ISOLATION OF BIOSURFACTANTPRODUCER BACTERIA FROM CENTRAL MUD TREATMENT FACILITY (CMTF) PT. BUMI SIAK PUSAKO, RIAU PROVINCE

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ABSTRACT

A study aims to find out and isolate biosurfactant producer bacteria originated from the Central Mud Treatment Facilities of the PT. Bumi Siak Pusako, Riau has been conducted by the end of 2008. Bacteria samples were isolated from mud and water samples. Sampling was conducted at once, in 2 sampling sites. The bacteria were then cultured using TSA, blood agar and MSM media. The presence of biosurfactant was tested using a Haemolysis Blood Agar Test (HBAT) and Index Emulsification Test (IET). Results is shown that there were 16 isolates, 8 in the mud and the other are in the water samples. Results of the IET indicate that all isolates have various level of biosurfactant production. There are 4 isolates that have 100% IET values, namely S_1AI_2 andS_2AI_2originated from water sample and S_1I_4 and S_2I_3 originated from soil sample. Using the HBAT, however, only 5 isolates from soil sample and 6 isolates from water sample shown biosurfactant production. It can be concluded that the IET method is more accurate to detect bacteria that are able to produce biosurfactant.

Key words: biosurfactant, mud treatment, oil waste, Index Emulsification Test

INTRODUCTION

Natural oil is a potential energy resources that is crucial for human life, especially for industrial needs, transportation and household activities (Kadarwati *et al*, 2004). Exploring the natural oil, however, may create environmental related problems as oil spill is negatively affect the environment (Verania, 2002). To reduce the risk of oil related pollution and to maintain the natiral resources, a proper environmental management should be applied

During this decade, handling of oil pollution has been conducted using several method. However, the methods applied may create another problem. For example, the use of synthetic surfactant may pollute the environment as it is toxic

(Ni'matuzahroh *et al*, 2006). The use of biosurfactant is a better alternative in handling the oil pollution, as it is naturally producad by bacteria, low toxicity, *biodegradable*, naturally present in the environment and it can be obtained cheaply (Kosaric,1992).

Suryatama (2006) stated that addition of *Azotobacter chroococcum* , a surfactant producer bacteria increase 54.98% of hydrocarbon biodegradation eficiency. Ni'matuzahroh *et al* (2006) proved that *Pseudomonas aeruginosa* IA7d biosurfactant increase the petrolium biodegradation in the Tanjung Perak harbor, Surabaya, it is 67.5 % higher than that of the synthetic surfactant *Tween*-80. Bodour *et al* (2004) conduct a emulsification test toward kerosene and achieve 100% of Emulsification Index.

Efforts to find out and isolate a new strain of biosurfactant producer has been conducted. Agustiani (2004) found 23 species of thermophylic isolates in the oil wells in Rumbai Riau. Among these isolates, 15 of them showing haemolytic activities and 12 of them are biosurfactant producers. Another study, Urum *et al* (2004) also proved that rhamnolipid bacteria is able to clean 80% of crude oil spill in the land. Kofli *et al* (2000) that used *Bacillus macerans* strain TS9-8 originated from PETRONAS Malaysia oil field (Murni, 1998) find out the optimum concentration of glycerol that can be used as carbon resources for biosurfactant producers, it is 2% of total media.

Riau Province is a potential oil resource in Indonesia, however, there is few information on biosurfactant producer bacteria originated from this area, from the oil wells as well as from the Dumai harbor that is polluted by oil industrial related waste. In order to find out and to isolate biosurfactant producer bacteria, this study is conducted. Bacteria samples were collected from in the Central Mud Treatment Facility (CMTF) of PT.Bumi Siak Pusako (one of oil companies present in Riau), Zamrud, Siak Sri Indrapura Regency.

MATERIALS AND METHODS

Time and place

This research was conducted for 6 months, in the Microbiology Laboratory, Biology Department, Math and Science Faculty, Riau University, Pekanbaru, Riau.

Materials and methods

Equipments used in this study are: glassware, inoculating lope, Bunsen burner, oven, autoclave, shaker, vortex, microscope, incubator, *hot plate*, micropipette, aluminum foil, cotton and cotton cloth. While materials used in this research are *Tripton Soya Agar* (TSA) (Merck), *Blood* Agar (Merck) and Mineral *Salt* Medium (MSM) media and kerosene.

Sampling

100 grams of mud/ soil and 100 grams water that are contaminated with oil pollutant were taken from 2 waste disposal ponds located in the Central Mud Treatment Facility (CMTF) of PT.Bumi Siak Pusako, Riau. In each pond, there were 3 sampling points, but the samples were kept in altogether in a sterile glass bottle (composite sample) and it is refrigerated (4°C) prior to bacteria isolation.

Bacteria culture media preparation

In this research, there are 3 types of media used, namely Tripton Soya Agar (TSA) (Murni, 1998) as a selective media, Blood Agar Media for bacteria screening and it is used to screen bacteria with blood haemolysis ability. During the media sterilization process, defibrinated lamb blood is added when this media reach 45•C (Jenning *et al*, 2000). *Mineral Salt Medium* (MSM) is used for fermentation process. The composition of this media is NH_4NO_3 0,05 g, KH_2PO_4 0,03 g, Na_2HPO_4 0,04 g, $MgSO_4$, $CaCl_2$, $FeSO_4$, Na_2 EDTA, Yeast extract 0,1 %, and glycerol 2 % (Cooper *et al*, 1981; Kofli *et al*, 2000).

Bacteria isolation

For bacteria isolation, 1 gram of mud or 1 ml of water sample was added with 10 ml of sterile NaCl 0,85 %. The liquid was then mixed (homogenized using a vortex) and was diluted into 10^{-4}. 10^{-3} and 10^{-4} diluted samples were then inoculated in the TSA media (*pour plate* method) and were incubated in room

temperature for 24 hours. To get single strain, each bacteria colony present was isolated and cultured in TSA media and were kept in the refrigerator.

Fermentation preculture preparation

An inoculum of pure/ single strain isolate was grown in the 50 ml of MSM. This culture was then kept in the *incubatorshaker* at room temperature, 200 rpm for 24 hours. Population of bacteria colony was then counted. This pre-culture was then used for fermentation inoculum (Tabatabaee *et al* 2005).

Preparation for Emulsification Test

1 ml of pre-culture was added into 50 ml of MSM liquid that is kept in the 250 ml Erlenmeyer flask. This liquid was then shake in room temperature (200 rpm) for 3 days (Tabatabaee *et al* 2005). This culture is then used for Emulsification test.

Screening of biosurfactant producer bacteria

There are 2 methods applied to identify the biosurfactant producer bacteria, namely 1) Haemolysis Test: using a Blood Agar media. Bacteria isolate that has ability in blood haemolysis is predicted to have ability in producing biosurfactant (Jenning *et al*, 2000). 2) Emulsification test: the ability of producing biosurfactant is predicted based on the 24 hours Emulsification Index (IE_{24}) and it can be counted by using a Cooper and Goldenberg (1987) formula. High IE_{24} value means high biosurfactant produced.

Data analysis

Data were obtained by dividing bacteria into 2 groups, the biosurfactant producer and non biosurfactant producer. Data were analyzed descriptively.

RESULTS AND DISCUSSION

Results

Bacteria isolation

Pure bacteria strain obtained from mud and water samples were coded and presented in Table 1, Figure 1 and Figure 2. There were 16 isolates found, 8 from the mud and 8 from the water.

Table 1. Isolated Bacteria Strain from Mud and Water Samples,
Based on Emulsification Test (ET) and Blood Haemolysis Test (BHT)

No	Isolate codes		ET (%)		Clear zone (BHT)			
					Mud		Water	
	Mud	Water	Mud	Water	Ø (cm)	Type	Ø (cm)	Type
1	S_1I_1	S_1AI_1	77,3	32	-	-	1,7	β
2	S_1I_2	S_1AI_2	47,8	100	-	-	2,3	β
3	S_1I_3	S_1AI_4	91	37,5	-	-	-	-
4	S_1I_4	S_2AI_1	100	17,5	2,2	β	1,3	β
5	S_1I_5	S_2AI_2	82	100	1,5	α	1,2	β
6	S_2I_1	S_2AI_3	95	5	1,5	β	1	β
7	S_2I_2	S_3AI_3	80	7,5	-	-	-	-
8	S_2I_3	S_3AI_4	100	10	2,0	β	1,1	β

Note : S= Sampling site, Number: number of sampling site … ; I= Isolate, Number: isolate number …;

A: water β = clear ; α = green ; - = negative

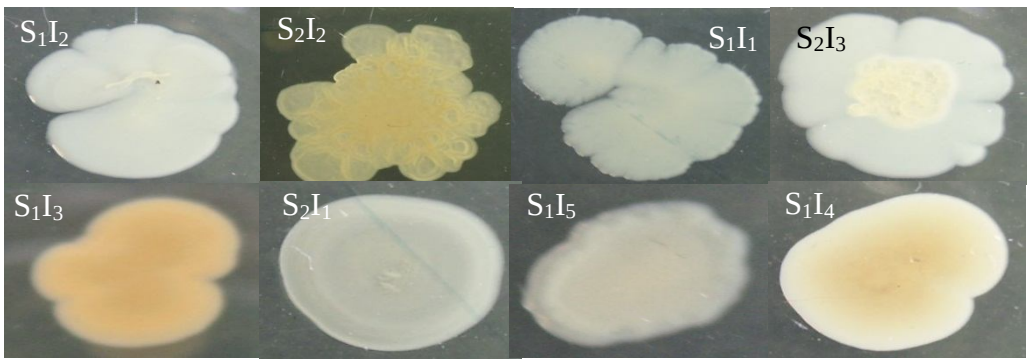


Figure 1. Bacteria Isolates from Mud Sample

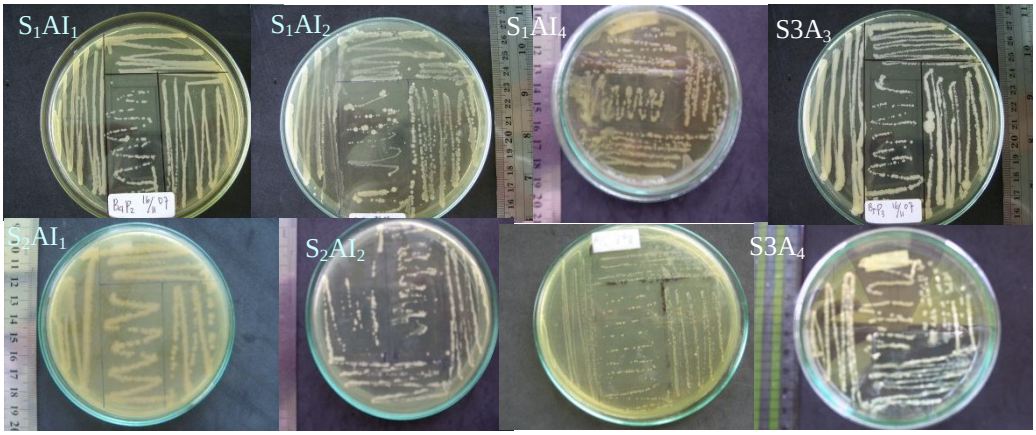


Figure 2. Bacteria isolates from water sample

Selection of Biosurfactant producer bacteria

Selection of biosurfactant producer bacteria was conducted by 2 different tests, namely the Emulsification Test (ET) and Blood Haemolysis Test (BHT). The ET is more specific in selecting the biosurfactant producer bacteria, but the BHT is the most simple test to detect bacteria that has abilty in producing biosurfactant (Jennings dan Tanner, 2000).

a. Blood Haemohysis Test (BHT)

This method aims to find out bacteria that has ability in blood haemolysis. The haemolysis activity is identified by the presence of clear zone around the colony and this zone is used as a basis to predict the ability of bacteria in producing biosurfactant (Chamanrokh *et al*, 2008). In this study, 4 isolates from mud sample and 6 isolate from water samples showing clear zone around the bacteria colony (Table 1)

b. Emulsification Test (ET)

Even though the ET process is more complicated than that of the BHT, it is able to provide more accurate results. In this method, all of bacteria isolates were fermented and then shake (200 rpm) for 3 x 24 hours prior to emulsification test. Results of ET are presented in Table 1. Different from that of the BHT, the results of ET shown that all of bacteria tested, from mud and water samples, have

ability in producing biosurfactant. The potential in producing biosurfactant, however, is various, as it is indicated by different level of biosurfactant produced.

Discussion

The number of bacteria isolates

The number of bacteria isolated from the mud and water samples is 16, 8 from each sample. Compare to a prior study, the number of bactaria isolated in this study is relatively low, Agustiani (2004) isolate 23 bacteria strains. This difference may be caused by the difference of physical factors applie during bacteria culture in the laboratory. Agustiani (2004) incubated the bacteria in the 50°C , a temperature that relatively same with the Rumbai's oil wells temperature where the sample was taken. In this study, however, the sample was taken from the area that have relatively high temperature (40 ° C), but the bacteria was incubated in room temperature (25 – 28 ° C). Due to this temperature difference, several specific bacteria may not be able to grow and as a result, the number of bacteria isolated is relatively low.

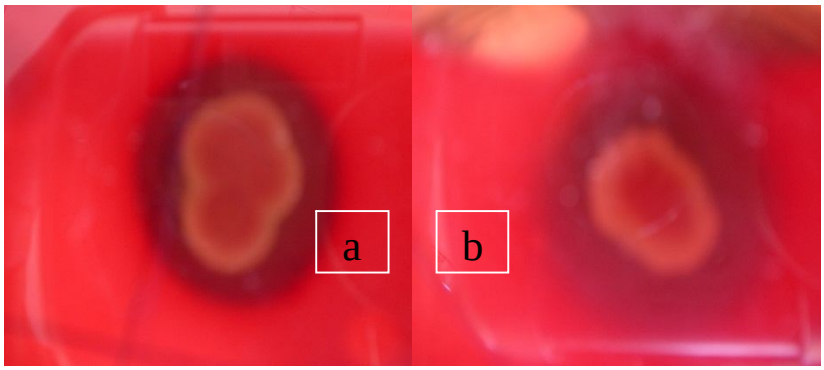
Selection of biosurfactant producer bacteria

Blood haemolysis Test (BHT)

Result of the HBT is showing that 4 isolates have clear zone around the each colony. Diameter of the S_1I_4 isolate is 2.2 cm, S_2I_3 is 2 cm, $S2I_1$ is 1.5 cm and that of the $S1I_5$ is 1.5 cm. S_1I_4, S_2I_3 and S_2I_1 isolates are grouped as beta haemolysis, as they are able to haemolyse all of red blood cells present in the media (Figure 2. a.). S_1I_5 isolate, however, is belonged to alpha haemolysis, as it is not able to haemolyse all of red blood cells available, as it is indicated by the presence of green zone around the colony (Figure 2. b.). The green color present as there is biliverdin that is formed when red blood cells are degraded (Anonim, 2007). Four other isolates are not able to haemolyse red blood present in the media, and they are grouped as "*non haemolytic bacteria*".

Results of water sample test indicated that 6 bacteria isolate shown positive signs. It means that these isolates may potential as biosurfactant producers, they are $S1AI_1$, $S1AI_2$, $S2AI_1$, $S2AI_2$, $S2AI_3$ and $S3AI_4$ .The diameter of

clear zones are 1.7; 2.3; 1.3; 1.2; 1; and 1.1 cm respectively. Two other isolates, however, are not potential for biosurfactant producers, they are $S1AI_4$ and $S1AI_3$.. Based on clear zone color, the biosurfactant producers bacteria isolated from the water sample can be categorized beta haemolysis, as they are able to degrade all of blood present in the media (Figure 2.).



According to Daud *et al* (2005) from Malaysia, the betha haemolysis bacteria may have high potential as biosurfactant producers. Ni'matuzahroh (1999) also proved that the betha haemolysis bacteria such as *Pseudomonas* sp. obtained from Surabaya waters is potential for biosurfactant producer. Moreover, Agustiani (2004) stated that among the 15 haemolysis positive isolates, 12 of them are biosurfactant producers. On the other hand, bacteria that is showing no haemolysis activity, may not be able to produce biosurfactant.

Even though 4 isolates shown haemolysis positive, other tests need to be conducted in order to prove the ability of the bacteria in producing the biosurfactant. Youssef *et. al.* (2004) stated that the HBT shown less correlation with reducing tegangan permukaan. So, the BHT method may not be sufficient for detecting the biosurfactent producer bacteria.

b.Emulsification Test (ET)

As presented in Table 1, results of the ET are different with that of the HBT. All isolates that are tested using ET method is shown positive signs or it means that all of isolate are able to produce various level of biosurfactant. The amount of stabile emulsion formed indicates the amount of biosurfactant produced. There are 4 isolates that have 100% Emulsification Index, namely

$S1I_4$and $S2I_3$ originated from mud sample and $S1AI_2$and $S2AI_2$ isolate from water sample. It means that all of hydrocarbon in the media can be emulsified properly.

Results of the BHT and ET are showing that they produce different results. Several previous studies indicates that haemolysis method may be used as a basis to early prediction of biosurfactant producer bacteria (Fatimah, 2007), (Tabatabaee, A. *et. al*, 2005). However, results of this study indicate that isolates with positive HBT results, may not produce biosurfactant. In contrast, isolate with negative BHT result may produce higher biosurfactant than those with positive result. So, it is clear that the Haemolysis can not be used as a basis to identify the biosurfactant producer bacteria. Similar results also found by Youssef, *et al.* (2004). To understand the exacts concentration of biosurfactant produced by bacteria, biosurfactant extraction should be conducted.

CONCLUSION AND SUGGESTION

Conclusion

1. There are 16 bacteria strain found in the mud and water samples obtained from Central Mud Treatment Facilities of the PT. Bumi Siak Pusako, Riau. Eight strains are found in each sample.
2. Results of 24 hours Index Emulsification Test (IE_{24}) shown that all of bacteria identified have various level of biosurfactant production. Four strains showing 100% of IE_{24}, they are S_1AI_2 and S_2AI_2 (from water sample) and S_1I_4 and S2I3 (from mud sample).
3. Blood Haemolysis Method may not sufficient for detecting biosurfactant producer bacteria. However, isolate with beta (β) positive result are able to produce biosurfactant.

Suggestion

As there are four isolates potential for producing biosurfactant and biosurfactant detection in this study is conducted by qualitative method only, a quantitative test should be conducted for detecting the concentration of biosurfactant produced.

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