ISOLATION AND IDENTIFICATION ON DEGRADATOR BACTERIUM OF PETROLEUM WASTE WHICH SYMBIONS WITH SPONGE FROM MELAWAI BEACH

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Abstract

The research has been done about isolation and identification on degradator bacterium of petroleum waste which symbions with sponge from Melawai Beach, Balikpapan. The research objectives; knowing sponge species, symbioning bacterium isolate and its ability of petroleum waste degradation. The research method were analysis of sponge Morphology analysis, isolation of symbions bacterium, identification, and activity testing at petroleum waste media which is coming from wells result of processing of PT. Chevron. The research result obtained the morphology analysis concluded that the sponges is *Callyspongia* sp. Bacterium symbion isolation obtained that two isolates which were given each symbols isolate A with the rounded shape and cream color whereas isolate B with yellow color and jagged shape. Coloring analysis of isolate obtained that isolate A is negative gram bacterium type and isolate B is positive gram bacterium type. Activity isolate testing at petroleum waste media, isolate A did not grow significantly at the media which marked with the existence of layer transparent on the media, isolate B growth significantly which marked with existence of solidity on the media, and the combination of isolate A+B also showed growth which marked with the existence of layer transparent and solidity on media. The conclusions research, sponge species is *Callyspongia* sp which contained two isolates bacterium namely negative gram bacterium and positive gram bacterium and both of the isolates can grow at media of petroleum waste.

Key word: sponge, symbion, bacterial, degradation, petroleum waste

1. INTRODUCTION

Environmental role of human life deep sea very of vital, not merely as transfortation medium, more than that sea represent assorted life place of biota and required to guarantee the continuity of being life is including human being. Besides at sea often found by minerals deposit, gas and petroleum which also very of vital for the human of life. Technological growth and make-up of requirement of human being force for the exploitation of is environmental of sea with exploitation and exploration various properties in it. As a result generate problems which actually earn life threatening and continuity of being life live not merely biota exist in depth but also itself human life.

Melawai Beach of Balikpapan is including one of the destination tourism and culinary visited by many people not merely simply see evening sundown but also as medium to enjoy 2 the coastal island which namely Island Twin of Tukung. Melawai Beach also represent place fisherman of boat it and place pamper eye seen oil tanker transfortation, mercantile marine and Pelni ship which by pass quickly, this situation happened every day which give contribution is make-up of petroleum volume which castaway in sea. This research target identifying spons and bacterium its Melawai Beach of Balikpapan, East Kalimantan as impure area of expected petroleum waste finding of sponge type and symbioning bacterium which can petroleum waste decomposed. This research contribution is to assist government in identifying sponge type and simbion bacterium which can decompose petroleum waste so that affect petroleum waste negative to damage of sea biota earn minimization.

2. THEORY

Melawai Beach up at distance around 1.500 m there are Refinery Balikpapan operating since year 1973 and 700 m. There are port of Semayang and also part of east there are Hospital Pertamina growing is vital of this Melawai Beach at the same time as source of contamination of heavy metal and petroleum waste which can menace life of being live the including human being and biota in it. Uniqly of differ from Melawai Beach is there are sea biota life owning big enough benefit, namely sponge ^[14, 19].

Research about heavy metal contamination like lead, copper, merkuri and arsenic at Melawai Beach of Balikpapan caused by many petroleum poured out from refinery activity, sea transfortation, port actifity, wash of hospital activity ship as well as human being activity ^[14].

Poured out petroleum effect not merely at damage influence of coastal esthetics nya, however also have an in with annoyed of life of biota go out to sea exist in depth which is on finally affect negativity at human life the core important which is many have interaction to with the Melawai beach ^[11].

Indonesia having spons type megadivercity, and often found by have symbiosis with microorganisme. Sponge is animals have pore to having the character of feeder filter so that become habitat to microorganisme to have a nest in its body. Microorganisme have two important role in biological system of sponge, that is first as source of food and both life have symbiosis to either through inter and also intra cellular, ^[3, 6, 22]. Some found by sponge type is symbiosis with bacterium, like: Spons *Theonella* sp., *Surassinoru Melophlus*, *Callyspongia* sp, *Ircinia* sp,

Stylissa Blabeli, *Lisoclinum* sp, *Clathria* sp, *Jaspis* sp, *P. nigra*, *X. Testudinaria* and *stellifera*,^[13, 15, 20].

Some microorganism sea (bacterium) known can aromatic hydrocarbon compound degrader with high toxicity for example: *Pseudomonas*, *Arthrobacter*, *Alcaligenes*, *Brevibacterium*, *Brevibacillus*, and *Bacillus*,^[2, 7, 21]. Fraction aromatic compound component degrader by bacterium, for example: *Macerans Bacillus*, *B. mycoides*, *B. megaterium*, *B. subtilis*, *B. pumilus*, *B. thermoleovorans*, *B. gordonae*, and *B. benzoovorans*^[18, 24], *Enterobacter*., *Bacillus* sp., *Clastiridium* Sp., *Arthrobacter* sp., *Shigella* sp., *Pseudomonas* sp., *Aeromonas* sp., and *Citrobacter* sp.,^[4], and most of the bacterium can symbioning with sea sponges^[15].

The importance of assorted exploration of bacterium which can PAH degradation, considering the nature of very toxic PAH, mutagenic and is carcinogenic, at the same time this petroleum waste besides pregnant many PAH compound also it contains some kinds of heavy metal which have potency add impure storey level of environmental of sea, and also process its decomposition need sufficient time^[1, 5, 16].

This research include to identify sponge type which is life at impure area of petroleum waste, symbion isolate bacterium, and activity test growth of symbion bacterium isolate at petroleum waste media. To reach the target hence conducted by some model namely analyse sponge morphology, symbion isolate bacterium of one of the anticipated by sponge type is symbioning with bacterium which can hydrocarbon decomposed, symbion bacterium isolate gram analysis through analysis and coloration growth of symbion isolate at petroleum waste media.

3. MATERIAL AND METHOD

Materials

Sample Sponge, CH₃OH p.a., sterile Water Sea, Phosphate Buffer Saline (PBS), Sea Water Complit (SWC), marine so that, glycerol 25%, bacterium isolate, petroleum waste, NaCl 0.9%, formalin 4%, Aquabidex, aquades, MTT reagent, DMSO.

Appliance

Scuba, camera in water, GPS, Balance (dissecting pan), scalpel, forceps, pial (toples), plastic sack; bag, ice box, Set microscope, Porcelain cup, pestle and lumpang, chemical glass, measure glass, volume pipette, drip pipette, Erlenmeyer, analytic balance, plate hot, funnel, rubber suck, electric waterbath, whatman paper, oven, freezer, BOD bottle, water thermometer, universal paper, salinometer, save reaction, solder cup, centrifugasi 1000 rpm and 4 rpm, stop watch, vortex, laminary irrigate flow, tail ose, prevarat glass, autoclave, NA media, filter 0.2 µ m, blender, haemositometer

Place and Research Time

Research time executed in May to September, 2014. Research place at Microbiological Laboratory of Pharmacy, Faculty of Pharmacy, Universitas of Hasanuddin, and Laboratory Ecology ITS

Sample and Location Sampling

Intake of potential Sample sponge done by 4 dot differ at Melawai Beach of Balikpapan, at deepness 2-7 m with diving scuba technique executed on 19 December 2013. After lifted from sea level, is immediately conducted by

photograph and taken by some of its networking, is later; then entered into medium 5% broth marine for the isolation of its bacterium way of (plating direct).

Figure 1 : Dot A : Code Sampel 1; Dot B : Code Sampel 2; Dot C : Code Sampel 3; Dot D : Code Sampel 4

Identify spongs done by noting special marking (colour, tekstur, form, size measure and deepness) intake moment. Later; Then systematic way and identify sponge morphology follow guide-book [9].

Sponge sampel taken by four dot with code of sample A, B, C, and D successively as sampel 1, 2, 3, and 4. Sample sponge sea taken by at impure area of petroleum sludge hydrocarbon waste around Melawai Beach, Balikpapan, East Kalimantan at deepness 2-7 m with diving technique (according to condition), is then entered into sterile plastic sack, bag of dark chromatic, is later then kept in box ice and brought to Laboratory to be analysed. Measured supporter data in each sampling dot coordinate point, temperature, deepness, salinity, and pH.

Analysis Method

Method of analysis conducted in 5 (five) work step: 1) spongs morphology analysis; 2) *Callyspongia* sp spongs symbions bacterium isolation; 3) purification of isolate; 4) Identify perception and bacterium microscopically; and 5) test growth of bacterium at media of petroleum waste.

1. Analysis Morphology sponge

Cell of morphology below MTT method inverted microscope, assay, (A) Culture Cell Before Treatment of inkubasi 24 clock, after 4 hour addition of MTT reagen, (B) Control cell, (C) concentration extract 960 µg/mL, (D) concentration extract 7,5 µg/mL, (E) control DMSO. Analysis Morphology spongs to know gender and its species is done in Laboratory ITS ecology, Surabaya.

2. Bacterium sponge symbion isolation

Sponge go out to sea sampel code 1 (deepness 2,7 m), *Callyspongia* sp species which have been taken to be sprayed at part of surface with sterile sea water with sponge size measure comparison 1 cm² : 5 sterile sea water mL, so that only bacterium with energy join just strong to sampling. Part of mesohyl taken of the size ± 1x1cm, is attenuated and thinned with Phospat Buffer Saline (Sterile PBS) with comparison 1:1 [12]. Isolation of bacterium from surface outside use sterile swab [23], which is stroked one wayly at external surface of spongs. Sterile Swab which have been stroked by at surface of sampel entered into containing thinning tube of Sterile PBS and the vorteks

Result of thinning disseminated into solder cup which have contained Sea Water Complit media (SWC) with composition 1 media litre consist of 5 g/L Bacto

pepton, 1 g/L Yeast extract, and 3 mL/L Glycerol, and the incubation at temperature 26° C during 24-36 hours, perceived by growth of its bacterium colony. Each; every bacterium colony which grows to be perceived its his, namely colour, size measure and colony form, is and also purified by using the same media.

3. Purification of Bacterium Symbionts

At plating directly the method moment in field, perceived by colony which grows after incubation during one till two weeks. To the number of colony counted, later; then colony which is form and its colour differed isolation. Each colony done by purification, that is by inscribing 1 colony on an inoculating loop at containing a sloop of media 100% marine-agar so that later; then the incubations at temperature 30° C during 1-2 days. After growing, colony just conducted by incision repeat at media 100% marine-agar is till got by single colony.

Rich method done with spreading of culture dilution to media 100% marine-agar to be, done by after incubations two weeks, hereinafter like plating direct method, incubated culture at temperature 30°C during one colony amount and week counted, dipurifikasi and isolation. Result depository of purification use 25% glycerol in media marine-agar.

4. Identify and perception isolate bacterium in microscopically

Process identify to cover coloration of gram, with intake of bacterium breeding 1 on at previous glass object have is free of fat with alcohol 96%, then in violet crystal dropped (Gram A) 1-3 drops made by hapused, hushed is later; then cleaned to use aquadest is then dried, is later; then dropped by lugol condensation (Gram B) 1-3 drops, made by hapusedly, hushed, later; then, cleaned and dried, is then dropped with acetate alcohol (Gram C) 1-3 drops is then made by hapused, hushed, and cleaned to use aquadest and dried, is later; then dropped by safranin (Gram D), made by hapusedly is later; then hushed, is then cleaned to use aquadest and dried, is afterwards perceived by below microscope with magnification 10X.

5. Growth test at Media of Waste Petroleum

The isolate obtained, is hereinafter analysed by ability in petroleum waste degradations, conducted by 15 liquid petroleum waste mL is PT. Chevron, Dumai, Riau in packing into 4 sloop cups differ, then bacterium isolate which grows to be made by suspension. Isolate A to suspension, isolate B suspension, and A+B mixture isolate suspension and positive control suspension of *Pseudomonas* sp, is successively dripped into petroleum waste media at A sloop cup (cawan petri), B, C, and D, each incubated at temperature 37° C during 48 hours, and perceived by its growth.

4. RESULT AND DISCUSSIONS

The data supporter of taken by is moment intake of spon samples in Melawai Beach of Balikpapan presented in Tables 1.

Table 1. Condition Of territorial water dot Sampling of sampel code 1 at Melawai Beach of Balikpapan

Parameter	Assess Measurement
Co-ordinate sampling	01° 16. 36'8" LS 116° 48. 23'6" BT
Temperature (°C)	29
Salinity	30‰
Ph	7
Deepness of sampling	2,70 m

There is found spon type at Melawai Beach of Balikpapan later on SP.1 sampel code is given; SP.2; SP.3; and SP.4.

Fourth physical characteristic of sponge of sampel type perceived direct is field, for the spon of Sp1 code: chocolate crem colour, long form and gang, owning spicula in the form of silicate and is fibrous of spongin. Sponge of Sp2 codes: fair purple, owning cavity and gang, silicate spicula with spongin fibre, spon of Sp3 code: green colour, domed form gang, spicula do not see, sharp-pointed back part, stiff harsh and also, while for the sponge of Sp4 code: easy brown, long form and disseminate, spicula in the form of calcite with spongin fibre

4.1. Morphology sample of sponge

Result of fourth morphology analysis of sponge sampel presented in Table 2, following:

Tables 2. Result Analysis Morphology sample of Sponge from Melawai Beach, Balikpapan

SP	Species	Family	Inventor
1	<i>Callyspongia</i> sp	<i>Callyspon-giidae</i>	Duchassaing Michelotti, 1964
2	<i>Coelocarteria singaporensis</i>	<i>Chalinidae</i>	Carter, 1883
3	<i>Callyspongia (Cladocalina) vaginalis</i>	<i>Callyspon-giidae</i>	Lamarck, 1814
4	<i>Haliclona (Haliclona) oculata</i>	<i>Chalinidae</i>	Pallas, 1766

Figure 2. Result analysis morphology of sampel of sponge code sample is Sp1, namely: species of *Callyspongia* sp., a) growth form massiva, raucous oscula as long as surface of sponge body; b) result of coloration with Etoh; c) tekstur result of compress; d) surface structure enlarged.

At the fourth of obtained sponge species from Melawai Beach of Balikpapan, after analysed morphology obtained by result there is 2 each set of relatives consist of 2 species, namely family of *Callyspongiidae* with species of *Callyspongia (Cladocalina) Vaginalis* and *Callyspongia* sp and also family of *Chalinidae* with species of *Haliclona (Haliclona) Oculata* and *Coelocarteria singaporensis*.

Bacterium of isolation symbions sponge done by just for *Callyspongia* sp species (sampel 1) from family of *Callyspongiidae* with reason that from is fourth of existing species only species of *Callyspongia* sp known to earn degradator of Polysiclic Aromatic Hydrocarbon (PAH).

4.2. Isolation Bacterium *Callyspongia* sp symbion of sponge

Isolation bacterium symbion at sponge of species of *Callyspongia* sp used media Sea Water Complit (SWC). Concentration thinning 10^{-1} until 10^{-5} done until 3 times series purification and each incubased during 1x24 clocks, is then conducted by macroscopic perception, that is colour, colony amount and form. perceived dominant colony that is white krem chromatic colony, big globularity with growth of meeting colony is later on named by A isolate and colony with

chocolate white colour, jagged form is and length growth of colony disseminate, hereinafter named by isolate B. Perception of this morphology each seeing clear at thinning 10^{-2} , 10^{-3} and 10^{-4} .

Result identifying of macroscopic of isolation sponge of *Callyspongia* sp obtained by two isolate. First Isolate with big globularity marking and crem chromatic- white is herein after referred to as isolate A and second isolate with jagged form marking, length, white chromatic is chocolate, herein after referred to as isolate B. Both this isolate is hereinafter purified by using ose needle and inscribed by at media of that marine-agar (MA 3). Purification of Isolate Bacterium *Callyspongia* sp which symbioning with sponge

4.3. Purification of Isolate Bacterium *Callyspongia* sp symbion of sponge

The result of perception obtained by isolate is hereinafter identified representing perception microscopically to obtained isolate from the thinning by doing painting of gram. Breeding microbe colony is hereinafter purified by incised at medium Marine-agar to beheeling as which specific medium. Using by breeding purification technique is method of contiyou incision

Media growth commutation and purification conducted meant recurring to ascertain perity of isolate, so that give certainty that the isolate is which isolate with dominant.

Purification of isolate bacterium aim to to dissociate result of inoculasi which consist of many different bacterium colony of type is got by pure bacterium colony in each bacterium breeding. Colony of taken bacterium to be purified by colony of dominant. Purification by using cup method incises. Isolate A and obtained to be isolate B, to be purified at media of Marine-Agar until thrice by recurring in each thinning aim to for the obtaining of really pure isolate and purification to four conducted by at media so that to be heeling, where both this isolate is identified can grow better at thinning 10^{-2} to 10^{-4} .

4.4. Identifications and perception microscopically of isolate bacterium

Table 3 Microscopic Examination with Coloration of Bacterium Gram Isolate A and Isolate B

Perception	Isolate A	Isolate B
Colour	red purple	Violet
Form	Bar	circular gang
Colourant	dissolve colour	colour remain to
Endospore	no spore	ill defined Spore
Node	negative gram	positive gram

Hereinafter identify to be donewith microscopic examination conducted with painting of gram which aim to for the, giving of paint A (crystal violet) at positive gram of bacterium will be permeated and bound by part of external cell. Gift paint

B (lugol) improved afinitas cordage of colour seent of vitamin by bacterium so that cordage of seen colour vitamin become is stronger.

At giving paint C (alcohol 96%), positive gram bacterium of chromatic remain to be purple because violet crystal compound complex and laktofenol remain tied at cell wall. At addition paint D (safranin), the do not cause change of positive gram bacterium colour, because complex compound of violet crystal and iodium remain tied at cell wall.

The after passing giving process paint A (violet crystal), paint B (lugol), paint C (alcohol 96%), and also paint D (safranin) in the reality isolate A of including into negative gram bacterium at microscope that is. thinning 10^{-2} to 10^{-4} got bacterium from all the is negativity gram bacterium maintaining safranin colour. This matter is caused by negative gram bacterium have lipid rate and high protein, [8]. Isolate B is positive gram bacterium, because at painting with safranin, do not cause change of colour in consequence of Crystal violet complex and iodium remain tied to cell wall.

4.5. Test Growth of Isolate Bacterium the symbion of sponge

Table 4 Test Growth of Isolate Bacterium Symbions Sponge *Callyspongia* sp at Media Of Petroleum Waste

Treatment	Isolate A	Isolate B	Isolate A + B	Control D
Media of petroleum waste	-	-	-	-
Incubation 1x24 clock	+	-	+	-
Incubation 2x24 clock	+	+	++	-

Boldness :

- (-) – there is no growth of symbion bacterium
(+) – there is growth of symbion bacterium

The analysis growth of isolate bacterium at media of petroleum waste media, where suspension of isolate A, suspension isolate B, suspension isolat mixture A+B and *Pseudomonas* sp bacterium suspension as positive control, each dripped by at petroleum waste in solder cup (cawan petri) and incubation at temperature 37° C during 2x24 hour. Obtained by result of isolate A, can grow at the media marked with the existence of solidity at solder cup, isolate B also can grow at petroleum waste media marked with formed its of transparent coat at surface of solder cup, while mix isolate A+B also can grow at the media marked with the existence of transparent coat at surface of solder cup as well as formed by solidity.

This matter indicate that both isolate is the, namely isolate A and B isolate which is isolations from spons as symbion species *Callyspongia* sp can adapt to its growth media or environment, including according to environmental temperature and pH of growth. Bacterium *Pseudomonas* sp as positive control do not show growth at media of petroleum waste. This matter is caused by growth media at

temperature 37⁰ C and pH 7 not such ideal environment for the growth of bacterium is *Pseudomonas* sp as control.

Is known that petroleum waste taken as media test growth of isolate can in the form of aliphatic hydrocarbon and aromatic indicating that one of the both types of the hydrocarbon or the two earning degradation by symbion bacterium isolate of spongs *Callyspongia* sp coming from Melawai Beach of Balikpapan East Kalimantan, marked with the existence of growth of isolate after incubation during 2 x 24 clock. Result of this research give information that at least there is 3 variable which can influence growth of isolate at media of petroleum waste, that is; 1) temperature,

2) growth media of pH and hydrocarbon type which implied in media of petroleum waste. Research the thereby can be done of continu by take third this variable to know furthermore related to ability of the isolate in degradation of petroleum waste. That way also to both the isolate can be identified by bacterium species through method of Polymerase Chain Reaction (PCR) Real Time.

5. CONCLUSIONS

1. Result analysis of Melawai Beach of Balikpapan at spongs of morphology, namely there is 2 set of relatives, that is first family is *Callyspongiidae* consist of 2 species namely *Callyspongia (Cladocalina) Vaginalis* and *Callyspongia* sp and both family is *Chalinidae* set of relatives and consist of 2 species namely *Coelocarteria Sinagporensis* and *Haliclona (Haliclona) Oculata*
2. The result identify macroscopic at isolation bacterium of symbion sponge at species *Callyspongia* sp obtained by 2 isolate. First of isolate A globularity, redish crem of colour and both isolate B is jagged, colour turn yellow is redish.
3. Identify microscopic for the isolate A is type bacterium of gram negative and isolate B is type positive gram bacterium isolate
4. The bacterium isolate A, isolate B and mixture isolate A+B can grow at media of petroleum waste, while positive control of *Pseudomonas* sp as pure bacterium suspension cannot grow.

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