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Deep-sea piezosphere and piezophiles: geomicrobiology and biogeochemistry

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The deep-sea piezosphere accounts for approximately 75% of the total ocean volume and hosts active and diverse biological communities. Evidence obtained thus far suggests that the microbial biomass present in the piezosphere is significant. Continued international interest in exploring the deep ocean provides impetus to increase our understanding of the deep-sea piezosphere and of the influence of piezophilic microbial communities on the global ocean environment and on biogeochemical cycling occurring in the deep sea. Here, we review the diversity, metabolic characteristics, geomicrobiology and biogeochemistry of the deep-sea piezophiles.

Deep-sea piezosphere and piezophiles

The deep-sea piezosphere [1] encompasses the volume of the deep sea at the depth of 1000 m and greater, with hydrostatic pressures of over 10 atm or 10 MPa (see Glossary). The temperature of the piezosphere for the most part is cold (2–3 °C) but can be very hot (up to 400 °C or higher) near hydrothermal vents. The deep sea accounts for approximately 75% of the total ocean volume and hosts ~62% of the global biosphere [2] (Box 1). Therefore, the deep-sea piezosphere represents a large biotope, possibly the largest, of the Earth [1]. Piezophiles are pressure-loving microorganisms, which reproduce preferentially or exclusively at pressures greater than atmospheric pressure [3,4]. Some piezophiles are able to grow at or slightly higher than atmospheric pressure (0.1–10 MPa) and are considered to be piezotolerant (Table 1; Figure 1). Others are classified as piezophilic or hyperpiezophilic, which require high pressure for optimal growth [2], 10–50 MPa and >50 MPa, respectively, for piezophiles and hyperpiezophiles. Hyperpiezophiles that grow at pressures as high as 130 MPa have been isolated [5]. Piezobiology is the field of studying organisms that live at high pressure [5].

Piezophiles have been found in the deep sea of all the major oceans except the Arctic Ocean (Figure 2). Yanoas [6] proposed a classification of piezophiles based on both the temperature ($T_{k_{max}}$) and pressure ($P_{k_{max}}$) for their optimum growth rate (Table 1). This classification scheme predicts that piezophiles can be found in various pressure and temperature regimes in the deep sea, including hydrothermal areas. Recent advances in genomic technology and renewed interests in the dark energy environment of the deep sea provide impetus to increase our understanding of the deep-sea piezosphere and of the influence of piezophiles on the global ocean environment and on biogeochemical cycling occurring in the deep sea. Here, we provide a concise summary of current knowledge of the field of microbial piezobiology and argue that piezophiles possess unique physiologies and metabolism that deserve special assessment of their roles in global biogeochemical cycles and that the study of piezophiles in the oceans has far-reaching significance in elucidating the origin and evolution of life and in searching for life in the deeper biosphere on Earth [7].

Diversity of deep-sea piezophiles

Psycho-piezotolerant, psycho-piezophilic and psycho-hyperpiezophilic bacteria

The majority of the piezophilic bacteria reported thus far are Gram-negative, facultative anaerobic species that

Glossary

Archaea: one of the three domains of life that contains prokaryotes with ether-linked (glycerol diether or diglycerol tetraether) isoprenoid membrane lipids and archaeal ribosomal RNA.

Bacteria: all prokaryotes that are not members of the domain Archaea. Bacteria contain primarily ester-linked phospholipids in their membranes and bacterial ribosomal RNA.

Biogeochemistry: the science that studies the biological processes and reactions that govern the chemistry of the environment.

Biomarker: a biochemical compound produced by an organism that retains structural remnants inherited from the source organism, which serves as a signature for the presence or activity of that organism or group of organisms.

Geomicrobiology: a hybrid science integrating geochemistry, microbiology, genomics and geology studying the interaction between microbes and their environment.

Hydrostatic pressure: the pressure exerted by water due to the force of gravity.

Isotope fractionation: the separation of isotopes of an element during naturally occurring physicochemical and biological processes as a result of the mass differences between their nuclei.

Metagenomics: the study of metagenomes of the entire communities of microbes in environmental samples.

Piezobiology: the field of studying biology at high pressure.

Piezophilic: a general term referring to the growth and reproduction of microorganisms under higher than atmospheric pressure.

Piezosensitive: microorganisms that grow optimally at atmospheric pressure but can also grow at higher pressures not more than 50 MPa.

Piezosphere: a term specifically referring to the volume of the deep sea having pressure greater than 10 MPa (or greater than 1000 m in depth).

Proteobacteria: a major phylum of the Bacteria. All Proteobacteria are Gram-negative. The Proteobacteria are divided into five subgroups (α, β, γ, δ and ε) based primarily on ribosomal RNA sequences.

Redox reaction: oxidation-reduction reaction, typically mediated by microorganisms.

Thiobrophic: a term describing an organism that oxidizes reduced sulfur compounds (i.e. elemental sulfur and hydrogen sulfide) as a major part of its metabolism.
belong to the domain Bacteria. Many of the cultured piezophiles are also psychrophilic and thus are psychro-piezotolerant, psychro-piezophilic or psychro-hyperpiezophilic (Table 1). These organisms belong to the Gammaproteobacteria class and are species from one of five main genera: Shewanella, Photobacterium, Colwellia, Moritella and Psychromonas [10].

Species of the genus Shewanella appear to be the most widely distributed piezophiles in the deep sea [11], at least based on those isolated in pure culture. They grow under a broad range of pressures with members ranging from piezotolerant to piezophilic and hyperpiezophilic. Representative species include Shewanella violacea strain DSS12, Shewanella benthica strain PT99 and strains...
DB1172F, DB1172R, DB21MT-2, DB5501, DB6101, DB6705, DB6906, WHB46, F1A, PT48 (Table S1 in supplementary material online). S. benthica strain DB21MT-2 is a hyperpiezophilic bacterium isolated from sediments of the Mariana Trench collected at a depth of 10,898 m [12]. Recently, a new hyperpiezophilic strain (KT99) was isolated from the Kermadec Trench (9856 m), representing the first piezophile from the Southern Hemisphere [13]. Phylogenetic analysis shows that this strain is closely related to S. benthica PT99 [13].

Four piezophilic bacterial isolates are *Moritella* species and include *Moritella japonica* DSK1, *Moritella yawanosii* DB21MT-5, *Moritella profundus* and *Moritella abyssii*. Strain DSK1 is piezophilic (10–50 MPa) and one of the most widely used model organisms for studies of piezophy. Strain DB21MT-5 is hyperpiezophilic and was isolated from sediment of the Mariana Trench and collected at a depth of 11,000 m [12].

There are several hyperpiezophiles that represent the genus *Colwellia*: *Colwellia* MT41 (100 MPa) and *Colwellia hadalensis* BNL-1 (93 MPa). Two new piezophilic strains (Y223G and Y251E), which apparently differ from other *hadalensis* BNL-1 (93 MPa). Two new piezophilic strains and *Psychromonas* species include *Psychromonas profunda* 2825T and *Psychromonas* CNPT3.

Recently, two new piezophilic strains, AT7 and AT12, were isolated from the Aleutian Trench at 2500 m [13]. These strains belong to the genus *Carnobacterium* and are closely related to *Carnobacterium pleistocenium*, a psychrophilic bacterium isolated from the Alaska permafrost [16]. Strains AT7 and AT12 grow at pressures from 0.1 to 60 MPa (optimal pressure for growth is 15 MPa) and can be classified as psychro-piezophilic [13]. These strains are the first Gram-positive piezophiles discovered and obviously not members of the five main Gram-negative genera previously discussed. This finding suggests that there is much piezophilic microbial diversity left to explore.

### Table 1. Classification scheme of piezophiles based on optimal growth temperature and pressurea

<table>
<thead>
<tr>
<th>Temperature/Pressure</th>
<th>Psychro-piezotolerant</th>
<th>Meso-piezotolerant</th>
<th>Thermo-piezotolerant</th>
<th>Hyperthermo-piezotolerant</th>
</tr>
</thead>
<tbody>
<tr>
<td>P&lt;sub&gt;m&lt;/sub&gt;max &lt; 10 MPa</td>
<td>&lt; 15 °C</td>
<td>15–45 °C</td>
<td>45–80 °C</td>
<td>&gt;80 °C</td>
</tr>
<tr>
<td>Piezotolerant (10–50 MPa)</td>
<td>Psychro-piezotolerant</td>
<td>Meso-piezotolerant</td>
<td>Thermo-piezotolerant</td>
<td>Hyperthermo-piezotolerant</td>
</tr>
<tr>
<td>Hyperpiezophilic (≥ 50 MPa)</td>
<td>Psychro-hyperpiezophilic</td>
<td>Meso-hyperpiezophilic</td>
<td>Thermo-hyperpiezophilic</td>
<td>Hyperthermo-hyperpiezophilic</td>
</tr>
</tbody>
</table>

*Modified from Ref. [2] and [5].

Both thermophilic and hyperthermophilic piezophiles have been found in the domains Bacteria and Archaea. Most of these organisms are from deep-sea hydrothermal vent areas and both heterotrophic and autotrophic species have been isolated. It is interesting to note that *Demacoccus abyssi* strain MT1.1<sup>T</sup>, a meso-piezophilic actinomycete (which grow optimally at 28 °C and 40 MPa), was not isolated from a hydrothermal vent site but from the Challenger Deep of the Mariana Trench [17].

Alain et al. [18] first reported the isolation of a thermopiezophilic sulfate-reducing bacterium, *Mariniloga piezophila* strain KA3<sup>T</sup>, from the East-Pacific Rise (EPR; 13°N) at 2630 m. Its optimal growth temperature and pressure were 65 °C and 40 MPa, respectively. Another thermopiezophilic sulfate-reducer, *Desulfovibrio hydrothermalis* strain AM13<sup>T</sup>, was also isolated from the EPR from approximately the same depth (2600 m) [19]. Strain AM13<sup>T</sup> is phylogenetically and genotopically similar to *Desulfovibrio profundus*, a piezophilic species isolated from deep sediment layers in the Japan Sea [20]. Strain AM13<sup>T</sup> uses sulfate, sulfite or thiosulfate as terminal electron acceptors [19]. Recently, two novel thermo-piezophilic, chemolithoautotrophic Proteobacteria from the Mid-Atlantic Ridge (MAR) were isolated that oxidize reduced sulfur compounds as electron donors [21]. These isolates appear to represent two novel genera of bacteria. One isolate, *Thioprofundum lithotrophic* strain 106, is phylogenetically associated with many of the theirotrophic endosymbionts found in deep-sea chemoautotrophic animals (mussels, clams and tubeworms) in the class Gammaproteobacteria. The other isolate, *Piezobacter thermophilus* strain 108, is phylogenetically affiliated with the family *Rhodobacteraceae* (Alphaproteobacteria class). No hyperthermo-piezophilic bacteria have been isolated and cultured thus far.

The hyperthermo-piezophiles and hyperthermo-hyperpiezophiles are archaea and are either chemolithoauto-
troths or chemoorganoheterotrophs. The hyperthermopiezophilic species *Pyrococcus abyssi* grows at 20–40 MPa [22] and was isolated from the Fiji Basin at 2000 m [23]. Furthermore, a hyperthermo-piezophilic archaeon, *Thermococcus barophilus*, was isolated from the MAR at a depth of 3550 m [24]. This species is chemoorganoheterotrophic and grows optimally at 85 °C and 40 MPa but can also grow at atmospheric pressure and a wide range of temperatures. The methanogen *Methanopyrus kandleri* strain 116 was isolated from the Central Indian Ridge and grows optimally at 105 °C under hydrostatic pressures of 20–30 MPa [25]. At 40 MPa, strain 116 can grow at 122 °C, the highest growth temperature recorded for any organism [25]. A chemoorganoheterotrophic, hyperthermo-hyperpiezophilic archaeaon, *Pyrococcus CH1*, was isolated from the MAR at a depth of 4100 m [26]. This species grows optimally at 98 °C and 52 MPa. These studies clearly show that by increasing the hydrostatic pressure, the upper temperature growth limit can also be increased, a phenomenon that has been previously observed [5,21].

Figure 2 shows the distribution of piezophiles in the global oceans, which to some extent reflects the history of the field of piezobiology. The field of piezobiology started with the pioneering work in the Pacific Ocean by ZoBell and associates in the 1950s and continued in the past decades in more or less the same geographic area by Yayanos and Bartlett at Scripps Institution of Oceanography and Horikoshi at the Japan Agency for Marine–Earth Science and Technology (JAMSTEC) [3]. The biogeography of piezophiles shown in Figure 2 might also shed light on the origin, evolution and adaptation of the piezophiles in the deep sea [4,13]. For instance, *Shewanella* piezophiles are found in the Western Pacific, South Pacific, Eastern Pacific, Weddell Sea and the North Atlantic, suggesting that piezophilic bacteria might be derived from shallow water psychrophiles in the Antarctic and that the distribution of piezophilic bacteria is influenced by global deep ocean circulation [4,13].

The microbial diversity of piezophiles might be seriously underestimated because of technical and other difficulties in sampling, isolating and culturing these organisms [21]. One problem might be the preferential use of rich, heterotrophic growth media in the isolation of piezophilic bacteria. Prokaryotes in the deep sea are probably never exposed to such high amounts of nutrients and many organisms (oligotrophs) are known to prefer or require very low amounts of nutrients. Thus, the use of this type of growth medium poses a bias in those organisms being cultured as this type of medium clearly favors the growth of heterotrophs that tolerate and can utilize the relatively high concentration of nutrients. This ultimately could result in a serious underestimation of the diversity of the cultured piezophiles [2]. For example, *Shewanella* species are the most abundant psycho-piezophilic isolates and belong to the five main Gram-negative genera of piezophiles discussed earlier (Table S1 in the supplementary material online; Figure 2) and could prefer rich to minimal growth medium during initial isolation resulting in their growth over many other organisms. Therefore, we strongly believe that many types of growth media should be used for the initial isolation of piezophiles.

In addition, in many cases, the currently used piezophile-culturing devices only reproduce the physical conditions (temperature and hydrostatic pressure) but not the chemistry (i.e. the energy and carbon source) of the organisms’ surrounding environment in the deep sea [21]. For example, it is difficult to simulate the dissolved gas composition and availability in the deep sea in current piezophile culturing devices. In most currently used pressure vessels, no gas space is left and some gases (e.g. oxygen) might be depleted during collection leading to the preferential isolation of anaerobes and facultative anaerobes [2]. This limitation has been only partially overcome by the use of liquid oxygen carriers such as Fluorinert FC-77 [27]. Therefore, it seems probable that the diversity of piezophiles will increase significantly as the use of new types of
growth media, novel culturing techniques [21,28], novel molecular sequencing techniques [28–31] and intact polar lipid analysis [32–34] are employed. The community genomics approach of DeLong et al. [29,35] is also very promising and allows simultaneous determinations of phylogeny, function, metabolic and community dynamics. There could indeed be a ‘rare biosphere’ in the ocean [30]. Using pyrosequencing, Sogin et al. [30] detected a large number of low-abundance phylotypes in the deep water of the North Atlantic, suggesting that microbial diversity in the oceans is much greater than previous, conventional molecular techniques have estimated. A similar ribosomal tag pyrosequencing survey along a depth gradient at the station ALOHA of the North Pacific Ocean identified ‘unexpected’ high phylogenetic and functional diversity of Bacteria, Archaea and micro-Eucarya [36]. The majority of the microorganisms in the rare biosphere are poorly represented in current databases simply because of a lack of breadth and scale in sampling, but these microorganisms might be responsible for key metabolic processes in global biogeochemical cycles [36]. Thus, the diversity and abundance of deep-sea piezophiles are probably much greater than what we know today and the relative abundance of piezophilic heterotrophic and autotrophic bacteria and archaea remains to be determined.

**Life in the slow lane: deep-sea microbial metabolism and energetics**

The deep sea in general is an oligotrophic environment with limited availability of usable organic matter. The
organic matter available in the deep sea mostly consists of refractory complex polymers [37,38]. Deep-sea heterotrophs are dependent mainly on the detrital ‘rain’ of organic matter from the euphotic zone. It has been suggested that deep-sea microbial activity is low because of low temperature, high pressure and low amount of nutrients present in the deep sea. However, recent findings challenge this notion in that the deep-sea crenarchaeota, which constitute one-third of the microbial biomass in the oceans, fix carbon autotrophically and provide an additional source of organic matter to the deep sea [39–41]. Nevertheless, deep-sea piezophiles probably have adapted to this extreme environment in many unique ways [2,31,36,42,43].

Recent studies have demonstrated that the piezophilic bacterium P. profundum SS9 degrades many refractory compounds such as chitin, cellulose and pullulan [44]. Cell-specific in situ measurements of microbial activities (ectoenzymatic activity, heterotrophic microbial production and dark fixation of CO₂) suggest that microbial metabolic rates in the deep sea are as much as 11-fold higher than previous estimates obtained under surface decompression conditions [45]. Specific metabolic genes related to the degradation of refractory pools of organic matter metabolic pathways have been recovered from the deep sea [29,46]. However, it is intriguing that the measured metabolic rates cannot be sustained by the available organic carbon measured in the Tyrrhenian Sea of the Mediterranean [47]. Temperature might be a factor in causing this as temperature in the deep part of the Mediterranean Sea is approximately 10 °C higher than the typical open ocean deep sea [46]. It is also possible that autotrophic fixation of inorganic carbon in the deep sea has been underestimated which would provide an additional source of organic carbon [41,47].

The dual flagellar motility systems observed in P. profundum SS9 and Shewanella piezotolerans WP3 is a manifestation of piezophilic bacterial adaptation to the deep-sea oligotrophic environment [48,49]. These studies revealed the presence of a large flexible gene pool for the production of the flagellar systems in strains SS9 and WP3 that are lacking in the shallow water strain P. profundum strain 3TCK [50]. These genes or gene clusters encode for nutrient acquisition, energy production and polyunsaturated fatty acid (PUFA) synthesis [48,49,51].

In a given area of the deep sea where the temperature is relatively constant and uniform, pressure influences microbial metabolic reactions to a greater extent [2]. Model simulations (Figure 3) show that, at constant temperature, increasing pressure reduces the energy yield ($\Delta G_r$) of all
redox reactions except those involved in Fe(III)-reduction where $\Delta G_r$ increases with pressure. This result suggests that iron-bearing minerals might play an important role in microbial metabolism and energetics under high pressure conditions in the deep sea [2]. However, lowering temperature and increasing pressure raises electron activity ($\pi(\pi')$) for some redox reactions commonly taking place at more positive redox potentials and lowers the $\pi(\pi')$ values for others that take place at more reducing conditions (Figure 4). Therefore, redox reactions prevailing at more oxidative conditions ($O_2$ and $NO_3^-/NO_2^-$ reduction) yield slightly more energy in the deep sea under low temperature–high pressure conditions, whereas those that dominate in more reducing conditions (sulfate reduction, iron reduction and CO$_2$ reduction) yield relatively less energy, compared to surface environments at 25°C and 0.1 MPa. Thus, we need to reevaluate microbial metabolism and energetics under high pressure conditions and their ecosystem significance for deep-sea and deep subsurface microbes in these biospheres.

Modern molecular analyses have proven useful in the discovery of new microbial metabolic capabilities. Recent metagenomic analysis of fosmid libraries of microbial communities in the ALOHA station of the North Pacific Subtropical Gyre and in deep Mediterranean water revealed the presence of metabolic genes related to catabolism, transport and degradation of complex organic molecules, as well as genes encoding carbon monoxide dehydrogenases (CODH; the cox genes) [29,46]. CODH genes were present in the photic zone but were more abundant in deep layers of the ALOHA water column [29,36]. These results suggest that microbial oxidation of carbon monoxide might be an important energy source for deep-sea microorganisms [46]. CO oxidizers in the ocean could include aerobic bacteria in the Alpha-, Beta- and Gammaproteobacteria classes and the Cytophaga–Flexibacter–Bacteroides group as well as anaerobic bacteria and archaea including acetogens, sulphidogens and methanogens [52]. It is clearly of contemporary importance that we need to elucidate unknown metabolic capabilities of piezophiles and reevaluate the known metabolic types under deep-sea high pressure conditions. New research tools such as those discussed in this section show great promise in this regard.

**Lipid biosynthesis and carbon isotope fractionation**

In our opinion, the most significant finding in studying the biochemistry of piezophilic bacteria is the discovery of *de novo* synthesis of PUFAs in these microorganisms [53,54]. The physiological and biogeochemical significance of PUFAs has been investigated in various studies [54–57]. Piezophiles contain all the major types of fatty acids commonly found in surface bacteria: C$_{14-18}$ saturated, monounsaturated, terminal methyl-branched, hydroxyl and cyclopropane fatty acids [58,59]. Piezophilic bacteria are unique in that they can synthesize long-chain PUFAs, either eicosapentaenoic acid (EPA; 20:5w3) or docosahexaenoic acid (DHA; 22:6w3) or both, neither of which are found in surface bacteria. The proportions of unsaturated fatty acids can be as much as 70% of the total fatty acids in piezophilic bacteria and increase with increasing growth pressure [53,54,58].

Carbon isotopes are important tools in geomicrobiology and biogeochemistry and can be used in conjunction with lipid analyses. Fang *et al.* [60] examined carbon isotope fractionation (the separation of isotopes of an element during naturally occurring physicochemical and biological processes) in the biosynthesis of fatty acids by piezophilic bacterium *Moritella japonica* strain DSK1. Bacterial biomass and fatty acids exhibited consistent pressure-dependent carbon isotope fractionation relative to growth substrate (glucose). Fatty acids became progressively more depleted in $^{13}$C with increasing pressure (Figure 5). The average carbon isotope fractionation effect was –5.7%o, –15.3%o and –18.3%o at 10, 20 and 50 MPa, respectively, as compared to cells grown at atmospheric pressure (0.1 MPa). A strong linear correlation between carbon isotope fractionation and hydrostatic pressure was observed. Based on results such as this, overall, heterotrophic piezophilic bacteria appear to fractionate carbon isotopes significantly (14–18%) more than surface heterotrophic bacteria [60].

Another characteristic of isotope geochemistry is that PUFAs have much more negative $\delta^{13}$C values than other short-chain saturated and monounsaturated fatty acids (Figure 5). The increased carbon isotope fractionation effect was attributed to the kinetics of hydrostatic pressure-affected enzymatic reactions and to the operation of two different fatty acid biosynthetic systems in piezophilic bacteria: the FAS (fatty acid synthase)- and PKS (polyketide synthase)-based pathways [59,60]. The FAS-based pathway is operational in surface bacteria and catalyzes...
the synthesis of typical short-chain bacterial fatty acids. The PKS-based biosynthetic pathway is fundamentally different than the FAS-based pathway and involves polyketide synthases that catalyze the biosynthesis of the long-chain PUFAs, EPA and DHA. The PKS-based pathway appears to be widely distributed in marine bacteria [61]. Thus, studies involving fatty acid (particularly EPA and DHA) analyses and carbon isotope signatures are becoming more informative in studying the geomicrobiology and biogeochemistry of deep-sea piezophiles [2,60].

The only reported study of carbon isotope fractionation in autotrophic archaea is that of Takai et al. [25]. Carbon isotope fractionation in methanogenesis and carbon fixation by the autotrophic, hyperthermo-piezophilic archaeon Methanopyrus kandleri strain 116 was examined [25]. In contrast to heterotrophic bacteria, carbon isotope fractionation in this autotroph decreases with growth pressure in cell biomass and methanogenesis (Figure 5). The reason for the reduced carbon isotope fractionation with pressure is not clear. The authors hypothesized that the decreased carbon isotope fractionation by hydrogeotrophic methanogen *M. kandleri* strain 116 is related to the increased H2 concentration in the liquid media resulting from the increased hydrostatic pressure [25]. If this is true, then carbon isotope fractionation by autotrophic piezophilic archaea (at least for *M. kandleri* strain 116) at elevated pressures is more an environment-dependent kinetic process rather than an organism-specific biological process. This is fundamentally different from the carbon isotope fractionation process observed in heterotrophic piezophilic bacteria [60]. However, at this time, definitive conclusions cannot be made as there are only two reports describing carbon isotope fractionation in piezophiles and these studies involve only one piezophilic bacterial species [60] and one archaeal species [25]. Nonetheless, studies involving lipid biosynthesis and carbon isotope fractionation by piezophiles under specific conditions might help to elucidate known and unknown biochemical pathways and metabolic features of these organisms.

**Box 2. Outstanding questions**

- What is the phylogenetic and physiological diversity of piezophilic bacteria and archaea in the deep sea? Are there functionally dominant piezophilic microbial communities in the deep sea?
- It is probable that the deep sea houses a large number of novel phylotypes of piezophiles with unique physiologies but low abundance. The question then is: what are the ecological roles of the high diversity, low abundance of microorganisms in global ocean biogeochemical cycles? Answers to these questions await further investigations. It appears that there are functionally dominant piezophilic bacterial populations in localized areas (e.g. [21]) but the potential ecological significance on the global scale is unknown [2].
- What new metabolic pathways are present in piezophiles? What are the physiological adaptations to the deep-sea low temperature, high pressure environment? How do the novel biosynthetic pathways and physiologies of piezophiles affect their biochemical signatures (e.g. lipid biosynthesis and carbon and hydrogen isotope fractionations) that are important in discerning their roles in global ocean biogeochemical cycles?
- Are there autotrophic piezophiles in the broad areas of the deep sea that are not associated with the energy-extensive areas (i.e. the cold seeps and hydrothermal vents)?

**Concluding remarks and future directions**

It is apparent how little is known regarding the microbial diversity and the geomicrobiological and biogeochemical potential of the piezophiles. Many fundamental questions about deep-sea piezophiles and the piezosphere remain unanswered (Box 2). Discoveries are continually being made about the microbial diversity, metabolic capability and biogeochemistry in general. Most of the currently known piezophiles are bacteria and only a few piezophilic archaea have been studied. Future studies should focus on characterizing piezophiles in areas not associated with cold seeps or hydrothermal vents. In these areas, there might be many phylotypes of relatively low abundance of piezophiles that play an important role in global ocean biogeochemical cycles. 'Low abundance' is misleading in that the piezosphere is so large that even these organisms play an important ecological and geochemical role in the ocean. The gene ecology approach [29] and intact phospholipid and isotope-based biogeochemical tools [32,33,41,60] will be useful in mapping the metabolic diversity, physiology, microbial biogeochemistry and environmental variability of both heterotrophic and autotrophic piezophiles in the piezosphere.

The inextricably interlinked deep-sea physical, biogeochemical and microbiological processes by which bioactive elements are cycled in the ocean could be one of the most dynamic systems on Earth. Our current understanding of the distribution and organization of piezophiles across spatial and temporal geochemical gradients in the deep sea remains sparse [2]. Comprehensive characterization of diversity, metabolic potential and ecophysiological functions of piezophiles in relation to geochemical gradients across different spatial and temporal scales is fundamentally important. We predict that by integrating culture-based and culture-independent biogeochemical, metagenomic and ecotype-level [29,30,46,62–64] techniques, new piezophilic species and novel metabolic capabilities will be discovered that will provide great insight into the ecological and biogeochemical roles of piezophiles in the deep-sea piezosphere. The opposite trends of carbon isotope fractionation observed in piezophilic bacteria [60] and archaea [25] are intriguing and warrant continued effort to characterize carbon isotope fractionation in lipid biosynthesis of piezophiles.

The deep sea is one of many dark energy environments [65] but is different than others (e.g. the deep subsurface) in that microorganisms in the deep sea continuously experience high hydrostatic pressure, as opposed to the lithostatic pressure in the subseafloor. Because of this, piezophiles probably possess unique physiologies and metabolism that deserve special assessment of their roles in global biogeochemical cycles. Finally, the study of piezophiles in the oceans has far-reaching significance in elucidating the origin and evolution of life and in finding life in the deeper Earth.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tol.2010.06.006.

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