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Polycyclic aromatic hydrocarbon degrading bacteria from the Indonesian Marine Environment

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Abstract. *Yetti E, Thontowi A, Yopi. 2016. Polyaromatic hydrocarbon degrading bacteria from the Indonesian Marine Environment. Biodiversitas 17: 857-864.* Oil spills are one of the main causes of pollution in marine environments. Oil degrading bacteria play an important role for bioremediation of oil spill in environment. We collected 132 isolates of marine bacteria isolated from several Indonesia marine areas, i.e. Pari Island, Jakarta, Kamal Port, East Java and Cilacap Bay, Central Java. These isolates were screened for capability to degrade polyaromatic hydrocarbons (PAHs). Selection test were carried out qualitatively using sublimation method and growth assay of the isolates on several PAHs i.e**.** phenanthrene, dibenzothiophene, fluorene, naphtalene, phenotiazine, and pyrene. The fifty-eight isolates indicated in having capability to degrade PAHs, consisted of 25 isolates were positive on naphthalene (nap) and 20 isolates showed ability to grow in phenanthrene (phen) containing media. Further, 38 isolates were selected for dibenzothiophene (dbt) degradation and 25 isolates were positive on fluorene (flr). On the other hand, 23 isolates presented capability to degrade in phenothiazine (ptz) and 15 isolates could grow in media with pyrene (pyr). Based on homology analysis of partial 16S rDNA gene, we obtained six taxonomy classes of PAH degrading bacteria, namely α-*Proteobacteria* (31%), γ-*Proteobacteria* (43%), *Firmicutes Bacilli* (12%), *Actinobacteria; Micrococcales* (9%), *Actinobacteria; Propionibacteriales* (2%), and *Bacteroidetes; Flavobacteriia* (3%). In this research, we obtained diverse PAH degrading bacteria from marine areas.

Keywords: Bacteria, degradation, marine environment, polyaromatic hydrocarbon

INTRODUCTION

Polycyclic aromatic hydrocarbon compounds (PAHs) is one of the oil component that has 37 % contribution of all (Baek et al. 2004). PAHs are aromatic compounds containing from two to eight conjugated ring systems which have properties cytotoxic, mutagenic, and carcinogenic. They can have a range of substituents such as alkyl, nitro, and amino groups in their structure. Nitrogen, sulfur, and oxygen atoms can also be incorporated into their ring system. PAHs are a concern environmental problem due to their persistency. Moreover, these compounds can stay in the environment for long periods of time. One of the most common ways of PAHs to enter the body is through breathing contaminated air (Crone and Tolstoy 2010).

PAHs in marine environment were distributed due to oil spill contamination noticed by some reports. Baumard et al. (1998) and Witt (1995) reported that PAHs are widespread in marine coastal sediments. Other researchers also found them in surface sediments of the Arctic Ocean with variable concentrations from the shelf to basin (Yunker and Macdonald 1995; Yunker et al. 2011; Zaborska et al. 2011).

Microorganisms play an essential role in the transformation of polycyclic aromatic hydrocarbons (PAHs) and their biological degradation is the main process of natural decontamination in ecosystems. It is well known that bacterial degradation plays an important role in PAH removal from marine environments. Many researchers reported about PAH degrading bacteria isolated both of marine and terrestrial areas. More recent advances, some researchers have revealed PAH degrading bacteria from marine such as *Cycloclasticus* (Dyksterhouse et al. 1995), *Marinobacter* (Hedlund and Staley 2001), *Pseudoalteromonas*, *Marinomonas* (Melcher et al. 2002), *Halomonas* (Melcher et al. 2002), *Sphingomonas* (Demaneche et al. 2004) and *Vibrio* (Hedlund and Staley 2001) found in coastal sediment. Further, biodiversity of PAH degrading bacteria from Indonesia marine environment have been reported by Thontowi and Yopi (2013) that focused in Pari Island. They concluded that *α-Proteobacteria* was the majority class of PAH degrading bacteria existed in Pari Island, Seribu Islands, Jakarta.

The purposes of this study were to screen marine bacteria as our collection in Laboratory of Biocatalyst and Fermentation (LBF), Research Center for Biotechnology, Indonesian Institutes of Sciences (LIPI) on PAHs, identify molecularly, and determine their biodiversity.

MATERIALS AND METHODS

Microorganisms, chemicals, and media

Isolates used in this study were our collection in Laboratory of Biocatalyst and Fermentation, Research Center for Biotechnology, Indonesian Institute of Sciences (LIPI), Cibinong-Bogor, West Java, Indonesia. We isolated these bacteria from several marine areas in Indonesia, i.e.

Pari Island, Jakarta, Kamal Port, East Java and Cilacap Bay, Central Java. The PAHs were purchased from Nacalai Tesque (phenothiazine, napthalene), TCL Tokyo Kasei (dibenzothiophene, fluorene), and Wako (phenanthrene, pyrene). Each PAH stock solution was prepared in dimethyl sulfoxide (DMSO) with a concentration of 3000 ppm. The growth medium, Marine Agar (MA), and Marine Broth (MB) were supplid by BD Difco, while Artificial Sea Water (ASW) that was used as screening media was prepared by PE, PET Japan.

Screening of marine bacteria

The screening test to select the best and the most potential strains were conducted using ASW agar by sublimation method as described by Alley and Brown (2000). The isolates were also growth in ASW broth medium contained 50 ppm of PAHs as carbon source (Juhasz et al. 1997. The isolates were incubated for 7-14 days to select the positive candidates. The candidates with ability to degrade PAHs from sublimation test was shown by one of followed indicator i.e. color change of media; clear zone appearance around the isolates; and both of them.

PCR amplification of the 16S rDNA genes and sequencing

The partial 16S rDNA genes of isolates were amplified from genomic DNA using the universal primer set 9f (5′GAGTTTGATYMTGGCTCAG-3′) and 1541r (5′-AAGGAGGTGWTCCARCC-3′). The thermal cycling parameters were a min, hot start at 95°C, 2 min (1cycle), followed by 95^{o}C, 2 min (1 cycle); 95^{o}C, 30 sec; 65^{o}C, 1min; 72^{o}C, 2 min (10 cycles); 95^{0}C, 30 sec; 55^{o}C, 1 min; 72^{o}C, 2 min (30 cycles); 72^{o}C, 2 min (1 cycle). The sequences were determined directly using conserved bacterial 16S rDNA sequencing primers by First Base.

Phylogenic tree analysis

The 16S rDNA sequences were aligned with published sequences from the GenBank database using the NCBI BLASTN comparison software. The program was run via internet through the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/blast/). Multiple alignment and phylogenetic trees were constructed by the neighbour-joining method using Mega 3.1 ABI *sequencer software* (Kumar et al. 2004). Nearly full-length 16S rDNA sequences of the most phylogenetically related strains were selected from the Gen Bank database as reference strains.

RESULTS AND DISCUSSION

Screening test

One hundred thirty two isolates were tested on six PAHs i.e. naphthalene, dibenzothiophene, fluorene, phenanthrene, phenothiazine, and pyrene. Due to their insolubility characteristic, the selection of PAH degrading bacteria on solid media was conducted by sublimation method as described by Alley and Brown (2000). With this test, qualitative indicators for candidates that have capability on PAHs degradation were clear zone and/or color change. Fifty eight isolates were selected as potential isolates for PAHs biodegradation.

Among candidates of PAHs degrading bacteria, 25 isolates were selected for naphthalene (naph) degradation and 20 isolates were able to degrade phenanthrene (phen). Further, 38 isolates could grow in dibenzothiophene (dbt) containing media, while 25 isolates were positive for fluorene (flr). Moreover, 23 isolates showed capability to degrade phenothiazine (ptz,) and 15 isolates were potential for pyrene (pyr) degradation. Results of sublimation performance of some isolates were shown in Figure 1, while growth test were performed in Figure 2.

Due to their difference performance, there were interesting phenomena showed by positive candidates of isolates possessing capability to degrade PAHs. According to some research, growth on PAHs in solid media was considered positive by the formation of a clear zone around the growing colonies or appearance of pigments (Johnsen et al. 2002; Kumar et al. 2006). In this research, the potential isolates for four PAHs degradation i.e. naphthalene, fluorene, phenanthrene, and pyrene, presented clear zone and/or color change of media from clear to yellow. On the other hand, dibenzothiophene (DBT) degrading bacteria showed on the plate as well as in liquid media the bacterium produced orange or reddish brown water-soluble product or metabolite (Kumar et al. 2006; Andreolli 2011). While, isolates candidates for phenothiazine degradation had clear zone and/or color change media to blue (Figures 1 and 2). The existence of color complex and clear zone on the media proved that the bacteria could utilize PAHs compound as a carbon source for their growth (Marino et al. 1998). The phenomenon shows that there has been metabolism of PAHs by these isolates. An example from observed the formation of a diffusible yellow color during microbial degradation of biphenyl and have shown that this color is caused by a meta-cleavage product (Ahmad et al. 1991).

Bacterial identification and phylogenis tree

Analysis of bacterial rRNA gene sequences revealed that the characterized isolates belong to 21 genera and six taxonomy classes within four phyla (*Proteobacteria*, *Firmicutes*, *Bacilli*, and *Actinobacteria*). Molecular identification of 58 isolates by homology analysis with BLAST search was provided completely in Table 1. Several isolates showed identity less than 96% such as LBF-1-0103, LBF-1-0108, LBF-1-0130, LBF-1-0136, and LBF-1-0137. The first tree isolates were identified as *Brachybacterium saurashtrense* strain JG 06 (94%), *Shewanella algae* strain KJ-W37 (95%), and *Janibacter limosus* strain DSM 11140 (95%). Whereas, the last two isolates were identified as *Halomonas cupida* strain NBRC 102219, respectively. The identity value less than 96% of isolate can be assumed as candidate of new species (Fox, et al. 1992), but definitely, these results need further assay such as morphological and biochemical test.

The relationship among 58 isolates as candidates of PAHs degrading bacteria was described by phylogenic tree as shown in figure 3. The phylogenetic tree was

constructed based on partial 16S rDNA gene fragments and MEGA 3.1 software using the maximum composite likelihood model with gamma-distributed rates and pairwise deletion.

Biodiversity of PAHs degrading bacteria

According to analysis of 16SrDNA gene, fifty eight isolates that selected as PAH degrading bacteria classified in six taxonomy classes designated as α-*Proteobacteria* (31%), γ-*Proteobacteria* (43%), *Firmicutes Bacilli* (12%), *Actinobacteria; Micrococcales* (9%), *Actinobacteria; Propionibacteriales* (2%), and *Bacteroidetes; Flavobacteriia* (3%) (Figure 4). According to the data, the majority of the isolates were members of *Proteobacteria* phylum (76%) followed by *Firmicutes* (12%), *Actinobacteria (9%),* and *Bacteroidetes* (3%), respectively.

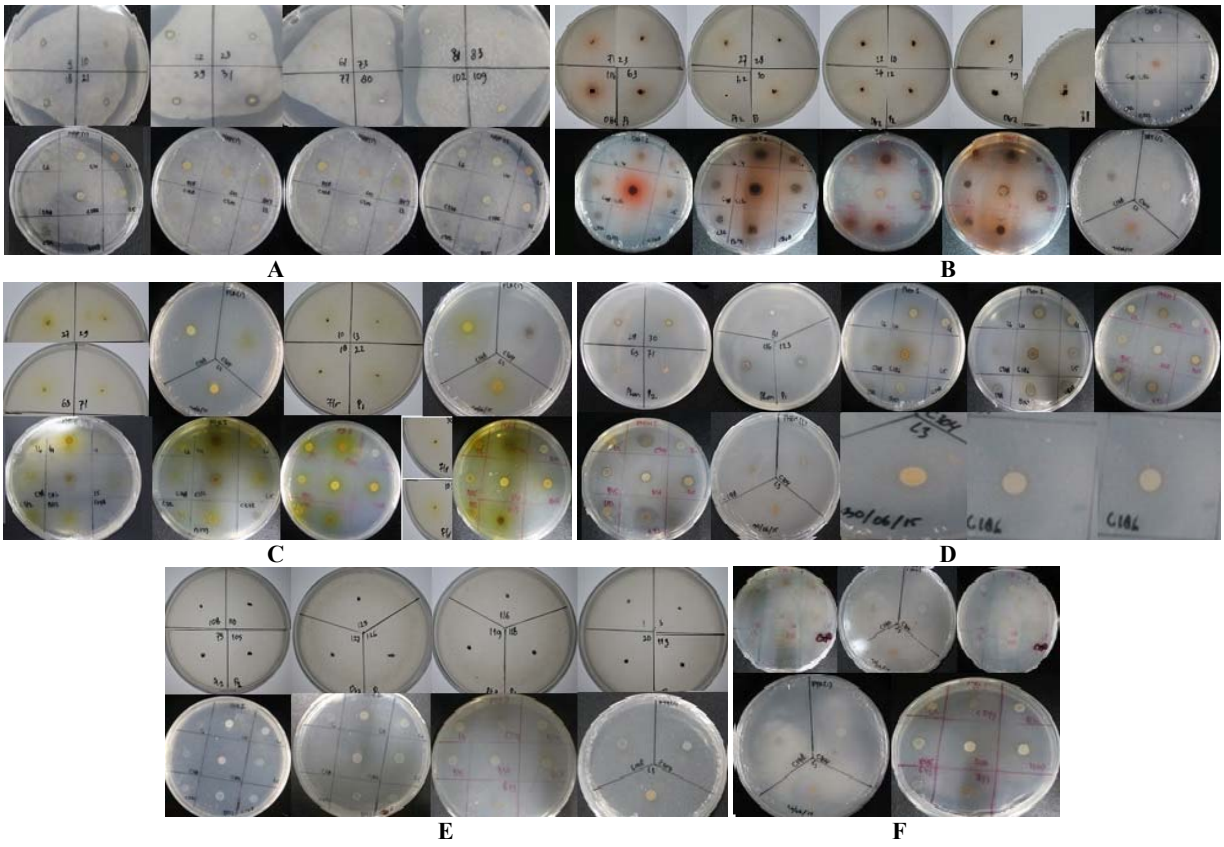


Figure 1. Screening result of marine bacteria on six PAHs by sublimation methods, A. Naphthalene, B. Dibenzothiophene, C. Fluorene, D. Phenanthrene, E. Phenothiazine, and F. Pyrene

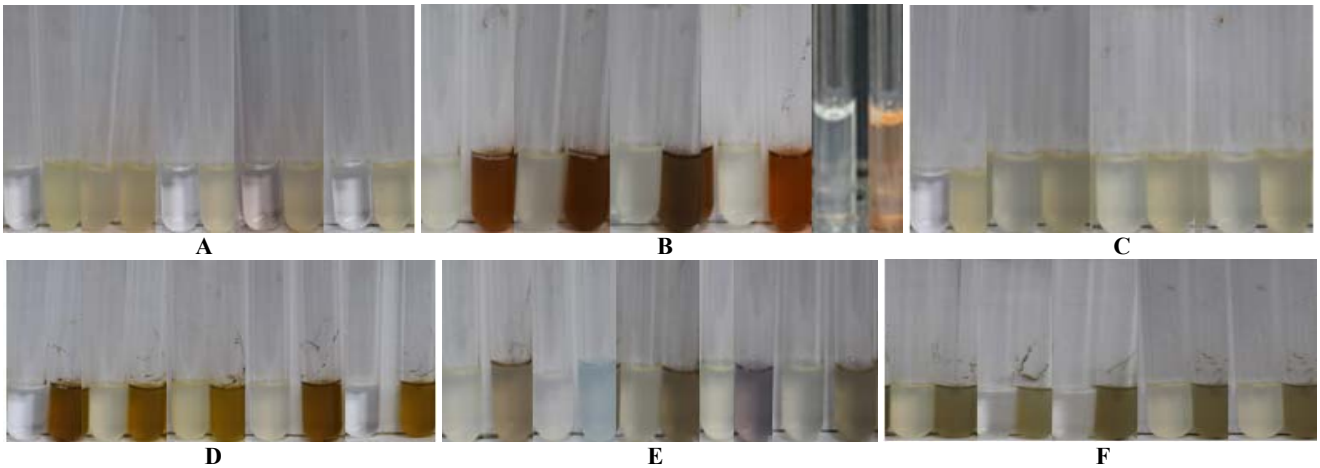


Figure 2. Screening results of marine bacteria on six PAHs by growth test in Broth medium; A. Naphthalene, B. Dibenzothiophene, C. Fluorene, D. Phenanthrene, E. Phenothiazine, and F. Pyrene

Table 1. Homology analysis of isolates with BLAST search

Isolate code	Closest strain	Class	Accession No	Similarity (%)
LBF-1-0001	*Labrenzia aggregata* IAM 12614	Proteobacteria; Alphaproteobacteria	NR-115659	99
LBF-1-0003	*Thalassospira permensis* strain SMB34	Proteobacteria; Alphaproteobacteria	NR_042909	99
LBF-1-0009	*Muricauda aquimarina* strain SW-63	Bacteroidetes; Flavobacteriia	NR_042909	100
LBF-1-0010	*Pseudoalteromonas shioyasakiensis* strain SE3	Proteobacteria; Gammaproteobacteria	NR_125458	99
LBF-1-0011	*Pseudoalteromonas shioyasakiensis* strain SE3	Proteobacteria; Gammaproteobacteria	NR_125458	99
LBF-1-0012	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0013	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0018	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0019	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0020	*Pseudoalteromonas shioyasakiensis* strain SE3	Proteobacteria; Gammaproteobacteria	NR_125458	99
LBF-1-0021	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0022	*Alcanivorax xenomutans* strain JC109	Proteobacteria; Gammaproteobacteria	NR_133958	99
LBF-1-0023	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR-116368	99
LBF-1-0024	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0026	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0027	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR-116368	99
LBF-1-0028	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0029	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0030	*Bacillus subtilis subsp. subtilis* strain OS-44.a	Firmicutes; Bacilli	NR_114997	99
LBF-1-0031	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0040	*Bacillus stratosphericus* strain 41KF2a	Firmicutes; Bacilli	NR_042336	98
LBF-1-0046	*Vibrio alginolyticus* strain NBRC 15630	Proteobacteria; Gammaproteobacteria	NR_113781	99
LBF-1-0050	*Brachybacterium conglomeratum* strain J 1015	Actinobacteria; Micrococcales	NR_104689	98
LBF-1-0054	*Microbacterium amylolyticum* strain X5	Actinobacteria; Micrococcales	KJ151779	99
LBF-1-0056	*Pseudomonas aeruginosa* strain KUN2	Proteobacteria; Gammaproteobacteria	KT966462	99
LBF-1-0057	*Pseudomonas aeruginosa* strain SNP0614	Proteobacteria; Gammaproteobacteria	NR_118644	99
LBF-1-0060	*Muricauda olearia* strain CL-SS4	Bacteroidetes; Flavobacteriia	NR_044579	99
LBF-1-0061	*Novosphingobium pentaromativorans* strain US6-1	Proteobacteria; Alphaproteobacteria	NR_025248	99
LBF-1-0062	*Pseudomonas balearica* strain SP1402	Proteobacteria; Gammaproteobacteria	NR_025972	99
LBF-1-0070	*Pseudomonas stutzeri* strain ISA12	Proteobacteria; Gammaproteobacteria	HQ189755	99
LBF-1-0072	*Nocardioides zeae* strain JM-1068	Actinobacteria; Propionibacteriales	NR_134102	98
LBF-1-0074	*Idiomarina zobellii* strain SBU4	Proteobacteria; Gammaproteobacteria	KF052992	99
LBF-1-0076	*Shewanella indica* strain 0102	Proteobacteria; Gammaproteobacteria	KP236237	99
LBF-1-0079	*Lysobacter* sp. 3070	Proteobacteria; Gammaproteobacteria	AM111012	99
LBF-1-0080	*Lysobacter concretionis* strain Ko07	Proteobacteria; Gammaproteobacteria	NR_041003	97
LBF-1-0082	*Stakelama pacifica* strain JLT832	Proteobacteria; Alphaproteobacteria	NR_116368	99
LBF-1-0101	*Sphingomonas* sp. 2MPII	Proteobacteria; Alphaproteobacteria	U90216	96
LBF-1-0102	*Brachybacterium* sp. XJ133-127-6NF2	Actinobacteria; Micrococcales	JX975428	97
LBF-1-0103	*Brachybacterium saurashtrense* strain JG 06	Actinobacteria; Micrococcales	NR_116516	94
LBF-1-0107	*Shewanella indica* strain KJW27	Proteobacteria; Gammaproteobacteria	NR_108899	98
LBF-1-0108	*Shewanella algae* strain KJ-W37	Proteobacteria; Gammaproteobacteria	JQ799131	95
LBF-1-0111	*Hydrothermal vent* strain TB66	Proteobacteria; Alphaproteobacteria	AF254109	97
LBF-1-0114	*Stenotrophomonas maltophilia* strain G7	Proteobacteria; Gammaproteobacteria	KC136824	98
LBF-1-0118	*Bacillus subtilis* strain A2	Firmicutes; Bacilli	KC433738	97
LBF-1-0122	*Bacillus stratosphericus* strain 41KF2a	Firmicutes; Bacilli	NR_042336	97
LBF-1-0124	*Bacillus aerius* strain 24K	Firmicutes; Bacilli	NR_118439	96
LBF-1-0125	*Bacillus altitudinis* strain LZLJ004	Firmicutes; Bacilli	KR018737	96
LBF-1-0128	*Alcanivorax dieselolei* strain B5	Proteobacteria; Gammaproteobacteria	NR_043106	97
LBF-1-0130	*Janibacter limosus* strain DSM 11140	Actinobacteria; Micrococcales	NR_026362	95
LBF-1-0131	*Pseudomonas stutzeri* strain ATCC 17588	Proteobacteria; Gammaproteobacteria	NR_041715	98
LBF-1-0133	*Pseudomonas stutzeri* strain W31	Proteobacteria; Gammaproteobacteria	KT380576	97
LBF-1-0134	*Pseudomonas aeruginosa* PAO1	Proteobacteria; Gammaproteobacteria	NR_074828	97
LBF-1-0135	*Bacillus zhanjiangensis* strain JSM 099021	Firmicutes; Bacilli	NR_117854	96
LBF-1-0136	*Halomonas cupida* strain NBRC 102219	Proteobacteria; Gammaproteobacteria	NR_114046	95
LBF-1-0137	*Halomonas cupida* strain NBRC 102219	Proteobacteria; Gammaproteobacteria	NR_114046	95
LBF-1-0141	*Alcanivorax xenomutans* strain JC109	Proteobacteria; Gammaproteobacteria	NR_133958	96
LBF-1-0142	*Marinobacter koreensis* strain DD-M3	Proteobacteria; Gammaproteobacteria	NR_043718	96
LBF-1-0143	*Marinobacter koreensis* NBRC 106396	Proteobacteria; Gammaproteobacteria	AB682412.1	96

Table 2. Classification of PAHs degrading bacteria based on their capability to degrade PAHs from sublimation test

Group	Isolates Code	Capability of PAHs degradation					
		LMW PAHs					HMW PAHs
		NAPH	DBT	FLU	PHEN	PTZ	PYR
I	LBF-1-0001	-	-	-	-	CC	-
	LBF-1-0003	-	-	-	-	CC	-
	LBF-1-0011	-	CC	-	-	-	-
	LBF-1-0012	-	CC	-	-	-	-
	LBF-1-0019	-	CC	-	-	-	-
	LBF-1-0020	-	-	-	-	CC	-
	LBF-1-0024	-	CC	-	-	-	-
	LBF-1-0026	-	CC	-	-	-	-
	LBF-1-0028	-	CC	-	-	-	-
	LBF-1-0040	-	-	-	-	CC	-
	LBF-1-0046	-	-	-	-	CC	-
	LBF-1-0050	-	-	-	-	CC	
	LBF-1-0054	-	CC	-	-	-	-
	LBF-1-0056	-	-	-	-	CC	-
	LBF-1-0057	-	-	-	-	CC	-
	LBF-1-0061	-	CZ, CC	-	-	-	-
	LBF-1-0072	CZ	-	-	-	-	-
	LBF-1-0074	-	-	-	-	CC	-
	LBF-1-0076	CZ	-	-	-	-	-
	LBF-1-0079	CZ	-	-	-	-	-
	LBF-1-0082	CZ, CC	-	-	-	-	-
	LBF-1-0107	-	-	-	-	-	-
	LBF-1-0111	-	-	-	-	CC	-
	LBF-1-0118	-	-	-	-	CC	-
	LBF-1-0124	-	-	-	-	CC	-
	LBF-1-0125	-	-	-	-	CC	-
II	LBF-1-0009	CZ	CC	-	-	-	-
	LBF-1-0010	CZ	CC	CC	-	-	-
	LBF-1-0013	-	CC	CC	-	-	-
	LBF-1-0018	CZ	CC	CC	-	-	-
	LBF-1-0021	CZ	CC	CC	-	-	-
	LBF-1-0022	CZ	CC	CC	-	CZ, CC	-
	LBF-1-0023	CZ	CC	-	-	-	-
	LBF-1-0027	-	CC	CC	-	-	-
	LBF-1-0029	CZ	CC	CZ, CC	CZ	-	-
	LBF-1-0030	-	CC	CC	CZ	-	-
	LBF-1-0031	CZ	CC	CC	-	-	-
	LBF-1-0060	CZ	-	CC	-	-	-
	LBF-1-0103	-	CC	CC	-	CC	-
	LBF-1-0070	-	CC	CC	CZ	-	-
	LBF-1-0131	CZ	CZ	CC	CZ, CC	-	-
	LBF-1-0141	CZ	CC	-	-	-	-
	LBF-1-0142	-	CZ, CC	CZ, CC	CZ, CC	-	-
III	LBF-1-0062	-	CC	CC	CZ	CC	CZ
	LBF-1-0080	CZ	-	-	CZ	-	CZ
	LBF-1-0101	CZ, CC	CC	-	CZ	CZ	CZ
	LBF-1-0102	CZ	CZ, CC	CC	CZ	CZ	CZ
	LBF-1-0114	CZ	CC	CC	CZ	CZ	CZ
	LBF-1-0122	-	CZ	-	CZ	-	CZ
	LBF-1-0128	CZ	CZ, CC	CC	CZ	-	CZ
	LBF-1-0129	CZ	CZ, CC	CC	CZ	CZ	CZ
	LBF-1-0130		CZ, CC	CC	CZ	CZ	CZ
	LBF-1-0133	CZ	CC	CZ, CC	CZ, CC	CZ	CZ
	LBF-1-0134	-	CZ, CC	CC	CZ, CC	-	CZ
	LBF-1-0135	-	CZ, CC	CC	CZ, CC	CZ	CZ
	LBF-1-0136	CZ	CC	CC	CZ, CC	-	CZ
	LBF-1-0137	-	CZ, CC	CZ, CC	CZ, CC	-	CC
	LBF-1-0143	CZ	CZ, CC	CZ, CC	CZ	CZ	CZ

Note: Tree indicator for positive candidates of PAH degrading bacteria namely Clear Zone (CZ), Colour Change (CC), and Both of them (CZ, CC)

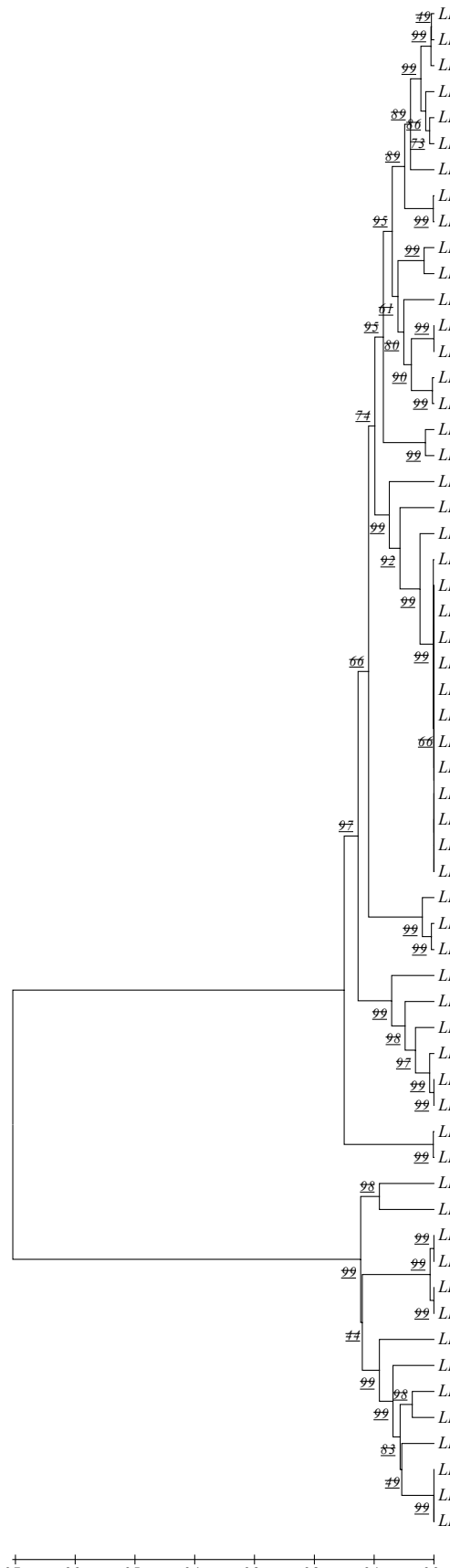
The information from this research confirm that *Proteobacteria* is a common phyla of PAH degrading bacteria from marine areas (Yuan et al. 2015; Isaac et al. 2013; Dong et al. 2015). The data of this research also supported several researches that informed *Proteobacteria* as dominant phyla of PAH degrading bacteria from Indonesia marine area (Yopi et. al. 2006; Harwati et al. 2007; Thontowi and Yopi 2013).

More specifically, *Stakelama, Pseudomonas,* and *Bacillus* were the predominant genera of PAHs degrading bacteria from tree marine areas i.e. Pari Island, Kamal Port, and Cilacap Bay with composition 22%, 12%, and 12%, respectively (Figure 5). Overall, *Stakelama* is the most dominant genera of PAH degrading bacteria from tree marine areas (Pari Islands, Kamal Port, and Cilacap). Actually, the bacteria from this genus is rarely reported as PAH degrader previously. Dong et. al. (2015) released that *Pseudomonas*, *Cycloclasticus*, and *Alcanivorax* are predominant genus of PAH degrading bacteria from deep-sea sediments of Arctic Ocean; while *Alcanivorax* is the most dominant in deep sea water of Southwest India (Yuan et al. 2015) .

The remaining other genera were *Alcanivorax, Pseudo-alteromonas, Shewanella, Halomonas, Brachybacterium, Marinobacter, Muricauda, Lysobacter, Novosphingobium, Labrenzia, Thallaspira, Hydrothermal, Vibrio, Nocardioides, Stenotrophomonas, Janibacter, Microbacterium,* and *Sphingomonas.* Previously, most of these genus have been reported as PAH degrader such as *Alcanivorax, Pseudo-alteromonas, Shewanella, Halomonas, Brachybacterium, Marinobacter, Muricauda, Lysobacter, Novosphingobium, Vibrio* (Hedlund and Staley 2001; Hedlund and Staley, 2006; Melcher et al. 2002; Demaneche et al. 2004; Dong et al. 2015). Particularly, *Alcanivorax* is also noteworthy because it has been recognized as one of of obligate marine hydrocarbon degraders (Yakimov et al. 2007). In addition, from this research, we also obtained several genus that are rarely reported as PAH degrader namely *Labrenzia, Thallaspira, Hydrothermal, Nocardioides, Steno-trophomonas, Janibacter,* and *Microbacterium*. Therefore, the result of this research revealed that PAH degrading bacteria from studied marine areas were very diverse.

Degradation characterization of PAH degrading bacteria

Interesting phenomena were shown in screening result using sublimation test. There were different indicators showed among positive isolates for PAHs degradation i.e clear zone, color change or both of them. Further, we also obtained information about diversity of PAH degrading bacteria corresponding with their capability in PAHs degradation (Figure 3). The phylogenic tree gave information of how far relationship among isolates. On the other hand, the tree also described how diverse the characteristics of each isolate in PAHs degradation. Although some isolates have close relationship even originated from same species, they have different characteristic in PAHs degradation. This information could be seen from some species such as *Pseudomonas aeruginosa*, *Stakelama pacifica, Bacillus stratosphericus*, and others.



Capability of PAHs Degradation					
LMW					**HMW**
NAPH	**DBZ**	**FLU**	**PHN**	**PTZ**	**PYR**
-	-	-	-	CC	-
-	-	-	-	CC	-
CZ	CC	CZ, CC	CZ, CC	CZ	CZ
-	CZ, CC	CC	CZ	CZ	CZ
-	CC	CC	CZ	CC	CZ
-	CC	CC	CZ	-	-
-	CZ, CC	CZ, CC	CZ, CC	-	-
CZ	CC	CC	-	CC, CZ	-
CZ	CC	-	-	-	-
-	-	-	-	CC	-
-	-	-	-	CC	-
-	-	-	-	CC	-
CZ	CC	CC	-	-	-
-	CC	-	-	-	-
CZ	-	-	-	-	-
-	-	-	-	CC	-
CZ	-	-	-	-	-
CZ	-	-	CZ	-	CZ
-	-	-	-	CC	-
-	-	-	-	CC	-
-	CZ, CC	-	-	-	-
CZ	CC	CC	-	-	-
CZ	CC	-	-	-	-
-	CC	-	-	-	-
CZ	CC	CC	-	-	-
-	CC	CC	-	-	-
CZ	CC	CZ, CC	CZ	-	-
-	CC	-	-	-	-
-	CC	-	-	-	-
CZ, CC	-	-	-	-	-
-	CC	-	-	-	-
-	CC	CC	-	-	-
CZ	CC	-	-	-	-
-	CC	-	-	-	-
-	CZ, CC	CC	CZ, CC	-	CZ
-	-	-	-	CC	-
-	CC	-	CZ	-	CZ
CZ	-	-	-	-	-
-	CC	-	-	-	-
CZ	CZ, CC	CC	CZ	CZ	CZ
-	-	-	-	CC	-
CZ	CZ, CC	CC	CZ	CZ	CZ
-	CC	CC	-	CC	-
CZ	CC	-	-	-	-
CZ	-	CC	-	-	-
CZ, CC	CC	-	CZ	CZ	CZ
-	-	-	-	CC	-
-	CC	CC	CZ		-
-	-	-	-	CC	-
-	-	-	-	CC	-
-	-	-	-	CC	-
CZ	CC	CC	CZ	CZ	CZ
CZ, CC	-	-	-	-	-
-	CZ	CC	CZ, CC	-	-
CZ	CZ, CC	CZ, CC	CZ	CZ	CZ
CZ	CZ, CC	CC	CZ	-	CZ
-	CZ, CC	CC	CZ, CC	CZ	CZ
CZ	CC	CC	CZ, CC	-	CZ
-	CZ, CC	CZ, CC	CZ, CC	-	CC

Figure 3. Phylogenetic tree derived from 16S rDNA gene sequence of 58 isolates and their capabilities for PAHs degradation. The NJ-tree was constructed using neighbour joining algorithm with Kimura 2 parameter distances in MEGA 3.1 software. Bar, 1% estimated sequence divergence. LMW: low molecular weight, HMW: high molecular weight, NAPH: naphthalene, DBT: dibenzothiophene, FLR: fluorene, PHEN: phenanthrene; PTZ: phenothiazine; PYR: pyrene; CC: colour change; CZ: clear zone

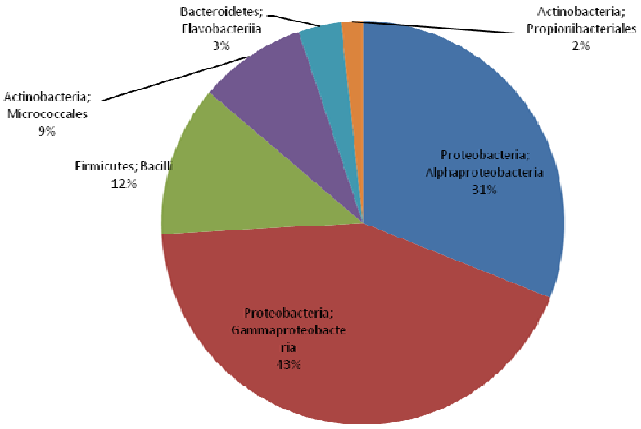


Figure 4. Biodiversity of PAH Degrading Bacteria from Marine Area in Indonesia. The 58 Selected Bacteria were divided into six Classes

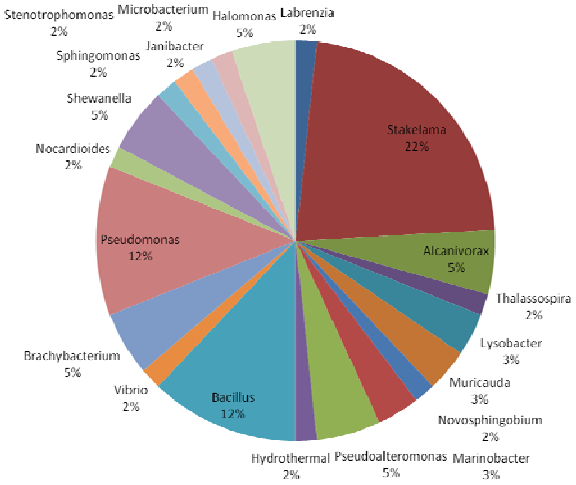


Figure 5. Biodiversity of PAH Degrading Bacteria from Marine Area in Indonesia. Analysis of bacterial rRNA gene sequences revealed that the characterized isolates belong to 21 genera

Furthermore, some data overview could be analyzed related to classification of PAHs used in this study. Naphthalene, dibenzothiophene, fluorene, phenanthrene, phenothiazine are categorized as Low Molecular Weight (LMW) PAHs, consisted of 2 ring benzene rings. Whereas, pyrene is High Molecular Weight (HMW), contained 3 (three) benzene rings (Gong et al. 2007). Otherwise, from the isolates, we could see that some isolates have capability to degrade 1 (one) or more PAHs. Therefore, from these data and information, we classified the isolates into 3 (three) group (Table 2). First, isolates that could degrade only 1 LMW PAHs (26 isolates); second, isolates that could degrade more than 1 LMW PAHs (17 isolates); and third, isolates that could degrade LMW and HMW PAHs (15 isolates).

Based on this study, we obtained six taxonomy classes and 21 genera of PAH degrading bacteria from marine areas in Indonesia within four phyla i.e. *Proteobacteria*, *Firmicutes*, *Bacilli*, and *Actinobacteria*. Therefore, we concluded that PAH degrading bacteria from studied areas were very diverse. These potential isolates have different characteristics in PAHs degradation. Furthermore, we classified these isolates into three groups designated as isolates that could degrade only 1 LMW PAHs (group I); isolates that could degrade more than 1 LMW PAHs (group II); and isolate that could degrade LMW and HMW PAHs (group III).

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