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CHARACTERIZATION OF PSYCHROTROPHIC BACTERIA FROM SEA WATERS OF MAKASAR STRAIT, INDONESIA

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ABSTRACT

In this study we isolated marine bacteria from seawaters of Makasar Strait, Indonesia and tested for low temperature adaptation profiles. A total of 27 bacterial isolates represented the most dominant colonies in ZoBell agar plates were selected and tested for low-temperature adaptation, in which all isolates were able to grow at 4 and 20°C incubation indicating that they are psychrotrophic bacteria. A rapid grouping by using repetitive PCR was carried to estimate the richness of the isolates. Following sequencings, it was shown that psychrotrophic bacteria belonged to the members of genera Psychrobacter, Pseudomonas and Vibrio.

Keywords: *psychrotrophic bacteria, deep-sea, Makasar strait, Psychrobacter, Pseudomonas, Vibrio*

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INTRODUCTION

Low temperature adapted bacteria have been classified based on the occurring of growth at 4°C and 20°C, i.e. psychrophiles were those able to grow at 4°C but unable to grow at 20°C, whereas psychrotrophs were those able to grow both at 4°C and 20°C incubations (Urakawa *et al*, 1999a; Radjasa *et al*, 2001).

Several studies have been carried out to determine the richness of low temperature adapted-bacteria from geographically different regions (Bowman *et al*, 2003; 1997, Urakawa *et al*, 1999a, 1999b, Radjasa *et al*, 2001; Knoblauch *et al*, 1999).

Rapid groupings of psychrotrophic bacteria based on molecular based-approaches have reported, such as RFLP (Restriction Fragment Length Polymorphism) (Urakawa *et al*, 1999a, 1999b; Radjasa *et al*, 2001).

Recent technique known as repetitive sequence-based PCR (rep-PCR) has been applied to group a numbers of bacterial isolates that produced complex fingerprint profiles from both gram positive and negative bacteria (Rademaker *et al*, 2005). However, this technique has not been employed to estimate the richness of marine

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psychrotrophic bacteria.

To my knowledge, there has no report been documented on the diversity of marine psychrotrophic bacteria from Indonesian waters. Studies regarding diversity of low temperature adapted-bacteria are important for understanding principal processes in the deep waters. Near Indonesia several deep-seas exist with diverse environmental conditions. However, up to now there has been no effort to study the microbial communities of the deep-sea environments by Indonesian scientists.

Microorganisms evolving in habitats with low temperatures need to be studied to understand their adaptation, the distribution within the ocean, and the role of in the biogeochemical processes as well as their biotechnological potentials.

In this work, we reported the richness of psychrotrophic bacteria isolated from the deep-sea waters of Makasar Strait, Indonesia assessed by 16S rDNA approach.

MATERIALS AND METHODS

Sampling and isolation of low temperature-adapted bacteria

Seawaters were collected by water samplers attached to CTD from Makasar Strait, Indonesia during INSTANT Cruise on R/V Baruna Jaya VIII. Preparation of water samples and isolation of bacterial isolates were carried out according to a method previously described (Radjasa *et al*, 2001). Isolation was performed onboard on R/V Baruna Jaya

Psychrophily test

Psychrophily test was conducted at the laboratory of Marine Microbiology, Department of Marine Science, Diponegoro University. Test was conducted to classify the low temperature-adapted bacteria based on the ability to grow at two different temperatures, 4^o C and 20^oC (Radjasa *et al*,

2001). Those grow only at 4^o are regarded as psychrophilic bacteria while those grow both at 4^o and 20^o are regarded as psychrotrophic bacteria, respectively (Morita, 1975).

Repetitive-PCR

Molecular based-works including DNA extractions, rep-PCR, and PCR were carried out at the laboratory of Marine Biotechnology, Department of Marine Science, Diponegoro University. Whereas, the DNA sequencings were performed at the Molecular Biology Laboratory, Agency for the Assessment and Application Technology (BPPT), in Jakarta, Indonesia.

For Rep-PCR, BOX A1R (5'-CTACggCAAggCgACgCTgACg-3') Versalovic *et al.*, 1994) were used. The REP 1R-I and REP 2-I primers contain the nucleotide inosine (I) at ambiguous positions in the REP consensus (34). PCR reaction contained of 1 µl DNA template (diluted 100x), 1 µl primer, 7,5 µl Megamix Royal dan sterile water up to total volume of 15 µl. Amplifications were performed with a thermal cycler model Gene Amp PCR System 9700 with the following temperature profiles: initial denaturation at 95 °C for 5 min; 30 cycles of denaturation (92 °C for 1 min), annealing (50 °C for 1,5 min), extension (68 °C for 8 min); and final extension at 68 °C for 10 min. Five µl aliquot PCR products were run using elektrophores on 6% acrilamide gel by using 1x TBE buffer.

Grouping of isolates

Grouping was carried out by making matrixes from the positions of bands on the gel which were then analyzed by using Free Tree program by using UPGMA method for constructing the tree. Resampling was performed by bootstrapping with 1000 replications.

DNA extraction, PCR amplification and sequencing of 16S rRNA gene fragments.

DNA extractions, PCR amplification of partial 16S rRNA gene of bacterial strains, purification of PCR products and subsequent sequencing analysis were performed according to the method of Radjasa et al (2007). The determined DNA sequences of strains were then compared for homology to the BLAST database

RESULTS AND DISCUSSION

Results

Rapid grouping of psychrotrophic isolates

Based on the repetitive-PCR results and constructed dendrogram of the isolates, 6 groups were created and 6 different isolates representing different groups (**Fig. 1**) were further selected for DNA sequencings.

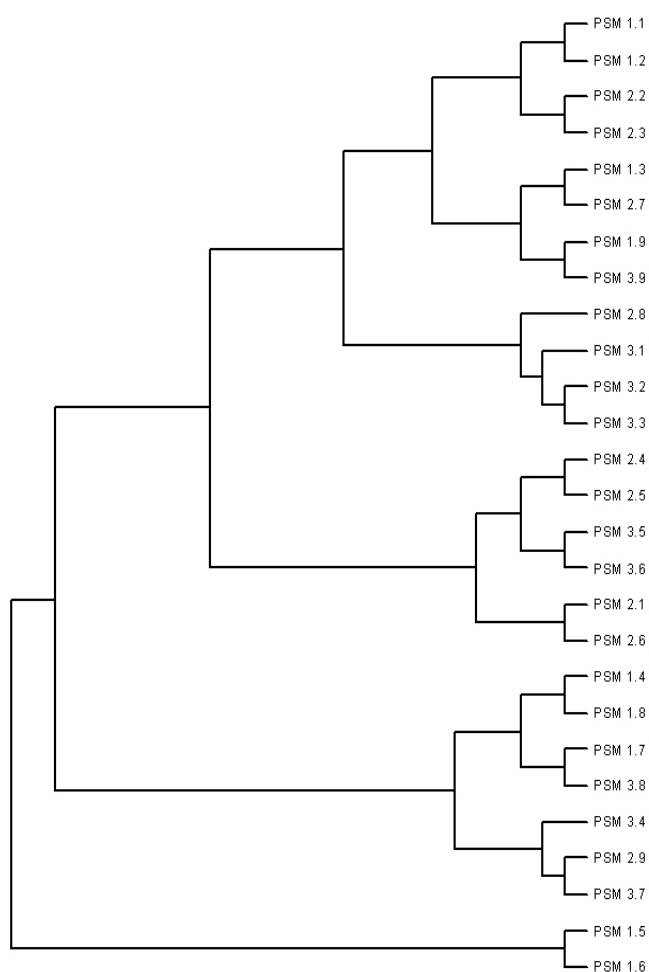


Fig. 1. Dendrogram of psychrotrophic isolates (PSM 1.5, PSM 2.2, PSM 2.4, PSM 2.7, PSM 2.8, PSM 3.4 were further selected for DNA sequencing)

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Psychrophilic isolates

therefore they were regarded as psychrotrophic bacteria (**Table 1**).

Psychrophily test indicated that all isolates were able to grow both at 4° and 20°C,

Table 1. Psychrotrophic isolates collected from different depths and stations

| No. | Station | Depth(m) | Isolate | Gram |
|-----|---------|----------|---------|------|
| 1 | | | PSM 1.1 | - |
| 2 | | | PSM 1.2 | - |
| 3 | | | | |
| 4 | | | | |
| 5 | | 400 | | |
| 6 | | | PSM 1.3 | - |
| 7 | | | PSM 1.4 | - |
| 8 | 1 | | PSM 1.5 | - |
| | | 600 | | |
| | | | PSM 1.6 | - |
| | | | PSM 1.7 | - |
| | | 800 | | |
| 9 | | | PSM 1.8 | - |
| | | | PSM 1.9 | - |
| 10 | | | PSM 2.1 | - |
| 11 | | | PSM 2.2 | - |
| 12 | | 400 | | |
| 13 | | | PSM 2.3 | - |
| 14 | | | PSM 2.4 | - |
| 15 | | | PSM 2.5 | - |
| 16 | | | | |
| 17 | 4 | | | |
| | | 600 | | |
| | | | PSM 2.6 | - |
| | | | PSM 2.7 | - |
| | | 800 | | |
| 18 | | | PSM 2.8 | - |
| 19 | | | PSM 2.9 | - |
| 20 | | | PSM 3.1 | - |
| 21 | | | PSM 3.2 | - |
| 22 | | | | |
| 23 | | 400 | | |
| 24 | | | PSM 3.3 | - |
| 25 | | | PSM 3.4 | - |
| 26 | | 600 | | |
| | | | PSM 3.5 | - |
| | | | PSM 3.6 | - |
| | 7 | | PSM 3.7 | - |
| | | | PSM 3.8 | - |
| | | 800 | | |
| 27 | | | PSM 3.9 | - |

- sign means Gram negative bacteria

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Sequencing of representative psychrotrophic isolates

repetitive approach are presented in the following table:

The results of DNA sequencing of the representative isolates based on PCR

Tabel 2. Characterization of representative psychrotrophic isolates

| No | Strain | Closest relative | Homology | Acc. Number |
|----|---------|---|----------|-------------|
| 1 | PSM 2.2 | <i>Psychrobacter alimentarius</i> | 99 | AY513645 |
| 2 | PSM 2.7 | <i>Psychrobacter</i> sp. 9B_7 | 99 | AY689064 |
| 3 | PSM 2.8 | <i>Psychrobacter</i> sp. TSBY-70 | 99 | DQ166171 |
| 4 | PSM 3.4 | Uncultured <i>Psychrobacter</i> sp. clone GAMMA4B | 98 | AY494611 |
| 5 | PSM 2.4 | <i>Pseudomonas</i> sp. WR7-2 | 98 | AY263480 |
| 6 | PSM 1.5 | <i>Vibrio splendidus</i> | 100 | AJ874367 |

Generic composition of psychrotrophic isolates

As indicated in the **Table 3**, the members of genus *Psychrobacter* are the most dominant group followed by *Pseudomonas* and *Vibrio* groups.

| No | Genus | Number of isolates | % |
|----|----------------------|--------------------|-------|
| 1 | <i>Psychrobacter</i> | 18 | 66,67 |
| 2 | <i>Pseudomonas</i> | 7 | 25,93 |
| 3 | <i>Vibrio</i> | 2 | 7.40 |

Discussion

In a study aimed at determining the diversity of low temperature-adapted bacteria, we investigated marine bacteria isolated from deep-sea waters of Makasar strait, Indonesia. Our attention was focused on the occurrence of low temperature-adapted bacteria followed by PCR-based approach for estimating the richness of psychrotrophic bacteria.

Understanding the indigenous low temperature-adapted bacteria has important

implications for analyses of microbial function and biogeochemical processes in the deep-sea environments as well as their biotechnological potentials.

It is interesting to note that 4 out of 6 representative strains chosen based on rep-PCR, belonged to the genus *Psychrobacter*. Members of the genus *Psychrobacter* are known to be obligate or facultative psychrophiles and have been isolated from a wide range of habitats (Shivaji *et al.* 2005), including food, clinical samples, skin, gills and intestines of fish, sea water, penguin colonies in Antarctica, Antarctic sea ice and Japan Trench sediments (Bozal *et al.* 2003;

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Bowman *et al.* 1997; Maruyama *et al.* 1997; Yumoto *et al.* 2003).

Romanenko *et al.* (2004) isolated two species of *Psychrobacter* from coastal sea ice and sediments of the Sea of Japan and proposed new species *Psychrobacter maritimus* and *Psychrobacter arenosus*. Furthermore, Shivaji *et al.* (2005) isolated the member of genus *Psychrobacter* from cyanobacterial mat samples collected from various water bodies in the McMurdo Dry Valley region of Antarctica and proposed new species, namely *Psychrobacter vallis* and *Psychrobacter aquaticus*, respectively.

One representative isolate obtained showed closest similarity to *Pseudomonas* sp. The genus of *Pseudomonas* is widely distributed in the marine environment and considered one of the most abundant group of marine γ -proteobacteria. Furthermore, Zeng *et al.* (2004) reported that 10 psychrotrophic bacteria isolated from the west Pacific deep-sea sediments were affiliated to the *Psychrobacter*, *Pseudoalteromonas*, and *Pseudomonas* genera in the γ -Proteobacteria group. Radjasa *et al.* (2001) also reported that *Pseudomonas* and *Halomonas*, specifically occurred in surface water of the North-western Pacific ocean.

The member of genus *Vibrio* was also found in the present study. *Pseudoalteromonas*, *Photobacterium*, and *Vibrio*, were common to surface and deep-sea waters of the North-western Pacific. Overall, the members of Vibrionaceae appear to be dominant in both habitats (Radjasa *et al.* 2001). Urakawa *et al.* (1999a), used Restriction fragment length polymorphism (RFLP) for grouping 136 natural isolates collected from inshore areas of Japan, mainly in winter, belonging to the family Vibrionaceae. Five RFLP groups (groups I to V) were obtained. Group I was found to be corresponded to *Vibrio splendidus*-like strains. It was confirmed that this group was not only found in Otsuchi Bay, but also in broad coastal areas of Japan.

Furthermore, Urakawa *et al.* (1999b) performed typing and identification of 60 marine psychrophilic and psychrotrophic vibrios isolated from the north-western Pacific Ocean and coastal environment of Japan performed by restriction fragment length polymorphism analysis. Fifteen operational taxonomic units (OTUs) by digestion with four restriction endonucleases (*Hha*I, *Dde*I, *Rsa*I, and *Sau*3AI) were obtained. Significant differences were observed in OTU composition between isolates from the deep sea and coastal areas. *Vibrio marinus* and *Photobacterium* species were the dominant culturable vibrios in the deep sea areas, while *Vibrio splendidus* like species were the dominant culturable vibrios in a coastal area of Japan.

In conclusion, the rep-PCR method is useful for rapid grouping of marine psychrotrophic bacteria from seawater of Makasar strait, Indonesia and offers an alternative technique for grouping of big numbers of marine bacterial isolates.

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