

Identification of marine sponges-symbiotic bacteria and their application in degrading polycyclic aromatic hydrocarbons

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Abstract. Marzuki I, Kamaruddin M, Ahmad R. 2021. Identification of marine sponges-symbiotic bacteria and their application in degrading polycyclic aromatic hydrocarbons. *Biodiversitas* 22: 1481-1488. Diverse and abundant microbial species that occupy marine sponges may make important contributions to host metabolism. Sponges are filter feeders and devour microorganisms from the seawater around them. Each microbe that endures the sponges' digestive and immune responses are related symbiotically. Marine sponges symbiont bacteria can comprise as much as 40% of sponge tissue volume, and these are known to exhibit a great potential on polycyclic aromatic hydrocarbons (PAHs) degradation. However, the potential use of marine sponges symbiont bacteria is unexplored. Therefore, we designed and conducted a study to identify bacterial isolates obtained from sponges. For this, we collected sponges samples (*Hyrtios erectus*, *Clathria (Thalysias) reinwardti*, *Niphates* sp., and *Callyspongia* sp.) from the Spermonde islands in Indonesia. We successfully found eight bacterial isolates from four sponges, as molecular identification based on 16S rRNA approach revealed bacterial isolates of SpAB1, SpAB2, SpBB1, SpDB1, and SpDB2 from three sponges (*Hyrtios erectus*, *Clathria (Thalysias) reinwardti*, *Niphates* sp.). Interestingly, these were closely related to *Pseudomonas*, and a bacterial isolate from *Callyspongia* sp. (SpCB1) showed similarity to *Bacillus*. *Bacillus* and *Pseudomonas* bacteria isolated from hydrocarbon-contaminated sponges exhibited degradation of naphthalene and pyrene PAHs.

Keywords: Naphthalene, polycyclic aromatic hydrocarbons, pyrene, sponges, sponges-associated bacteria

INTRODUCTION

Sponges-associated bacteria play a significant role in digestion, waste removal, and chemical defense for the sponge hosts (Taylor et al. 2007). Symbiont and symbiotic words refer to two or more species that have been continuously observed to live together for a long period (Angelard and Bever 2013). In fact, sponge-associated microorganisms are of great interest because of their potential role in producing several compounds (Montalvo and Hill 2011; Moitinho-Silva et al. 2017). Several studies of marine sponges-related bacteria have identified novel bacterial groups, such as the candidate phylum Poribacteria (Lafi et al. 2009). Moreover, bacteria also play a significant role in eliminating polycyclic aromatic hydrocarbons (PAHs) from contaminated habitats but, the potential use of marine sponges with symbiont bacteria is still unexplored (Marzuki 2018).

It is a well-known fact that polycyclic aromatic hydrocarbons that include carcinogenic and mutagenic compounds are dangerous (Khabouchi et al. 2020; Marzuki et al. 2020a) with two or more fused benzene rings that originate from both natural sources and anthropogenic activities. Generally, PAHs are ubiquitously distributed on the Earth and the Sea (Bendouz et al. 2017). The marine environment is the most vulnerable region to PAHs sludge exposure by human activities and dynamics at sea, a significant threat to marine life (Cajthaml et al. 2008).

Along with industrial growth and the global dynamics of today, the accumulation of PAHs waste into the sea continues because of the contribution of waste from the oil refining industry and marine transport activities (Adkins et al. 2002; Marzuki et al. 2015a). If this condition persists, it may cause serious problems for the lives of different forms of marine life (Marzuki et al. 2015b; Nursid et al. 2017).

Maintaining the protection of the marine environment and the conservation of the growth and production of different marine biota happens naturally, but active human intervention is important because the natural ability of the sea to sustain its ecological balance is very limited due to the massive pollutants that reach the sea (Harpere et al. 2001). Marzuki et al. (2015b) noted that some dramatically exhibit an ability to defend themselves and adapt to the environment and various threatening disturbances; one of which are sponges.

Interestingly, the sponge is a filter feeder by sucking and extracting the sludge, and it provides space for the aggregation of different microorganisms in the sponge body (Lee et al. 2001; Montalvo and Hill 2011; Moitinho-Silva et al. 2017). Melawaty et al. (2014) suggested that marine sponges have bioindicator properties for heavy metals. Their study also indicated that marine sponges symbionts were capable of biodegrading to components of petroleum hydrocarbons (Melawaty et al. 2014). In the context of marine natural resources, the different conditions described above require research that can provide new

information about the ability of marine biota and other materials that can be used to preserve environmental balance. Research on tracing various marine natural resources, such as mangroves, seagrass, seawater and sediment, as well as sponges and their symbiont bacteria that are potential as biomaterials in the PAHs-type waste biodegradation process (Marzuki et al. 2016).

MATERIALS AND METHODS

Sponge sample collection, isolation, and purification sponge-symbiotic Bacteria

The sponge samples were collected from four small islands (Lae-lae, Samalona, Barang Caddi, and Kodingareng Keke) in the administrative area of Makassar city, South Sulawesi Province, Indonesia. These four islands are part of the Spermonde Archipelago area (Figure 1). Sponge SpA has been obtained around Lae-lae Island, sponge SpB around the island of Samalona, sponge SpC around Barang Caddi Island, and sponge SpD has been obtained around Kodingareng Keke Island. Sampling locations have been documented as a basis for geographic distributions (Marzuki et al. 2020c). The position of the

coordinates, the ecological, and physical characteristics of each sponge was provided in Table 1.

Thus, the sponge samples were directly grouped and marked by location at which they were collected. Mesohyl portion of each sponge was taken 1 x 1 centimeter in size, washed with 5 milliliters of sterilized seawater, crushed, and diluted with 1:1 sterilized Phosphate Buffer Saline (PBS) ration (Trindade-Silva et al. 2012). Sponge-associated Bacteria were isolated by using a sterilized swab which was rubbed into one direction on the outer surface of the sponge (Taylor et al. 2007). The sterilized swab was then placed in a dilution tube that contained sterilized PBS and vortexed. The dilution results (1 mL) were spread on a petri-dish containing Sea Water Complete (SWC) media. Bacterial colonial growth was observed for 24-36 hours at 26°C by analyzing colony morphology (shape, color, cell distribution, and endospore). The bacteria which grew were then purified on SWC media for 1-2 weeks (Yetti et al. 2015). For each colony grown on the SWC media, transferred to Marine Agar (MA) media and incubated for 1-2 days at 30°C. The same procedure applied to new colonies growing up till getting a single colony (Zafra et al. 2016; Medic et al. 2020).

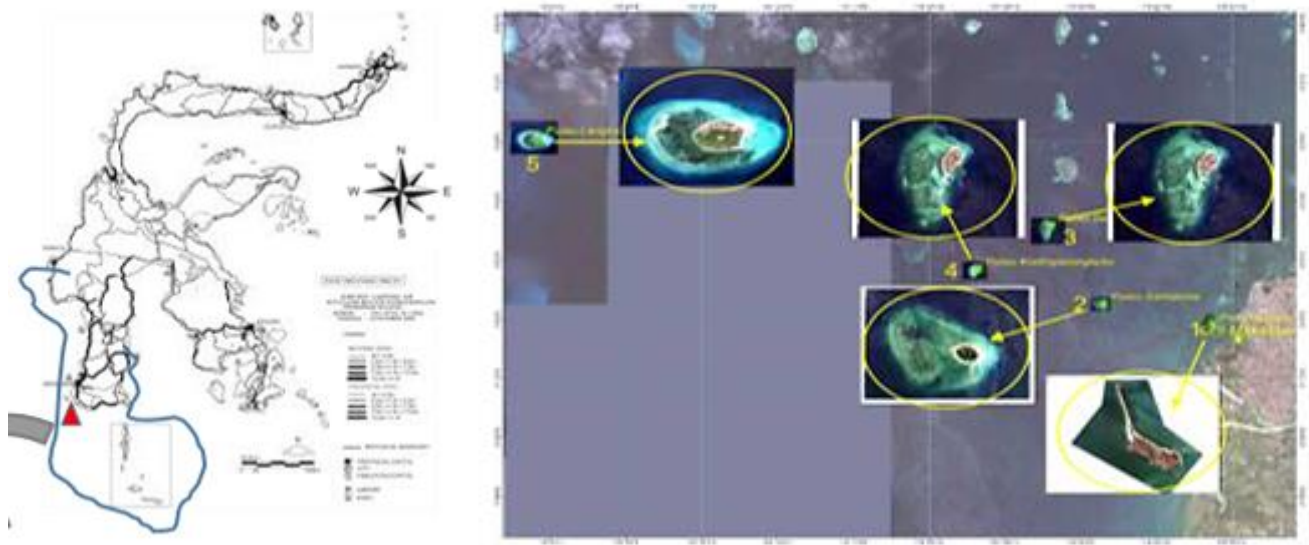


Figure 1. Location of sponge sampling points on four small islands as part of the Spermonde Archipelago area. Point 1. Lae-lae Island ($5^{\circ}11'14.743''\text{S}$, $119^{\circ}23'31.170''\text{E}$), point 2. Samalona Island ($5^{\circ}2'31.975''\text{S}$), point 3. Barang Caddi Island ($5^{\circ}4'52.272''\text{S}$, $119^{\circ}19'14.376''\text{E}$), and point 4. Kodingareng Keke Island ($5^{\circ}6'21.423''\text{S}$, $119^{\circ}17'19.399''\text{E}$)

Table 1. Data of collected sponges and sampling points

Sponges	Location	Surface	Altitude (m)	Latitude	Longitude
<i>Hyrtios erectus</i>	Lae-lae Island	Rough	4.2	$5^{\circ}11'14.743''\text{S}$	$119^{\circ}23'31.170''\text{E}$
<i>Clathria</i>	Samalona Island	Rough	4.8	$5^{\circ}2'31.975''\text{S}$	$119^{\circ}20'37.061''\text{E}$
<i>Niphates</i> sp.	Barang Caddi Island	Smooth	3.9	$5^{\circ}4'52.272''\text{S}$	$119^{\circ}19'14.376''\text{E}$
<i>Callyspongia</i> sp.	Kodingareng Keke Island	Smooth	4.6	$5^{\circ}6'21.423''\text{S}$	$119^{\circ}17'19.399''\text{E}$

Sponge-symbiotic bacteria phenotype analysis

The mesohyl portion of each sponge was cut cross-sectional immediately after the sponge was immersed in 70% methanol, and observed under a hemocytometer phase-contrast microscope. The sponge sample was crushed in a blender, then the suspension was analyzed using a hemocytometer phase-contrast microscope to see the types of cells, and skeletons sponges (Ismet et al. 2011; Marzuki et al. 2016). Phenotype analysis of symbiont bacteria was carried out using bacterial isolates biochemical test applied a typical biochemical reagent assay (Kurniawan 2012). Gram stain method (Nguyen and Thomas 2018), and the results were observed under a 10x magnification microscope.

DNA extraction, PCR, and sequencing analysis

Genomic DNA was extracted from symbiont bacterial isolates using the DNAzol® reagent (Thermo Fisher Scientific, Waltham, MS, USA) according to the manufacturer's instructions with some modifications (Kamaruddin et al. 2014). Total DNA of extracted symbionts was amplified with universal primers of 16S rRNA gene with forward sequence was 5'CCAGCAGCCGCGTAATACG 3' attached to the nucleotide base of 518-537, and reverse sequence was 5'ATCGG(C/T)TACCTTGTTACGACTTC 3' attached to the nucleotide base of 1513-1491 (Kamaruddin et al. 2020; Marzuki et al. 2020; Minarti et al. 2020). The PCRs were performed in a final volume of 20 µl using DreamTag buffer (Fermentas, MA), approximately 1µg of genomic DNA as templates, each primer at 0.2µM, each deoxynucleoside triphosphate (dNTP) at 2.5 mM and 2.5 mM enzyme under the following condition: a denaturation step of 94°C for 1 min followed by 30 cycles of 95°C for 5 min, annealing at 54°C for 1 min 20 seconds, and extension at 72°C for 2 min. The post-extension was completed at 72°C for 10 min. The target bands were then excised from the gel and purified using the FastGene® gel/PCR extraction kit (Nippon Genetics, Tokyo, Japan) according to the manufacturer's instructions. The direct sequencing of each purified PCR product was conducted with the ABI Prism BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies Japan, Tokyo, Japan) on an Applied Biosystems 3130 Genetic Analyzer (Life Technologies). All nucleotide sequences were confirmed using both the forward and reverse reading data, and ambiguous nucleotides were precisely confirmed by conducting the sequencing procedure repeatedly.

Phylogenetic analysis and nucleotide sequence accession numbers

In total 9 reference sequences of the 16S rRNA region of marine sponge symbiont bacteria were obtained from the NCBI GenBank, and analyzed using ClustalW (Rua et al. 2018). The phylogenetic reconstruction was conducted using the neighbor-joining method with MEGA 6 (Tamura et al. 2013) based on the multiple sequence alignments, and the bootstrap statistical value was calculated with 1,000 replications to estimate the reliability of the tree. The newly

identified sequences of 16S rRNA region were deposited in the GenBank nucleotide sequence databases under accession numbers of MW025974-MW025979.

Biodegradation activity of sponge-symbiotic bacteria

Naphthalene and pyrene were the PAHs components used, each in the form of a solution with a 5000 mg/L concentration (term as PAHs-contaminated waste). The isolated bacteria cells used per vial were 2.62 to 3.45x10⁴ (based on the turbidity of bacterial suspension and PAHs), thus they were suspended with a 0.9% physiological NaCl solution, then 10 mL were applied to each 50 mL falcon tubes which were adapted by 1 x 24 hours of incubation. Add 2 mL of PAHs-contaminated waste for each falcon tubes. The interaction between bacterial suspension and PAHs-contaminated waste was maximized at 100 rpm, room temperature, and 15-day incubation by aeration with a shaker incubator. Each treatment was repeated 3 times. The parameters for biodegradation were observed and recorded every 3 days (Taylor et al. 2007; Marzuki et al. 2015b; Pawar and Al-Tawaha 2017).

RESULTS AND DISCUSSION

Sponge symbiont bacteria isolation

We isolated 14 bacterial isolates from 4 types of mesohyl sponges that collected from different islands of the Spermonde Archipelago area, Makassar district (South Sulawesi province, Indonesia).

Among the initially bacterial isolated, only limited isolates such as two bacterial isolates from sponge SpA (SpAB1; SpAB2), a SpB sponge bacterial isolate (SpBB1), a SpC sponge bacterial isolate (SpCB1), and SpD sponge bacterial isolates (SpDB1; SpDB2). Additionally, some reference 16S sequences that were added in the phylogenetic analysis have been identified through direct sequencing of the 16S rRNA gene.

Morphology and phenotype analysis of sponge-symbiotic bacteria

Depending on the host where the bacteria did symbiosis, the morphological examination of bacteria collected from sponges has remarkable differences. Morphology (shape, color, cell distribution, and endospore) analysis of bacterial isolates (SpAB1 and SpAB2) from sponge SpA showed similar morphology. They were rounded in shape, bluish beige color, clustered distribution, and their endospores were invisible. The SpB sponge symbiont bacteria have similarities in the form of serrated stem, brown color, separated distribution. However, the endospore was less visible in SpBB1 isolate, while the endospore was visible in SpBB2 isolate. The bacteria SpCB1 and SpCB2 isolates have morphological differences from each other. Isolate SpCB1 had such a serrated rod and brown color. Meanwhile, the isolate SpCB2 was slightly round in shape and bluish color. The two isolates, however, had endospores and clustered-distribution (Marzuki 2018). Equally, with sponge SpD, both SpDB1 and SpDB1

bacterial isolates had the same shape and color (round and brown), but differed in cell distribution and availability of endospores. Isolate SpDB1 was distributed separately and the endospore was less visible, while isolate SpDB2 was clustered and the endospore was clear. The Gram stain test revealed that 5 isolates (SpBB2, SpCB1, SpCB2, SpDB1, and SpDB2) of the sponge symbiont bacteria were Gram-positive (+), and the remaining 3 isolates (SpAB1, SpAB2, and SpBB1) were Gram-negative (-) (Table 2).

Sponge-symbiotic bacteria phenotype analysis used standard biochemical reagents with fourteen types of activity test reactions were presented in Table 3.

Identification and phylogenetic analysis of sponge symbiont bacteria

Among the 14 sponge-symbiotic bacteria isolated in this study, only 6 bacterial isolates (948-955 bp) were identified phylogenetically (Figure 2) revealed that bacterial isolates of SpAB1, SpAB2, SpBB1, SpDB1 and SpDB2 from three sponges: *Hyrtios erectus*, *Clathria*

(*Thalysias reinwardti*), *Niphates* sp. were closely related to *Pseudomonas*, one bacterial isolated from *Callyspongia* sp. (SpCB1) showed similarity to *Bacillus*.

Biodegradation activity of sponge-symbiotic bacteria

Several types of sponge-symbiotic bacterial isolates have been tested for PAHs biodegradation activity on a laboratory scale. Waste modified with PAHs of the form naphthalene (2-ring aromatic) and pyrene (4-ring aromatic) were prepared separately. Biodegradation of PAH components by bacterial suspension by immediate contact with naphthalene and pyrene in liquid water, each with a concentration of 5000 mg/L for 15 days in falcon tubes. Biodegradation parameters, as shown in Tables 4 and 5, which air bubbles or gas, the odor of fermentation were typical of fermentation reactions as an indication of the breakdown of the molecular structure of PAH (Naphthalene/Pyrene) resulting in degradation products in the form of basic organic compounds and CO₂ gas.

Table 2. Morphology analysis and Gram staining test of sponge-symbiotic bacteria

Sponge Species	Symbiont bacteria isolates	Morphology of Sponge-symbiotic bacteria	Gram staining test
SpA	SpAB1	Rounded shape, bluish beige color, clustered distribution, less visible endospore	Gram-negative (-)
	SpAB2	Slightly round-shape, bluish beige color, clustered distribution, less visible endospore	Gram-negative (-)
SpB	SpBB1	Serrated stem-shape, brown color, separated distribution, less visible endospore	Gram-negative (-)
	SpBB2	Serrated stem-shape, brown color, separated distribution, visible endospore	Gram-positive (+)
SpC	SpCB1	Serrated stem-shape, brown color, clustered distribution, visible endospore	Gram-positive (+)
	SpCB2	Slightly round-shape, bluish color, clustered distribution, visible endospore	Gram-positive (+)
SpD	SpDB1	Round-shape, brown color, separated distribution, less visible endospore	Gram-positive (+)
	SpDB2	Round-shape, brown color, clustered distribution, visible endospore	Gram-positive (+)

Table 3. Characterization of marine sponge-symbiotic bacteria using biochemical research approach

Biochemical reagents	Media	Sponge symbiont bacteria							
		SpAB1	SpAB2	SpBB1	SpBB2	SpCB1	SpCB2	SpDB1	SpDB2
Starch Hydrolysis	starch agar	basic	basic	basic	basic	basic	basic	basic	basic
Casein Hydrolysis	milk agar	acidic	acidic	acidic	acidic	acidic	acidic	acidic	acidic
Gelatin Hydrolysis	nut. gelatin	-	-	-	-	+	+	-	-
Nitrate reduction	nitrate broth	-	-	-	-	-	-	-	-
Indole	tryptone broth	-	+	-	-	+	-	-	-
H ₂ S	H ₂ S agar	-	-	+	-	+	-	-	-
Red Methyl	R-VP broth	+	-	+	+	+	+	+	+
Voges-Proskauer	R-VP broth	+	+	+	+	+	+	+	+
Citric	CSA	+	+	-	-	+	+	-	-
Urease	urea broth	-	-	+	-	-	-	-	-
Glucose	glucose catalase	-	-	-	-	-	-	-	-
Lactose	lactose catalase	+	+	-	+	+	+	+	+
Sucrose	sucrose catalase	-	+	-	-	-	-	-	-
Mannitol	mannitol catalase	-	-	-	-	-	-	-	-

Note: (+) indicates there is a reaction, (-) means no reaction

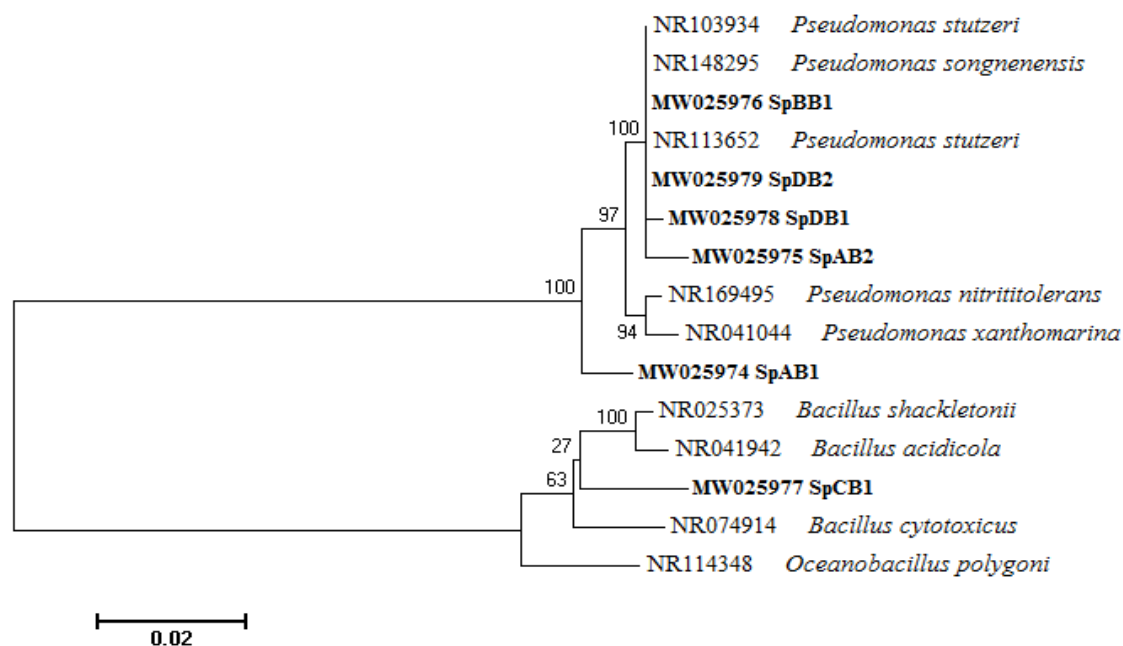


Figure 2. Representative neighbor-joining tree reconstructed with the 16S rRNA sequences of marine sponge symbiont bacteria

Table 4. Sponge-symbiotic bacteria activity of modified-naphthalene waste

Biodegradation parameters	Contact time (days)	Interaction between sponge symbiont bacteria and anthracene							
		SpAB1	SpAB2	SpBB1	SpBB2	SpCB1	SpCB2	SpDB1	SpDB2
Optical density (absorbance λ : 600 nm)	0 d	0.062	0.069	0.071	0.067	0.072	0.062	0.068	0.073
	15 d	0.212	0.176	0.163	0.343	0.373	0.434	0.483	0.356
pH	0 d	7.0	7.1	7.0	7.1	7.1	7.0	7.1	7.1
	15 d	6.9	6.8	6.7	6.5	6.2	5.9	5.9	6.3
Air bubble	0 d	-	-	-	-	-	-	-	-
	15 d	√	√	√	√	√√	√√	√√	√√
Fermented flavor	0 d	-	-	-	-	-	-	-	-
	15 d	Δ	Δ	Δ	Δ	Δ Δ	Δ Δ	Δ Δ	Δ Δ
Activity	0 d	-	-	-	-	-	-	-	-
	15 d	++	+	+	++	+++	+++	+++	++

Note: √ : there are a few air bubbles forming, √√: a lot of air bubbles forming, Δ: fermentation flavor was not strong, Δ Δ: fermentation flavor was strong, +: low biodegradation activity, ++: moderate biodegradation activity, +++: strong biodegradation activity

Table 5. Sponge-symbiotic bacteria activity of modified-pyrene waste

Biodegradation parameters	Contact time (days)	Interaction between sponge symbiont bacteria and pyrena							
		SpAB1	SpAB2	SpBB1	SpBB2	SpCB1	SpCB2	SpDB1	SpDB2
Optical density (absorbance λ : 600 nm)	0 d	0.065	0.063	0.075	0.071	0.072	0.064	0.069	0.071
	15 d	0.112	0.102	0.113	0.262	0.311	0.351	0.326	0.274
pH	0 d	7.1	7.0	7.0	7.0	7.0	7.0	7.0	7.1
	15 d	6.9	6.9	6.9	6.8	6.6	6.3	6.4	6.5
Air bubble	0 d	-	-	-	-	-	-	-	-
	15 d	√	-	-	-	√	√	√√	√
Fermented flavor	0 d	-	-	-	-	-	-	-	-
	15 d	Δ	-	-	Δ	Δ	Δ	Δ Δ	Δ
Activity	0 d	-	-	-	-	-	-	-	-
	15 d	+	+	-	+	++	++	+++	++

Note: √ : there are a few air bubbles forming, √√: a lot of air bubbles forming, Δ: fermentation flavor was not strong, ΔΔ: fermentation flavor was strong, +: low biodegradation activity, ++: moderate biodegradation activity, +++: strong biodegradation activity

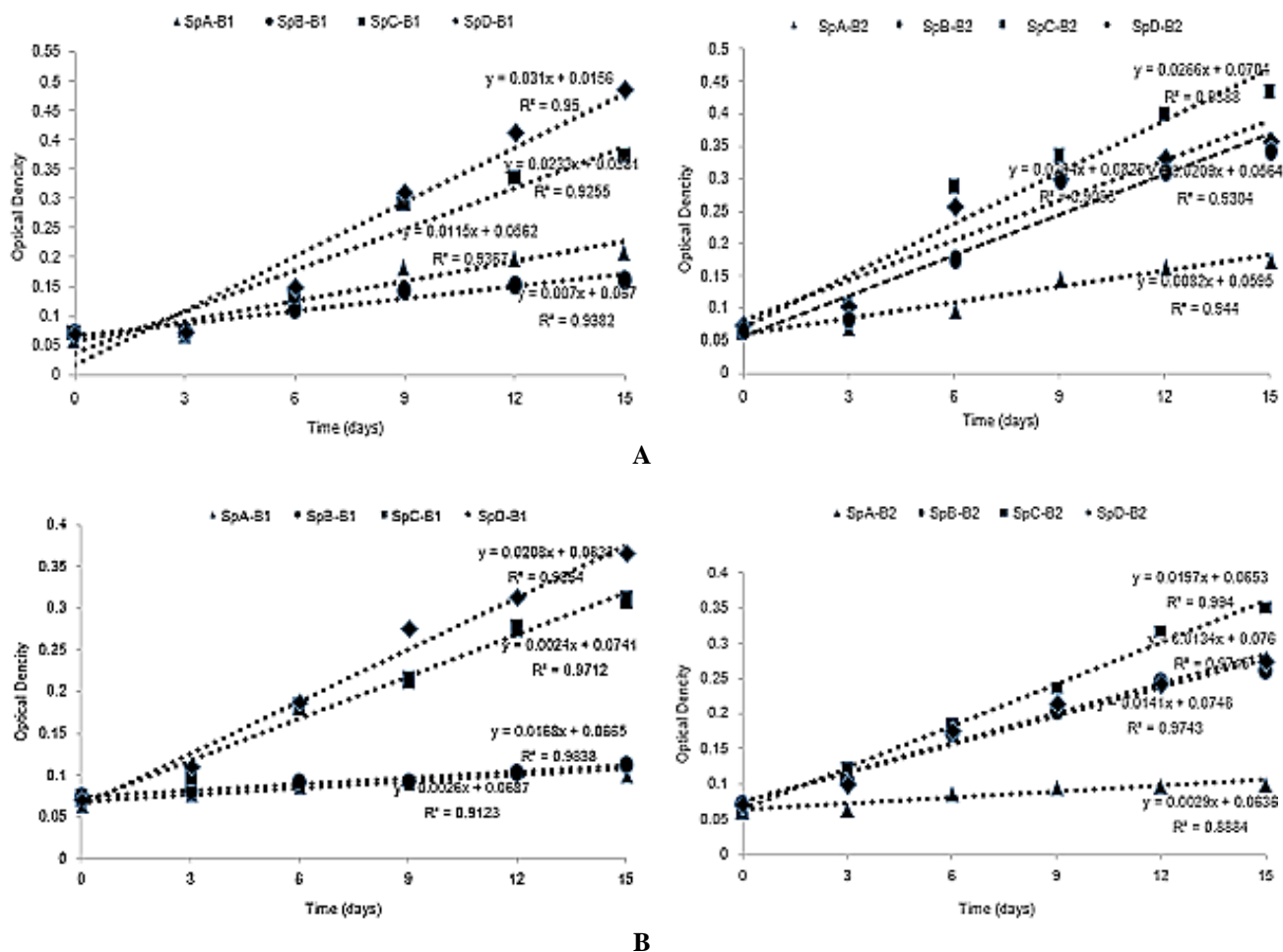


Figure 3. Biodegradation activity of sponge symbiont bacteria against PAHs. A. Activity of sponge symbiont bacteria isolates on Naphthalene; B. Activity of sponge symbiont bacteria isolates on Pyrene

In particular, the media become more acidic by measuring the pH of the media using the pH universal indicator paper. In order to determine the type of simple organic compounds biodegradation products of the bacteria against Naphthalene/Pyrene, it should be measured using GC-MS so that the abundance of PAH is determined if it has decreased or is constant and can also determine the type of organic compounds formed by biodegradation. Suggested that all bacterial isolates have biodegradation activity against naphthalene and pyrene, but vary in frequency of activity.

The bacterial isolates of SpCB1, SpCB2 and SpDB1 were found to have the highest biodegradation activity against naphthalene compared to other isolates. While biodegradation activity against pyrene was the strongest suggested by SpDB1 isolate.

Discussion

The identification of marine sponge-symbiotic bacteria has done in this study showed that morphology and Gram staining of each bacteria is different depending on the host where the bacteria did symbiosis. This can be used as a reference to the biodegradation of polycyclic aromatic

hydrocarbons by these marine sponge symbionts. Marzuki et al. (2015a; 2020b) has developed morphological analysis and Gram stain tests of sponge-symbiotic bacteria to determine if there is a relationship between the morphology of bacteria and the activity of PAH biodegradation (Marzuki et al. 2015b; Marzuki et al. 2020a). Phenotype data for sponge-symbiotic bacteria is also one of the variables that can be used as a reference in the tracking of prospective bacteria for biodegradation of PAH components in waste. Previous studies by (Ismet et al. 2011; Wang et al. 2018) have explained that bacteria that respond positively to the Methyl Red (MR) reagent are bacteria that ferment glucose into acidic products. If bacteria reacted positively with Voges-Proskauer (VP), it shows that the bacteria have the ability to reduce the molecular structure of simple chemical components based on their ability to ferment butanediol (Akinde and Iwuozor 2012). Another theory reports that the positive reactions of the bacteria to catalase suggest that they have enzymes capable of breaking down peroxides in aerobic metabolism (Marzuki et al. 2015b; Bibi et al. 2016) stated that the positive reactions of the lactose-reactive bacteria indicate that the bacteria are capable of producing enzymes that can

break down the lactose molecules into glucose (Marzuki et al. 2020b).

Considering the phenotype analysis results on 8 bacterial isolates (Table 4), this information indicates that only 5 isolates (SpBB2; SpCB1, B2; and SpDB1, B2) have the ability to degrade PAH components. Meanwhile, 3 other isolates (SpAB1, B2, and SpBB1) did not all react positively with MR, VP, catalase, and lactose reagents.

The reconstructed phylogenetic tree of sponge-symbiont bacteria 16S rRNA consisted of 3 well-resolved clades supported by moderate bootstrap value shown in Figure 2 that the first clade included SpAB2, SpBB1, SpDB1, B2 that clustered with *Pseudomonas stutzeri* and *Pseudomonas songnenensis* with a homology of 97% to 99%. The second clade consisted of SpAB1, which was positioned as a daughter clade of the *Pseudomonas* species. The third clade is SpCB1 that clustered with *Bacillus* species (96%).

This study's findings are very useful in evaluating PAHs biodegradation activity related to phenotypic characteristics of marine sponge symbiont bacteria. Some comparable data, such as morphology, phenotype, and characterization of bacteria should be considered in the search for possible bacteria in PAHs biodegradation (Lloyd et al. 2010; Marzuki et al. 2016). Several factors explain the difference in the biodegradation activity of bacteria to PAHs, including 1) the properties of PAHs with a higher toxicity level of pyrene than naphthalene have an effect on the biodegradability of bacteria; 2) the characteristics and adaptability of bacteria to predators are different; and 3) the number of bacterial cells, the supply of nutrients and oxygen have contributed to the growth, development activity, and strength of the cells (Medić et al. 2020). The amount of bacterial biodegradation activity against PAHs is determined on the basis of the biodegradation parameters. Increasing the optical density of the bacteria in the media indicated that the bacterial cells were growing and evolving. The higher the optical density of the degradation medium, the more bacterial cells that expand and divide. This indicates that sponge-symbiotic bacterial isolates can adapt to naphthalene and pyrene's polluted environment (Figure 3).

Changes in media pH have been shown to be more acidic, suggesting that bacteria have biodegradation activity against PAHs. The cause of media acidity to increase from the initial stage was due to the contribution of PAH components to the destruction of simple acid organic compounds in biodegradation process. These findings were consistent with previous research (Akinde and Iwuozor 2012), which stated that a simple organic acid compound was one of the products of bacterial biodegradation of hydrocarbons. The increase in the acidity of the biodegradation medium also represents the efficiency of the enzymatic reaction of the bacteria to the PAHs substrate (Marzuki et al. 2015a).

The formation of air bubbles and the fermentation flavor are both unique features and part of the enzyme biodegradation process. In this case, the enzyme was produced by bacteria as a signal for the presence of PAHs toxins. The formation of this enzyme is a bacterial reaction

as it protects itself from the toxic material of PAHs. The ability of bacteria to produce enzymes was based on the results of the biochemical tests (Table 4) in which the isolates used in this biodegradation react to multiple reagents (Lu et al. 2019).

Based on the data in Tables 4 and 5, it can generally be assumed that the 8 sponge-symbiont bacterial isolates used have biodegradation activity against naphthalene and pyrene, while the strength of their activities differs from each other. Order of strength of biodegradation activity of sponge-symbiont bacteria to naphthalene:

SpDB1=SpCB1=SpCB2>SpDB2=SpBB2
= SpAB1> SpAB2=SpBB1.

In comparison to pyrene:

SpDB1>SpDB2=SpCB1,B2>SpAB1,B2=SpBB1

In conclusion, eight marine sponge symbiont bacteria have biodegradable activity against naphthalene and pyrene. The biodegradation activity of bacteria against naphthalene tends to be greater than that of pyrene. There is a tendency for bacteria isolated from sponges with a slimy body surface to have a greater biodegradation activity than bacteria from sponges with clean, and rough body surface regarding sponges from which bacterial isolates were collected. There is, therefore, a relationship between the morphology of the sponge, the features of phenotypic isolation, and the biodegradation strength of PAHs symbiont bacteria.

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