CHARACTERISTICS OF DEEP-SEA ENVIRONMENTS AND BIODIVERSITY OF PIEZOPHILIC ORGANISMS

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Contents

Summary

Piezophilic organisms, which are specially adapted to a high-pressure environment, live in the deep sea. Due to the pressure barrier, few studies of piezophile biology and

diversity were carried out until 20 years ago. Since then, several excellent tools, including deep-sea submersibles, support vessels, and high-pressure cultivation systems, have been developed to investigate the deep-sea environment and sample organisms living there, and piezophile research has made great progress. Scientists first succeeded in recovering living piezophilic microorganisms and transferring them to the laboratory in 1979. Although the amount of information on the physiology and characteristics of piezophilic microorganisms is increasing, it is still very difficult to recover living deep-sea animals (multicellular organisms) because of their largely unknown life systems. In this article, the authors focus on descriptions of deep-sea piezophilic bacteria and their current studies on taxonomy and diversity. The features of their molecular characterizations related to high-pressure adaptation are also described.

1. Investigation of Life in a High-Pressure Environment

It has been suggested that life may have originated in the deep sea some 3.5 to 4 billion years ago, where it was protected from the damaging effects of ultraviolet light. The deep sea is a particularly high-pressure environment, and hydrostatic pressure would have been very important stimuli for early stages of life. Recently, scientists have proposed that life might have originated in deep-sea hydrothermal vents, and thus it seems possible that high-pressure-adapted mechanisms of gene expression could represent a feature present during the early forms of life. Recently, it has been reported that the primary chemical reactions involved in the polymerization of organic materials (i.e., amino acids) could have occured in such an environment. Thus, the study of deep-sea microorganisms may not only enhance our understanding of particular adaptations to abyssal and hadal ocean realms, but may also provide valuable insights into the origin and evolution of all life.

The effects of pressure on biological systems have been analyzed, and the physicochemical basis of these effects is well established. Several studies have recently been published on high-pressure tolerance mechanisms in the yeast *Saccharomyces cerevisiae*, focusing on the role of intracellular trehalose and Hsp104 proteins. Authors have analyzed the physiological effects of non- lethal (less than 100 MPa) levels of hydrostatic pressure in yeast and they showed that elevated pressure promotes the acidification of vacuoles. The acidification that occurs under elevated pressure is ascribed to hydration and ionization of carbon dioxide generated through ethanol fermentation, which leads to the accumulation of large numbers of protons in the cytoplasm. To maintain a favorable cytoplasmic pH, the yeast vacuole may serve as a proton sequestrant to allow survival under conditions of high-pressure stress. In the case of *Escherichia coli*, three types of pressure responses were observed, categorized as pressure-inducible, pressure-independent, and pressure-repressible responses. *E. coli* is closely related to deep-sea high-pressure-adapted bacteria. Thus, these responses under pressure are very interesting to consider in relation to the development of life systems, e.g., microorganisms at high pressure.

To analyze the molecular mechanisms of the pressure response in deep-sea bacteria, Bartlett and his coworkers have been studying the pressure-inducible outer membrane protein OmpH in the high-pressure-adapted bacterium *Photobacterium profundum* strain SS9. Pressure-sensing mechanisms in strain SS9 have been elucidated, and it was found that ToxR/S proteins play an essential role in the recognition of environmental stress and in the control of the expression of the *ompH* gene. Additional studies are needed to fully understand the general mechanisms of the pressure response in deep-sea bacteria, but so far relatively few high-pressure-adapted microorganisms have been identified taxonomically. It appears necessary to search for other suitable bacteria to be studied in more detail.

In 1949, Zobell and Johnson started work on the effect of hydrostatic pressure on microbial activities. The term "barophilic" was first used by them, defined as optimal growth at a pressure higher than 0.1 MPa or a requirement for increased pressure for growth. Recently, the term "piezophilic" was proposed to replace "barophilic" as the prefixes "baro" and "piezo," derived from the Greek, meaning "weight" and "pressure," respectively. Thus, the word "piezophilic" may be more suitable than "barophilic" to describe bacteria that grow better at high pressure than at atmospheric pressure. In this article, therefore, the authors have opted to use the term "piezophilic bacteria," meaning high-pressure-loving bacteria. The history of piezophile studies is shown in Table 1.

1949	Definition of piezophiles (barophiles)
1950~	Several people started to isolate piezophiles Study of protein synthesis, cell division etc. Physiological analysis (lipid components etc.)
1979	1st Isolation of piezophiles
1981	Isolation of obligatory piezophiles
1985	Piezophilic bacteria *Shewanella benthica* was defined
1988	Piezophilic bacteria *Colwellia hadaliensis* was defined
1989	Pressure-regulated outer membrane protein
1995	Analysis of pressure-regulated genes
1998	Piezophiles, *Photobacterium profundum, Shewanella violacea,* and *Moritella japonica* were defined
1999	Extremely piezophiles *Moritella yayanosii* was defined
2000	Analysis of the pressure-regulated transcription mechanisms
2002	Obligatory piezophiles *Psychromonas kaikoae* was defined
2002~	The study is in progress.

Table 1. History of pressure microbiological study

2. JAMSTEC Exploration of the Deep-Sea High-Pressure Environment

The development of manned and unmanned submersibles has supported the approaches used for investigating deep-sea environments, and has thereby contributed to research on the mechanisms of deep-sea bacterial adaptation. One of these is the manned submersible *SHINKAI 6500* (Figure 1A), which is operated by the Japan Marine Science and Technology Center (JAMSTEC) and can submerge to a depth of 6 500 m. The *SHINKAI 6500* system has been used for scientific investigations since 1991, and around 700 deep-sea dives had been performed by 2001. Many piezophilic bacteria have been isolated from samples obtained with this submersible, and it represents the most powerful tool available for studying the microbial diversity of the deep-sea environment.

JAMSTEC also constructed the unmanned submersible *KAIKO* (Figure 1B), which dove to a depth of 10 911 m in the Mariana Trench in March 1995. In 1996, the deepest sediment samples from the Mariana Trench were collected with no microbial contamination using the *KAIKO* system. JAMSTEC researchers succeeded in isolating extremely piezophilic bacteria from the samples and elucidated the microbial diversity in the deepest ocean. These submersibles are so useful in studying the deep-sea environment and life forms, that fierce competition occurs for the acceptance of diving proposals.

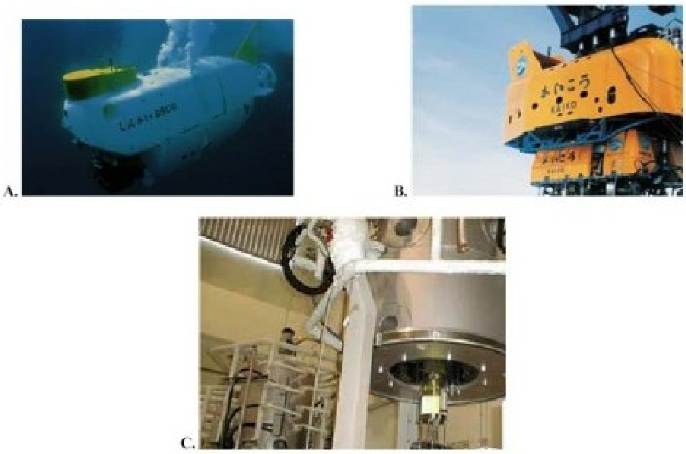

Figure 1. JAMSTEC facilities (A) the manned submersible *SHINKAI 6500* (B) the unmanned submersible *KAIKO* (C) the high-pressure microbial cultivation DEEPBATH system

JAMSTEC researchers have also developed the unique high-pressure microbial sampling and cultivation system called “DEEPBATH,” derived from “DEEP Sea BArophiles and THermophiles Collecting and Cultivating System” (Figure 1C). This system is composed of four elements: 1) a pressure-retaining sediment sampler, 2) dilution device, 3) isolation devices, and 4) cultivation chambers. These elements are interconnected, and samples can be maintained at high-pressure and low-temperature conditions during the procedures so that no physical change in the samples occurs. The authors have isolated many novel piezophilic bacteria from the deep-sea environment using those systems, as described in the following section.

3. Taxonomic Identification of Piezophilic Bacteria

3.1. Isolation of Piezophiles and their Growth Properties

Bacteria living in the deep sea have several unusual features that allow them to thrive in their extreme environment. Numerous piezophilic and piezotolerant bacteria have since been isolated and characterized by the DEEPSTAR group of JAMSTEC from deep-sea cold sediments at depths ranging from 2 500 m to 11 000 m obtained using sterilized mud samplers on the submersibles *SHINKAI 6500* and *KAIKO*. Some of the isolated piezophiles are listed in Table 2. Most of the isolated strains are not only piezophilic, but also psychrophilic and cannot be cultured at temperatures above 20°C.

Comparison of the deep-sea piezophilic strains isolated by Yayanos and those isolated by the DEEPSTAR group shows that the effects of pressure and temperature on cell growth are similar, in that all strains become more piezophilic at higher temperatures. The data indicate that all piezophilic isolates are obligately piezophilic at temperatures higher than that at which growth occurs at atmospheric pressure. This means that the upper temperature limit for growth can be extended by high pressure. Similarly, piezophilic bacteria reproduce more rapidly at lower temperature (such as 2°C) when the pressure is less than that at their capture depth. It also appears to be a general rule that the pressure at which the rate of reproduction at 2°C is maximal may reflect the true habitat depth of an isolate. The reproduction rate of piezophiles at pressures near that at the depth of capture increases with increasing temperature within the range of 6–10°C.

Organism	Optimal growth condition	Research on
Colwellia hadaliensis BNl-1	75-94 MPa at 2°C	Physiology
Moritella japonica DSK1	50 MPa at 15°C	Physiology
Moritella yayanosii DB21MT-5	80 MPa at 10°C	Membrane lipids
Photobacterium profundum SS9	28 MPa at 9°C	Gene expression, Membrane protein Membrane fatty acids
Photobacterium profundum DSJ4	10 MPa at 10°C	Physiology
Psychromonas kaikoae JT7304	50 MPa at 10°C	Physiology
Shewanella benthica strains	50- 70 MPa at 10-15°C	Physiology, Gene expression Respiratory system Diversity
Shewanella violacea DSS12	30 MPa at 8°C	Physiology Gene expression Respiratory system
Other unidentified strains	41-62 MPa at 3°C	Physiology

Table 2. List of psychrophilic piezophilic microorganisms isolated from deep-sea environments

Why do piezophilic cells grow best at temperatures that one would think might never be encountered in the cold deep sea? There are three possible explanations. One is that this behavior is simply a consequence of adaptation to high pressure and low temperature. Second, the response may have been inherited by the bacteria from ancestors that lived in a warmer environment. A third explanation is that bacteria of the cold deep sea periodically encounter warmer temperatures.

3.3 Taxonomic Characterization and Phylogenetic Relations

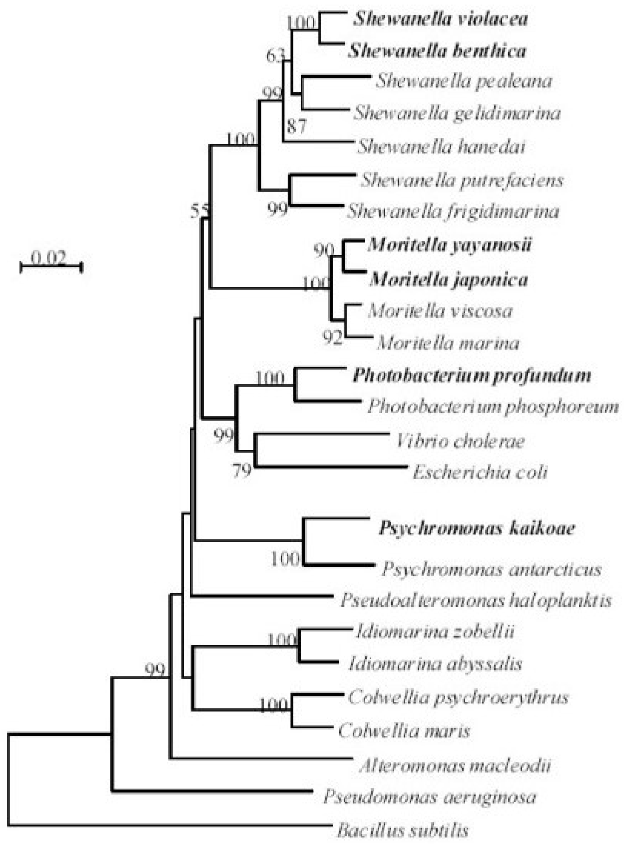

Figure 2. Phylogenetic tree showing the relationships between isolated deep-sea piezophilic bacteria (in bold) within the gamma-subgroup of proteobacteria, as determined by comparing 16S rDNA sequences using the neighbor-joining method. The scale represents the average number of nucleotide substitutions per site. Bootstrap values (%) are shown for frequencies above the threshold of 50%.

Many deep-sea piezophilic bacteria have been shown to be members of the gamma-Proteobacteria through comparison of 5S and 16S rDNA sequences. The G+C content of chromosomal DNA from *Vibrio sp*., which belongs to the gamma-Proteobacteria, is between 40–50% and is considered to be typical of this subgroup. The G+C content of chromosomal DNA from the piezophiles isolated in our laboratory was found to be similar, at 40–50%. As a result of a taxonomic study based on 5S rDNA sequences, it was reported that the obligately piezophilic bacterium *Colwellia hadaliensis* belongs to the Proteobacteria gamma-subgroup. DeLong and his colleagues have also documented the existence of piezophilic and psychrophilic deep-sea bacteria that belong to this subgroup, as indicated by 16S rDNA sequences. It is interesting to note that the 16S rDNA sequences of the piezophilic strains DB6906, DB172F, and DB172R and the psychrophilic and moderately piezophilic strain DSS12 show the highest degree of similarity of all, indicating that these strains are very closely related in the genus *Shewanella*. These data suggest that most of the deep-sea, high-pressure-adapted piezophilic bacteria that can be readily cultured belong to the Proteobacteria gamma-subgroup, and that these may not be widely distributed within the domain Bacteria. Figure 2 shows the phylogenetic relations between the identified piezophile species (shown in bold characters) and other bacteria within the gamma-subgroup of Proteobacteria.

It has been reported that eleven cultivated psychrophilic and piezophilic deep-sea bacteria are affiliated with one of five genera within the gamma-subgroup: *Shewanella*, *Photobacterium*, *Colwellia*, *Moritella*, and an unidentified genus. The only deep-sea piezophilic species of two of these genera were identified as *S. benthica* in the genus *Shewanella* and *C. hadaliensis* in the genus *Colwellia* prior to the reports by the DEEPSTAR group. We have identified five new piezophilic species within those genera based on the results of chromosomal DNA–DNA hybridization studies and several other taxonomic properties. Both previously described and new species of bacteria have been identified among the piezophilic bacterial isolates.

Photobacterium profundum, a new species, was identified through studies of the moderately piezophilic strains DSJ4 and SS9. *P. profundum* strain SS9 has been extensively studied with regard to the molecular mechanisms of pressure regulation. *P. profundum* is the only species within the genus *Photobacterium* known to display piezophilia and the only one known to produce the long-chain polyunsaturated fatty acid (PUFA) eicosapentaenoic acid (EPA). No other species of *Photobacterium* produces EPA.

The moderately piezophilic strain DSS12 isolated from the Ryukyu Trench at a depth of 5 110 m was identified as *Shewanella violacea*, a novel species within the *Shewanella* piezophile branch. Other *Shewanella* piezophilic strains, PT-99, DB5501, DB6101, DB6705, and DB6906, DB172F and DB172R, and DB21MT-2 were all identified as members of the same species, *S. benthica*. The piezophilic and psychrophilic *Shewanella* strains, including *S. violacea* and *S. benthica*, also produce EPA, and thus the production of this PUFA is a property shared by many deep-sea bacteria.

S. violacea strain DSS12 has been studied extensively, particularly with respect to its molecular mechanisms of adaptation to high pressure. This strain is moderately piezophilic, with a fairly constant doubling time at pressures between 0.1 MPa and 70 MPa, whereas the doubling times of most piezophilic *S. benthica* strains change substantially with increasing pressure. As there are few differences in the growth characteristics of strain DSS12 under different pressure conditions, this strain is a very convenient deep-sea bacterium for use in studies on the mechanisms of adaptation to high-pressure environments. Studies using this strain include analyses of the pressure regulation of gene expression and of the role of *d*-type cytochromes in the growth of cells under high pressure. The molecular mechanisms of gene expression have been analyzed, focusing on a cloned pressure-regulated promoter, and more detailed studies are in progress, as described in the following section.

Strain DSK1, a moderately piezophilic bacterium isolated from the Japan Trench, was identified as *Moritella japonica*. This is the first piezophilic species identified in the genus *Moritella*. The type strain of the genus *Moritella* is *M. marina*, previously known as *Vibrio marinus*, one of the most common psychrophilic organisms isolated from marine environments. *M. marina* is closely related to the genus *Shewanella* on the basis of 16S rDNA data, and is not a piezophilic bacterium. The extremely piezophilic bacterium strain DB21MT-5 isolated from the worlds deepest sea bottom, the Mariana Trench Challenger Deep at a depth of 10 898 m was identified as a *Moritella* species and designated *M. yayanosii*. The optimal pressure for the growth of *M. yayanosii* strain DB21MT-5 is 80 MPa; this strain is unable to grow at pressures of less than 50 MPa, but grows well at higher pressures as high as 100 MPa.

Production of the long-chain PUFA docosahexaenoic acid (DHA) is a characteristic property of the genus *Moritella*. The fatty acid composition of piezophilic strains changes as a function of pressure, and in general greater amounts of PUFAs were synthesized at higher growth pressures. Approximately 70% of the membrane lipids in *M. yayanosii* are PUFA, which is a finding consistent with its adaptation to very high pressures.

Strains JT7301 and JT7304, newly isolated from the Japan Trench at a depth of 7 434 m, are novel obligatory piezophilic bacteria, identified as *Psychromonas kaikoae*. The hybridization values for DNA–DNA relatedness between these strains and the known *Psychromonas* species, reference strain *Psychromonas antarctica*, were significantly lower than that accepted as the phylogenetic definition of a species. The optimal temperature and pressure for growth of the isolates were 10°C and 50 MPa, respectively, and both EPA and DHA are produced in the membrane layer. Based on the observed taxonomic differences, the isolated strains appear to represent a novel obligatory piezophilic *Psychromonas* species, called *Psychromonas kaikoae*. This is the first proposed species of obligatory piezophilic bacteria of the genus *Psychromonas*. DeLong and co-workers stated that strain CNPT-3 is involved in an unidentified genus, and that this strain is closely related to *Psychromonas kaikoae*. Thus, the genus *Psychromonas* is affiliated with the fifth piezophilic genus within the gamma-subgroup of Proteobacteria.

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Bibliography

Abe F. & Horikoshi K. (1998) Analysis of intracellular pH in the yeast *Saccharomyces cerevisiae* under elevated hydrostatic pressure: a study in baro-(piezo-) physiology. *Extremophiles* **2**, 223–228. [This review article indicated that the vacuole acidification was the result of the change of glycolysis pathway under pressure conditions in yeast; theauthors developed the new field, piezophysiology].

Bartlett D.H., Chi E. & Welch T.J. (1996) High pressure sensing and adaptation in the deep-sea bacterium *Photobacterium* species strain SS9. *In*: *High Pressure Bioscience and Biotechnology* (Eds. Hayashi R. & Balny C.), Elsevier Science BV, Amsterdam, pp. 29–36. [This proceedings review demonstrated the pressure-sensing mechanisms using *ompH* gene expression].

DeLong E.F., Franks D.G. & Yayanos A.A. (1997) Evolutionary relationship of cultivated psychrophilic and barophilic deep-sea bacteria. *Appl. Environ. Microbiol*. **63**, 2105–2108. [This paper indicated that the piezophilic species were divided into five genera in the Proteobacteria gamma subgroup].

Kato C. & Nogi Y. (2001) Correlation between phylogenetic structure and function: examples from deep-sea *Shewanella*. *FEMS Microbiol. Ecol*. **35**, 223–230. [This paper showed that the genus *Shewanella* can be separated in to two taxonomic groups, and that group 1 *Shewanella,* the cold temperature- and pressure-adapted species, might be related by global deep-ocean circulation].

Kato C. & Qureshi M.H. (1999) Pressure response in deep-sea piezophilic bacteria. *J. Mol. Microbiol. Biotechnol*. **1**, 87–92. [This review showed the respiratory model of deep-sea *Shewanella* under pressure conditions].

Kato C., Sato T. & Horikoshi K. (1995) Isolation and properties of barophilic and barotolerant bacteria from deep-sea mud samples. *Biodiv. Conserv*. **4**, 1–9. [This paper described the isolation and properties of piezophiles from deep-sea environment using the manned submersible, *SHINKAI 6500*].

Li L., Kato C. & Horikoshi K. (1999) Bacterial diversity in deep-sea sediments from different depths. *Biodiv. Conserv*. **8**, 659–677. [This paper demonstrated the microbial diversity using the 16S rDNA sequences directory isolated from deep-sea sediments at different depths.]

Nakasone K., Ikegami A., Kato C., Usami R. & Horikoshi K. (1998) Mechanisms of gene expression controlled by pressure in deep-sea microorganisms. *Extremophiles* **2**, 149–154. [This review article demonstrated the molecular mechanisms of pressure-regulated transcription in piezophilic *Shewanella violacea*].

Nakasone K., Ikegami A., Kawano H., Usami R., Kato C. & Horikoshi K. (2002) Transcriptional regulation under pressure conditions by the RNA polymerase ^{54} factor with a two components regulatory system in *Shewanella violacea*. *Extremophiles* **6**, 89-95. [This review article demonstrated that two component systems (NtrB and C) should have an important role in pressure-regulated transcription by sigma 54 promoter].

Nogi Y. & Kato C. (1999) Taxonomic studies of extremely barophilic bacteria isolated from the Mariana Trench, and *Moritella yayanosii* sp. nov., a new barophilic bacterial species. *Extremophiles* **3**, 71–77. [This paper demonstrated the taxonomic identification of novel species, *Moritella yayanosii*].

Nogi Y., Masui N. & Kato C. (1998a) *Photobacterium profundum* sp. nov., a new, moderately barophilic bacterial species isolated from a deep-sea sediment. *Extremophiles* **2**, 1–7. [This paper demonstrated the taxonomic identification of a novel species, *Photobacterium profundum*].

Nogi Y., Kato C. & Horikoshi K. (1998b) Taxonomic studies of deep-sea barophilic *Shewanella* species, and *Shewanella violacea* sp. nov., a new barophilic bacterial species. *Arch. Microbiol.* **170**, 331–338. [This paper demonstrated the taxonomic identification of a novel species, *Shewanella violacea*].

Nogi Y., Kato C. & Horikoshi K. (1998c) *Moritella japonica* sp. nov., a novel barophilic bacterium isolated from a Japan Trench sediment. *J. Gen. Appl. Microbiol*. **44**, 289–295. [This paper demonstrated the taxonomic identification of a novel species, *Moritella japonica*].

Nogi Y., Kato C. & Horikoshi K. (2002) *Psychromonas kaikoae* sp. nov., a novel piezophilic bacterium from the deepest cold-seep sediments in the Japan Trench. *Int. J. Syst. Evol. Microbiol*. **52**, in press. [This paper discusses the taxonomic identification of a novel species, *Psychromonas kaikoae*].

Tamegai H., Kato C. & Horikoshi K. (1998) Pressure-regulated respiratory system in barotolerant bacterium, *Shewanella* sp. strain DSS12. *J. Biochem. Mol. Biol. Biophys.* **1**, 213–220. [This paper showed that the cytochrome *bd* complex appeared only at high pressure condition].

Yanagibayashi M., Nogi Y., Li L. & Kato C. (1999) Changes in the microbial community in Japan Trench sediment from a depth of 6,292 m during cultivation without decompression. *FEMS Microbiol. Lett*. **170**, 271–279. [This paper demonstrated the DEEPBATH cultivation of the Japan Trench sediment under high-pressure conditions from various sampling stages].

Yayanos A.A. (1995) Microbiology to 10,500 meters in the deep sea. *Annu. Rev. Microbiol*. **49**, 777–805. [This review article mentioned that "piezophile" is a better term to use to mean high-pressure adapted organisms than "barophiles"].

Zobell C.E. & Johnson F.H. (1949) The influence of hydrostatic pressure on the growth and viability of terrestrial and marine bacteria. *J. Bacteriol*. **57**, 179–189. [This is a first article to indicate that high-pressure loving microorganisms (piezophiles) exist at deeper sea bottom].

Biographical Sketches

Chiaki Kato received an MSc degree in biological chemistry in 1978 from Rikkyo University in Japan and was appointed a researcher in the Central Research Institute of ZERIA Pharmaceutical Co. Ltd. In 1982, he started to study biotechnology at RIKEN Institute, and then continued to study Extremophiles at Prof. Horikoshi SuperBugs Project from 1986. He got his Ph.D. in 1984 from the University of Tokyo with a thesis focusing on the excretion of protein from *Escherichia coli* using alkaliphiles genes. In 1988-90, he was a post-doc research fellow in Dept. Microbiology-Immunology, Northwestern University Medical and Dental Schools and Dept. Pediatric Dentistry, the University of Texas Health Science Center at San Antonio (USA). From 1991, his research activities were centered on the deep-sea microbes, their isolation and molecular adaptation to the high-pressure environment, as a group leader of deep-sea Extremophiles research program, Japan Marine Science and Technology Center. He participated in several cruises in the deep-sea investigations using submersibles. In 1998, he was appointed Professor of the Department of Bioinformatics at Tokyo Institute of Technology, and he was promoted to senior scientist in the Department of Marine Ecosystems Research at JAMSTEC, in 2002. He is the author of 180 publications and has been a secretariat of the International Society for Extremophiles since 2001.

Koki Horikoshi received a Ph.D. degree in agricultural chemistry in 1963 from the University of Tokyo in Japan and was appointed an Assistant Professor at the RIKEN Institute. In 1966, he was invited as an Associate Professor to California University at Davis (USA). He then became an Associate Professor in RIKEN in 1970. He discovered a new type of microbe, "Alkaliphiles", which could grow well in alkaline environments. In 1974, he became a Professor in the Department of Applied Microbiology, RIKEN, and then he focused on studying the alkaliphilic microbial world, and discovered plenty of useful enzymes for industrial applications from alkaliphilic microbes. In 1984, he was selected as a director of the Super Bugs Project, ERATO, for five years, and many studies of "Extremophiles" were done under the aegis of this project. In 1988, he was appointed a Professor of the Department of Bio-Engineering at Tokyo Institute of Technology, and in 1990, he started a new long-term project called the "DEEPSTAR project", which studied deep-sea Extremophiles, as a program director at JAMSTEC. In 1993, he was appointed Professor at Toyo University, and then he worked as a dean of the College of Bioscience at Tokyo University in 1997. His current position is Professor Emeritus of the Tokyo Institute of Technology (1993-), Professor Emeritus of RIKEN Institute (1993-), Program Director of DEEPSTAR, JAMSTEC (1990-), and Professor of Toyo University (1993-). He has received many Awards, for example, an award from the Agricultural Chemical Society in Japan (1966, 1989), The Purple Ribbon Medal from the Japanese Emperor (1987), the Gold Medal of the International Institute of Biotechnology at The Royal Society, London (1991), the Honda Prize (1993), and is Professor Emeritus, Kent Univetsity, UK (2001). He is also President of the International Society for Extremophiles and the Japanese Society of Extremophiles, and is a chief editor of the journal *Extremophiles*. He has published more than 500 scientific papers, 30 books, and 300 patents.