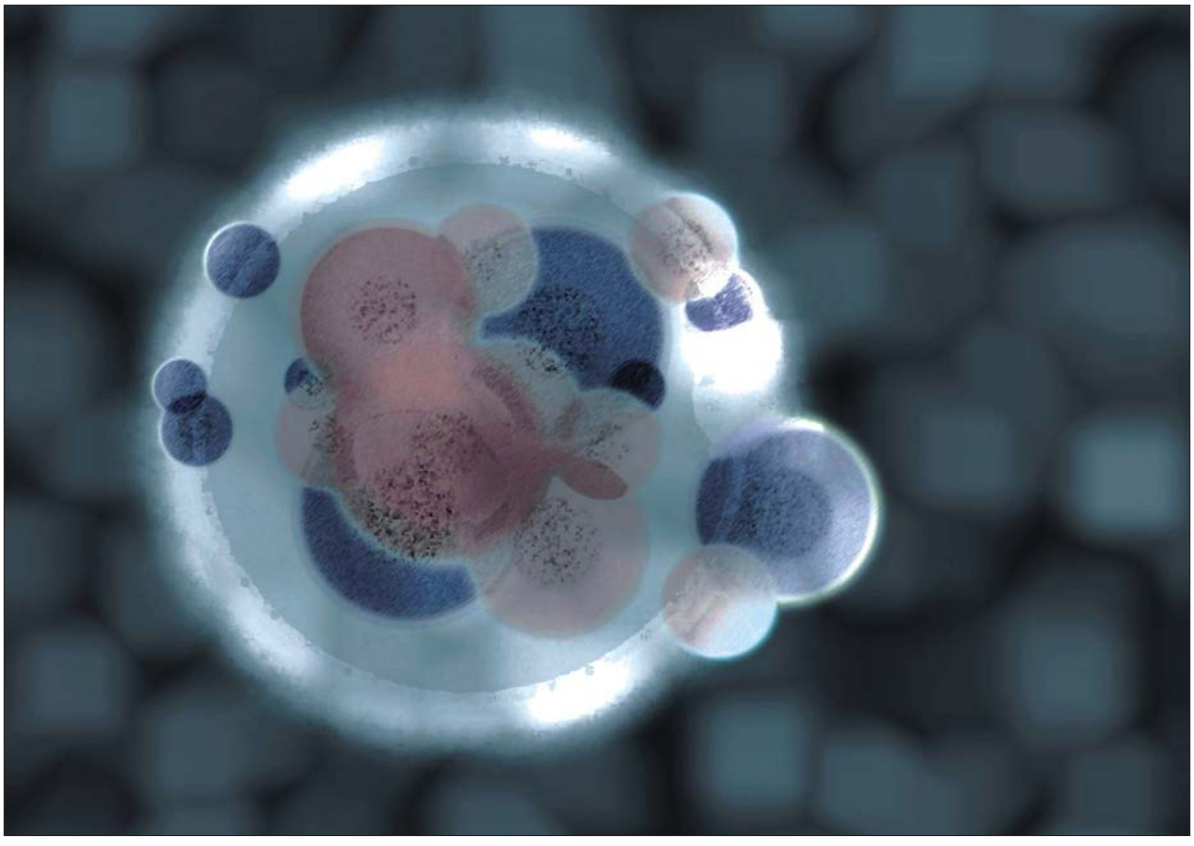

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 7 (4): 658-662, 2007
ISSN 1727-3048

Characterization of Psychrotrophic Bacteria from Sea Waters of Makasar Strait, Indonesia

[1,2]Ocky Karna Radjasa, [2]Dewi Nasima, [1,2]Agus Sabdono, [3]Kumiko Kita-Tsukamoto and [4]Kouichi Ohwada
[1]Department of Marine Science, Diponegoro University,
Semarang-50275, Central Java, Indonesia
[2]Center for Tropical Coastal and Marine Studies, Diponegoro University,
Widya Puraya, Semarang-50275, Central Java, Indonesia
[3]Marine Microbiology Division, Ocean Research Institute, University of Tokyo,
Minamidai 1-15-1, Nakano-ku, Tokyo 164, Japan
[4]Department of Environmental and Symbiotic Sciences,
Prefectural University of Kumamoto, Kumamoto, Japan

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*.

Key words: Psychrotrophic bacteria, deep-sea, Makasar strait, *Psychrobacter*, *Pseudomonas*, *Vibrio*

INTRODUCTION

Low temperature adapted bacteria have been classified based on the occurring of growth at 4 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 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,b; 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,b; 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 and de Bruijn, 1997). However, this technique has not been employed to estimate the richness of marine 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 bio-geochemical processes as well as their biotechnological potentials.

In this study, 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 (Fig.1) during INSTANT Cruise on R/V Baruna Jaya I.

Corresponding Author: Ocky Karna Radjasa, Department of Marine Science, Diponegoro University, Semarang-50275, Central Java, Indonesia Tel: 62-24-7460038/7474698 Fax: 62-24-7460039/7474698

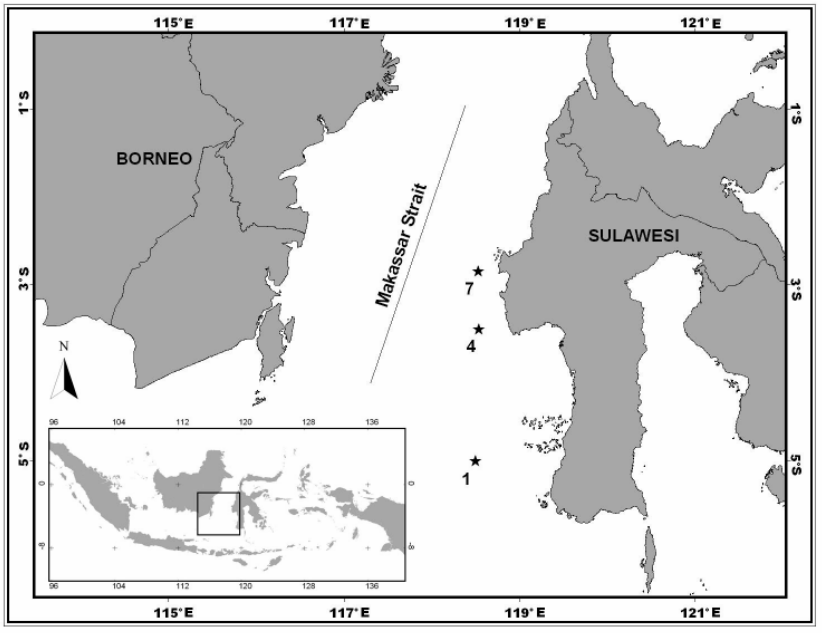

Fig. 1: Sampling sites of deep-sea waters at makasar strait, Indonesia

Isolation of bacterial isolates: 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 and 20°C (Radjasa *et al*., 2001). Those grow only at 4°C are regarded as psychrophilic bacteria while those grow both at 4 and 20°C 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'-CTACggCAAggCg AcgC Tg ACg-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 microliter aliquot PCR products were run using elektrophores on 6% acrilamide gel by using 1×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

Temperature profiles: As seen in the Fig. 2, there were temperature drops in the depth of 400 m and below that indicated the presence of psychrosphere having water temperatures lower than 10°C.

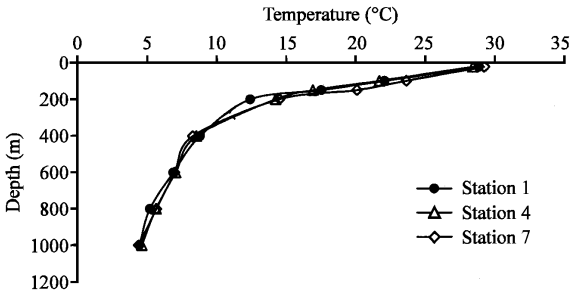

Fig. 2: The temperature profile of waters from different depths and location

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	1	400	PSM 1.3	-
4			PSM 1.4	-
5			PSM 1.5	-
6		600	PSM 1.6	-
7			PSM 1.7	-
8			PSM 1.8	-
9		800	PSM 1.9	-
10			PSM 2.1	-
11		400	PSM 2.2	-
12			PSM 2.3	-
13	4		PSM 2.4	-
14			PSM 2.5	-
15		600	PSM 2.6	-
16			PSM 2.7	-
17			PSM 2.8	-
18		800	PSM 2.9	-
19			PSM 3.1	-
20			PSM 3.2	-
21		400	PSM 3.3	-
22			PSM 3.4	-
23			PSM 3.5	-
24		600	PSM 3.6	-
25			PSM 3.7	-
26			PSM 3.8	-
27	7	800	PSM 3.9	-

Sign means gram negative bacteria

Psychrophilic isolates: Psychrophily test indicated that all isolates were able to grow both at 4 and 20°C, therefore they were regarded as psychrotrophic bacteria (Table 1).

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. 3) were further selected for DNA sequencings.

Sequencing of representative psychrotrophic isolates: The results of DNA sequencing of the representative isolates based on PCR repetitive approach are presented in the following Table 2.

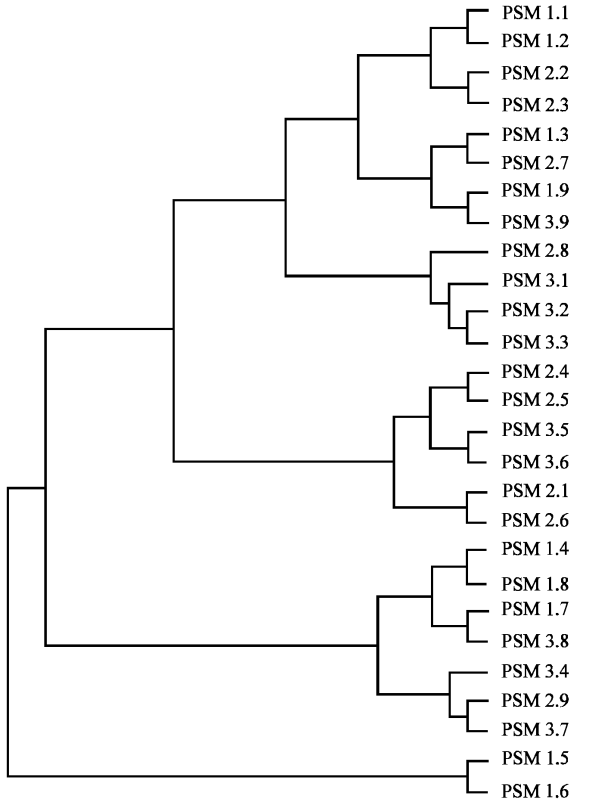

Fig. 3: Dendrogram of psychrotrophic isolates (PSM 1.5, PSM 2.2, PSM 2.4, PSM 2.7, PSM 2.8, PSM 3.4 were further selected for DNA sequencing)

Table 2: Characterization of representative psychrotrophic isolates

Strain	Closest relative	Homology	Acc. No.
PSM 2.2	*Psychrobacter alimentarius*	99	AY513645
PSM 2.7	*Psychrobacter* sp. 9B_7	99	AY689064
PSM 2.8	*Psychrobacter* sp. TSBY-70	99	DQ166171
PSM 3.4	Uncultured *Psychrobacter* sp. clone GAMMA4B	98	AY494611
PSM 2.4	*Pseudomonas* sp. WR7-2	98	AY263480
PSM 1.5	*Vibrio splendidus*	100	AJ874367

Table 3: Grouping of *Psychrotrophi* isolates

Genus	No. of isolates	(%)
Psychrobacter	18	66.67
Pseudomonas	7	25.93
Vibrio	2	07.40

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.

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

Romanenko *et al*. (2004) isolated two species of *Pyschrobacter* 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 • -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 • -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 pyshcrotrophic bacteria from seawater of Makasar strait, Indonesia and offers an alternative technique for grouping of big numbers of marine bacterial isolates.

ACKNOWLEDGMENTS

This work was partly supported by the grants from INSTANT project and Ministry of Research and Technology, Indonesia. Special thanks to the Captain and crews of R/V Baruna Jaya I, for their skillful helps during the sampling.

REFERENCES

Bowman, J.P., S.A. McCammon, M.V. Brown, D.S. Nichols and T.A. McMeekin, 1997. Diversity and association of psychrophilic bacteria in Antarctic sea ice. Applied Environ. Microbiol., 63: 3068-3078.

Bowman, J.P., S.A. McCammon, J.A.E. Gibson, L. Robertson and P.D. Nichols, 2003. Prokaryotic metabolic activity and community structure in antarctic continental shelf sediments. Applied Environ. Microbiol., 69: 2448-2462.

Bozal, N., M.J. Montes, E. Tudela and J. Guinea, 2003. Characterization of several Psychrobacter strains isolated from Antarctic environments and description of *Psychrobacter luti* sp. nov. and *Psychrobacter fozii* sp. nov. Int. J. Syst. Evol. Microbiol., 53: 1093-1100.

Knoblauch, C., B.B. Jørgensen and J. Harder, 1999. Community size and metabolic rates of psychrophilic sulfate-reducing bacteria in Arctic marine sediments. Applied Environ. Microbiol., 65: 4230-4233.

Maruyama, A., R. Taniguchi, H. Tanaka, H. Ishiwata and T. Higshihara, 1997. Low-temperature adaptation of deep-sea bacteria isolated from the Japan Trench. Mar. Biol., 128: 705-711.

Morita, R.Y., 1975. Psychrophilic bacteria. Bacteriol. Rev., 39: 144-167.

Rademaker, J.L.W. and F.J. de Bruijn, 1997. Characterization and Classification of Microbes by Rep-PCR Genomic Fingerprinting and Computer-assisted Pattern Analysis. In: DNA Markers: Protocols, Applications and Overviews. Caetano-Anolles, G. and P.M. Gresshoff (Eds.). J. Wiley and Sons, New York, pp: 151-171.

Radjasa, O.K., H. Urakawa, K. Kita-Tsukamoto and K. Ohwada, 2001. Characterization of psychrotrophic bacteria in the surface and deep-sea waters from northwestern Pacific Ocean based on 16S ribosomal DNA approach. Mar. Biotechnol., 3: 454-446.

Radjasa, O.K., T. Martens, H.P. Grossart, T. Brinkoff, A. Sabdono and M. Simon, 2007. Antagonistic activity of a marine bacterium *Pseudoalteromonas luteoviolacea* TAB4.2 associated with coral *Acropora* sp. J. Biol. Sci., 7: 239-246.

Romanenko, L.A, A.M. Lysenko, M. Rohde, V.V. Mikhailov and E. Stackebrandt, 2004. *Psychrobacter maritimus* sp. nov. and *Psychrobacter arenosus* sp. nov., isolated from coastal sea ice and sediments of the Sea of Japan. Int. J. Syst. Evol. Microbiol., 54: 1741-1745.

Shivaji, S., G.S.N. Reddy, K. Suresh, P. Gupta, S. Chintalapati, P. Schumann, E. Stackebrandt and G.I. Matsumoto, 2005. *Psychrobacter vallis* sp. nov. and *Psychrobacter aquaticus* sp. nov., from Antarctica. Int. J. Syst. Evol. Microbiol., 55: 757-762.

Urakawa, H., K. Kita-Tsukamoto and K. Ohwada, 1999a. 16S rDNA restriction fragment length polymorphism analysis of psychrotrophic vibrios from Japanese coastal water. Can. J. Microbiol., 45: 1001-1007.

Urakawa, H., K. Kita-Tsukamoto and K. Ohwada, 1999b. Restriction fragment length *Polymorphism* analysis of psychrophilic and psychrotrophic *Vibrio* and *Photobacterium* from the north-western Pacific Ocean and Otsuchi Bay, Japan. Can. J. Microbiol., 45: 67-76.

Versalovic, J., M. Schneider, F.J. de Bruijn and J.R. Lupski, 1994. Genomic fingerprinting of bacteria using repetitive sequence based-Polymerase Chain Reaction. Meth. Cell. Mol. Biol., 5: 25-40.

Yumoto, I., K. Hirota, Y. Sogabe, Y. Nodasaka, Y. Yokota and T. Hoshino, 2003. *Psychrobacter okhotskensis* sp. nov., a lipase-producing facultative psychrophile isolated from the coast of the Okhotsk Sea. Int. J. Syst. Evol. Microbiol., 53: 1985-1989.

Zeng, Z., X. Xiao, P. Wang and F. Wang, 2004. Screening and characterization of psychrotrophic, lipolytic bacteria from deep-sea sediments. J. Microbiol. Biotechnol., 14: 952-958.