against several pathogens either with inhibition or destruction mechanism. From these results, it can be concluded that marine bacteria are great potential sources of novel antibiofilm compounds.

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