PIEZOPHILES: MICROBIAL ADAPTATION TO THE DEEP-SEA ENVIRONMENT

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Keywords: Archaea, barophile, cell division, *cis*-vaccenic acid, deep sea, eicosapentaenoic acid, *E. coli*, FabF (KAS II), membrane lipids, omega-3 polyunsaturated fatty acids, osmolytes, outer membrane proteins, *Photobacterium*, piezophile, pressure, pressure-inducible proteins, psychrophile, *PTk* diagram, RecD, RseC, *Shewanella*, thermophile, ToxR, unsaturated fatty acids

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Summary

The study of microorganisms isolated from deep-sea habitats is providing insight into the ecology and evolution of life in high pressure environments. Elevated hydrostatic pressure is an important thermodynamic parameter that has greatly influenced the physiological and biochemical adaptations of marine organisms inhabiting different depths. At the molecular level, the responses of microorganisms that are not high-pressure adapted to elevated pressures have revealed fundamental differences in cellular metabolism and regulatory processes as compared to microorganisms that specifically thrive under elevated pressures (piezophiles). Analysis of nonpiezophilic microorganisms at elevated pressures hint at pressure-sensitive cellular phenomena which piezophilic microorganisms must modify for high-pressure adaptation. Investigations with piezophilic isolates have identified genes required for growth at

elevated pressure, pressure responsive genes and gene products, and general categories of cellular processes which, if disrupted, result in specific growth impairment at high pressure. Examples of these processes include cell division and membrane homeostasis. The focus of this chapter is to review the genetic, physiological, and biochemical evidence regarding microbial adaptation to high pressure.

1. Introduction

Since the middle of the twentieth century, hydrostatic pressure has emerged scientifically as a significant environmental parameter influencing the ecology and evolution of marine organisms. Whereas the roots of piezobiology began to be established in the late nineteenth century by the efforts of Certes and coworkers, progress in the field lay relatively dormant until the mid-twentieth century. Around this time, the question of whether life existed in the deepest portions of the world's oceans remained a mystery. In the early 1950s, the Danish ship *Galathea* lowered dredges into the deepest ocean trenches and hauled up a variety of invertebrates. It is with credit to the *Galathea* expedition that the modern field of deep-sea microbiology was born. ZoBell and Morita, participants onboard *Galathea*, showed that bacteria could be cultivated under *in situ* pressure and temperature conditions from 10 400 m depth. However, despite these findings, the question of whether true high pressure-adapted bacteria existed was still unresolved. In 1979, the first pure culture of a pressure-adapted bacterium was reported by Yayanos *et al.* and served to underscore the relevance of pressure as a selective influence on the evolution of life. Today, improvements in oceanographic sampling and laboratory instrumentation have allowed researchers to begin to uncover molecular processes important for growth and survival under conditions present in the deep sea. In this chapter, an overview of the diversity of deep-sea environments and their resident microflora will be discussed along with physiological, biochemical, and genetic data related to mechanisms of deep-sea (primarily high pressure) adaptation.

2. Deep-Sea Habitats

Terrestrial habitats with pressures of one atmosphere or lower account for <1% of the total volume of the biosphere. The oceans, which cover ~70% of Earth's surface, have an average depth of 3 800 m and an average pressure 380 times that present at Earth's surface. Thus, high-pressure and low-temperature deep-sea environments occupy the largest fraction of the biosphere in terms of volume, with the notable exception of deep subsurface environments, the precise biologically relevant volume of which having yet to be determined. The deep sea can broadly be characterized by the presence of high hydrostatic pressures (up to 1 100 atm or 110 megapascal (MPa)), generally low temperatures ~2 °C except in regions of hydrothermal activity (up to 380 °C), the absence of light, and general oligotrophy. As such, the deep sea can be regarded as an extreme environment. Throughout the spectrum of physicochemical parameters encountered in the deep sea, microbial life exists.

The nature of deep-sea habitats is determined by numerous factors including input of surface derived nutrients, geochemical and geothermal influences, and physical oceanographic and hydrological regimes in addition to the pressure and the temperature.

These factors in turn govern the local community structure and biodiversity of the habitat. Microbiologically relevant high pressure environments encountered on Earth are listed in Table 1.

Environment	Approximate Pressure
Deep-sea water column/surface sediments	112 MPa
Deep-sea invertebrates	108 MPa
Deep-sea fish	63 MPa
Deep-sea brines	15 MPa
Hydrothermal vents	41 MPa
hale falls	41 MPa
Lake Baikal, Siberia[a]	16 MPa
Lake Vostok, Antarctica[a]	41 MPa
Deep marine sediments	14 MPa
Deep basaltic rock	67 MPa
Deep granitic rock	55 MPa
Deep oil reservoirs	31 MPa

[a]No microbiological studies have yet been done on samples from these deep freshwater environments.

Table 1. High-pressure microbiological environments on Earth and their documented approximate upper pressures

Chemosynthetic communities of microorganisms, often associated with invertebrate hosts, exist in the deep sea at hydrothermal vents and cold seeps. Hydrothermal vents are fissures in the ocean floor that leak hot, acidic water. Cold seeps are additional sites of fluid release from the seafloor that are frequently present along the borders of continental plates. Adult animals living near hydrothermal vents have been found to require high pressure for survival, and their larvae require high pressure for development. Whale falls in the deep sea have been found to also harbor chemosynthetic communities of microorganisms and animals similar to those found at cold seeps and hydrothermal vents. Another high-pressure environment where chemosynthetic communities are also found is that of methane hydrates. Under appropriate conditions of high pressure, low temperature, and sufficient methane concentration, methane in the seafloor can combine with water to form solid methane hydrate. These structures support a unique microbial assemblage, which is apparently capable of utilizing the methane as an energy source under anaerobic conditions.

In addition to deep-sea environments, high pressure can be considered an important environmental parameter within other habitats. For example, Lake Baikal in Siberia is the deepest surface exposed freshwater lake in the world, possessing a maximal depth of 1 600 m. Recent evidence suggests that Lake Vostok, a large freshwater lake located 3 to 4 km beneath the East Antarctic Ice Sheet contains bacteria in relatively high concentrations. Microbes existing in these habitats could also be adapted for optimal growth and survival under elevated pressure conditions. If we allow our consideration of possible biospheres to extend outside of Earth, high pressure will remain an important parameter. For example, if life exists within the potentially watery

environment of the Jovian moon Europa, then it may need to be adapted down to the lower liquid water depths of 80–170 km. Taking into account Europa's surface gravity of ~13% of that of Earth, hydrostatic pressures could reach 200 MPa, roughly twice that of earthly deep-sea environments.

An active area of current research involves assessing the extent of the deep subsurface biosphere. Piezophilic sulfate reducing bacteria have been isolated from greater than 500 m below the seafloor in the Pacific Ocean, and evidence for microbial activity in deep basaltic or granitic rock has also been obtained. The extent to which life within the deep subsurface exists is likely to be determined not by the pressures encountered but rather the extremes of temperature. Currently, the upper limit for microbial growth is 113 °C. For oceanic crust, where the temperature rises ~15 °C per kilometer of depth, tolerance of microbial life may extend to ~7 km below the seafloor. For continental crust, where the temperature is near 20 °C at the surface and typically increases by ~25 °C per kilometer, life should extend ~4 km into the subsurface. It is predicted that the largest number of prokaryotes in the biosphere is likely to reside within the subsurface environment.

3. Isolation and Characterization of Piezophiles

3.1. Nomenclature

ZoBell and Johnson first coined the term barophile to describe organisms that grow optimally at increased pressures. Later, the change from barophile to piezophile was proposed for etymological reasons (the prefix *baro* is from the Greek word for "weight" whereas the prefix *piezo* is from the Greek word for "pressure"). Piezophiles are broadly defined as those organisms that exhibit optimal reproduction rates at pressures >0.1 MPa. Hyperpiezophiles can be defined as those organisms which display optimal growth rates at pressures >60 MPa. Such definitions give an operational framework within which to describe high pressure-adapted organisms. Just as all organisms can be categorized according to their growth temperature ranges (psychro-, meso-, thermo-, hyperthermo-philes), so can piezophilic species. Following the temperature convention, the vast majority of piezophilic species in culture would be classified as piezopsychrophiles or hyperpiezopsychrophiles. Currently, no examples of hyperpiezomesophiles have been characterized. The most piezophilic bacterium yet obtained is an isolate from the Mariana Trench whose pressure limit for growth is ~130 MPa.

3.2. Isolation and Cultivation

The majority of research conducted on deep-sea piezophiles has been performed on isolates collected from the cold deep sea. This is primarily a reflection of the relative ease with which many of these organisms are capable of laboratory-based cultivation in nutrient-rich media. Hence, although significant advances have been made in the isolation and culturing of piezothermophiles, our emphasis here will be placed on the piezopsychrophiles. Pressure-adapted microorganisms have been successfully collected from water, sediment, and animal samples. An important consideration when sampling the deep sea is the maintenance of appropriate *in situ*-like conditions. This usually

entails the use of appropriate isopiestic (pressure retaining) and isothermal (temperature retaining) samplers. The final consideration is the maintenance of collected samples in the dark. It has been shown that deep-sea bacteria are extremely sensitive to ultraviolet radiation. It follows to reason that autochthonous organisms present in the deep sea are specifically adapted to deep-sea conditions.

Many microorganisms that are not specifically adapted to deep-sea conditions may be introduced into this environment as a result of their association with sinking phytoplankton debris or as spores. Even piezosensitive sporeforming thermophiles have been recovered at colony forming units between 100 and 1 000 colonies per gram of dry sea mud. Eurythermal *Clostridium* species related to *C. bifermentans* have been obtained from sediments within the Japan Trench at a water column depth of 7.3 km. The vegetative cells of these microbes were very pressure sensitive, whereas their spores were very pressure resistant. It has also been possible to isolate *Clostridium perfringens* (an indicator of sewage contamination) from a temporary deep-sea dump site located off the continental slope about 200 km off the coast of New Jersey at water depths of ~2 500 m.

The isothermal recovery of deep-sea samples is the key consideration when attempting to retain autochthonous deep-sea bacteria within a sample, while limiting the proliferation of allochthonous invader species. Loss of viability of deep-sea psychrophiles due to warming must therefore be prevented. Depending on the variety of samples to be collected a host of sampling devices are available which provide ample insulation to meet these requirements. For example, Niskin bottles are ideal in the collection of water samples because of their insulation properties. In addition, numerous box and gravity coring devices to obtain sediment samples and animal traps have been used with success which are sufficiently insulated to avoid large temperatures fluctuations.

With regard to maintenance of *in situ* pressure conditions during sampling, a key question is the decompression sensitivity of the target organisms. This is somewhat problematic since the spectrum of loss of viability due to decompression of deep-sea organisms is not entirely understood. For example, numerous deep-sea piezophiles exhibit facultative piezophily meaning they are capable of growth at atmospheric pressure as well as increased hydrostatic pressures. For such strains, decompression is not a lethal event. However, certain isolates such as MT-41 (isolated from the Mariana Trench at a pressure of 100 MPa) do not survive decompression for extended periods of time. For these reasons, care should be taken to preserve deep-sea samples under *in situ* pressure conditions.

The use of pressure-retaining animal traps have been used with particular success for obtaining piezophilic isolates. The first piezophile to be characterized, strain CNPT3, was isolated from an animal trap. Deep-sea metazoans appear to be particularly reliable sources for the isolation of piezopsychrophiles. These include scavenging amphipods, filter-feeding holothurians, and various fish species. Investigations of the pressure growth responses of the intestinal microflora of abyssal fish species have revealed facultative and obligate piezophiles to be the dominant representative isolates with non pressure-adapted and piezotolerant species playing a minor role. The use of animal traps

for the recovery and isolation of deep-sea microorganisms is particularly useful in the study of metazoan/bacterial symbioses, an area of investigation yet to be fully explored.

Advances in deep-sea research technologies have greatly facilitated investigations of deep-sea bacterial adaptation. In particular, the use of manned and unmanned submersibles has notably aided in the efficient sampling of deep-sea microorganisms. Operated by the Japan Marine Science and Technology Center (JAMSTEC), the manned submersible Shinkai 6500 and the unmanned submersible Kaiko are two prime examples. Shinkai 6500 is capable of diving to a depth of 6 500 m whereas Kaiko has recorded dives to nearly 11 000 m in the Mariana Trench. In 1996, Kaiko obtained sediment samples from a depth of 10 897 m from the Challenger Deep of the Mariana Trench. From this sample, numerous microbes were isolated composed of diverse lineages including actinomycetes, yeasts, and a variety of various piezophilic and nonpiezophilic extremophiles.

Laboratory cultivation and analysis of deep-sea isolates requires suitable pressure vessels for the maintenance of working cultures and experimental incubations. Commonly employed is the use of a pin-retained piston closure vessel in conjunction with a quick-connect coupler to secure the vessel to a high-pressure pump (Figure 1). Such vessels are practical owing to their rapid compression/decompression time and their relatively inexpensive fabrication cost. Various techniques of cultivation and colony formation at high pressure have been recently reviewed by Yayanos. The use of heat-sealable sterile transfer pipettes has proven ideal for routine batch culturing of isolates for example in performing high-pressure growth curves.

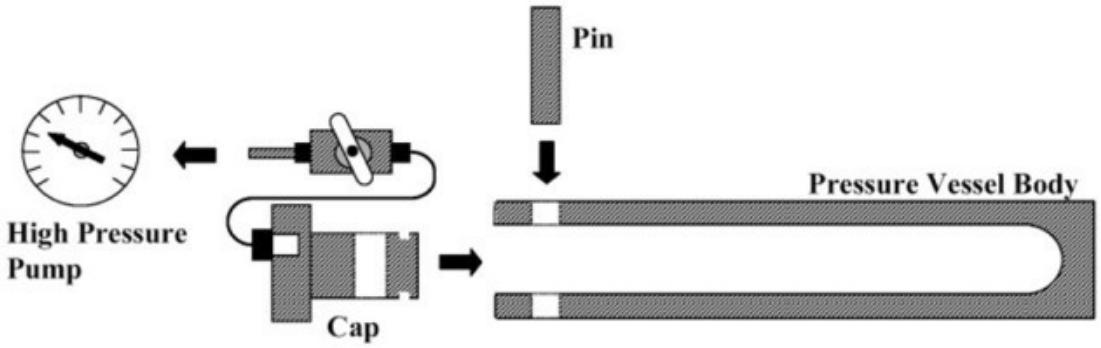

Figure 1. Schematic showing a pin-retained piston closure pressure vessel commonly employed in routine cultivation of high-pressure microbiological samples
Diagram template provided by Professor A. Aristides Yayanos, Scripps Institution of Oceanography, University of California–San Diego.

Analysis of deep-sea thermophiles is complicated by the simultaneous requirements of high pressures, high temperatures, and strictly anaerobic conditions. Characterization of such strains has been significantly aided by the engineering of large-scale cultivation systems that are capable of maintaining these parameters. One such system, the DEEP-BATH system (deep-sea baro/thermophiles cultivation system), has been developed by the Japan Marine Science and Technology Center. This high-pressure/high-temperature bioreactor is designed to work within a 0–300 °C range and at pressures up to 68 MPa.

In addition, it is suitable for continued sampling without sample perturbation. Such systems are particularly well suited for analysis of microbial assemblages isolated from hydrothermal vent habitats.

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Bibliography

Allen E.E. and Bartlett D.H. (2000). FabF is required for piezoregulation of cis-vaccenic acid levels and piezophilic growth of the deep-sea bacterium *Photobacterium profundum* strain SS9. *Journal of Bacteriology* 182, 1264–1271. [This paper demonstrates a role for a specific enzyme involved in fatty acid biosynthesis in high-pressure adaptation.]

Allen E.E., Facciotti D., and Bartlett D.H. (1999). Monounsaturated but not polyunsaturated fatty acids are required for growth of the deep-sea bacterium *Photobacterium profundum* SS9 at high pressure and low temperature. *Applied Environmental Microbiology* 65, 1710–1720. [This paper demonstrates a role for monounsaturated but not polyunsaturated fatty acids in high-pressure adaptation.]

Bartlett D.H. (1992). Microbial life at high pressures. *Science Progress* 76, 479–496. [This review provides a large amount of background information on high-pressure microbiology.]

Bartlett D.H. (1999.) Microbial adaptations to the psychrosphere/piezosphere. *Journal of Molecular Microbiology and Biotechnology* 1, 93–100. [This review emphasizes progress made in understanding the roles of fatty acids and DNA recombination in high-pressure adaptation.]

Bartlett D.H. and Bidle K.A. (1999). Membrane-based adaptations of deep-sea piezophiles. *Enigmatic Microorganisms and Life in Extreme Environments*, (ed. J. Seckbach), pp. 501–512. Dordrecht: Kluwer. [This paper provides an overview of membrane-based processes that are critical to high-pressure adaptation.]

Bartlett D.H., Kato C., and Horokoshi K. (1995). High-pressure influences on gene and protein expression. *Research in Microbiology* 146, 697–706. [This review summarizes pressure effects on gene expression in piezophilic and piezosensitive microorganisms.]

Gross M. and Jaenicke R. (1994). Proteins under pressure—the influence of high hydrostatic pressure on structure, function and assembly of protein complexes. *European Journal of Biochemistry* 221, 617–630. [This review addresses important concepts in pressure effects on proteins.]

Kato C. and Bartlett D.H. (1997). The molecular biology of barophilic bacteria. *Extremophiles* 1, 111–116. [This review presents information on the taxonomy of piezophiles and their pressure-regulated genes.]

Kato C., Li L., Nogi Y., Nakamura Y., Tamaoka J., and Horikoshi K. (1998). Extremely barophilic bacteria isolated from the Mariana Trench, Challenger Deep, at a depth of 11 000 meters. *Applied Environmental Microbiology* 64, 1510–1513. [This paper presents an example of the important work being done by Professor Horikoshi and colleagues on the isolation and characterization of piezophiles.]

Marteinsson V.T., Birrien J.L., Reysenbach A.L., Vernet M., Marie D., Gambacorta A., Messner P., Sleytr U., and Prieur D. (1999). *Thermococcus barophilus sp. nov.*, a new barophilic and

hyperthermophilic archaeon isolated under high hydrostatic pressure from a deep-sea hydrothermal vent. Int. J. Syst, Bacteriol. 49, 351–359. [This paper is a good example of work on piezohyperthermophiles.]

Miller J.F., Shah N.N., Nelson C.M., Lulow J.M., and Clark D.S. (1988). Pressure and temperature effects on growth and methane production of the extreme thermophile *Methanococcus jannaschii. Applied and Environmental Microbiology* 54, 3039–3042. [This paper is another good example of work being done on piezohyperthermophiles.]

Somero G.N. (1992). Adaptations to high hydrostatic pressure. *Annual Revue Physiology* 54, 557–577. [This paper reviews high pressure adaptation in metazoans.]

Welch. T.J., Farewell A., Niedhardt F.C., and Bartlett D.H. (1993). Stress response of *Escherichia coli* to elevated hydrostatic pressure. *Journal of Bacteriology* 175, 7170–7177. [This paper provides a detailed analysis of pressure stress-induced proteins.]

Yayanos A.A. (1986). Evolutional and ecological implications of the properties of deep-sea barophilic bacteria. *Proceedings of the National Academy Sciences (USA)* 83, 9542–9546. [This paper describes some of the fundamental properties of piezophile growth.]

Yayanos A.A. (1995). Microbiology to 10 500 meters in the deep sea. *Annual Revue of Microbiology* 49, 777–805. [This is a comprehensive review of deep-sea microbiology; history, equipment, and biophysical considerations.]

Biographical Sketches

Eric Allen received a BSc degree in Biology from the University of Oregon in 1995 where he worked for several years as a research assistant investigating presynaptic vesicle fusion proteins in frog saccular hair cells and later analyzing phytoplankton assemblages collected from the Arabian Sea. In 1996, he began his PhD work in Marine Biology at the Scripps Institution of Oceanography, University of California, San Diego in the laboratory of Professor Douglas H. Bartlett. His thesis research involves analysis of membrane-based adaptations of deep-sea piezophilic bacteria. Focused on microbial adaptation to high pressure and low temperature, his research has included deep-sea collection cruises to the Japan Sea and participation in the United States Antarctic Program. In 2000, he was a student in the National Science Foundation sponsored Antarctic Biology Training Course where he spent 5 weeks at McMurdo Station conducting research on Antarctic organisms. In 2002, he will pursue postdoctoral research on the molecular genetics and biochemistry of microbial natural products with primary interest in the mechanism of bacterial omega-3 polyunsaturated fatty acid synthesis.

Douglas Bartlett received a BSc degree in biology in 1979 from Valparaiso University in Indiana, and his PhD degree in molecular biology from the University of Illinois at Chicago in 1985, with a thesis focusing on the identification and characterization of gene products involved in motility and chemotaxis in Escherichia coli. Later in 1985, he traveled out west to the Agouron Institute in La Jolla, California. to work with Dr. Michael Silverman as a postdoctoral scholar and then later as a Research Scientist on various genetic analyses of marine bacteria. Since 1989, he has been at Scripps Institute of Oceanography, University of California, San Diego, where he is now an Associate Professor. His research has focused on mechanisms of high pressure sensing and adaptation in deep-sea bacteria. More recently, he has also been examining the microbiology of methane hydrates in marine sediments and the adaptations of the human pathogen Vibrio cholerae to the marine environment. He is the author of 40 original publications, the organizer of the San Diego Microbiology Group, and a member of a number of scientific societies including the International Society for Extremophiles.