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Review

DEEP- SEA BACTERIA AND THEIR BIOTECHNOLOGICAL POTENTIALS

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ABSTRACT

Deep-sea environment characterized by its low temperature, high hydrostatic pressure, limited nutrient contents, and constant darkness has been a hostile environment for most marine microorganisms. Therefore it is a home for low temperature and high pressure-loving bacteria believed to functionally over shallow water intruders.

Deep-sea has been regarded as rainforests in terms of their microbial diversity. Thus, the deep-sea should commend itself to microbiologists and biotechnologists alike as source of novel microorganisms and exploitable properties.

Near Indonesia several deep-sea trenches exist with diverse environmental conditions. However, up to now there has been no effort to study the microbial communities of those deep-sea environments by Indonesian scientists. This is mainly because of the lack of knowledge in the field of deep-sea microbiology in Indonesia.

Key words: Deep-sea bacteria, barophilic, psychrophilic, resources, Indonesia

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INTRODUCTION

Constant darkness, temperatures below 4°C, and hydrostatic pressure increasing every 10 m by 1 bar make deep sea an extreme, hostile environment for most of the marine microorganisms. However, it has been proven, that in the deep-sea pressure-loving (barophilic, piezophilic) and low temperature-loving (psychrophilic) bacteria exist which are at least functionally dominant over shallow water intruders at abyssal depth (Weyland and Helmke 1989).

According to Zo Bell (1968), deep-sea is arbitrarily taken as 3800 m or more, and 3800 m being approximately the mean depth of the sea. The term abyssal is applied to water masses, bottom sediments and to organisms occurring at depths of 3800 m or more. By this definition, about 60% of the area of the sea floor is abyssal. On the other hand, hadal is a term sometimes used to describe super-oceanic depths exceeding 6000 m.

The pressurized deep-ocean is considered as the largest of accessible extreme environments on the planet (Deming, 1998) and its natural history of the inhabitants is fragmentary because of the need to use ships, submersibles, and uncommon instruments. Microorganisms evolving in habitats with low temperatures and high pressures need to be studied to understand the physical limits of life, the distribution of life within the ocean, and the role of organisms in the bio-geochemical processes.

To my knowledge, there has no report been documented on the diversity of deep-sea barophilic bacteria from Indonesian deep-sea trenches. Studies regarding diversity of barophilic populations are important for understanding principal processes in the deep-sea and today, deep-sea processes are also in the centre of interest concerning climatic changes. In addition, the significance of deep-sea as sink or source for CO_2 is still under discussion.

Near Indonesia several deep-sea trenches 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. This is mainly because of the lack of knowledge in the field of deep-sea microbiology in Indonesia.

The main purpose of the present review in the context of the above-mentioned description, is to address the issue on phylogenetic diversity of deep-sea bacteria, mechanisms of adaptation and their biotechnological potentials. The current status of the unexplored diversity of marine bacteria from Indonesian deep-sea environments is also discussed.

DIVERSITY OF DEEP-SEA BACTERIA

Understanding the indigenous low temperature-adapted bacteria (psychrophilic) and high pressure-adapted bacteria (barophilic) diversity has important implications for analyses of microbial function and biogeochemical processes in the deep-sea environments as well as their biotechnological potentials.

De Long et al. (1997) described that in the context of understanding the genetic diversity of marine organisms, their biogeography, and their evolutionary relationships, application of molecular phylogenetic techniques is having substantial impact in advancing the field. Just recently, the diversity of psychrophilic and barophilic communities in the deep-sea environment have been characterized, mainly by molecular phylogenetic techniques using 16S rDNA sequences.

Morita (1975) classified low temperature adapted bacteria 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. On the other hand, high pressure adapted bacteria have commonly been categorized into barotolerant and barophilic types. Barotolerant type is described as capable of growing at atmospheric and elevated pressures, whereas barophilic type is defined as bacteria that need elevated pressure to achieve optimal growth. In other word, barophilic type does not grow at atmospheric pressure.

Since DeLong et al. (1997) reported a phylogenetic affiliation of cultivated deep-sea psychrophlic and barophilic bacteria based on 16S rDNA (Figure 1), there has been not much changes, except the addition of four deep-sea species: barophilic *Moritella yayanosii*, and barotolerant *M. japonica, M. viscosa*, and *Photobacterium profundum* (Kato, 1999). In a more recent work, Radjasa (2001), described a new affiliated group among deep-sea psychophilic and barophilic bacteria, i.e. MTW-13 group (Figure 2), which represent the deep-sea isolates closely related to uncultured deep-sea clones, NB1-c and NB1-d from Japan Trench (Yanagibayashi et.al, 1999).

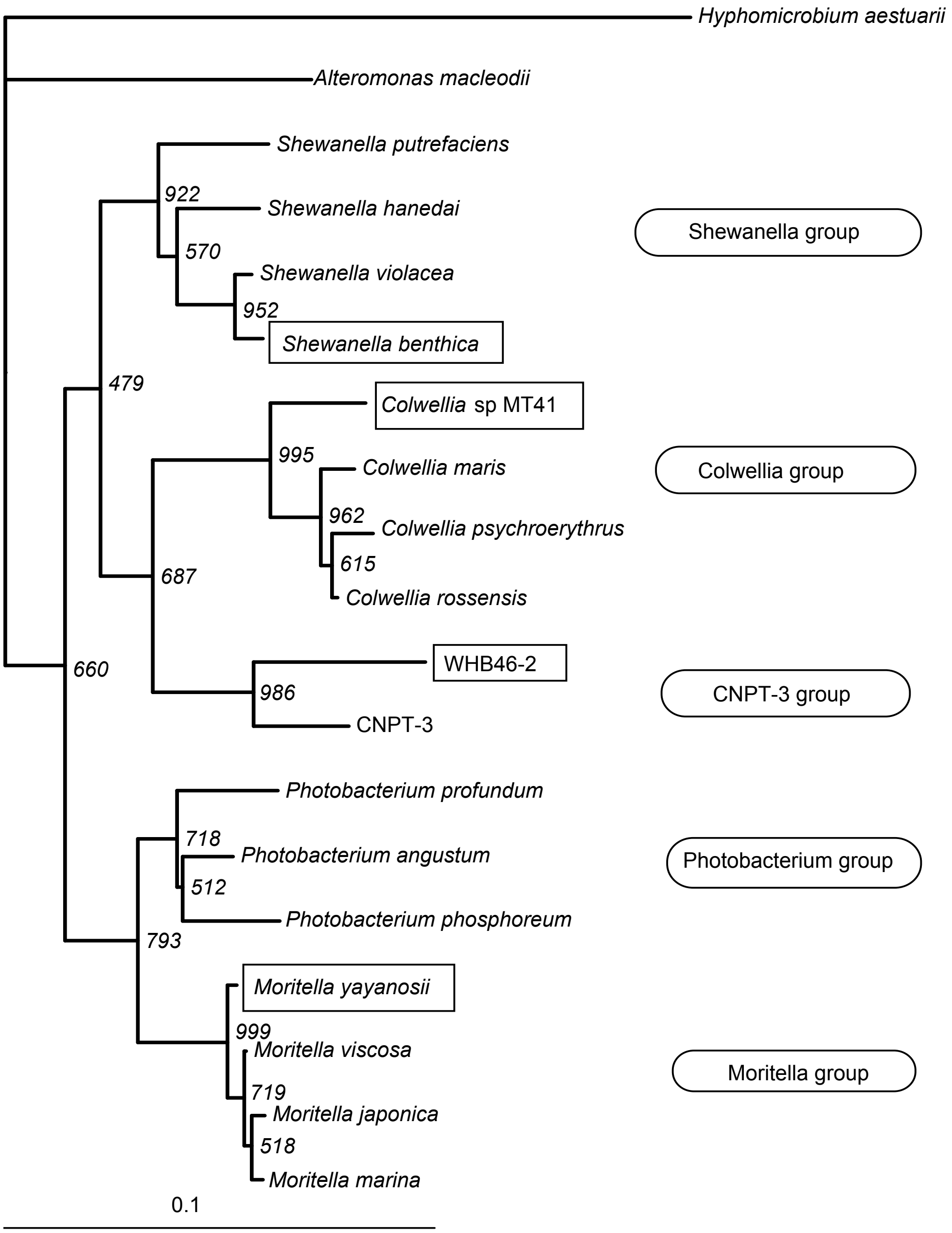

Fig 1. Phylogenetic relationship of cultivated deep-sea psychrophilic and barophilic bacteria (Modified from Delong et al., 1997). Boxed strains indicate the obligate barophilic bacteria.

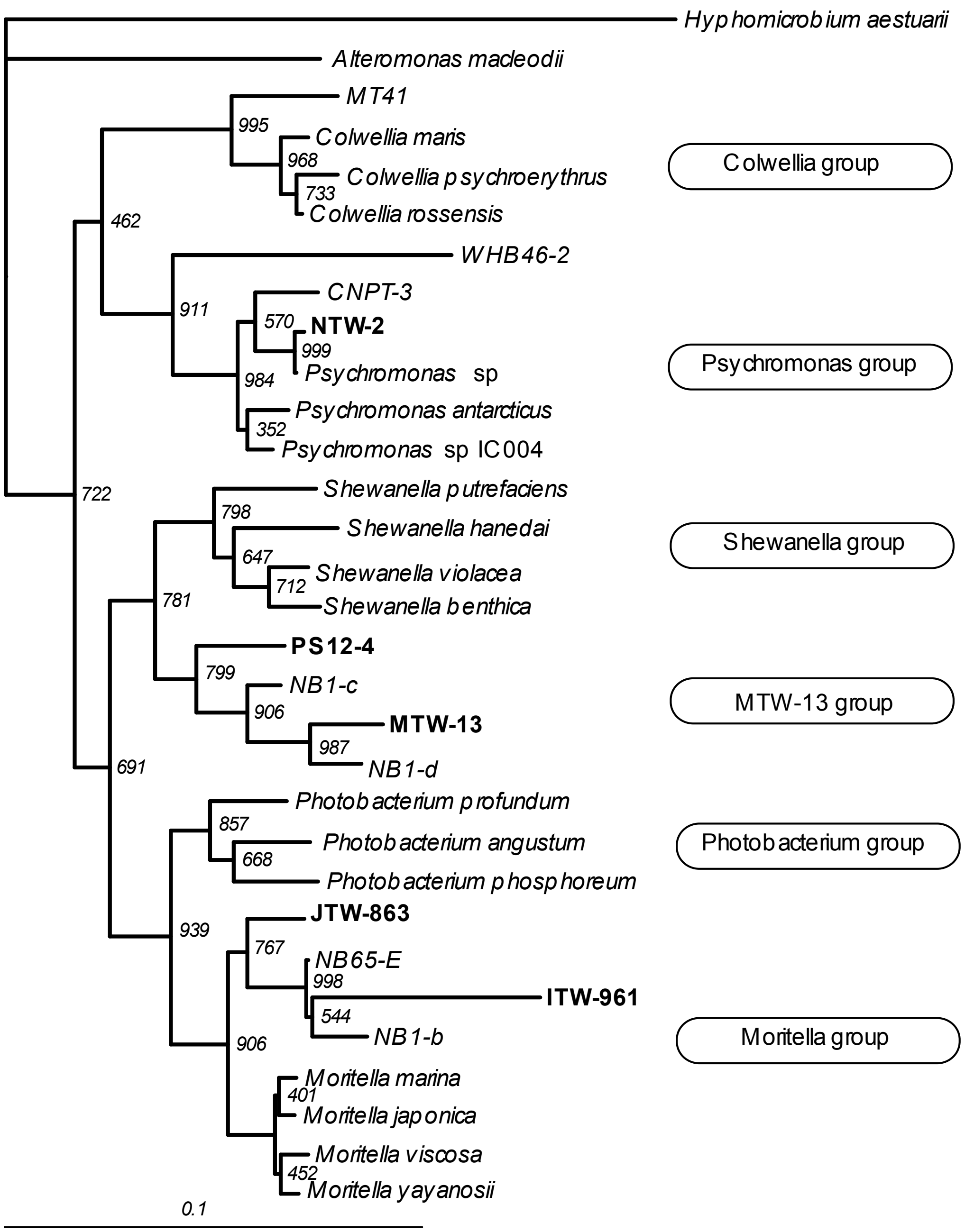

Fig. 2. New phylogenetic affiliation of psychrophilic and barophilic bacteria.*Hyphomicrobium aestuarii* was used as outgroup. Bar indicates 10% sequence dissimilarities.

MECHANISMS OF ADAPTATION

The mechanisms by which marine bacteria adapt to high pressures are very inadequately understood, but pressure-regulated gene expression and its relationship to barophily and barotolerance are gradually being determined. Pressure-regulated genes are believed to aid pressure acclimatization in marine bacteria that are exposed to large vertical changes in the water column, but they are also found in bacteria that are not subject to pressure changes as a result of overlapping effects of pressure and other environmental stresses (Bartlett et al, 1995).

To date most work on deep-sea barophilic bacteria has concerned taxon within the Proteobacteria: *Colwellia, Moritella, Photobacterium*, and *Shewanella* and an unidentified genus (DeLong et al, 1997). Some of these bacteria are extremely barophilic, such as the newly described species *Moritella yayanosii* isolated from the Challenger Deep of the Mariana Trench, which grows at 60 to 100 MPa and has an optimum of 70 MPa (Nogi and Kato, 1999).

Promising progress has been made on molecular mechanism of deep-sea *Photobacterium* and *Shewanella* strains. Reverse-pressure regulation of outer membrane proteins has been shown in the moderate barophile *Photobacterium profundum* SS9. A 10- to 100-fold increase in the expression of the OmpH protein occurs at high pressures (28 MPa), while at 0.1 MPa the OmpL protein is produced in greatest quantity. Furthermore, a third pressure-regulated protein, OmpI, is expressed at 40 MPa. The OmpH protein is believed to be a relatively nonspecific porin (Welch and Bartlett, 1998) that may facilitate nutrient uptake under increasingly oligotrophic conditions of the deep-sea. More recently it has been demonstrated that RecD function is required for high-pressure growth and maintenance of plasmid stability in *P. profundum* SS9 (Bidle and Bartlett, 1999).

The Japan Marine Science and Technology Center (JAMSTEC) group, whose main focus has been on deep-sea *Shewanella* strains, has distinguished a "barophilic branch" of *Shewanella benthica* strains from moderately barophilic and barotolerant strains (Li et al, 1998).

Kato et al, (1995) reported that a pressure-regulated operon unidentified ORFs (ORF1 and ORF2) of the barophilic *S. benthica* to have a sequence similar to that of the OmpH promoter of *P. profundum*. In addition, a second pressure-regulated operon (ORF3 and ORF4) is located downstream from the first operon; ORF3 encodes the *Cyd*D protein which is required for the assembly of the cytochrome *bd* complex (Kato et al, 1996). A truncated respiratory chain has been proposed for *S. benthica* at high pressure in which quinol oxidase acts as the terminal oxidase (Kato, 1999).

BIOTECHNOLOGICAL POTENTIALS OF DEEP-SEA BACTERIA

Several world wide oceanographic institutions have carried out exploration of deep-sea microorganisms, such as JAMSTEC Japan, Monterey Bay Aquarium Institute (MBARI), Woodshole Oceanographic Institution (WHOI), Scripps Institute of Oceanography (USA) and Ifremer of France.

So far, from the cold deep-sea environments there have come new findings of unexpected microbial diversity and the promise of industrially useful enzymes or secondary metabolites. New classes of predictive models are emerging to guide future exploration of microbial diversity in the deep ocean. It is expected that still quite a few parts of culturable psy-chrophilic and barophilic microorganisms are explored in the deep-sea environments. Thus, the discoveries of novel deep-sea bacteria possibly become sources of novel natural products, including lipids, pigments, pharmaceuticals and low temperature adapted enzymes.

Microbial communities that thrive in spite of pronounced biotic pressure as it is among deep-sea bacteria, can come to some degree be expected to contain metabolites that are also of interest bioactive prospector searching the oceans. It is then reasonable for marine microbiologists and biotechnologists to look into marine environment, and the deep-sea in particular as a source of novel microorganisms and exploitable properties. If deep-sea bacteria are indeed the producers of bioactive metabolites of interest, transfer of gene clusters responsible for the biosynthesis of the respective natural products to a vector suitable for large-scale fermentation could perhaps provide alternative strategy thereby avoiding the foreseeable difficulties in culturing bacteria.

Low temperature adapted bacteria have been known to produce enzymes with special interest such as protease, lypase, galactosydase, pectinase, catalase and glucose oxidase (Ohgiya et al, 1999), in addition to another potential, new pigments for food additives.

Lipid membrane of barophilic bacteria contains almost 70% of poly unsaturated fatty acids (PUFA) which are very useful to decrease the chollesterol, prevent cardiovascular disease, and reduce the risk of cancers (Kelly, 1991). PUFA are also needed for normal embrionic development, in particular for optical and neural (Craigschmitdt et al, 1996; Farkas et al, 1996; Linko and Hiyakawa, 1996). PUFA from marine bacteria also serve as the alternative sources in aquaculture field (Nichols et al, 1996).

A work by Kojima et al (1998) was carried out as an effort to find alternative sources for synthetic surfactants which are widely used by industries. These surfactants in a large amount could affect the quality of ecosystems. Studies dedicated to obtaining sources of environmentally friendly and degradable surfactants were conducted in the area of Japan, Palau and Mariana Trenches.

INDONESIA'S YET UNEXPLORED DEEP-SEA BACTERIA

As a world center of diversity with 81,000 km of coastline and 5.8 million km^2 of marine area, Indonesia's important marine resources represent a significant management challenge to achieve sustainable levels of development.

The Indonesian terrain is divided into three main parts, western, central and eastern basins. The Western Basin has shallow sea that covers the southern part of the Strait of Malacca, the southern end of the South China Sea, Karimata strait, Galasa Strait, Bangka Strait, Sunda strait and the Java Sea. The Eastern Basin also has shallow sea which covers the Arafuru Sea and the off shore of other parts of Irian Jaya. The Central basin has deeper sea of about 4,000 meter. Trenches with a depth of more than 6,000 meters include the Halmahera, the Banda and the Sulawesi Trenches.

Moreover, most of the deep-sea environments are oligotrophic. Nevertheless, complex media with high amounts of peptone, yeast extract etc. are commonly used for culture enrichment and isolation approaches of barophiles (DeLong et. al, 1997; Kato et. al, 1995, 1998; Nogi et. al, 1998; Wirsen and Molyneaux, 1999; Radjasa et. al, 2001). This practice may be one of the reasons, why the barophilic strains are quite similar and why most of them were obtained from nutrient rich habitats.

It is clear that the currently known culturable deep-sea microbial diversity is strongly affected by the cultivation conditions. However, up to now, the influence of quality and concentration of substrates on the diversity of barophilic isolates has not been studied.

Samples from Indonesian deep trenches are especially suited for such comparative studies with other deep-sea regions as well as polar regions either Arctic or Antarctic, especially due to the Arctic and Antarctic deep-seas differ from other deep-sea areas by its nutrient supply. The Arctic and Antarctic Oceans are covered by sea ice the entire year, while Indonesian waters are typical productive tropical waters that normally the deep-sea areas will get nutrients by vertical transports. Therefore, in the Arctic and Antarctic regions, vertical transport processes are limited which supply the "normal" deep sea with nutrients. The major transport processes in the Arctic and Antarctic Oceans are lateral, bringing nutrients from the shelves, therefore partly of terrestrial origin, into the deep-sea. Thus, specific deep sea populations can be assumed to exist in the Indonesian deep-sea waters.

In the year of 2002, a Java-trench expedition was jointly carried between Indonesian and Japanese scientists by using a manned-submersible Shinaki 6500 owned by Japan Marine Science and Technology Centre (JAMSTEC), however, no research was directed toward the exploration of barophilic communities.

CONCLUDING REMARKS

Today, the diversity and taxonomical structure of bacterial communities in deep-sea environments are determined by means of molecular biological methods without the limiting step of cultivation. However, these results give only a general overview about all the taxonomic groups present. It is not possible by means of these results to differentiate between specific physiological groups like indigenous barophilic deep-sea bacteria and barosensitive deep-sea intruders as well as to determine their biotechnological prospects. Therefore, enrichment cultures and/or isolations under deep sea conditions are necessary to get information about the real deep-sea inhabitants and their biotechnological potentials.

Exploring the richness of deep-sea microbial population from Indonesian deep-sea environments is a great challenge that will require not only human resources which has been lacked in Indonesia, but also knowledge in the area of deep-sea microbiology, institutional capacity, and popular support to be effective. On the other hand, marine institutes in Indonesia have been equipped with research vessels with the facilities to work on deep-sea samples. It is now the time to start exploring the deep-sea micro-organisms and to get the benefit from them once we could cultivate them in the pure forms.

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REFERENCES

Bartlett, D.H., C. Kato., and K. Horikoshi. 1995. High pressure influences on gene and protein expression. *Res. Microbiol.* 146: 697-706.

Bidle, K.A., and D.H. Bartlett. 1999. RecD function is required for high-pressure growth of a deep-sea bacterium. *J. Bacteriol*. 181: 2330-2337.

Craigschidt, M.C., K.E. Stieh and E.L. Lien. 1996. Retinal Fatty acids of piglets fed docosahexaeonoic and arachidonic acids from microbial sources. *Lipids* .3: 53-59.

DeLong EF, Franks DG, Yayanos, AA .1997. Evolutionary relationships of cultivated psychrophilic and barophilic deep-sea bacteria. *Appl. Environ. Microbiol*. 63: 2105-2108.

Deming, J.W. 1998. Unusual extreme high-pressure marine environments. In : Manual of environmental microbiology. ASM Press, Washington D.C.

Farkas K. I., A.J. Ratchford., R. Noble and B.K. Speake. 1996. Change in the size and docosahexaenoic acid content of adipocytes during chick embryo development. *Lipids*. 31: 313-321.

Kato, C., M. Smorawinska, T. Sato., and K. Horikoshi. 1995. Cloning and expression in *Escherichia coli* of a pressure-regulated promoter region from a barophilic bacterium, strain DB6705. *J. Mar. Biotechnol*. 2: 125-129.

Kato, C., M. Smorawinska, T. Sato., and K. Horikoshi. 1996. Analysis of a pressure-regulated operon from the barophilic bacterium strain DB6705. *Biosci. Biotechnol. Biochem*. 60: 166-168.

Kato, C. 1999. Barophiles(Piezophiles). In Extremophiles in deep-sea environments. K. Horikoshi and K. Tsujii (Ed).pp. 91-111. Springer-Verlag Tokyo.pp. 175-210.

Kato, C., L. Li., Y. Nogi., Y. Nakamura, J. Tamaoka and K. Horikoshi. 1998. Extremely barophilic bacteria isolated from the Mariana Trench Challenger Deep, at a depth of 11.000 meters. *Appl. Environ. Microbiol*. 64: 1510-1513

Kelly, F.J. 1991. The metabolic role of n-3 PUFA : Relationship to human disease. *Comp. Biochem. Physiol.* 98: 581-585

Kojima, T.Y. Takaki A. Inoue, R. Usami and K. Hirokoshi 1998 Isolation of biosurfactant producing bacteria from deep-sea sediment. Abstracts of International Congress on Extremophiles. Yokohama, Japan p. 332.

Li. L.,C. Kato and K. Hirokoshi. 1998. Distribution of the pressure regulated operons in deep-sea bacteria. *FEMS Microbiol. Lett.* 159: 159-166

Linko, Y.Y. and K. Hiyakawa. 1996. Docosahexaenoic acid a valuable nuetraceutical. *Trends. Food. Sci. Tech.* 7: 59-63

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

Nichols, D., S.P. Hart., P.D. Nichols and T.A. McMeekin. 1996. Enrichment of the rotifier *Brachionus plicatilis* fed Antarctic bacterium containing polyunsaturated fatty acids. *Aquaculture.* 147:115-125

Nogi, Y., C. Kato and K. Horikoshi 1998a. Taxonomic studies of deep-sea barophilic *Shewanella* species and *Shewanella violacea* sp.nov, a new barophilic bacterial species. *Arch. Microbiol*. 170: 331-338

Nogi, Y., C. Kato and K. Horikoshi 1998b. *Moritella japonica*, sp.nov. a novel barophilic bacterium isolated from a Japan Trench sediment. *J. Gen. Appl. Microbiol*. 44 : 289-295

Nogi, Y and C. Kato. 1999. Taxonomic studies of extremely barophilic bacteria isolated from the Mariana Trench and description of *Moritella yayanosii* sp. nov., a new barophilic bacterial isolate. *Extremophiles*.3: 71-7.

Ohgiya, S., T. Hoshino., H. Okuyama., S. Tanaka and K. Ishizaki. 1999. Biotechnology of enzymes from cold adapted microorganisms.In : Biotechnological applications of cold–adapted organisms. Margesin, R. and F Schinner (Eds), Springer Tokyo.

Radjasa OK, Urakawa H, Kita-Tsukamoto K and Ohwada K. 2001. Characterization of pscyhrotrophic bacteria in the surface and deep-sea waters from the northwestern Pacific Ocean based on 16S ribosomal DNA analysis. *Mar. Biotechnol.* 3:454-462.

Radjasa, OK. 2001. Occurrence of psychrophiles in cold deep sediments from Southwestern Pacific Ocean. *Indon J. Mar. Scie*. 24:283-287.

Welch, T.J., and Bartlett, D.H. 1998. Identification of a regulatory protein required for pressure-responsive gene expression in the deep-sea bacterium *Photobacterium* species strain SS9. *Mol. Microbiol.* 27:.977-985.

Weyland, H. and Helmke, E. 1989. Barophilic and psychrophilic bacteria in the Antarctic Ocean. In: Recent advances in microbial ecology. Eds. Hattori, T, Ishida, Y., Maruyama, Y., Morita, R. Uchida, A. pp.43-47.

Wirsen, C.O and Molyneaux, S.J. 1999. A Study of Deep-Sea Natural Microbial Populations and Barophilic Pure Cultures Using a High-Pressure Chemostat. *Appl. Environ. Microbiol.* 65: 5314–5321.

Yanagibayashi M, Nogi Y, Li L, Kato C. 1999. Changes in the microbial community in Japan Trench sediment from a depth of 6292 m during cultivation without decompression. *FEMS Microbiol. Lett*. 170: 271-279

Zobell, C.E. 1968. "Bacterial life in the deep-sea". In : *Proceedings of the US-Japan Seminar on the marine microbiology*. Kadota, H and N. Taga (Ed).