Journal of Coastal Development ISSN: 1410-5217
Volume 5, Number 1, October 2001 : 35-40 Accredited: 69/Dikti/Kep/2000

PHYLOGENETIC RELATIONSHIP OF BAROPHILIC BACTERIA FROM NORTHWESTERN PACIFIC OCEAN

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Received July 10, 2001; accepted July 26, 2001

ABSTRACT

In order to elucidate the role of hydrostatic pressure on the distribution of marine microorganisms, deep-sea water samples from north-western Pacific Ocean were collected and used as the source of barophilic bacteria. One isolate, JTW-863 was obtained from a depth of 6000 m, while two isolates, MTW-1 and MTW-13, were obtained from a depth of 10,500 m.

Phylogenetic analysis based on PCR-amplified 16S rDNA revealed that JTW-863 belonged to Moritella, while MTW-1 was mostly related to Shewanella. On the other hand, MTW-13 was affiliated with the uncultured clone NB1-d from deep environment.

Key words: Barophilic, phylogenetic, northwestern Pacific Ocean

INTRODUCTION

Ten meters of depth change in the ocean equals to a hydrostatic pressure change of approximately 0.1 MPa. In marine environment, hydrostatic pressure ranges from 0.1 to 110 MPa with average pressure in the ocean is of the order of 38 MPa. The pressurized deep-ocean is considered as the largest of accessible extreme environments on the planet, and its natural history of the inhabitants is still fragmentary. However, deep-sea environment is a home to pressure preferring or barophilic bacteria, it is believed to be functionally dominant over shallow-water intruders at abyssal depths.

Determining the role of hydrostatic pressure for marine microorganisms has been a challenge in the field of marine microbiology. One strategy, which has widely been employed to elucidate high pressure-related adaptation, is to determine the distribution of microorganisms inhabiting deep-sea environments. Yayanos (1986) suggested that barophilic is a characteristic of bacteria at depths greater than 2000 m.

Understanding the diversity of

indigenous high pressure-adapted bacteria has important implications for analyses of microbial function, biogeochemical processes, and biotechnological potential. Just recently, the diversity of barophilic communities in the deep-sea environment is only beginning to become well characterized, mainly through the use of molecular phylogenetic techniques using 16S rDNA based-approach.

To address the question that high pressures of the deep ocean serve as a barrier to colonization of marine bacteria, the present study was initiated to describe the diversity of barophilic bacteria from seawater column of northwestern Pacific Ocean.

MATERIALS AND METHODS

Sampling protocols

Seawater samples were collected by using Niskin butterfly water samplers (General Oceanics, Florida) from the Japan Trench during KT-00-9 cruise by R/V Tansei Maru, and the Mariana Trench during KH-98-2 by R/V Hakuho Maru of Ocean Research Institute, University of Tokyo. A description of the sampling sites is presented in the table 1.

Table 1. Sampling site and depth of seawater column as sources of obligate barophilic during the course of study

Latitude	Longitude	Depth(m)	Isolates
14’59.786S	162’00.941E	6000	JTW-863
19’53.100S	161’58.224E	10,500	MTW-1; MTW-13

Seawater samples were immediately transferred to sterile glass bottles immersed on ice on board. One thousand ml seawater samples were filtered using nuclepore filters of 0.2 μ m pore size, and each filter was placed on small polyethylene plastic bags (Whirl-Pak, Nasco, USA) in which 1/5 ZoBell 2216E broth medium was previously added, and re-pressurized at their captured depths at 60 and 100 MPa, respectively. All pressure chambers were then incubated at 4^{o}C.

Isolation and growth studies.

Isolation was carried out as described by Sakiyama and Ohwada (1997). Barophily test was performed among deep-sea strains at 0.1, 60 and 100 MPa based on their captured depths at 4 ^{o}C for 30 days. Screening of strict barophiles followed the

ISSN: 1410-5217
Accredited: 69/Dikti/Kep/2000

definition of Yayanos (1986). Those that grow only at high pressure when tested at the temperature of their presumed habitat, were regarded as true barophilic bacteria.

DNA extraction and PCR amplification

Cells were harvested by centrifugation from cultures grown at 4 ^{o}C on 1/5 strength ZoBell 2216E broth medium under 60 and 100 Mpa, respectively. DNAs were extracted with proteinase K (1 mg ml^{-1}) (Sigma Chemical Co, St. Louis, MO, USA), then 50 µl reaction mixtures were prepared and 30 cycles amplifications were performed as described by Urakawa et al. (1997). Amplified DNA was examined by 1% agarose gel in TAE electrophoresis buffer with 1µl aliquots of PCR product.

DNA sequencing and phylogenetic analysis

PCR products were purified using Microcon-100 micro-concentrators (Amicon, Beverly, MA, USA) and were prepared using a SequiTherm Long- Read Cycle Sequencing Kit (Epicentre Technologies, Madison, WI, USA) for subsequent sequencing on an automated sequencer (Pharmacia LKB Biotech, Uppsala, Sweden). Sequences were alignedby using the CLUSTAL W program (Thompson et al., 1994), and were then manually aligned using MacClade program (Ver.3.06). The phylogenetic tree was constructed based on Neighbour-joining method (Saitou and Nei 1984). Boostrap confidence analysis was carried out on 1000 resamplings.

RESULTS AND DISCUSSION

An attempt to elucidate the role of hydrostatic pressure in shaping the distribution of marine bacteria, phylo-genetic analysis of high pressure adapted bacteria from northwestern Pacific was carried out. Barophily test among the colonies resulted in 3 barophilic isolates, 1 from a depth of 6000 m and 2 isolates from a depth of 10,500 m.

Jannasch (1987) suggested that barotolerance and barophilism, thus defined and expressed by growth responses of microbial culture, vary greatly among the reported deep-sea bacteria (Delong *et al*., 1997; Kato *et al.*, 1995, 1998; Nogi *et al.*, 1998). One possible explanation for this result is that a continuous transport of surface-born microorganisms attached to sinking particles to the deep-ocean resulted

in the dominant presence of less pressure adapted forms and in selection for adaptation to higher pressure. Another explanation was that deepwater upwellings may contribute to the mixed occurrence of different types of pressure adaptation in microorganisms of the water column (Jannasch ,1987).

Studies of 16S rDNA sequences obtained from PCR-amplified DNA for assessing the microbial relationships have become a common standard in microbial ecology. The existence of extensive DNA-data base of cultured and uncultured strains has served as a reference for comparison of sequences retrieved from different habitats, and from different parts of the world. The information obtained from DNA-database reveals approximate relationships between naturally occurring microorganisms and cultured strains, and it allows us to determine the presence of novel types of microorganisms.

Table 2. The closest neighbors of barophilic isolates deduced from DNA-database

Depth (m)	Isolate	Closest neighbors	Similarity (%)
6000	JTW-863	*Moritella yayanosii*	97
10,500	MTW-1	*Shewanella benthica*	95
10,500	MTW-13	Clone NB1-d	95

As shown in the Table 2, the determined sequence of strain JTW-863 showed best conformity to the previously reported sequence(Kato et al, 1998) of *Moritella yayanosii* (97%), which was isolated as a new obligate barophilic species *Moritella* from the sediment of Mariana Trench and was designated as *Moritella yayanosii*. This is the first existence of barophilic *Moritella yayanosii* out of Mariana Trench and confirmed the wide distribution of barophile *Moritella yayanosii*.

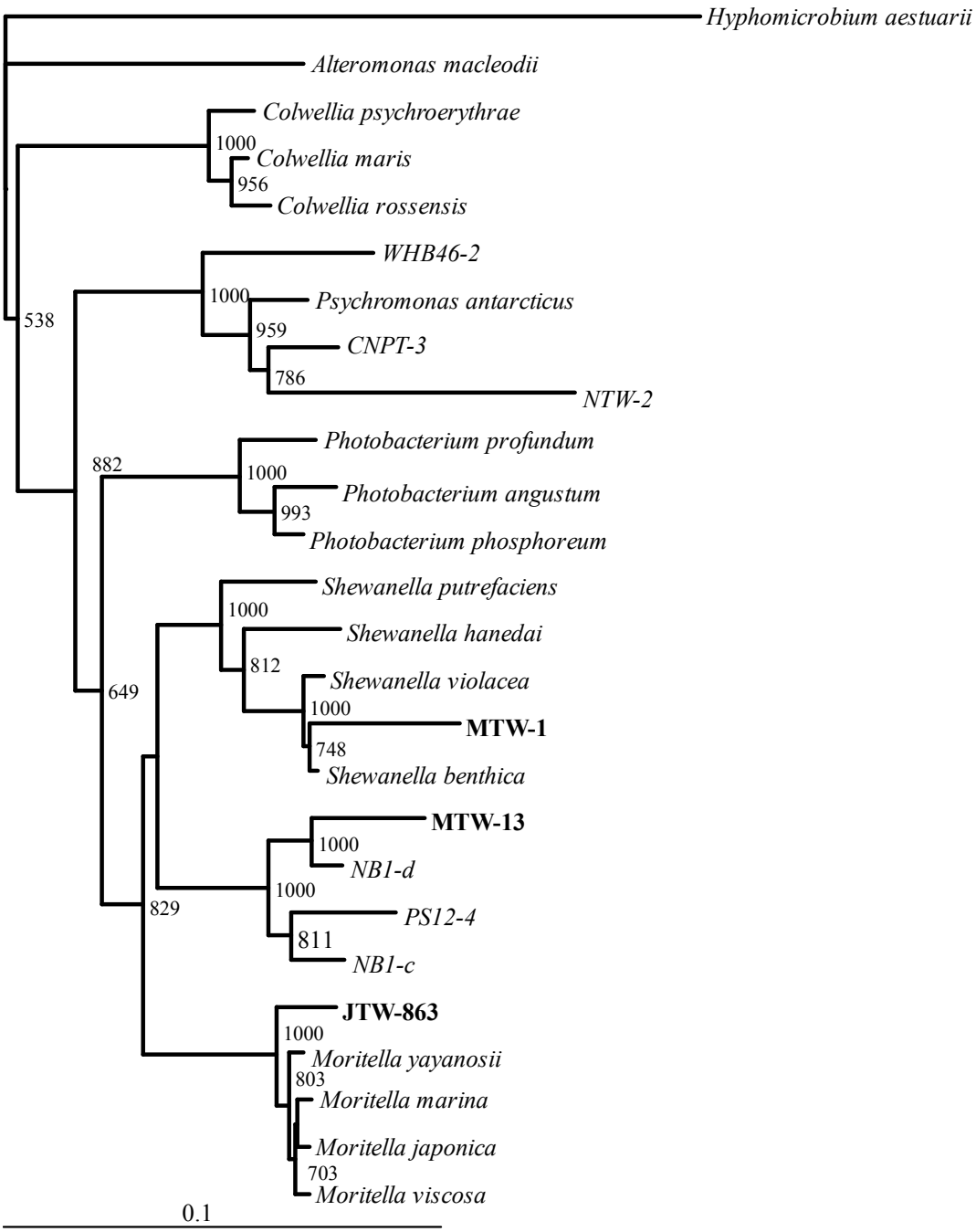

Figure 1. Phylogenetic affiliation of barophilic bacteria from deep-sea waters. (*Hyphomicrobium aestuarii* was used as out group). Bar indicates 10% sequence dissimilarities.

ISSN: 1410-5217
Accredited: 69/Dikti/Kep/2000

High pressure adapted bacteria were also successfully obtained from a depth of 10,500 m under 100 MPa pressure using 1/5 ZoBell culture medium containing low melting agarose gel. Growth studies also revealed that those isolates were barophiles as indicated by their ability to grow at the elevated pressure but failed to grow at atmospheric pressure. The determined sequences of the representative strain MTW-1 showed high similarity of 95% to the barophile *Shewanella benthica* isolated by Kato *et al.* (1998) from the deep-sea sediment of the Mariana Trench.

On the other hand, MTW-13 showed highest similarity only to environmental clones NB1-d and NB1-c, 95% and 94% respectively. Both clones were obtained from DNAs extracted from deep-sea sediment of the Japan Trench at a depth of 6294 m using a pressure retaining sampler at 65 MPa (Yanagibayashi *et al*., 1999), and have been assigned to as unidentified sequences. The isolation of strain MTW-13 has significantly proven the presence of natural bacterial community which previously detected only using the culture-independent technique.

From the phylogenetic tree, it is clear enough that strain MTW-13 was affiliated with clones NB1-c and NB1-d, and occupied separated lineage. From this perspective, this result supports the views that many new groups of deep-sea barophiles remain to be discovered and described. Furthermore, it was clear that the current phylogenetic groups among cultivated deep-sea psychrophilic and barophilic bacteria (DeLong *et al*., 1997) cannot accommodate our new isolate, MTW-13, and an establishment of a new group in which the isolate will become most accommodated in the suitable phylogenetic affiliation is required.

ACKNOWLEDGEMENT

The author would like to thank the captains and crews of the R/V Hakuho Maru and R/V Tansei Maru for their technical helps during the samplings. This work was supported by grants in aid from the Ministry of Education, Culture, Sports, Science and Technology of the Japanese government No. 11460085 and 10556043 (1998-2000) and a Monbusho doctoral thesis grant to author.

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