PIEZOPHILY: PROKARYOTES EXPOSED TO ELEVATED HYDROSTATIC PRESSURE

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Contents

Summary

All living organisms, and particularly prokaryotes, which colonize the most extreme environments, have their physiology controlled by a variety of physicochemical parameters whose different values contribute to the definition of biotopes. Hydrostatic pressure is one of the major parameters influencing life, but its importance is limited to only some environments, especially the deep sea. If the deep sea is defined as water layers below one kilometer depth, this amount of water, which is exposed to pressures up to 100 MPa, represents 62% of the volume of the total Earth biosphere. A rather small numbers of investigators have studied the prokaryotes that, alongside invertebrates and vertebrates, inhabit this extreme environment. Deep-sea prokaryotes show different levels of adaptation to elevated hydrostatic pressure, from the barosensitive organisms to the obligate piezophiles. Piezophily is frequent among deep-

sea psychrophilic prokaryotes and thermophiles belonging to the Bacteria or Archea domains. Recent microbiological investigations of other extreme environments exposed to elevated hydrostatic pressure also revealed living prokaryotic communities in deep sediments, aquifers, porous rocks, or oil reservoirs. All these data indicate that Earth's biosphere is larger than was previously thought, but all these new biotopes are exposed to high pressure. This novel concept of a deep biosphere is important to our knowledge of our planet, but also has major applications to the search for life (fossil or actual) on other planets and bodies, such as Mars and Europa.

1. Introduction

In addition to availability of energy and carbon sources and electron acceptors, the physiology of prokaryotes, and consequently their distribution on Earth, is dependent on a series of major physicochemical parameters, including temperature, salt concentration, pH, light radiation, and redox potential. Each biotope may be characterized by a combination of particular values of all these different parameters. However, some environments may be influenced by other specific parameters, such as hydrostatic pressure. Hydrostatic pressure can be defined as the weight of liquid above a given surface. This parameter is generally expressed in kilograms per centimeter squared, atmospheres (atm), bars, or MPa (megaPascals). Roughly, 1 kg cm^{-2} = 1 atm and 1 bar = .1 MPa. Hydrostatic pressure increases by .1 MPa for every 10 m of water; at a depth of 2000 m, hydrostatic pressure is 200 bars or 20 MPa. This last parameter is important for many environments as will be seen later, but was mostly studied in the case of the deep sea. The deep sea corresponds to the water layers and bottom sediments of the oceans that are located below a depth of 1000 m. Earth, the blue planet, has 70% of its surface area covered by oceans, and 88% of this surface corresponds to the deep sea. The deepest trenches have a depth of more than 10 km (10 790 m in the Mariana Trench), and the average ocean depth is about 3800 m. It has been calculated that this huge amount of water corresponds to 75% of the total oceanic volume and 62% of the Earth biosphere. In terms of volume, the deep sea represents the largest environment on Earth. In other terms, 62% of the biosphere (by volume) is exposed to hydrostatic pressure above 10 MPa. Exploration of the deep sea was made difficult essentially by hydrostatic pressure, which prevents animals (including human beings) with an atmospheric respiration from permanently thriving in this environment. However, several diving birds and marine mammals can dive for a rather long time down to several hundred meters depth (it is known that the sperm whale *Physeter catodon* can reach at least one kilometer). But, although several sophisticated technical devices were designed, human beings (obviously only well trained and equipped divers) have not been able to reach depths below a few hundred meters, and that only for a limited amount of time. Only the protection of the steel or titanium hulls of the deep-sea manned submersibles allowed engineers and scientists in the second half of the twentieth century to watch the ocean floors at depths of several kilometers. Actual life on Earth is driven by energy from the sun, and consequently photosynthetic organisms (i.e., plants and algae) that are able to fix carbon dioxide into organic carbon are then used as food by the primary consumers. In aquatic environments, light cannot penetrate beyond a few hundred meters depth, so obviously photosynthesis and photosynthetic organisms cannot occur in the deep sea. This does not necessarily rule out the existence of animals, but, for a long time, it was believed that the deep sea was not inhabited permanently by animals, either in the water column or in sediments under deep water. Until the second half of the nineteenth century, the existence of animals living at a depth of more than 500 m was not definitely proven. The first certain answers came from the observation of electric or telephone cables that came up colonized with benthic deep-sea invertebrates after being immersed in the deep sea for a long period. After these pioneer observations, many oceanographic expeditions were organized to describe the composition, physiology, and functioning of deep-sea communities. Before 1977, the deep sea was considered a poor oligotrophic environment with low density animal communities, living slowly in coldness and darkness under elevated hydrostatic pressure. But in 1977, a major discovery was made with the first observation of deep-sea hydrothermal vents and their fascinating associated animal communities, occurring at a depth of 2000 m in the Galapagos Islands area. Two years later, the "black smokers," which emit hydrothermal fluids at temperatures of about 350 °C (fluids remain liquid at these elevated temperatures because of hydrostatic pressure), were discovered and induced a novel research effort on extremophilic prokaryotes.

2. Deep-Sea Microbiology

2.1. A Brief History

Deep-sea microbiology initially started in the late nineteenth century with the report of the French microbiologist Certes demonstrating the presence of bacteria in samples collected in 1882–1884 by the oceanographic expeditions of the "Travailleur" and the "Talisman." However, with the exception of Fisher's work published in 1894, the real start occurred in the second half of the twentieth century when Zobell published the first works on effect of elevated hydrostatic pressure on deep-sea bacteria. One of the first questions investigated by deep-sea microbiologists was the decomposition of organic matter under *in situ* conditions. To address this, microbiologists carried out numerous experiments to measure heterotrophic activity within samples collected in the deep sea and processed shipboard under atmospheric or *in situ* pressure conditions, on decompressed or undecompressed samples. Most of these experiments concluded that heterotrophic activity of deep-sea bacteria was very slow under deep-sea conditions, and that probably no free-living bacteria adapted to deep-sea conditions existed. The problem is that a sample of sediment from the surface of the sea floor may contain bacteria permanently living on the sea floor, but also bacteria associated with particles sinking from the surface, which were shown to lose their activity when reaching the cold and pressure-exposed deep waters. A major breakthrough came from the suggestion that piezophilic bacteria could thrive within the digestive tracts of deep-sea invertebrates. Effectively, analysis of these permanently pressure-exposed habitats revealed that true piezophilic bacteria really existed within amphipod, holothurian, or fish digestive tracts. From there, many piezophilic bacteria were isolated, characterized by their physiology, taxonomy, phylogeny, and adaptations to pressure conditions. Until 1977 all deep-sea bacteria described were only psychrophilic or psychrotolerant organisms, since the deep oceanic waters are generally cold (about 2 °C). The discovery of deep-sea hydrothermal vents, where temperature varies from 2 °C for the cold deep sea to 350 °C for the hot fluids, allowed scientists to find bacteria and archea with optimal temperatures from a few degrees up to 110 °C, some of them showing a true piezophilic behavior.

2.2. Deep-Sea Psychrophiles

2.2.1. General Features

The most significant research effort on piezophilic bacteria was carried out by Yayanos and his group since 1978. Although it was recently suggested that the term piezophile was more correct, the term barophile is still used very often for bacteria that grow better under hydrostatic pressure or require hydrostatic pressure for growth. The most remarkable result of these investigations is the isolation of strain MT 41 from a decomposing amphipod captured at a depth of 10476 m. This organism was the first obligate piezophile, growing optimally at 2 °C and 69 MPa, with a doubling time of 25 hours, but unable to grow under pressures below 38 MPa. From the study of a set of strains isolated from various depths, Yayanos and coworkers concluded that piezophily is a general feature of bacteria from cold deep seas. They also reported that a threshold exists at 2000 m, with piezotolerant bacteria in the upper layers and increasingly piezophilic bacteria in the deeper layers. However, optimal pressure for growth, although being an indicator of the original depth of a strain, does not correspond exactly to the capture depth. More precisely, optimal pressure for growth at 2 °C of a strain isolated from a sample collected, for instance, at 5000 m is not 50 MPa as expected, but rather 30 or 35 MPa. A qualification of the statement that piezophily is a general feature is that this feature was originally found only for heterotrophic psychrophiles, isolated from nutrient-rich environments (digestive tracts). Demonstration of piezophily for oligotrophs was recently obtained, but reports of piezophilic chemolithoautotrophs are still required to definitely establish that piezophily is an essential feature of deep-sea bacteria.

2.2.2. Adaptations to Elevated Hydrostatic Pressure

The availability of piezophilic deep-sea bacteria in pure culture under laboratory conditions allowed for many experiments investigating how they are adapted to elevated pressure conditions. The activity of various extracellular enzymes from piezophilic bacteria was studied under atmospheric and *in situ* pressure conditions by several authors, but the data obtained did not clearly demonstrate piezophilic activities. For instance, in the case of chitinase, it was reported that chitinase synthesis was inhibited by high pressure (40 MPa), and that chitinase activity measured on cell extracts was rather piezotolerant. However, data obtained on enzymatic activities appeared rather different when performed with whole cells, using a modified commercially available API ZYME assay kit, which allows the detection and approximate quantification of 19 enzymatic activities. Experiments carried out with 10 facultative piezophilic strains showed that nine of them had their phenotype modified (activity was detected under pressure only) for at least one enzyme, particularly esterase–lipase or beta-galactosidase. An explanation for these apparently contradictory observations came from the study of the molecular mechanisms involved in adaptations to elevated hydrostatic pressure. The increase of polyunsaturated fatty acid synthesis, demonstrated for several strains, or the expression under pressure of a porine-like protein, for a facultative piezophile, clearly indicate the major role of the membrane in the response of a bacterial cell to hydrostatic pressure. This may explain why the enzymatic activity data obtained with whole cells favor piezophily more than those obtained with cell extracts or purified enzymes. In 1989, genes involved in this adaptation were cloned and sequenced, and some appear to be organized within pressure-regulated operons. These features have been particularly found for a subbranch of the genus *Shewanella* within the gamma sub-class of Proteobacteria, called the "Shewanella barophile branch." Moreover, since demonstration of piezophily is difficult for deep-sea chemoautotrophic organisms that certainly have a very slow growth rate, utilization of molecular methods based on detection of genes involved in pressure adaptations may allow proof of the ubiquity of barophily.

2.3. Deep-Sea Hydrothermal Vents

Deep-sea hydrothermal vents are a direct consequence of plate tectonics. Oceanic plates are demarcated by oceanic ridges, with a total length of 60 000 km. In these areas, seawater penetrates though cracks on the seafloor and approaches the magma chamber, where it is heated. There, seawater leaches the surrounding basalts and is enriched with heavy metals and various substances such as hydrogen, hydrogen sulfide, and methane. The hot fluid then rises because of its low density, reaches the seafloor, and vents out at temperatures of up to 350 °C. Vent temperature depends on the mixing of hot fluid coming up with cold water coming down. If mixture occurs, fluids vent out at temperatures from 10 to 40 °C. These areas are colonized by vent animal communities that depend on symbiotic chemolithoautotrophic bacteria for their food. If mixture does not occur, hot, acidic, anoxic fluids vent out at very high temperatures. When discharged into cold oxygenated deep-sea waters, minerals contained in the fluid precipitate. The result of this precipitation is the construction of spectacular mineral structures made mostly of calcium sulfate and polymetallic sulfides, from which the particle-rich fluids vent out; these are called hydrothermal chimneys or black smokers. After the discovery of hydrothermal vents in 1977 in the Galapagos area, and black smokers in 1979 on the East Pacific Rise, hydrothermal vents were explored in many places in the eastern and southwestern Pacific and on the mid-Atlantic Ridge, at depths ranging from 800 to 3500 m. Microbiology studies on deep-sea hydrothermal vents mostly dealt with invertebrate-associated symbionts, and thermophilic or hyperthermophilic organisms. Although mesophilic habitats exist, only a few organisms were characterized, and very few studied for their response to hydrostatic pressure.

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Biographical Sketch

Daniel Prieur received a Masters degree in Biological Oceanography from the University of Paris VI in 1971, and defended successively a "Thèse de 3ème cycle" and a "Thèse de Doctorat es Sciences Naturelles" at the University of Brest (France) in 1974 and 1981, respectively. After a contractual position at the University of Brest, he was appointed as Senior Scientist by CNRS (National Center for Scientific Research) in 1976, still in the same university. From 1976 to 1988, he developed research activities concerning the relationships between bivalve molluscs and bacteria in the marine environment, and, during the later part of this period, concerning microbial communities in coastal waters. In 1984, he started a new project on the microbiology of deep-sea hydrothermal vents, and participated in his first diving cruise in the eastern Pacific. In 1988, he moved to the Roscoff Biological Station (France) and set up a research group on thermophilic prokaryotes from deep-sea hydrothermal vents, focusing on the following areas: metabolic and phylogenetic diversity; adaptation to extreme conditions, temperature, pressure, radiations; and genetic elements of hyperthermophiles. In 1990, he became "Directeur de Recherche CNRS" and received in 1995 a CNRS silver medal award. In 1996, he moved back to the University of Brest as a professor, where he developed several microbiology teaching units and became head of the Microbiology PhD program. During his "thermophilic" period he participated in several deep-sea cruises and dove several times aboard the manned submersibles Cyana, Nautile, and Alvin. He signed (or co-signed) about 160 publications and was elected Vice-President of the University, in charge of the Scientific council, for the period 1998–2002. Still involved in deep-sea microbiology, he now actively participates in several Exo-Astrobiology committees and working groups organized by CNES, ESA, and NASA.