Isolation and Screening of Surfactant-producing Bacteria from Indonesian Marine Environments and Its Application on Bioremediation

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Abstract

Isolation and screening have been undertaken on oil-degrading microbes from Indonesian marine environments. During screening process it has been found many bacterial isolates capable of degrading crude oil. Hence, study has been focused on the biodiversity of biosurfactant-producing bacterial species in Indonesian marine environment and its function for remedial the pollutant in marine and soil areas. A total of 103 out of 463 isolates showed positive surfactant-degrading properties. By means of partial 16S rRNA gene analyses, it has been found that the majority of taxa are related to *Alcanivorax*, *Pseudomonas, Bacillus*, *Bortetela*, *Brucella, Acenitobacter*, *Staphia*, *Lysobacter*, and *Talasosophira*. Biosurfactant properties assay showed that they were capable of lowering the surface- and interfacial water tension from 74 mN/m to 40-65 mN/m and from 24 mN/m to 6-10 mN/m, respectively. In addition, most of the surfactants were capable of emulsifying hydrocarbon (crude oil) of 0.01 to 0.15 units, comparable to 0.08 units of synthetic surfactant (20% Tween). Further observation showed that the majority of the surfactants were able to degrade a long chain of alkane, but not branched alkane, with a recovering rate of 20-80%. The application of the surfactant towards oil polluted model beach was done in laboratory scale and showing the surfactant obtained from microbial broth cultures capable for recovering the oil pollutant significantly, compared to the control (without addition microbial broth).

Keywords: bacteria, biosurfactant, screening, improvement soil texture

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Introduction

Marine microorganisms synthesize a wide range of surface-active agents (biosurfactants) from low-molecular-weight that lowering the surface and interfacial tensions efficiently to high-molecular-weights compounds that emulsifies tightly to surface. Their chemical structures and the surface properties are diver's therefore reasonable to assume that different groups of biosurfactants have different natural roles in their environments. The most immediate application of biosurfactants are for the environment remediation technologies, because the product purity are negligible, non-toxic and have particular functions. A number of studies showed that biosurfactants were effective for the abatement of marine oil pollution with better biodegradability and lower toxicity than synthetic surfactants due to their biogenetic origin (Poremba & Gunkel, 1990; Finnerty, 1991; Garcia-Junco *et al*., 2001; Makkar & Cameotra, 2002).

It's well known, that petroleum hydrocarbons are the major pollutants of marine environment as a result of domestic waste run-off, offshore oil production, shipping activities, refuse from coastal oil refineries and accidental spillage of fuels. Process of degradation by evaporation and photo-oxidation are playing an important role in oil recovery, while ultimate and complete degradation is accomplished mainly by marine microflora, bacteria being dominant in this function. However, natural microbial degradation occurs relatively slowly in marine environment due to the low temperatures, limited availability of nutrients such as nitrogen and phosphorous salts, and because a large compounds in the marine environment. Therefore, original marine bacteria with

surfactant-producing properties are helpful for eliminating pollutant in marine environment.

The objectives of the study, therefore, include: 1) screening surfactant producing bacteria, 2) preliminary characterization of surfactant type, and 3) bioactivities and properties of the surfactant. These fundamental information are essential for clarification of their physiological roles in the natural environment and for their practical application in marine field.

Materials and Methods

Screening for Biosurfactant Producing Bacteria. Water samples were collected from Jakarta bay and its surroundings, and Seribu Island (Jakarta) which often got the oil-spill pollution from domestic, industrial waste run off, and shipping activities. The bacteria were isolated by enrichment methods for crude oil and PAH (polycyclic aromatic hydrocarbon) degradaters in liquid medium. Isolation, purification and screening were done by the sublimation techniques (Alley & Brown, 2000). Sampling and streaks were conducted in sequences of 0, 3, 7, 14, 21 and 30 days. The single colonies were chosen for further observations, for surfactant assay. Positive candidates were used for further studies.

16S rRNA Gene Sequence Determination. Total genomic DNA was isolated from late-exponential-phase cells in marine agar medium containng 1000 ppm (w/w) Arabian crude oil using kit of quick-qiagen. PCR amplifcation of 16S rRNA genes was obtained using the forward primer 16F27 (5'-AGAGTTTGATCMTGGCTCAG-3') and the reverse primer 16R1492 (5'-TACGGYTACCTTGTTACGACTT-3'). Direct sequence determination of PCR-amplifed DNA was carried out using an Automated DNA Sequencer and Taq cycle sequencing reactions according to the protocols of the manufacturer (Perkin-Elmer, Applied Biosystems, 2005). Sequence data were aligned initially with 16S rRNA and rDNA sequences using the e-mail servers at the Ribosomal Database Project (RDP) of Clustal-x program (www.ebi.ac.uk/clustalw). Phylogenetic relationships were estimated using NJ plot of the Phylogeny Inference Package (Phylip version 3.4, 2005). Homologies were searched by gene-bank database of NCBI (www.ncbi.nlm.nih.gov).

Characterization and Determination of Surfactant Properties. The supernatant of cultures were evaluated for their characters and compositions. The surface active tension and interfacial tension were analyzed using the drop shape analysis (DSA) equipment (Kruss DSA100, 2006, Germany). The emulsification activity was done following the Navon-Vanezia methods (1995). The sugar content was determined by total sugar analysis using phenol-sulfuric assay (Taylor, 1995), lipid/fat was analyzed by Bligh-Dryer methods (1956), and protein was determined by Bradford (1976).

Assay for the Biodegradation of Hydrocarbon and Crude Oil. Analysis of hydrocarbon and crude oil recovery was performed by Gas Chromatography/mass spectrophotometry (GC/MS) with a 6890/5973 instrument using PETRSCA1 methods as described by Harayama *et al*. (1996). The 2 ml cell cultures were extracted by 3 ml dichloromethane (Merck, USA) followed by shaking for a few minutes and degassing. Further, the shaking reciprocally for 2 min and stand for few minutes, the lower phase was transferred into a new tube, and the extraction was repeated until the yellow phase disappears. A spoonful of sodium sulfate (Merck, USA) was applied for removing the water followed by concentration under nitrogen gas stream to a final volume of about 250 µL and then transferred into GC-vial, the samples were ready for measurement in the GC/MS. HP19091B-102 with silica column was used with the following conditions: initial oven temperature 250°C, injection temperature 320°C, flow rate of the mobile phase 54.1 mL/min and running time 50 min.

Preliminary Test for *ex situ* Application. The sand coated with Arabian crude oil was prepared for the sand-beach simulation. The treated sand was added into the sea water (negative control), sea-water containing broth cultures of selected strains, and positive control of sea water containing Tween 2%. Gentle shaking was employed while crude oil disappearance was observed by visual and GC/MS analysis. While an treatment of polluted soil was prepare by mixing the soil

with arabian crude oil. The treated soil was added with whole selected cultures and the disappearance of crude oil was analyses by GC. In addition the texture of soil was performed by the character of soil porosity and their form. *Alcanivorax*, *Pseudomonas* and *Lysobacter* were mixed in ratio 1:1:1 volume of cultures after 24 h incubation.

Results and Discussion

Recently, there is an increasing interest on safe and clean products for sustainable development. In this respect, biological production systems using microorganisms have distinct advantages, since they are biodegradable. Surfactants produced or synthesized by microbes- biosurfactants have met the expectation because they are environmental friendly and do not generate pollutants. To explore the biodiversity of biosurfactant-producing microorganisms of Indonesian marine environments, study on the synthesis, characterization and function of these compounds compared to the synthetic one (Tween) was conducted.

Screening of Marine Biosurfacant-Producing and Hydrocarbon-Degrading Bacteria

First isolation step from natural samples were focused on the oil-degrading bacteria only by using specific media treatment strategy. Thereafter, during the characterization and screening the function of isolates there are several bacteria seems producing the surfactant appearing by clear off the addition oil. Using the quick screening by plate assay technique a total of 103 isolates out of 463 tested had been obtained. Out of these 103 isolates, 57 isolates showed double capability for hydrocarbon removal and surfactant production based on the speed and size of halo formation (Table 1). This indicates the strength of surfactant properties such as the surface active or interfacial tensions activity. They should have correlation with the surfactant function towards the hydrocarbon compounds, for instance in breaking the hydrocarbon chain, lowering surface and interfacial tensions, enlarge the movement area of the bacteria or to make soluble the compounds into water phase. There is an interaction of biosurfactant with the bacterial cells. In the biodegradation works, there is a tendency they follow two mechanisms: first, it can solubilize hydrophobic compounds within micelle structures, effectively increasing the apparent aqueous solubility of the organic compound and its availability for uptake by cells. Second, it can cause the cell surface to become hydrophobic, thereby increasing the association of the cell with slightly soluble substrate (Pembrey *et al*., 1999; Al-Tahhan *et al*., 2000).

Because the enrichment samples were conditioned for isolating degrading hydrocarbon bacteria, the majority of isolates of surfactant-producer obtained play an important role for the *Alcanivorax, Stapia, Pseudomonas, Bacillus, Bordetella, Lysobacter* and *Acenitobacter* (Table 1). In this regard, the phenomenon was revealed by strong elimination of oil-smeared capability and probability of several agar plate that have the occurrences. Some of the isolates hydrocarbon degradation such as were capable for eliminating crude oil and remove several PAH compounds. *Alcanivorax* is a ubiquitous typical marine petroleum oil-degrading bacterium with an unusual physiology specialized for alkane metabolism. This hydrocarbonoclastic bacterium degrades an exceptionally broad range of alkane hydrocarbons but few other substrates. *Pseudomonas*, *Bacillus*, and *Acenitobacter* are famous for the ability to synthesize the extracellular compounds around the cells, which possibly polysaccharides, lipid and protein which have properties as surfactant-like depending on the substrate sources and the environment they are living (Noordman *et al*., 2002; Bodour *et al*., 2003). On the other hand, *Stappia*, *Bordetella*, and *Lysobacter* are not reported as a surfactant producer bacteria. *Stappia* is a gram negative bacteria that capable for ring-cleaving dioxygenase-producing bacteria and for CO-oxiding bacteria (Pujalte *et al*., 2005). Whereas *Bordetella* is a genus of small, Gram-negative coccobacilli of the phylum *Proteobacteria*. *Bordetella* species, with the exception of *Bordetella petrii*, are obligate aerobes, as well as highly fastidious, or difficult to culture and reported as a contagious bacteria (Staveley, 2003). *Lysobacter* is gram negative gliding bacteria that has potency as biocontrol and source of anti-infectives (Wang *et al*., 2013). Further study on the surfactant excretion

capability, several (around 66) selected isolates were examined.

Surfactant Properties of Selected Isolates

The surfactant was defined by their surface active molecules which exhibited the emulsifying activity, reducing surface and interfacial tension, and their critical micelle concentration. In these researches surface active tension, interfacial tension and emulsifying activities are observed. Surface active tension of the surfactant compound is important for attachment surface media between two or more substances. i.e. oil surface and water surface. Surface active tension values of selected isolates were variable from 40 mN/m to 65 mN/m, and water tension of 74 mN/m (Figure 1). Some of the highest activity was shown by *Pseudomonas* since the strains possess the ability to synthesize various surfactants such as rhamnolipid, in their late-exponential and stationary phase of growth under the limiting of nitrogen and iron concentration (Oschner & Reiser, 1995). Those value were some cases lower when compare to the activity of synthetic surfactant (Tween). In concentration of 20% v/v Tween could reduce the water tension up to 40 mN/m. However, the bacterial surfactants possibly were less toxic than the Tween and have selective functions.

The interfacial tension observations of selected strains (15 strains) that performed the strong activities of surface active tension were exhibited relatively comparable to the synthetic surfactant of 1-20% v/v Tween (Figure 2A). Surfactants that lower interfacial tension are particularly effective in mobilizing bound hydrophobic molecules and making them available for biodegradation. It is suggested that surfactant synthesized by *Pseudomonas* and *Bacillus* have low-molecular-weight, of low critical micelle concentration for increasing the apparent solubility of hydrocarbon in hydrophobic cavities of micelles (Ron & Rosenberg, 2001; Bodour *et al*., 2003).

The hydrocarbon emulsifying activities of the selected isolates were comparable to the chemical surfactant of 0.1-20% v/v Tween, i.e. around 0.04-0.28 units. The highest activities were shown in groups of *Bordetella*, *Pseudomonas* and *Alcanivorax* (Figure 2B). Emulsifying activities is indicating that the compound capable for emulsifying others substances, such as preservatives agent that require a complex compound. Those results suggest that these groups of bacteria may synthesize the high-molecular-weight biosurfactants. The exocellular polymeric surfactant composed of polysaccharides, proteins, lipopolysaccharides, peptides, lipoproteins and their mixtures. These complex of biopolymers were possibly produced by bacteria (Lin *et al*., 1994; Oschner & Reiser, 1995; Al Tahhan *et al*., 2002).

Monitoring of Hydrocarbon Removal of Selected Cultures

Most of the selected strains could remove crude oil with a recovery rate of 60-90% of the base concentration. Some of them can remove long chain alkanes, several PAH in a rate of 20-80% and branch alkanes with a rate of 2-20%, respectively (Table 2). These showed the specific function of each strain or isolate some seem to have function for emulsifying but does not degrade it others tend to degrade a particular of long chain alkanes, PAH or branched alkanes, while others have no emulsifying nor degrading hydrocarbon activity, just producing biosurfactants. Those phenomena are exhibited interesting cases, although, the bacteria same genus or species however they produce different compounds and function, might be due to differences of their function in natural habitat. Major physiological role of biosurfactant is to permit microorganisms to grow in water immiscible substrates by reducing the surface tension at the phase boundary, thus making the substrate more readily available for uptake and metabolised. In addition, biosurfactant may also be involved in the adhesion of the microbial cells into the hydrocarbon substrates in the waters (Sugiura, 1997; Ron & Rosenberg, 2001).

Preliminary Characterization of the Surfactant Products

Characterization of surfactant compound was used the selected strain of *Alcanivorax* and *Lysobacter* that have high excretion amount of surfactant, strongly eliminate the crude oil and could consuming hydrocarbon in wide range. Preliminary characterization results showed that some of the surfactant compounds positively contain lipids, proteins and sugars or their mixture (Table 3). These indicated that various types of surfactants were

obtained only from one genus. Further studies on their specificity such as their chemical structures and function in nature, particularly in the biodegradation of hydrocarbon in marine environment, should be done.

Table 2. Monitoring the recovery rate of selected strains by GC/MS determination

No.	Isolate	Genus/Species Name	Recovery Rate (%)	
			Remaining concentration of Alkanes	Remaining concentration of PAHs
1	ID05-RI-506	*Nocardioides alkalitolerans*	NS	70-80
2	ID05-RI-509	*Alcanivorax dieselolei*	C13-C34 (18-83)	70-80
3	ID05-RI-518	*Alcanivorax dieselolei*	C13-C34 (9-62)	67-80
4	ID05-RI-520	*Alcanivorax dieselolei*	C13-C32 (23-65)	70-80
5	ID05-RI-521	*Alcanivorax dieselolei*	NS	NS
6	ID05-RI-531	*Alcanivorax dieselolei*	C13-C32 (6-36)	70-80
7	ID05-RI-545	*Alcanivorax dieselolei*	C13-C34 (3-71)	NS
8	ID05-RI-549	*Alcanivorax dieselolei*	C13-C34 (6-67)	NS
9	ID06-RI-571	*Pseudomonas aeruginosa*	NS	NS
10	ID06-RI-581	*Lysobacter* sp.	NS	NS
11	ID06-RI-636	*Cytophaga* sp.	NS	NS
12	ID06-RI-1070	*Pseudomonas aeruginosa*	C13-C33 (47-75)	Naphtalene (15)
13	ID06-RI-1072	*Pseudomonas aeruginosa*	C13-C37 (2-72)	22-80
14	ID06-RI-1079	*Bacillus pumilus*	NS	NS
15	ID06-RI-1086	*Alcanivorax* sp.	C13-C31 (12-33)	NS
16	ID06-RI-1088	*Bordetella petrii*	C13-C32 (12-71)	NS
17	ID06-RI-1091	*Pseudomonas aeruginosa*	C13-C33 (12-33)	NS
18	ID06-RI-1100	*Alcanivorax* sp.	C13-C34 (5-61)	NS
19	ID06-RI-1102	*Pseudidiomarina taiwanensis*	C13-C33 (6-56)	NS
20	ID06-RI-1145	*Halomonas* sp.	NS	NS

Note: NS (Not significant), C (Carbon chain)

Table 3. Preliminary assay for determination the biosurfactants from genus *Alcanivorax* and *Lysobacter*

Strain	Assays		
	Carbohydrate content (ppm)	Protein content (ppm)	Lipid content (%/dry weight biomass)
Alcanivorax dieselolei ID05-IR-509	22.1875	226.8182	0.955
Alcanivorax dieselolei ID05-RI-518	16.1458	140.4545	-
Alcanivorax dieselolei ID05-RI-531	16.5938	340.4545	0.71
Alcanivorax dieselolei ID05-RI-532	21.3958	122.2727	0.39
Alcanivorax dieselolei ID05-RI-545	33.4688	167.7273	-
Alcanivorax dieselolei ID05-RI-549	16.2813	213.1818	0.275
Lysobacter sp. ID06-RI-581	13.9688	99.5455	-
Alcanivorax sp. ID06-RI-1055	7.4479	135.9091	0.7

Preliminary Test for *ex situ* Application

Further study on application of surfactant product obtained from selected bacteria on remediation process has conducted. In this preliminary test, the cells number of bacteria were ignored, the observed parameters are time removal of oil pollutant and the alkenes contain in both model of marine water and soil. Around one or two weeks incubation, crude oil pollutant was disappear in both of treated sea water and soil by mixture of *Alcanivorax* (ID05-RI-545), *Pseudomonas* (ID06-RI-580) and *Lysobacter* (ID06-RI-581). The remaining concentration of alkenes substances also significantly eliminate from the media compared to the control of treated sand or soil only, almost comparable with tween 2% ability. The results also indicated that removal oil in the treated soil with synthetic surfactant of tween (2%) was only remove the oil substances however the alkenes compound did not remove yet (Table 4). Evaluation the capability of microbes for soil and sea water reclamation needs various

parameters such as nitrogen content, dissolved oxygen, solid suspended substances, soil characters, water current and other environmental factors (Lefrancois *et al*., 2010; Masciandaro *et al*., 2014; and Sumiardi *et al*., 2012).

These studies exhibited that diversity of marine oil degrading bacteria in tropical areas has a wide range of genus or species with specific function for degrade the hydrocarbon compounds. Some of bacteria have function for eliminating the long chain hydrocarbon (PAHs), remove the alkenes pollutant and surfactant producer. In the model application of reducing the oil pollutant in marine water and soil, exhibited that whole cultures treatment significantly capable remove the pollutant. Bacteria community has shaped the subsequent development of life on earth ever since their first appearance, the metabolic processes that they carry out in the transformation of elements, degradation of organic matter, and recycling of nutrients play a central role in innumerable activities that affect the support and maintenance of all other forms of life.

Table 4. Preliminary test on remediation crude oil pollutant using oil degrading bacterial agent (mixture of *Alcanivorax* (ID05-RI-545), *Pseudomonas* (ID06-RI-580), and *Lysobacter* (ID06-RI-581))

Subject	Treatment	Time of oil elimination (days)	Remaining concentration of Alkenes (%)
A. Marine water	1. Treated Sand, sea water	30~	80-90
	2. Treated sand, sea water, bacteria broth cultures	14-17	17-28
	3. Treated sand, sea water, tween 2 % (total concentration)	May 11, 2014	Sep 16, 2014
B. Soil	1. Treated soil	60~ (soil remain in aggregate form)	73-81
	2. Treated soil, bacteria broth cultures	15-21 (soil became more sandy)	26-33
	3. Treated soil, tween 2% (total concentration)	11-25 (soil became more sandy)	56-75

Conclusion

Surfactant-producing bacteria were isolated from Indonesian marine environment and characterized by their capability of degrading hydrocarbon and PAH compounds (60 out of 103 strains). Evaluation on surfactant properties showed various types of surfactant were isolated, for instance lipid-, protein-, carbohydrates-containing or their mixtures. Analysis showed that some of these surfactants were able to eliminate hydrocarbon, PAH or both. It is suggested that each strain and its surfactant production potential have specific functions in marine environment. Further elucidation and psychochemical analyses of each surfactant are needed to find out their functions in oil bioremediation in nature. Simulation the bacterial consortia for eliminating the oil pollutant in the marine and soil areas also exhibited the mixture of consortia could degrade the alkenes compound in shorter time than control.

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Table 1. Plate assay for oil elimination and information of selected isolates

No	Code number	Private number	Sources	Origin Area	Plate assay	BLAST search result of 16S rRNA gene sequence	Homology (%)
1	ID05-RI-501	ID05-P-001Bt	Gravel	Pari islands	***	AY639889.1 *Stappia aggregata* strain CHLG 11	100
2	ID05-RI-502	ID05-P-002Bt	Gravel	Pari islands	*	DQ831000.1 *Novosphingobium* sp. FND-3	95
3	ID05-RI-503	ID05-P-003Bt	Gravel	Pari islands	*	DQ831000.1 *Novosphingobium* sp. FND-3	95
4	ID05-RI-504	ID05-P-004Bt	Gravel	Pari islands	*	AY621063.1 *Pseudoalteromonas* sp. NJ6-3-1	100
5	ID05-RI-505	ID05-P-005Bt	Gravel	Pari islands	*	DQ659435.1 *Thalassospira* sp. DBT-2	99
6	ID05-RI-506	ID05-P-006Bt	Gravel	Pari islands	*	AY633972.1 *Nocardioides alkalitolerans* strain KSL-12	96
7	ID05-RI-509	ID05-P-009Bt	Enrichment napthalene	Pari islands	***	AY683531.1 *Alcanivorax dieselolei* strain NO1A clone 1	99
8	ID05-RI-510	ID05-P-010Bt	Enrichment napthalene	Pari islands	*	AB189306.1 *Chromohalobacter* sp. IS-Ch1	100
9	ID05-RI-511	ID05-P-011Bt	Enrichment napthalene	Pari islands	*	DQ659435.1 *Thalassospira* sp. DBT-2 16S	99
10	ID05-RI-512	ID05-P-012Bt	Enrichment napthalene	Pari islands	*	AY683531.1 *Alcanivorax dieselolei* strain NO1A clone 1	99
11	ID05-RI-518	ID05-P-018Bt	Enrichment phenotiazine	Pari islands	***	AY683537.1 *Alcanivorax dieselolei* strain B-5	99
12	ID05-RI-519	ID05-P-019Bt	Enrichment phenotiazine	Pari islands	*	AY683537. *Alcanivorax dieselolei* strain B-5	99
13	ID05-RI-520	ID05-P-020Bt	Enrichment phenotiazine	Pari islands	***	AY683537.1 *Alcanivorax dieselolei* strain B-5	99
14	ID05-RI-521	ID05-P-021Bt	Enrichment phenotiazine	Pari islands	*	AY683531.1 *Alcanivorax dieselolei* strain NO1A	99
15	ID05-RI-522	ID05-P-022Bt	Enrichment phenotiazine	Pari islands	*	AY683531.1 *Alcanivorax dieselolei* strain NO1A	99
16	ID05-RI-523	ID05-P-023Bt	Enrichment dimethylnaphtalene	Pari islands	***	AY639889.1 *Stappia aggregata* strain CHLG 11	100
17	ID05-RI-524	ID05-P-024Bt	Enrichment dimethylnaphtalene	Pari islands	*	AY639889.1 *Stappia aggregata* strain CHLG 11	100
18	ID05-RI-525	ID05-P-025Bt	Enrichment dimethylnaphtalene	Pari islands	*	AY683531.1 *Alcanivorax dieselolei* strain NO1A clone 1	99
19	ID05-RI-526	ID05-P-026Bt	Enrichment dimethylnaphtalene	Pari islands	*	AY445074.1 *Muricauda flavescens* strain SW-74	98
20	ID05-RI-529	ID05-P-029Bt	Enrichment penanthrene	Pari islands	*	AY683531.1 *Alcanivorax dieselolei* strain NO1A	100
21	ID05-RI-530	ID05-P-030Bt	Enrichment penanthrene	Pari islands	*	AY526861.1 *Idiomarina fontislapidosi*	99
22	ID05-RI-531	ID05-P-031Bt	Enrichment penanthrene	Pari islands	***	AY683531.1 *Alcanivorax dieselolei* strain NO1A	99
23	ID05-RI-532	ID05-P-032Bt	Enrichment penanthrene	Pari islands	***	AY683531.1 *Alcanivorax dieselolei* strain NO1A	99
24	ID05-RI-538	ID05-P-038Bt	Enrichment penanthrene	Pari islands	*	AY683531.1 *Alcanivorax dieselolei* strain NO1A	99
25	ID05-RI-545	ID05-P-045Bt	Enrichment penanthrene	Pari islands	***	AY683531.1 *Alcanivorax dieselolei* strain NO1A clone 1	99
26	ID05-RI-546	ID05-P-046Bt	Enrichment penanthrene	Pari islands	*	AY683537.1 *Alcanivorax dieselolei* strain B-5	99
27	ID05-RI-547	ID05-P-047Bt	Enrichment penanthrene	Pari islands	*	AY669169.1 *Marinobacter aquaeolei*	99
28	ID05-RI-548	ID05-P-048Bt	Enrichment penanthrene	Pari islands	*	AY683531.1 *Alcanivorax dieselolei* strain NO1A	100
29	ID05-RI-549	ID05-P-049Bt	Enrichment penanthrene	Pari islands	***	AY683531.1 *Alcanivorax dieselolei* strain NO1A	99
30	ID05-RI-550	ID05-P-050Bt	Enrichment penanthrene	Pari islands	*	AY669169.1 *Marinobacter aquaeolei* isolate OC-9	99
31	ID05-RI-551	ID05-P-051Bt	Enrichment penanthrene	Pari islands	*	AY683531.1 *Alcanivorax dieselolei* strain NO1A	99
32	ID05-RI-553	ID05-P-053Bt	Sea water	Pari islands	*	AM294944.1 *Thalassospira lucentensis*	99

No	Code number	Private number	Sources	Origin Area	Plate assay	BLAST search result of 16S rRNA gene sequence	Homology (%)
33	ID05-RI-555	ID05-P-055Bt	Enrichment crude oil	Pari islands	*	DQ312361.1 *Erythrobacter* sp. GY-2	99
34	ID05-RI-556	ID05-P-056Bt	Enrichment crude oil	Pari islands	*	AY683531.1 *Alcanivorax dieselolei* strain NO1A	100
35	ID05-RI-563	ID05-P-063Bt	Enrichment penanthrene	Pari islands	*	AY647305.1 *Haererehalobacter ostenderis* strain MSU3710	98
36	ID05-RI-568	ID05-P-068Bt	Enrichment penanthrene	Pari islands	*	AY683531.1 *Alcanivorax dieselolei* strain NO1A	100
37	ID06-RI-570	ID06-KWF.3Bt	Enrichment Fluoranthene	Kamal bay	***	AJ295007.1 *Acinetobacter venetianus*	98
38	ID06-RI-571	ID06-KWF.4Bt	Enrichment Fluoranthene	Kamal bay	***	|DQ493895.1 *Pseudomonas aeruginosa* strain XJU-1	99
39	ID06-RI-580	ID06-KWF.13Bt	Enrichment Fluoranthene	Kamal bay	***	DQ666628.1 *Pseudomonas aeruginosa* strain RsB-29	96
40	ID06-RI-581	ID06-KWD.14Bt	Enrichment dibenzothiophene	Kamal bay	***	AM111012.1 *Lysobacter* sp.	96
41	ID06-RI-636	ID06-KWPh.104Bt	Enrichment penanthrene	Kamal bay	*	AB188214.1 Cytophaga sp. TUT1213	99
42	ID06-RI-651	ID06-KWPh.44Bt	Enrichment penanthrene	Kamal bay	*	U82826.1 *Castellaniella denitrificans* strain NKNTAU	98
43	ID06-RI-669	ID06-KWPh.139Bt	Enrichment penanthrene	Kamal bay	*	AB188214.1 *Cytophaga* sp. TUT1213	99
44	ID06-RI-679	ID06-BWPh.13Bt	Enrichment penanthrene	Bogasari Beach	*	DQ854982.1 *Bacillus* sp.	100
45	ID06-RI-690	ID06-BWPh.30Bt	Enrichment penanthrene	Bogasari Beach	*	DQ837546.1 *Pseudomonas aeruginosa* strain XJU-2	100
46	ID0 RI-709	ID06-BWPy.2Bt	Sea water	Bogasari Beach	*	AY707779.1 *Aerococcus viridans* strain ATCC 700406	99
47	ID06-RI-1066	ID06-070O	Enrichment crude oil	Marina	*	AB055207.1 *Alcanivorax* sp. TE-9 gene	99
48	ID06-RI-1070	ID06-074O	Enrichment crude oil	Marina	***	AE004091.2 *Pseudomonas aeruginosa* PAO1	100
49	ID06-RI-1072	ID06-076O	Enrichment crude oil	Marina	***	DQ837546.1 *Pseudomonas aeruginosa* strain XJU-2	100
50	ID06-RI-1079	ID06-083O	Enrichment crude oil	Jak-Bay 2	***	DQ523500.1 *Bacillus pumilus* strain B402	99
51	ID06-RI-1080	ID06-084O	Sediment	Muara Baru	**	AY669169.1 Marinobacter aquaeolei isolate OC-9	99
52	ID06-RI-1081	ID06-085O	Sand	Pari islands	***	DQ523500.1 *Bacillus pumilus* strain B402	99
53	ID06-RI-1082	ID06-086O	Sand	Pari islands	***	AB242987.1 *Bacillus* sp. Pd-E-(s)-l-D-8(3)	99
54	ID06-RI-1085	ID06-089O	Enrichment crude oil	Jak-Bay 1	*	AY394865.1 *Alcanivorax* sp. EPR 6	99
55	ID06-RI-1088	ID06-093O	Enrichment crude oil	Pari	***	AJ870969.1 *Bordetella petrii*	99
56	ID06-RI-1102	ID06-109O (pyr 60)	Enrichment penanthrene (active eliminate pyrene)	Pramuka	*	DQ118948.1 *Pseudidiomarina port taiwanensis* strain PIT1	97
57	ID06-RI-1145	ID06-164O (phe 107)	Enrichment dibenzothiophene (active eliminate phenanthrene)	Jak-Bay 2	*	AB167060.1 *Halomonas* sp. SB J85	98

Abbreviation: BWPh (Bogasari Water Sublimation by phenantrhene), BWD (Bogasari Water Sublimation by Dibenzothiophene), Bt (Biotechnology), CO (crude oil), ID (Identity), JB1 (Jakarta Bay 1), JB2 (Jakarta Bay 2), KWF (Kamal Sea Water Sublimation by Fluorene), KWD (Kamal Sea Water Sublimation by Dibenzothiophene), KWPh (Kamal Sea Water Sublimation by Phenanthrene), P (Pari Island), Ph/PHE (Phenanthrene), RI (Republic Indonesia), O (Oceanography). Jak, Jakarta

Notes: *, low strengthness; **, medium strengthness; ***, high strengthness

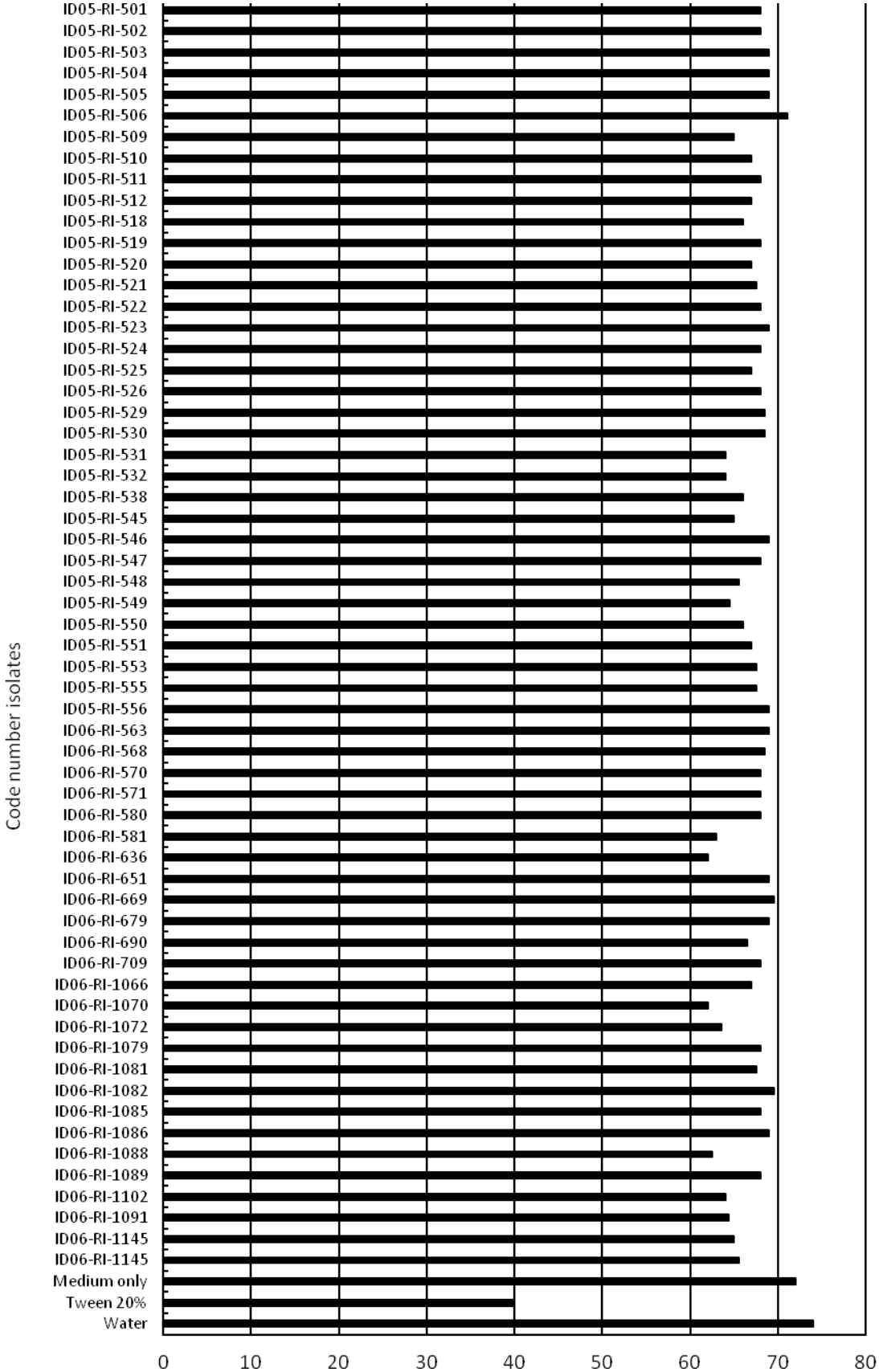

Figure 1. Surface tension activities of selected isolates
Notes: values are average of triplicates surface active tension measurements

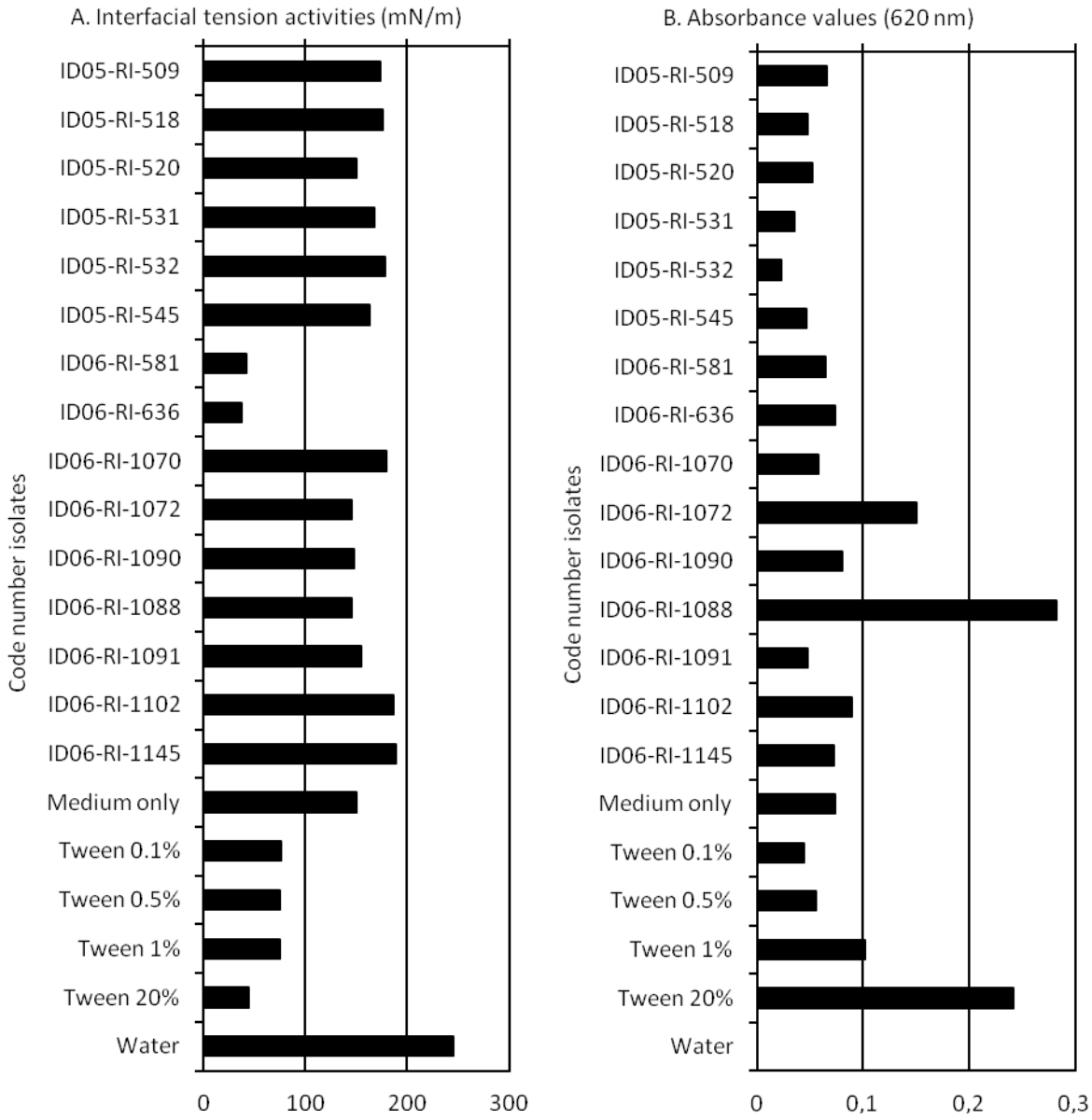

Figure 2. Surface tension activities (A) and emulsifying activities (B) of selected isolates
Notes: values are average of triplicates interfacial active tension measurements