Proc. NIPR Symp. Polar Biol., **11**, 1–7, 1998

EFFECT OF HYDROSTATIC PRESSURE ON THE GROWTH OF DEEP-SEA BACTERIAL COMMUNITIES

Tokuki SAKIYAMA and Kouichi OHWADA

Marine Microbiology Division, Ocean Research Institute, University of Tokyo, 15–1, Minamidai 1-chome, Nakano-ku, Tokyo 164-8639

Abstract: In order to observe the effect of hydrostatic pressure on the deep-sea bacterial population, growth experiments were conducted with water samples collected from depths of 0, 2000, 4000 and 6000 m at two locations in the northwest Pacific Ocean. When the water samples were incubated under different pressures at 2°C, good growth was observed at pressure levels of the depths where the samples were collected. These results suggest that the bacterial population at each depth of the deep-sea is well adapted to the environmental conditions, as has been suggested by A. A. YAYANOS (Proc. Natl. Acad. Sci. U.S.A., **83**, 9542, 1986) with pure culture isolates from the deep-sea.

key words: hydrostatic pressure, deep-sea, bacterial community, growth, adaptation

Introduction

Bacteria are the smallest unit of life and are known to be distributed widely on earth wherever life can survive. They are considered ecologically as mineralizers of organic matter as well as converters of particulate organic matter to higher levels in the marine ecosystem. The deep-sea bacteria, which are considered adjusted to its extreme environment with high hydrostatic pressure, low temperature and low concentrations of organic material, are not an exception (KINNE, 1972; MACDONALD, 1975).

Live bacterial cells can be recovered from all depths of the ocean. Some bacteria are also known to be able to tolerate or conform to high hydrostatic pressure (ZOBELL and JOHNSON, 1949; ZOBELL and MORITA, 1957). In particular, high pressure adapted bacteria, called as barophilic bacteria, or barophiles, are known to grow better under pressure than at atmospheric pressure. Many barophilic strains have been isolated from various depths and locations (DEMING and COLWELL, 1981; JANNASCH and WIRSEN, 1984; NAKAYAMA *et al.*, 1994; WEYLAND and HELMKE, 1989; YAYANOS *et al.*, 1979). Obligate barophilic bacteria, barophiles that cannot grow at certain pressure levels and atmospheric pressure, have also been isolated (DEMING *et al.*, 1988; SAKIYAMA and OHWADA, 1997; YAYANOS *et al.*, 1981). The physiological characteristics of barophiles are gradually being elucidated (CHASTAIN and YAYANOS, 1991; DELONG and YAYANOS, 1985; JANNASCH and WIRSEN, 1984; KATO *et al.*, 1995; YAYANOS and DELONG, 1987).

Conversion of pressure units. 1 MPa = 10 bars = 9 869 atmospheric pressures = 145.38 psi.

Since barophilic bacteria were discovered in the late 19th century, many experiments have been conducted to determine the ecological significance of the deep-sea bacterial community. Some experiments have suggested that the deep-sea bacterial population shows barophilic activity (SEKI *et al.*, 1974; SCHWARTZ *et al.*, 1976), while other reports have suggested that microbial activity is inhibited, when compared with the control incubation at atmospheric pressure (JANNASCH and WIRSEN, 1973; TABOR *et al.*, 1982; WIRSEN and JANNASCH, 1976). At present, the role of barophiles in the deep-sea is still unclear (JANNASCH and TAYLOR, 1984; OHWADA, 1992; MORITA, 1988; ZOBELL, 1968).

In this paper, we describe the results of experiments on the effect of hydrostatic pressure levels on the growth of bacteria at different depths in the northwest Pacific Ocean.

Materials and Methods

Seawater samples were collected from the northwest Pacific Ocean during the cruise KH-95-2 by R/V HAKUHO-MARU, University of Tokyo. Details of the sampling locations are described in Table 1. All samples were collected by using the Niskin Butterfly sterile water samplers (General Oceanics, Florida, USA) in order to avoid contamination from outside.

Bacteria in the seawater samples were counted according to HOBBIE *et al.* (1977). Each water sample was fixed with filtered formaldehyde (Millipore, pore size 0.22 μm) with a final concentration of 2% and preserved under cold temperature (4°C) until counting. Bacterial cells were stained with acridine orange, collected on a black-stained Nuclepore filter (pore size 0.2 μm) and were counted under a fluorescence microscope. All the samples were counted within a month after fixation.

Seawater samples retrieved from different depths, 0 m, 2000 m, 4000 m and 6000 m, were transferred immediately to sterile polyethylene bags (Whirl Pak, 20–30 m*l* each), and incubated in 1/25 strength ZoBell 2216E medium (ZOBELL, 1941) at temperature 4° and 20°C, and hydrostatic pressure 0.1, 20, 40, 60 and 80 MPa (ZOBELL and OPPENHEIMER, 1950). The incubation is schematically summarized in Fig. 1. At intervals

Table 1. Sampling locations and abundance of bacterial cells in seawater samples. Two sampling locations located in the northwest Pacific Ocean The temperature was measured by CTD Cell abundance was counted under a fluorescent-microscope

	Date (GMT)	Position (Long./Lat.)	Depth (m)	Temperature (°C)	Cell abundance (cells/m*l*)
Stn 11	July 16, 1995	19°59′N/131°00′E	0	30.1	3.4×10^5
			2000	2.05	2.6×10^4
			4000	1.58	1.5×10^4
			6000	1.76	1.2×10^4
Stn 13	July 17, 1995	17°59′N/130°59′E	0	30.9	3.2×10^5
			2000	2.13	2.0×10^4
			4000	1.58	1.5×10^4
			6000	1.77	1.3×10^4

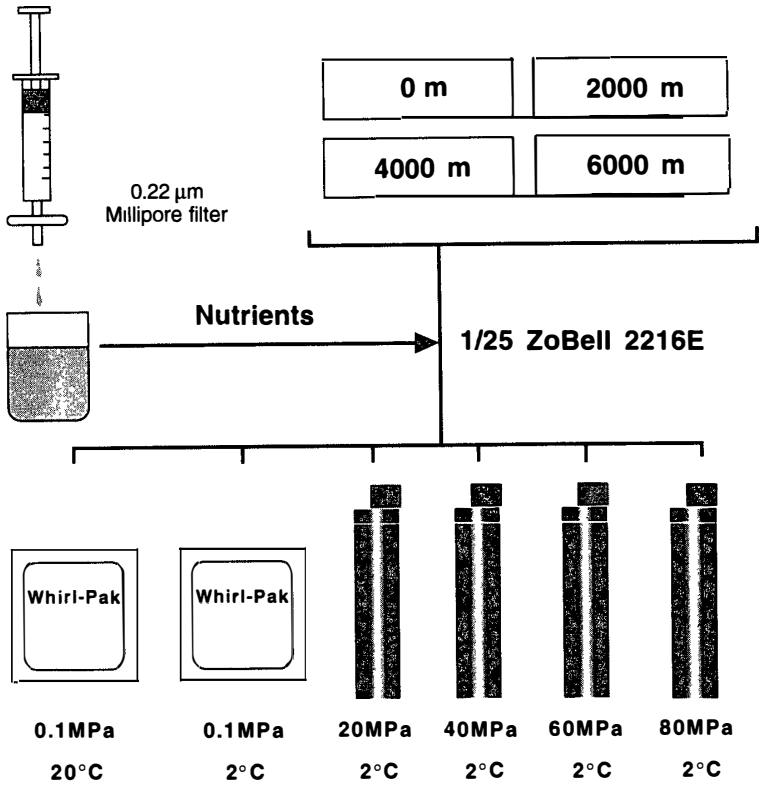


Fig. 1. A schematic representation of incubation. All the samples collected were put into sterile polyethylene bags, supplied with sterile nutrients and incubated under six different conditions (0.1 MPa/20°C, 0.1 MPa/2°C, 20 MPa/2°C, 40 MPa/2°C, 60 MPa/2°C and 80 MPa/2°C). All incubation was done simultaneously in the dark.

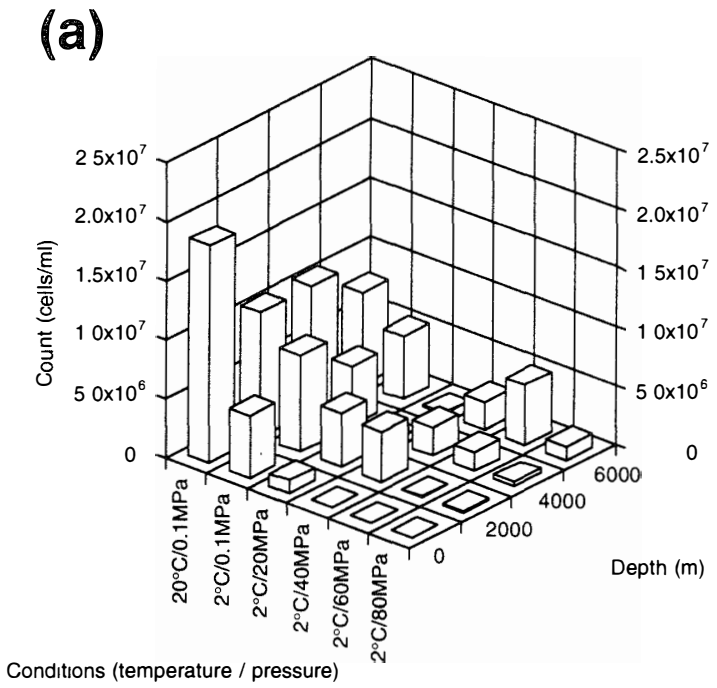
during incubation, small samples were taken, and cells in the samples were counted.

Results

The numbers of bacterial cells counted in the seawater samples are summarized in Table 1. These numbers are used as controls to compare with the numbers of the bacterial growth during incubation at different pressures and temperatures. Bacterial growths during 14 days of incubation are summarized in Fig. 2a, b. Both figures are described in the same coordinates: the incubation temperature (°C) and pressure (MPa) (x axis), the depths in meters (y axis) and the bacterial counts in number per ml (z axis). The figures are summaries of growth after 14 days of incubation of samples collected from different depths at two locations in the northwest Pacific Ocean.

The bacterial population at each depth appeared to be well adapted to the *in-situ* pressure and temperature. For example, the bacterial population taken from 6000 m showed good growth at 2°C/60 MPa and 2°C/80 MPa (Fig. 2b), and showed some growth at 2°C/40 MPa (Fig. 2a, b). The bacterial population taken from 2000 m did not grow at all under the very deep-sea conditions of 2°C/60 MPa and 2°C/80 MPa, but grew well from 2°C/0.1 MPa to 2°C/40 MPa (Fig. 2a, b).

The surface bacterial population, originally counted as 3.2 and 3.4×10^5 ml^{-1}, only



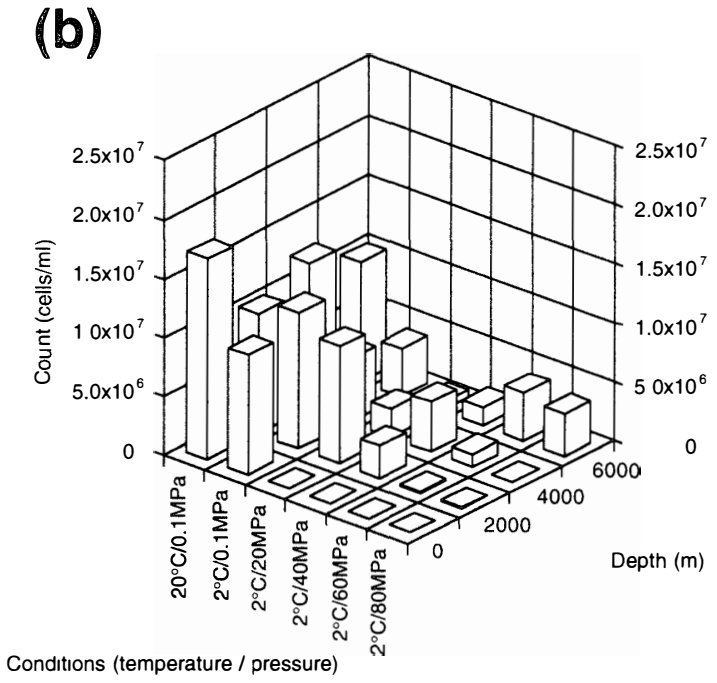


Fig. 2 Summary of increased bacterial cell numbers in the samples collected from Stn 11 (a) and Stn 13 (b), after 14 days of incubation. Both figures are described in the same categorical coordinates: the incubation conditions described in temperature and pressure (x axis), the sampled depth described in meters (y axis) and the values of bacterial count described in numbers per ml (z axis).

showed limited growth at 20 MPa, and did not show any growth at all or showed negative growth at higher pressure conditions at 2°C.

Bacteria taken from all depths showed much faster growth at 20°C/0.1 MPa and 2°C/0.1 MPa than at the conditions typical of greater depth.

Discussion

Figure 2 indicates that bacteria from 2000 m grew better under higher hydrostatic pressures than at the surface. Bacteria from 4000 m showed similar or higher growth at higher pressures, such as 40 and 60 MPa, than those from 2000 m. The results of 6000 m samples showed good growth at higher pressures, such as 40 and 60 MPa at Stn 11 and 60 and 80 MPa at Stn 13. These results suggest that the barophily of the bacterial communities increased with increasing depth. These characteristics were previously reported by YAYANOS (1986) with pure isolates from different depths.

Figure 2 also indicates that bacterial growth of the surface water samples was significantly restricted in most pressure conditions, which suggests that bacteria living in the surface layer cannot proliferate in the deep-sea.

Our observations of the bacterial population support the result of YAYANOS (1986), using isolated pure cultures from different depths, that deep-sea bacteria from different depths have several characteristics, presumably evolutionally derived, distinguishing them from each other and from bacteria living under atmospheric-pressure. Pressure plays a significant role in determining the distribution of oceanic life, and pressure-adapted bacteria are easily recovered from and ubiquitous in the deep ocean.

Though every sample contained bacteria which were able to grow under atmospheric pressure, and also had the ability to dominate in the atmospheric pressure condition, it might be suggested that the barophilic populations of the deep-sea samples were simply masked by the growth of bacteria that could grow under atmospheric pressure. Our observations indicate that surface living bacteria in the ocean are always transported slowly to deeper water attached to sinking particles and suspended matter, or through activity of vertically migrating organisms. These microorganisms never proliferate again unless they return to the surface by upwelling or by being sampled. It has been observed that spore-forming species of thermophilic bacteria usually living in the intestines of terrestrial mammals, *Bacillus stearothermophillus*, have been found in deep ocean sediments (ZOBELL, 1968). They don't grow well in deep-sea environments (YAYANOS and DIETZ, 1983).

Marine bacteria play an important ecological role as the mineralizers of organic matter as well as producers of particulate organic matter that is consumed by higher levels in the marine ecosystem. To clarify bacterial roles, it is important to study the biomass, growth, metabolic activity and composition of microbial communities. In conclusion, we have to always take into account that increase in hydrostatic pressure with depths will have a strong effect on bacterial growth, metabolic activity and bacterial community structure.

Acknowledgments

This work was supported by a grant-in-aid from the Ministry of Education, Science, Sports and Culture of Japan. We would also like to thank Mr. Genta YASUNAGA (University of the Ryukyus) for his kind help during the experiment.

References

CHASTAIN, R. A. and YAYANOS, A. A. (1991): Ultrastructural changes in an obligately barophilic marine bacterium after decompression. Appl. Environ. Microbiol., **57**, 1489–1497.

DELONG, E. F. and YAYANOS, A. A. (1985): Adaptation of the membrane lipids of a deep-sea bacterium to changes in hydrostatic pressure. Science, **228**, 1101–1103

DEMING, J. W. and COLWELL, R. R (1981): Barophilic bacteria associated with deep-sea animals. BioScience, **31**, 507–511

DEMING, J. W., SOMERS, L. K., STRAUBE, W L , SCHWARTZ, D. G. and MACDONELL, M. T (1988): Isolation of an obligately barophilic bacterium and description of a new genus, Colwellia gen. nov System Appl Microbiol , **10**, 152–160.

HOBBIE, J E., DALLEY, R. J. and JASPER, S. (1977) Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol , **33**, 1225–1228

JANNASCH, H. W and TAYLOR, C. D. (1984): Deep-sea microbiology Ann. Rev. Microbiol , **38**, 487–514

JANNASCH, H. W. and WIRSEN, C. O. (1973) Deep sea microorganisms: *In situ* response to nutrient enrichment. Science, **180**, 641–643

JANNASCH, H. W and WIRSEN, C O. (1984)· Variability of pressure adaptation in deep sea bacteria Arch. Microbiol., **139**, 281–288.

KATO, C., SATO, T and HORIKOSHI, K. (1995) Isolation and properties of barophilic and barotolerant bacteria from deep-sea mud samples Biodiversity Conserv , **4**, 1–9.

KINNE, O (1972). Pressure. Marine Ecology, ed by O. KINNE. London, Wiley-Interscience, 1323–1360.

MACDONALD, A. G. (1975). Physiological Aspects of Deep Sea Biology. London, Cambridge Univ. Press, 450 p

MORITA, R Y. (1988). Bioavailability of energy and its relationship to growth and starvation survival in nature. Can J Microbiol., **34**, 436–441

NAKAYAMA, A., YANO, Y. and YOSHIDA, K (1994): New method for isolating barophiles from intestinal contents of deep-sea fishes retrieved from the abyssal zone. Appl. Environ. Microbiol., **60**, 4210–4212

OHWADA, K. (1992): History and present status of research of deep-sea microorganisms. Gekkan Kaiyo (Kaiyo Monthly), **24**, 595–599 (in Japanese)

SAKIYAMA, T and OHWADA, K. (1997): Isolation and growth characteristics of deep-sea barophilic bacteria from the Japan Trench. Fisheries. Sci., **63**, 228–232

SCHWARTZ, J. R., YAYANOS, A. A and COLWELL, R. R. (1976): Metabolic activity of the intestinal microflora of a deep-sea invertebrate. Appl. Environ. Microbiol., **31**, 639–649

SEKI, H., WADA, E., KOIKE, I and HATTORI, A. (1974): Evidence of high organotrophic potentiality of bacteria in the deep ocean. Mar. Biol , **26**, 1–4.

TABOR, P S , DEMING, J W., OHWADA, K. and COLWELL, R. R. (1982). Activity and growth of microbial populations in pressurized deep-sea sediments and animal gut samples. Appl Environ. Microbiol., **44**, 413–422.

WEYLAND, H and HELMKE, E. (1989) Barophilic and psychrophilic bacteria in the Antarctic Ocean. Recent Advances in Microbial Ecology, ed. by Japan Scientific Society Press. Tokyo, 43–47.

WIRSEN, C. O. and JANNASCH, H. W. (1976). Decomposition of solid organic materials in the deep sea Environ. Sci Technol , **10**, 880–887.

YAYANOS, A. A (1986). Evolutional and ecological implications of the properties of deep-sea barophilic bacteria. Proc. Natl Acad. Sci U S.A , **83**, 9542–9546.

YAYANOS, A A. and DELONG, E F. (1987). Deep-sea bacterial fitness to environmental temperatures and pressure. Current Perspectives in High Pressure Biology, ed by H. W. JANNASCH, *et al* London, Academic Press, 17–32

YAYANOS, A A. and DIETZ, A S (1983) Death of hadal deep-sea bacterium after decompression. Science,

220, 497–498.

YAYANOS, A. A., DIETZ, A. S. and VAN BOXTEL, R. (1979): Isolation of a deep-sea barophilic bacterium and some of its growth characteristics. Science, **205**, 808–810.

YAYANOS, A. A., DIETZ, A. S. and VAN BOXTEL, R. (1981): Obligately barophilic bacterium from the Mariana Trench. Proc. Natl. Acad. Sci. U.S.A., **78**, 5212–5215.

ZOBELL, C. E. (1941): Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. J. Mar. Res., **4**, 42–75.

ZOBELL, C. E. (1968): Bacterial life in the deep sea. Proceedings of the U.S.-Japan Seminar on Marine Microbiology, ed. by H. KADOTA and N. TAGA. Kyoto, Kyoto Univ., 77–96.

ZOBELL, C. E. and JOHNSON, F. H. (1949): The influence of hydrostatic pressure on the growth and variability of terrestrial and marine bacteria. J. Bact., **57**, 179–189.

ZOBELL, C. E. and MORITA, R. Y. (1957): Barophilic bacteria in some deep sediments. J. Bact., **73**, 563–568.

ZOBELL, C. E. and OPPENHEIMER, C. H. (1950): Some effects of hydrostatic pressure on the multiplication and morphology of marine bacteria. J. Bact., **60**, 771–781.

(Received March 10, 1997, Revised manuscript accepted July 3, 1997)