ISOLATION AND CHARACTERIZATION of BACTERIA DEGRADING PYRENE from PORT PAOTERE

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ABSTRACT

The aim of this study was to obtain bacterial isolates originating from Port Paotere waters capable of degrading pyrene compound. From the results of the isolation and molecular characterization of the 16S rRNA gene was obtained 3 types of bacteria is Alcaligenes faecalis to isolate A, Sphingobacterium sp. to isolate B, Bacillus Cereus to isolate C. BLAST results for the third isolates achieve a high degree of similarity genus, reaching 99%.

Keywords: Polycyclic Aromatic Hydrocarbons (HAP), pyrene, isolation of bacteria, biodegradation

1. INTRODUCTION

Oil is one of the major energy sources for industry, transport and households [4]. Industrial activity, both located near the coast or in the offshore area has increased lately, this could increase pollution in the sea. These activities include drilling, refining, production and transportation process that can produce waste oil in the ground or at sea [7].

Paotere is the largest fish auction center in Makassar. Loading and unloading activities of fishing vessels has the potential to increase the discharge vessel, particularly those containing oil, causing the oil waste around the coast. One cause damage to coastal ecosystems is caused by oil spills polluting because they contain compounds that Polycyclic Aromatic Hydrocarbons (PAH). As a pollutant, PAH whereabouts should always be monitored because it can cause mutation of genetic material and cause cancer [5].

Polycyclic Aromatic Hydrocarbons (PAH) is a contaminant that is often found at sea in relatively high concentrations compared to anthropogenic input [1]. The presence of Polycyclic Aromatic compounds Hidokarbon (PAH) in the environment pose a serious threat to health, because it is toxic, mutagenic karsiogenik and [11]. One PAH compounds that are carcinogens that pyrene. PAH pyrene is a compound commonly found as pollutants in air, water and soil, are carcinogenic and harmful to health and can increase the risk of skin cancer and damage to the lungs [10]. LH Decree 128 in 2003 to enter pyrene as a list of contaminants. US Environmental Protection Agency (EPA) sets pyrene as a very dangerous and toxic substances [3].

Patient management efforts in the environment can be done biologically, that bioremediation using microbial degradation potential pyrene. One technique that bioremediation is biodegradation process of decomposition by microbial activity resulting in the transformation of the structure of a compound that changes the molecular integrity and toxicity of these compounds is reduced or becomes toxic at all [7].

In Indonesia research on search pyrene degrading bacterial isolates still limited. Based on the above background, it will be carried out research on "Isolation and Characterization of Compounds Degrading Bacteria Piren of Water Port Paotere".

2. METHOD

2.1 Sampling

The research sample of sea water in the form of waste are taken at the fish auction Paotere Makassar harbor waters.

Seawater samples taken at three points each taking as much as 500 mL of the surface, put into glass bottles and labeled. The sample is introduced into the cooler. Before the water samples were taken, the first measurement is pH, temperature and salinity.

2.2 Enrichment Degrading Bacteria

Enrichment of degrading bacteria was performed using selective media in aseptic. Selective media was made of 0.4 g peptone, yeast extract 0.2 g, was dissolved in 1000 mL of sterile sea water. Selective media were homogenized, sterilized and supplemented with 50 mg/L pyrene. Media then poured into the erlenmeyer each volume 25 mL. This medium is used for the enrichment of degrading bacteria first stage, the second and third.

Enrichment first stage is done by adding 25 mL samples of sea water taken from polluted waters at the fish auction harbor waters Paotere, entered into erlenmeyer containing selective medium and incubated in a rotary shaker at room temperature for 7 x 24 hours with a speed of 150 rpm. Before and after the incubation period, was observed OD (optical density) at a wavelength of 600 nm. The controls on enrichment first stage is minimal media without addition of seawater samples.

The next stage is the second stage of enrichment. Inoculum source is the first stage of enrichment seven days old. Bacteria cultures taken 25 mL and cultured into erlenmeyer containing 25 ml of minimal new media. Bacterial growth at this stage is also detected as the first stage. The last stage is the third stage of enrichment. The method used is equal to the second enrichment and a source of inoculum used was the second enrichment stage aged 7 days [7].

2.3 Bacterial Isolation and Characterization of Bacteria

Isolation of bacteria was conducted using pour (pour plate) aseptically. Source isolates originating from the third stage of enrichment. The medium used is a medium PCA (5.0 g peptone, 2.5 g of yeast extract, 1.0 g of glucose and 14.0 g that in 1000 ml of sterile distilled water) that has been sterilized. Petri dish containing bacterial isolates were then incubated in an incubator at 37°C for 2 x 24 hours.

Single isolates that grow on the surface of the media to the new media purified by the method of scratch and incubated 1 x 24 hours. Purification is done gradually to isolate pure cultures obtained through microscopic observation [7].

Bacteria are identified by the character biokimiawinya in stages (step ways) according to the dichotomous key Bergey's manual of determinative bacteriology. Isolates were used for biochemical tests is pure culture and incubated at 37°C for 1 x 24 hrs., Then performed a DNA analysis using PCR.

2.4 Degrading Bacteria Growth Piren

Isolate pure already characterized allowed back in the media. Previously created first starter 1 x 24 hours old. Taken 10 mL starter included in 100 mL of medium and incubated in a rotary shaker at room temperature for 7 x 24 hours. During the incubation period, the growth of isolates measured by optical density at a wavelength of 600 nm every 24 hours. There are two control at this stage, control (+) and control (-). Control (+) is a bacterial isolates were grown on selective media without pyrene. While the control (-) is minimal media with pyrene, but without bacteria. Results of measurement of growth compared to the control (+) and (-) is then used to create a growth curve [2].

3. RESULTS AND DISCUSSION

3.1 Enrichment Degrading Bacteria

The growth of bacteria in week 1, 2 and 3 are shown in Table 1 where a decline OD (Optical Density) from week 1 to week 3. This shows that the bacteria in the media has been selected and is thought to be bacteria that are able to use pyrene sebaga source nutrition.

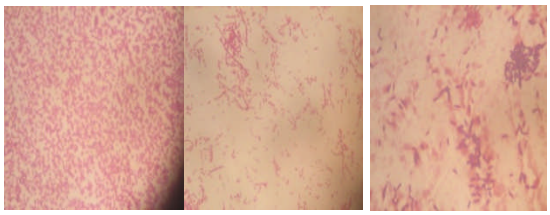
Table 1. Results of Measurement OD enrichment Bacteria

Sample	Week 1		Week 2		Week 3	
	OD day 0	OD day 7	OD day 0	OD day 7	OD day 0	OD day 7
A	0.043	0.601	0.372	0.469	0.286	0.303
B	0.107	0.776	0.445	0.542	0.369	0.392
C	0.142	0.378	0.214	0.367	0.155	0.210
Control	0.015	0.095	0.039	0.051	0.021	0.029

3.2 Bacterial Isolation and Characterization of Bacteria

3.2.1 Gram stain

Of the isolation process in three sample points, obtained three different bacterial isolates. Purity isolates detected using 1000X magnification microscope by observing the shape and color of the cell. Microscopic gram stain results are shown in Figure 1.



(a) (b) (c)

Figure 1. The results of microscopic analysis with gram staining isolates a= isolate A, b = isolate B, c = isolate C

The results of microscopic analysis in Figure 1 can be seen isolate A and B are gram-negative isolates, and isolate C is a gram positive. Based on the data from staining gram, where after the observation with a microscope looks red in the A and B isolates, while the isolates to C blue-purple.

3.2.2 Test Biochemistry

Results of the third characterization of bacteria through biochemical tests are shown in Table 2.

Table 2. Results of characterization of bacterial isolates

Parameter Tes		Isolate A	Isolate B	Isolate C
TSIA	Slant	Acid	Acid	Acid
	Butt	Base	Base	Acid
	H_2S	-	-	-
MRVP	MR	-	-	+
	VP	-	-	-
Indole		-	-	-
Citrat		-	+	-
Urease		-	-	-
Glukose		+	-	+
Laktose		-	-	-
Sukrose		-	-	-
Mannitol		-	-	-

Biochemical test results in Table 2 is based on test TSIA (Triple Sugar Iron Agar) against isolates of A, B, and C in the area of media slant becomes red color means that these isolates are acidic. In areas A and B isolates butt alkaline media isolates C turns yellow. Formation of fermentation gases of H_2 and CO_2 can be seen from the outbreak in order. H_2S gas formation characterized by the deposition of black color. At isolates A, B, and C do not form H_2S gas.

In the test of MRVP for the analysis of MR isolate C shows positive results because the media turns red after adding methyl red. While A and B isolates showed negative results because the color red approaching media. For the analysis of the third VP isolates showed a negative result because no red color is formed after adding α naphtol and KOH.

At the indole test against isolates A, B and C were negative because it does not form a pink ring. Based on citric test to isolate the A and C results negative reaction

because it does not change color in the media. While isolates B positive results because the color changes from green to blue.

Urease test results on the reaction of the isolates A, B and C were negative because it does not change color in the media. The result of fermentation using a reagent lactose, sucrose and mannitol to the three isolates gave negative results because it does not change color from red to yellow, while for reactants glucose isolates A and C showed positive results because the color changes from red to yellow can be seen in Figure 2.

a b c

Figure 2. Biochemical test: a = isolates A, b = isolates b, c = isolate C

3.2.3 Molecular analysis of 16S rRNA gene bacterial isolates

Molecular characterization of bacterial 16S rRNA gene is done by identifying the DNA sequence of the gene molecule pair through the method of Polymerase Chain Reaction (PCR). DNA amplification product of bacterial isolates is shown in Figure 3.

Figure 3. Results of amplification isolates A, B, and C

Molecular universal 16S rRNA gene in bacteria, can generally be compared with samples of RNA sequences [2,6]. DNA sequences of 16S rRNA gene is analyzed using BLAST (Basic Local Alignment Search Tool) from NCBI (National Center for Biotechnology Information). Results of the sample sequence alignment with GenBank sequences showed high similarity homologous sequence, which is shown in Table 3.

Table 3. Results of BLAST (Basic Local Alignment Search Tool) bacteria

Sample	Sequent Sampel	Sequent *GenBank*	Identity (%)	Species
A	6-933	18-945	918/928 (99%)	*Alcaligenes Faecalis*
B	6-939	541-1477	926/927 (99%)	*Sphingobacterium sp.*
C	1-894	512-1408	891/898 (99%)	*Bacillus Cereus*

BLAST results that achieve a high degree of similarity genus when reaching above 80% homologous, [8]. Identification by PCR and DNA sequencing of bacteria through the BLAST program is more convincing as compared with bacteria in GenBank DNA data containing tens of millions of existing bacterial DNA data. 16S rRNA analysis allows the discovery of new pathogenic bacteria, because it can identify the bacteria that can not be cultured [9].

3.3 Degrading Bacteria Growth Piren

Media used in the growth of pyrene degrading bacteria is the same media with the media selection. There are two control at this stage, control (+) and control (-). Control (+) is a bacterial isolates were grown on selective media without pyrene controls (-) is a selective medium with pyrene without bacteria. Here is the growth curve of bacteria degrading pyrene.

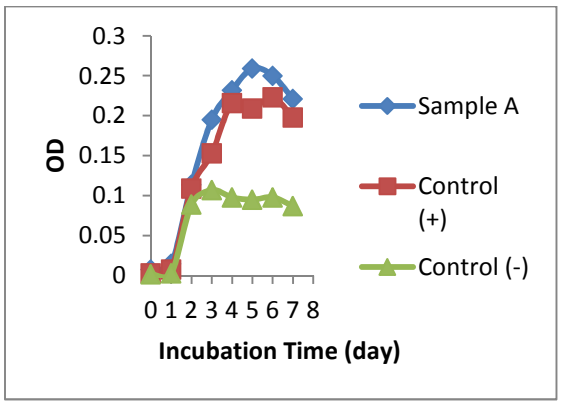


Figure 4. The growth curve of bacteria that degrade pyrene in bacteria Alcaligenes faecalis

Based on Figure 4 isolates A capable of degrading pyrene. It can be seen from a comparison of the value of Optical Density (OD) between the growth media with positive control (+) and negative control (-). Media bacteria grow better growth compared to control (+) and control (-). This means that these isolates are likely able to utilize pyrene as a carbon source. The optimum time the growth of bacteria that is on the 5th day of incubation and decreased on day 6, but on day 7 still seen many bacteria living. This suggests that these bacteria have a long growth period.

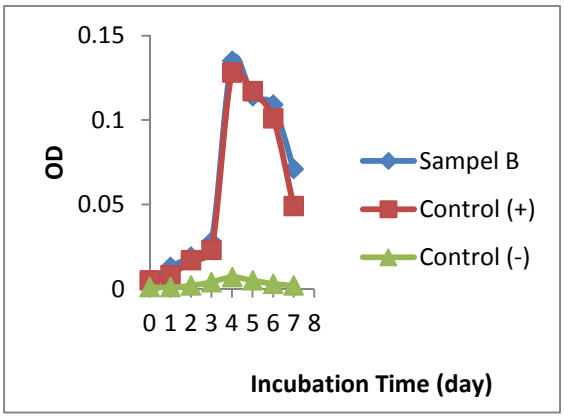


Figure 5. The growth curve of bacteria that degrade pyrene in Sphingobacterium sp.

Based on Figure 5 isolates B are less able to degrade pyrene. It can be seen from a comparison of the value of Optical Density (OD) between the growth media with positiif control (+) and negative control (-). Bacterial growth on growth media to grow less well. The growth curve of bacteria that grow on the control (+) is almost equal to the growth curve this suggests the bacteria can grow by utilizing the yeast extract as a source of carbon. Contamination that occurs at this stage is small, can be seen from Figure 4 the value of OD in controls (-) low. The optimum time of growth, namely on day 4 and decreased on day 5 of incubation.

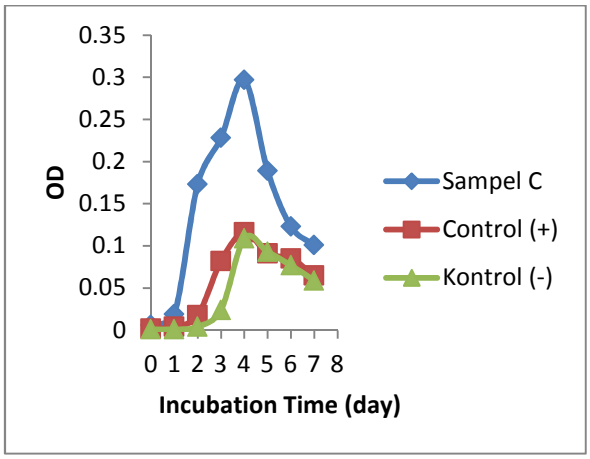


Figure 6. The growth curve of bacteria that degrade pyrene on Bacillus Cereus

Based on Figure 6 isolates C capable of degrading pyrene. It can be seen from a comparison of the value of Optical Density (OD) between the growth media with positiif control (+) and negative control (-). Media bacteria grow better growth compared to control (+) and control (-). This means that these isolates are likely able to utilize pyrene as a carbon source. Contamination that occurs at this stage is high, it can be seen from Figure 6 in the negative control OD values high. The optimum time is the growth of bacteria on day 4 of incubation and decreased on day 5 while the decline in the number of bacteria caused by the accumulation of toxic materials, the nutrients are very limited, so many dead cells.

4. CONCLUSION

Three degrading bacteria pyrene compounds of Water Port Paotere is Alcaligenes faecalis, Sphingobacterium 21, Bacillus Cereus. Results BLAST isolates achieve a high level of similarity genus, reaching 99%

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