Malaysian Journal of Microbiology, Vol 13(2) June 2017, pp. 92-99

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Inhibition of marine biofouling by aquatic Actinobacteria and coral-associated marine bacteria

Diana Elizabeth Waturangi[1*], Jessen Purwa Hariyanto[1], Windy Lois[1], Rory Anthony Hutagalung[1], Jae Kwan Hwang[2]

[1]Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Jenderal Sudirman no. 51, 12930 Jakarta, Indonesia.
[2]Biomaterial Research Laboratory, Department of Biotechnology, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-749, Korea.
Email: diana.waturangi@atmajaya.ac.id

Received 4 May 2016; Received in revised form 26 September 2016; Accepted 12 October 2016

ABSTRACT

Aims: Biofouling is a common biology phenomenon occuring on ship surface. This phenomenon has become serious threat in marine industries because of great economic loss. Tributyltin has been used to prevent biofouling, but it turned to cause the environmental problem. Therefore, the discovery of alternative environment-friendly compound is necessarily needed.
Methodology and results: Five Actinobacteria isolates and fourteen marine bacteria isolates were tested against the biofilm formation of eight biofouling bacteria isolates that isolated from boat surface and the attachment of three biofouling diatoms (*Amphora*, *Navicula*, *Nitzschia*). Actinobacteria CW17 supernatant showed the broad spectrum activity against all fouling bacteria, whereas BC 11-5 supernatant was the only marine bacteria that capable to inhibit biofilm formation of *V. neocaledonicus.* Moreover, three representative diatoms attachment could be inhibited by the bioactive compounds produced by Actinobacteria and marine bacteria. CW01 supernatant showed broad spectrum and high activity against all three representative diatoms which is very promising. Molecular identification based on 16S rDNA gene sequence showed eight fouling bacteria isolates were biofilm-forming bacteria.
Conclusions, significance and impact of study: This research showed aquatic Actinobacteria and coral-associated marine bacteria have the potential to prevent biofouling formation. Further studies are needed to purify and characterize these antibiofouling compounds for environmental application.

Keywords: Biofouling, antibiofouling, Actinobacteria, marine Bacteria, diatom

INTRODUCTION

Marine transportations and structures are easily colonized by fouling organism in a process called biofouling. This is a serious problem for marine industries all over the world that creates great disadvantages and economic loss. Fouling organism form a complex layer on submerged substrates, like ship hulls, which increases the surface roughness, resulting in increased frictional resistance and fuel consumption because the top speed and the range of the ship is decreased (Müller *et al.*, 2013). Biofouling also causes the distribution of non-indigenous species (NIS) by ship transport (Hong and Cho, 2013). To prevent this biological phenomenon, antifouling coatings had been developed for water-exposed surfaces. Copper oxide and tributyltin oxide (TBT) were found to be the most effective agents against biofouling. Unfortunately, these compound not environmentally friendly due to the fact that they are not quickly degraded naturally and attack both target and non-target species (Müller *et al.*, 2013). This led the International Maritime Organization (IMO) to ban their application on ships since 2008 (Qian *et al*., 2010). Since then, the demand for new antibiofouling compounds that environmentally friendly is increased.

Biofouling is formed by the adhesion and interaction of fouling organism, which consist of microfoulers (i.e. bacteria and diatoms) and macrofoulers (i.e. barnacles, mussels, polychaete worms, bryozoans, and seaweed). The process of biofouling formation is divided into four main steps: (i) formation of conditioning film composed of organic materials (such as protein, polysaccharide, and proteoglycan) on water-exposed surface, (ii) the settlement of microfoulers, (iii) formation of biofilm, and (iv) attachment of marcofoulers larvae. Many organisms involved in biofouling makes it hard to removed (Cao *et al.*, 2011; Müller *et al.*, 2013).

***Corresponding author**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Bacterial and diatom biofilm formation was the initial step biofouling formation. Biofilm consists of extracellular polymeric substance (EPS) secreted by bacteria and diatom. Biofilm leads to irreversible bacteria adhesion and stronger diatom attachment (Cao *et al.*, 2011). Moreover, biofilm formation will stimulate the attachment of invertebrates and algae to submerged marine surfaces. Microbial biofilms in particular provide biochemical signals that larvae employ in selecting a settlement site, attaching to it, and undergoing metamorphosis (Zardus *et al.*, 2008). Therefore, inhibiting formation of the bacteria biofilm was the one of the important thing to prevent the biofouling formation.

Diatom was the dominant eukaryotic marine fouling organisms. Diatom does passive movement to approach on a surface because their lack of flagella. Electrostatic interactions such as coulomb attraction and van der Waals force was involved in diatom attachement. After the diatom land on the substrate, it will secret EPS and reorient themselves along the surface into better positions, this movement called diatom gliding. EPS of diatom is composed of carboxylated or sulfated acidic polysaccharides. Diatom would secret mucilage strand at their central pore to tightly bind on the substrate (Cao *et al.*, 2011). Diatom attachment would be prevent because when they are abundant, it can promote bio-corrosion of the surface (Silva-Aciares and Riquelme 2008).

Actinobacteria are the group of filamentous bacteria which are recognized as source of bioactive metabolites. According to Bérdy (2012), about 13,700 microbial metabolites are reported derived from this group of bacteria. Many metabolites have been reported to have antibiotics, antivirals, and anticancer activity. However, aquatic and marine Actinobacteria are not explored widely. Therefore, many novel bioactive compounds can be harvested (Kumaran *et al.*, 2011). Marine bacteria group also have been reported produce many type of bioactive compounds. Satheesh *et al.* (2012) isolated coral-associated bacteria with antifouling activity from *Sigmadocia* sp.

In the present study, antibiofouling compounds from Actinobacteria and marine bacteria are still scarce. There are more novel Actinobacteria and marine bacteria which have bioactive metabolites that have not studied yet which may have potential activity against biofouling.

MATERIALS AND METHODS

Biofouling bacteria isolation

Biofouling sample was collected by scrapping everything that covered the fisherman boat surfaces in Segara Ayu Beach, Sanur, Bali, Indonesia and transported to laboratory. One gram of biofouling sample was serially diluted using sterile seawater. 10^{-3} to 10^{-5} dilutions were spread to Marine Agar (Difco™) and incubated at 28 °C for 1-3 days. Morphologically-different bacterial colonies were selected, purified, and sub-cultured (Gopikrishnan *et al.*, 2013). Each biofouling bacteria isolates were tested for biofilm formation activity using static biofilm assay (detailed explanation in section 2.5). Biofilm-forming isolates were used for further assay and identification.

Molecular identification of biofouling bacteria

Biofouling bacteria isolates were identified using polymerase chain reaction (PCR) amplification of 16S rDNA gene. The method is optimized from Marchesi *et al.* protocol (Marchesi *et al.*, 1998). The PCR master mix and conditions are described in Tables 1 and 2, respectively. Samples were sent to 1st Base Sequencing, Malaysia for DNA sequencing analysis. DNA sequences then were processed with SeqTrace 0.9.0 software for basic local alignment search tool (BLAST) purpose. The 16S rDNA gene sequences were submitted into the GenBank.

Table 1: Master mix for 16S rDNA amplification.

Solution	Volume (µL)
GoTaq Green Master Mix	12.5
Primer 63f'	1
Primer 1387r'	1
Nuclease free water	9.5
DNA template	1
Total volume	**25**

Table 2: Polymerase chain reaction condition for 16S rDNA.

Steps	Time (min)	Temperature (°C)
Pre-Denaturation	7	95
Denaturation	0.5	95
Annealing	0.5	55
Elongation	1	72
Post-Elongation	20	72
Hold	∞	4
Cycles	30 cycles	

Diatom culture

Three representative biofouling species of diatom (*Nitzschia* sp., *Navicula* sp., and *Amphora* sp.) were obtained from the culture collection of Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia. The diatoms were isolated from soft coral *Dendronephthya* sp. and identified morphologically that based on size, form, and color characteristics (Hutagalung *et al.*, 2014). The cultures were grown in f/2 media (Guillard 1975) at room temperature with 24 h light, maintained in 5 mL glass tubes and sub-cultured every two weeks.

Crude extract production

Five Actinobacteria isolates (CW01, CW17, SW03, SW12, and TB12) were obtained from previous study, which isolated from various aquatic environments (Table 3). Each isolates were sub-cultured in Glucose Yeast Malt Extract Starch Agar (GYMS) (glucose 4 g/L, yeast extract 4 g/L, malt extract 10 g/L, starch 20 g/L, $CaCO_3$ 2 g/L,

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

and bacteriological agar 12 g/L) and incubated at 28 °C for 7 days. Fermentation was done using Tryptone Soy Broth (Oxoid) at 28 °C 120 rpm for 7 days. After that, the broths were centrifuged at 7,798 ×g, 4 °C for 15 min. Then, fourteen coral-associated marine bacteria (BB 08-1, BF 04-2, BF 06-2, BF 08-2, BF 09-2, BF 13-4, BF 14-2, BF 15-2, BB 07-6, BC 10-1, BC 11-5, BC 12-4, BC 13-2, and BF 05-4) from previous studies were used. These specimens were isolated from hard coral and soft coral in Indonesia (Table 4). Each marine bacteria isolates were inoculated in Marine Broth (Oxoid) and incubated at 28 °C, 125 rpm for 3 days. The cultures were centrifuged at 8,000 rpm, 4 °C for 20 min. Collected supernatants were kept under 4 °C until further assay were performed.

Table 3: Actinobacteria isolates origin and genus.

Isolates	Origin	Genus/Species	Accession Number
CW01	CuncaWulang River at West Flores	*Arthrobacter* sp.	JX434848
CW17	CuncaWulang River at West Flores	*Streptomyces* sp.	JX434845
SW03	Paddy Field at Gancahan 8 Village, Sleman	*Streptomyces* sp.	JX434841
SW12	Paddy Field at Gancahan 8 Village, Sleman	*S. carpaticus*	JX434849
TB12	TelagaBiru Lake at Green Botanical Garden, Cibodas	*A. mysorens*	JX434842

Table 4: Marine bacteria isolates origin

Isolates	Coral Species	Origin
	Soft Coral	
BB 08-1	*Lobophytum* sp.	Bali
BF 04-2	*Scleronephthya* sp.	Lampung
BF 06-2	*Sinulariamollis*	Lampung
BF 08-2	*Scleronephthya* sp.	Kapuran
BF 09-2	*Heteroxenia*	Bali
BF13-4	*Studeriotes* sp.	Kapuran
BF 14-2	*Nephthyigorgia sp.*	Kapuran
BF15-2	*Sinularia lobata*	Seribu Island
	Hard Coral	
BB 07-2	*Tubastrea micrantha*	Cilegon
BC 10-1	*Acropora simplex*	Lombok
BC 11-5	*A. desalwi*	Bali
BC 12-4	*A. echinata*	Kendari
BC 13-2	*Haliclona* sp.	Karawang
BF 05-4	*Brotyllus* sp.	Kapuan

Biofilm inhibition assay

Biofilm-forming bacteria were grown in Brain Heart Infusion Broth (Oxoid) with 1% glucose supplementation and incubated overnight at 28 °C, 125 rpm. Bacterial densities were measured until reach absorbance value OD_{600} = 0.132 (McFarland 0.5) using spectrophotometer and dilution were done if needed. Biofilm inhibition assay were done using static biofilm assay using 96-well microplate (IWAKI). Each well contains 200 µL suspensions with 10% (v/v) supernatants. After two days of incubation at 28 °C, spent medium was discarded, and rinsed twice using sterile distilled water. Adherent biofilm was then stained with crystal violet solution for 30 min, and subsequently rinsed five times using sterile distilled water and air dried. Crystal violet solutions were then solubilized with 200 µL absolute ethanol. Then, 200 µL solubilized crystal violet were transferred to new microplate, and the optical density were determined at 595 nm using microplate reader Biorad 680 Microplate Reader (Stepanović *et al.*, 2007). Biofilm inhibitory activities of each supernatant were determined with Equation (1).

$$\% \text{ Activity} = \frac{\text{positive control absorbance} - \text{sample absorbance}}{\text{positive control absorbance} - \text{negative control absorbance}} \times 100\% \quad (1)$$

Note:

1. Positive control is biofouling bacteria growth in medium without supernatants added.
2. Negative control is 200 µL medium.

Diatom attachment inhibition assay

Assay of diatom attachment inhibition were followed Hong and Cho (Hong and Cho, 2013) with some modifications. The initial cell density of diatom cell suspension was counted and necessary dilutions using f/2 medium were made to obtain 1×10^5 cells/mL. Thirty milliliter of diatom suspension was transferred to 50 mL centrifugal tube and 10% (v/v) supernatants were added. Then, sterile object glass (2.5 × 7.5 cm) was placed in the tube for facilitating the attachment. The tubes were lied down statically and incubated at room temperature with 24 h light conditions. The object glass was completely drowned and not moved. After three days of incubation, the object glasses were removed and the cells attached on the object glasses were scraped, then it was diluted in 1 mL of aquades. The cells were counted using hemocytometer. Diatom attachment inhibition activities of the supernatants were determined with Equation (2).

$$\% \text{ Activity} = 1 - \frac{\text{Sample total cell count}}{\text{Positive control total cell count}} \times 100\% \quad (2)$$

RESULTS

Isolation and identification of biofouling bacteria

Eight of eleven biofouling isolates showed the ability to form biofilm. Molecular identification using BLASTN revealed that those bacteria are closely related (99% similarity) with members of genus *Vibrio*, *Pseudomonas* and *Shewanella* (Table 5). This result showed Gram-negative bacteria, especially *Vibrio*, were dominant bacteria in biofuling community.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 5: Biofouling biofilm-forming bacteria molecular identification using PCR 16S rDNA gene.

Isolate	Closest relatives	Similarity (%)	Accession Number
FB1	*Vibrio neocaledonicus*	99	KP744374
FB2	*V. neocaledonicus*	99	KP744375
FB3	*Pseudomonas stutzeri*	99	KP744376
FB5	*V. neocaledonicus*	99	KP744377
FB6	*V. alginolyticus*	99	KP744378
FB7	*V. natriegens*	99	KP744379
FB8	*Shewanella algae*	99	KP744380
FB9	*Shewanella algae*	99	KP744381

Biofilm inhibition activity

Supernatants extracted from Actinobacteria showed inhibition activity against attachment of fouling bacteria (Figure 1). CW17 supernatant was the only one who had the inhibition activity against all fouling bacteria biofilms, followed by CW01 (no inhibition against FB7) and TB12 (no inhibition against FB6). However, the overall activity of CW17 was lower than TB12 supernatant, except against FB6 and FB9.

Supernatants from fourteen marine bacteria showed different inhibitory activity against 8 fouling bacteria (Figure 2). The highest inhibition (95%) of supernatant was shown by BC 13-2 against biofilm formation of FB3 and (94%) BC 11-5 against biofilm formation of FB 2. On the other hand, BC 13-4 showed the lowest inhibition activitiy (3.15%) against FB3 biofilm formation. Meanwhile, inhibition against FB7 biofilm formation was shown by the most marine of bacteria; i.e., BB 08-1, BF 04-2, BF 06-2, BF 08-2, BF 09-2, BF 13-4, BF 14-2, and BF 15-2. This result showed that bioactive compounds from these marine bacteria have antibiofilm activity against specific fouling bacteria.

Diatom attachment inhibition activity

Five Actinobacteria and 14 marine bacteria supernatants showed inhibition activity against *Amphora, Navicula,* and *Nitzchia*. These species are common fouling diatoms found on biofouling surface (Yang *et al.*, 2014). From the five isolates, CW01 and CW17 supernatants showed broad spectrum activity, whose activity was above 60%, especially for CW01 having the highest inhibition against *Amphora* (83.7%). However, the highest inhibition activity against *Navicula* (98.35%) and *Nitzschia* (99.28%) were generated by TB12 supernatant (Figure3). Following to the result, almost all of the marine bacteria supernatants could inhibit the three representative diatoms attachment on the substrate. Highest attachment inhibition activity (91.8%) was shown by BB 08-1 against *Nitzchia,* while BF 13-4 showed the lowest attachment inhibitory activity (19.96%) against Amphora (Figure 4).

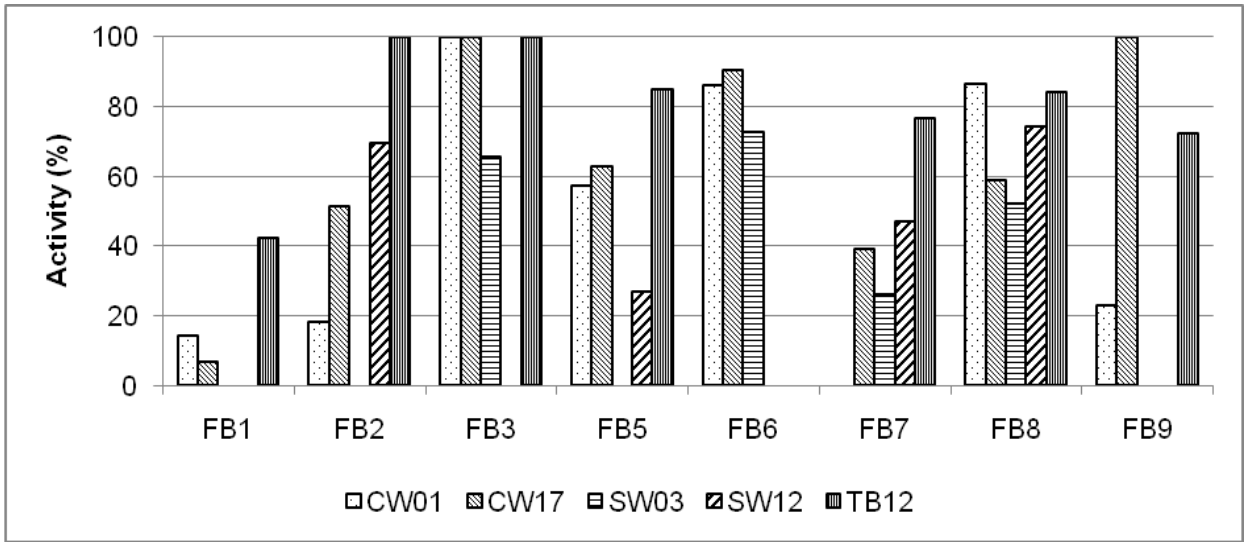

Figure 1: Biofilm inhibition activity of 10% (v/v) Actinomycetes extracts against fouling bacteria isolates.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

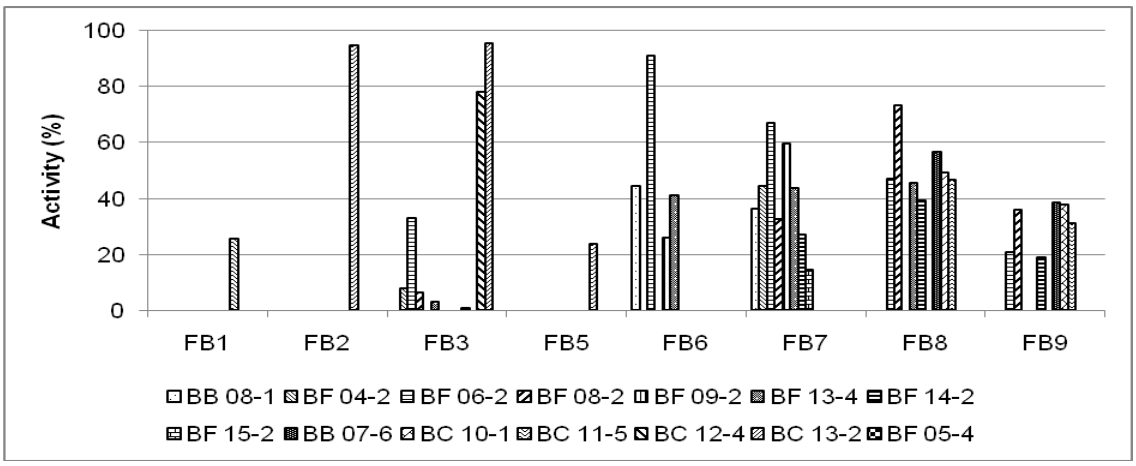

Figure 2: Biofilm inhibition activity of 10% (v/v) marine bacteria extracts against fouling bacteria isolates.

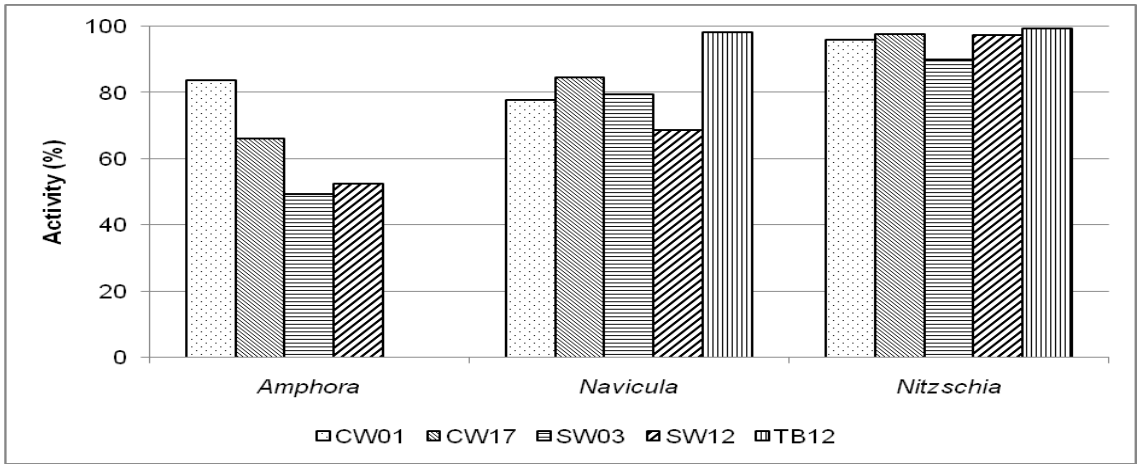

Figure 3: Attachment inhibition activity of 10% (v/v) Actinobacteria extract against fouling diatoms.

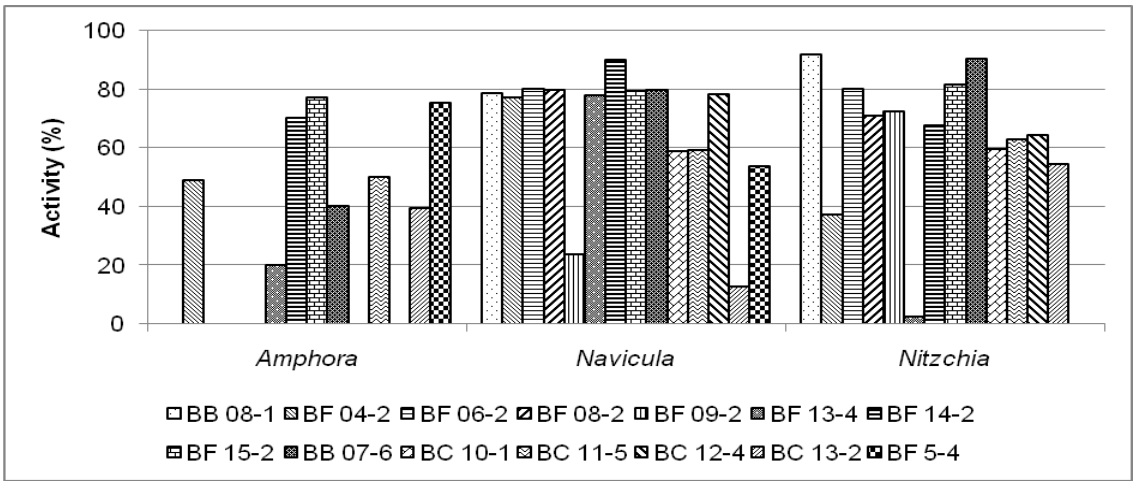

Figure 4: Attachment inhibition activity of 10% (v/v) marine bacteria extract against fouling diatoms.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

DISCUSSION

The eight isolated and identified biofouling bacteria (FB1, FB2, FB3, FB5, FB6, FB7, FB8, and FB9) were known to have biofilm-forming activity (Casey *et al.*, 2000; Snoussi *et al.*, 2008; Chalkiadakis *et al.*, 2013; Martín-Rodríguez *et al.*, 2014), except *Pseudomonas stutzeri.* However, *P. stutzeri* has flagella and pili structure that help in bacteria attachment (Lalucat *et al.*, 2006). This leads to false perception of biofilm formation by FB3. Some members of genus *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Vibrio*, *Aeromonas*, *Micrococcus*, *Alcaligenes*, *Proteus,* and *Shewanella* have been successfully isolated from marine biofouling samples (Dhanasekaran *et al.*, 2009; Kumaran *et al.*, 2011; Gopikrishnan *et al.*, 2013). All of our bacterial isolates are belongs to those genera. Moreover, *S. algae* was reported as contributors in biofouling formation (Martín-Rodríguez *et al.*, 2014). Therefore, these isolates can represent biofouling bacteria as model organisms.

There were three supernatants could inhibit FB3 completely. This might related to the inability of *Pseudomonas stutzeri* to form biofilm (Lalucat *et al.*, 2006). The absence of biofilms on FB3 might allow the inhibition of the adhesion directly (Lalucat *et al.*, 2006).

FB8 isolate could be inhibited by every Actinobacteria supernatants, while FB9 could not. FB1, FB2, and FB5, which share same similarity, also showed different inhibition results between them. This can be deduced that they have different properties in mechanism or structure involved in biofilm formation. Therefore, further researches are needed to reveal the distinct properties between bacteria, which will lead into strain classification.

CW17, SW03, and SW12 are belongs to *Streptomyces* genus, while CW01 and TB12 are *Arthrobacter* genus. Chen *et al.* (2013), reported that *Streptomyces* sp. and *Arthrobacter* sp. had the ability to interfere quorum sensing, which is known as quorum quenching. They could produce acyl homoserine lactone (AHL)-degrading enzyme, like acylase and lactonase. Some strains producing those enzymes have been identified in *Streptomyces*, while in *Arthrobacter* only lactonase-producer was found (Chen *et al.*, 2013). In other study, *Streptomyces libani* and several marine Actinobacteria were screened to have inhibition activity against biofouling bacteria (Kumaran *et al.*, 2011). *Streptomyces filamentous* also has been reported to have good antifouling activity (Bavya *et al.*, 2011). Molecular analysis should be done to reveal their shared traits and genetic diversity, which leads to find a specific mechanism in antifouling activity.

Several marine Actinobacteria were found to produce furanone compounds as biofilm inhibition mechanisms. 2-furanone structure in the compounds responsible in interrupting quorum sensing, which is correlated with biofilm formation (Xu *et al.*, 2010). Other mechanism was reported from two Actinobacteria members, *Streptomyces akiyoshinensis* and *Actinobacterium* sp. They produced potent biofilm inhibitor of *Streptococcus pyogenes* by reducing cells surface hydrophobicity, which plays important role in cell adhesion and colonization (Nithyanand *et al.*, 2010). These characteristics may be used as screening method for novel Actinobacteria with antibiofouling activity.

As for marine bacteria, only BC 11-5 supernatant could inhibit the biofilm formation of *V. neocaledonicus* while the other marine bacteria crude extract did not show that activity. *Vibrio neocaledonicus* is a new member of the *Vibrio* genus bacteria, recent study showed *V. neocaledonicus* could produce a different type of extra polymeric substance (EPS). This EPS exhibits a high N-acetyl-hexosamines and uronic acid content with a low amount of neutral sugar. The different EPS structure of *V. neocaledonicus* may happen due to the evolution. It is lead to the better defense mechanism that refer to the result showed limited bacteria can inhibit this bacteria (Chalkiadakis *et al.*, 2013).

Many types of coral-assosciated bacteria was isolated from *Acroporadigitifera* which *Bacillus* genus was represent the most abundant (Thenmozhi *et al.*, 2009). Their *Bacillus* genus crude extract show promising result that can be used for antibiofilm and quorum quenching agent. In fact, marine bacteria with antifouling activity were often found associated with coral (Thenmozhi *et al.*, 2009). These coral-associated bacteria mostly play an important role for the coral and sponge. The sponges, as sessile filter-feeder animals, could not produce antibiofouling agent by itself. Positive symbiotic with coral-associated bacteria that produce antibiofouling agent is evolved antifouling strategies to protect themselves against micro- and subsequent macrobiofouling processes (Müller *et al.*, 2013). Many bioactive compounds of marine bacteria with antibiofouling activity have been characterized and identified. Biosurfactant was the common bioactive compound that marine bacteria produce to inhibit the formation biofilm. Biosurfactant can reduce the surface or interfacial tension for resist attachment of biofilm in the surface (Dusane *et al.*, 2011).

Amphora was the least inhibited diatoms by Actinobacteria and marine bacteria crude extracts. This result might be appropriate with the properties of *Amphora*, which has two raphes on one side (ventral surface), while *Navicula* and *Nitzschia* have single raphes on ventral and dorsal side of the cell (Arce *et al.*, 2004; Wigglesworth-cooksey and Cooksey, 2005; Jin *et al.*, 2013). This might lead to stronger attachment for *Amphora* to the substrate. The varieties of diatom morphological structure could generate different mechanism of attachment in every diatom (Hilaluddin *et al.*, 2011).

Our study did not analyse the mechanism or determine the spesific compound that responsible for inhibition of diatom attachment. Recent study reported that two furanone derivatives had been successfully isolated from *Streptomyces violaceoruber* SCH-09 and showed antifouling activities against *Navicula annexa* and other fouling organism. This marine Actinobacteria was isolated from seaweed *Undaria pinnatifida* surface. The result also showed these compounds had no effect against non-target organism at the same concentration (Hong and Cho, 2013). Coral-associated bacteria was the

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

one natural potential for prevent the diatom attachment on substrate. Polybrominated diphenyl ethers had been identified in a marine sponge (genus *Dysidea*) causing an inhibition of diatom growth (Ortlepp *et al.*, 2008).

CONCLUSION

Extracts with broad spectrum activity against biofouling bacteria was extracted from *Streptomyces* sp. (CW 17), whereas *Arthrobacter* sp. (CW 01) against diatoms, and extracts with overall highest activity was extracted from *Arthrobacter mysorens* (TB 12). Extracts of marine bacteria BC 11-5 had the most promising ability to inhibit biofilm among others. Even though our study has not discovered either the compound or mechanism of the inhibition activity, the promising results showed they have great potential, especially in inhibiting marine biofouling pioneer organisms, which will become the alternative solution against biofouling. Hence, further study is needed to characterize these compounds, in order to find out the inhibition mechanism, and screen inhibition activity with other organism contributed to the formation of biofouling.

ACKNOWLEDGEMENT

Authors appreciated the help of Cindy Ciputra for collecting the samples. Authors also thanked Atma Jaya Catholic Univerisity of Indonesia for providing the research facility. This work was supported in part by the Ministry of Land, Transport and Maritime Affairs, South Korea and Hibah Kompetensi research grant 2014 from Directorate General of Higher Education, Indonesia.

REFERENCES

Arce, F. T., Avci, R., Beech, I. B., Cooksey, K. E. and Wigglesworth-Cooksey, B. (2004). A live bioprobe for studying diatom-surface interactions. *Biophysical Journal* **87, 4284-4297**.

Bavya, M., Mohanapriya, P., Pazhanimurugan, R. and Balagurunathan, R. (2011). Potential bioactive compound from marine Actinomycetes against biofouling bacteria. *Indian Journal of Marine Sciences* **40, 578-582.**

Bérdy, J. (2012). Thoughts and facts about antibiotics: Where we are now and where we are heading. *The Journal of Antibiotics* **65, 385-395.**

Cao, S., Wang, J. D., Chen, H. S. and Chen, D. R. (2011). Progress of marine biofouling and antifouling technologies. *Chinese Science Bulletin* **56, 598-612.**

Casey, E., Glennon, B. and Hamer, G. (2000). Biofilm development in a membrane-aerated biofilm reactor: Effect of intra-membrane oxygen pressure on performance. *Bioprocess Engineering* **23, 457-465.**

Chalkiadakis, E., Dufourcq, R., Schmitt, S., Brandily, C., Kervarec, N., Cotanea, D., Amir, H., Loubersac, L., Chanteau, S., Guezennec, J., Dupont-Rouzeyrol, M. and Simon-Colin, C. (2013). Partial characterization of an exopolysaccharide secreted by a marine bacterium, *Vibrio neocaledonicus* sp. nov., from new caledonia. *Journal of Applied Microbiology* **114, 1702-1712.**

Chen, F., Gao, Y., Chen, X., Yu, Z. and Li, X. (2013). Quorum quenching enzymes and their application in degrading signal molecules to block quorum sensing-dependent infection. *International Journal of Molecular Sciences* **14, 17477-17500.**

Dhanasekaran, D., Thajuddin, N., Rashmi, M., Deepika, T. L. and Gunasekaran, M. (2009). Screening of biofouling activity in marine bacterial isolate from ship hull. *International Journal of Environmental Science & Technology* **6, 197-202.**

Dusane, D. H., Matkar, P., Venugopalan, V. P., Kumar, A. R. and Zinjarde, S. S. (2011). Cross-species induction of antimicrobial compounds, biosurfactants and quorum-sensing inhibitors in tropical marine epibiotic bacteria by pathogens and biofouling microorganisms. *Current Microbiology* **62, 974-980.**

Gopikrishnan, V., Pazhanimurugan, R. and Shanmugasundaram, T. (2013). Bioprospecting of Actinobacteria from mangrove and estuarine sediments for antifouling compounds. *International Journal of Innovative Research in Science, Engineering and Technology* **2, 2726-2735.**

Guillard, R. R. L. (1975). Culture of phytoplankton for feeding marine invertebrates. *In*:*Culture of Marine Invertebrate Animals*. Smith, W. L. and Chanley, M. H. (eds.). Plenum Press. New York, USA. **pp. 29-60.**

Hilaluddin, F., Leaw, C. P. and Lim, P. T. (2011). Morphological observation of common pennate diatoms (*Bacillariophyceae*) from Sarawak estuarine waters. *Annals of Microscopy* **11, 12-23.**

Hong, Y. K. and Cho, J. Y. (2013). Effect of seaweed epibiotic bacterium *Streptomyces violaceoruber* SCH-09 on marine fouling organisms. *Fisheries Science* **79, 469-475.**

Hutagalung, R. A., Sukoco, A. E., Soedharma, D., Goreti, L. M., Andrean, I., Elshaddai, B. and Mulyono, N. (2014). Isolation, identification and growth optimization of microalgae derived from soft coral *Dendronephthya* sp. *APCBEE Procedia* **10, 305-310.**

Jin, C., Xin, X., Yu, S., Qiu, J., Miao, L., Feng, K., and Zhou, X. (2013). Antidiatom activity of marine bacteria associated with sponges from San Juan Island, Washington. *World Journal of Microbiology and Biotechnology* **30, 1325-1334.**

Kumaran, S., Radakrishnan, M. and Balagurunathan, R. (2011). Bioactive compound from marine actinomycetes againts biofouling bacteria. *Advanced Biotechnology* **10, 22-26.**

Lalucat, J., Bennasar, A., Bosch, R., García-Valdés, E. and Palleroni, N. J. (2006). Biology of *Pseudomonas stutzeri*. *Microbiology and Molecular Biology Reviews* **70, 510-547.**

Marchesi, J. R., Sato, T., Weightman, A. J., Martin, T. A., Fry, J. C., Hiom, S. J. and Wade, W. G. (1998). Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

16S rRNA. *Applied and Environmental Microbiology* **64, 795-799.**

Martín-Rodríguez, A. J., González-Orive, A., Hernández-Creus, A., Morales, A., Dorta-Guerra, R., Norte, M., Martín, V. S. and Fernández, J. J. (2014). On the influence of the culture conditions in bacterial antifouling bioassays and biofilm properties: *Shewanella algae*, a case study. *BMC Microbiology* **14, 102.**

Müller, W. E. G., Wang, X., Proksch, P., Perry, C. C., Osinga, R., Gardères, J. and Schröder, H. C. (2013). Principles of biofouling protection in marine sponges: A model for the design of novel biomimetic and bio-inspired coatings in the marine environment? *Marine Biotechnology* **15, 375-398.**

Nithyanand, P., Thenmozhi, R., Rathna, J. and Pandlan, S. K. (2010). Inhibition of *Streptococcus pyogenes* biofilm formation by coral-associated actinomycetes. *Current Microbiology* **60, 454-460.**

Ortlepp, S., Pedpradap, S., Dobretsov, S. and Proksch P. (2008). Antifouling activity of sponge-derived polybrominated diphenyl ethers and synthetic analogues. *Biofouling* **24, 201-208.**

Qian, P. Y., Xu, Y. and Fusetani, N. (2010). Natural products as antifouling compounds: Recent progress and future perspectives. *Biofouling* **26,223-234.**

Satheesh, S., Soniamby, A. R., Sunjaiy Shankar, C. V., and Punitha, S. M. J. (2012). Antifouling activities of marine bacteria associated with sponge (*Sigmadocia* sp.). *Journal of Ocean University of China* **11, 354-360.**

Silva-Aciares, F. and Riquelme, C. (2008). Inhibition of attachment of some fouling diatoms and settlement of *Ulva lactuca* zoospores by film-forming bacterium and their extracellular products isolated from biofouled substrata in northern Chile. *Electronic Journal of Biotechnology* **11, 1-11.**

Snoussi, M., Noumi, E., Cheriaa, J., Usai, D., Sechi, L. A., Zanetti, S., and Bakhrouf, A. (2008). Adhesive properties of environmental *Vibrio alginolyticus* strains to biotic and abiotic surfaces. *New Microbiologica* **31, 489-500.**

Stepanović, S., Vuković, D., Hola, V., Bonaventura, G. D., Djukić, S., Ćirković, I. and Ruzicka, F. (2007). Quantification of biofilm in microtiter plates: Overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. *APMIS* **115,891-899.**

Thenmozhi, R., Nithyanand, P., Rathna, J. and Pandian, S. K. (2009). Antibiofilm activity of coral-associated bacteria against different clinical M serotypes of *Streptococcus pyogenes*. *FEMS Immunology and Medical Microbiology* **57, 284-294.**

Wigglesworth-cooksey, B. and Cooksey, K. E. (2005). Use of fluorophore-conjugated lectins to study cell-cell interactions in model marine biofilms. *Applied and Environmental Microbiology* **71, 428-435.**

Xu, Y., He, H., Schulz, S., Fusetani, N., Xiong, H., Xiao, X. and Qian, P. Y. (2010). Potent antifouling compounds produced by marine Streptomyces. *Bioresource Technology* **101, 1331-1336.**

Yang, C., Wang, J., Yu, Y., Liu, S. and Xia, C. (2014). Seasonal variation in fouling diatom communities on the Yantai coast. *Chinese Journal of Oceanology and Limology* **33, 439-446.**

Zardus, J. D., Nedved, B. T., Huang, Y., Tran, C. and Hadfield, M. G. (2008). Microbial biofilms facilitate adhesion in biofouling invertebrates. *Biological Bulletin* **214, 91-98.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538