5.1 Distribution of Piezophiles

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Numerous cold deep-sea adapted microorganisms (piezophilic, formerly referred to as "barophilic" bacteria) have been isolated using deep-sea research submersibles and/or several sediment/animal sampling systems. Many of the isolates from cold sea bottom are novel psychrophilic bacteria, and we have identified several new piezophilic species, that is, *Photobacterium profundum*, *Shewanella violacea*, *Moritella japonica*, *Moritella yayanosii*, *Psychromonas kaikoi*, and *Colwellia piezophila*. These piezophiles involve five genera in gamma-Proteobacteria subgroup and produce significant amounts of unsaturated fatty acids in their cell membrane fractions to maintain the membrane fluidity in cold and high-pressure environments. Piezophilic microorganisms have been identified in deep-sea bottoms of many of the world's oceans. Therefore, these microbes are well distributed on our planet. This chapter focuses on the distribution and taxonomy of the piezophilic microorganisms and their growth habitats.

Piezophiles are Adapted to the Deep-Sea Environment

It has been suggested that life may have originated in the deep sea some 3.5–4 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 could have been a very important stimulus for early forms of life. Scientists have proposed that life might have originated in deep-sea hydrothermal vents (Stetter 1993) and thus it appears possible that high-pressure-adapted mechanisms of gene expression could represent a feature present in early forms of life (Kato and Horikoshi 1996). It has recently been reported that the primary chemical reactions involved in the polymerization of organic materials (i.e., amino acids) could have occurred in such high-pressure and high-temperature environments (Imai et al. 1999). Thus, the study of deep-sea microorganisms may not only enhance our understanding of specific adaptations to abyssal and hadal ocean realms but may also provide valuable insights into the origin and evolution of life on our planet.

In 1949, Zobell and Johnson began investigating the effects of hydrostatic pressure on microbial activities (Zobell and Johnson 1949). They first used the term "barophilic" to define organisms whose optimal growth occurred at pressures higher than 0.1 MPa or a requirement for increased pressure for growth. The term "piezophilic" has been proposed to replace "barophilic," as the prefixes "baro" and "piezo," derived from Greek, mean "weight" and "pressure," respectively (Yayanos 1995). Thus, the word "piezophilic" is more suitable than "barophilic" to describe bacteria that grow better at high-pressure than at atmospheric pressure. Therefore, we have opted to use the term "piezophilic" bacteria, meaning high-pressure-loving bacteria. The definitions of "piezophilic" are shown in *Fig. 5.1.1*.

The Deepest Bottom, Mariana Trench Challenger Deep

The Mariana Trench, Challenger Deep (11°22′N. 142°25′E), is the deepest ocean bottom in the world. In 1996, the sediment samples have been obtained from this extreme environment by the unmanned submersible KAIKO (Kyo et al. 1995) in three dives, numbers 21, 22, and 23, at the depth of 11,000 m (*Fig. 5.1.2*, Kato et al. 1997). It seems likely that these were the first sediment samples recovered from the world's deepest point without any microbiological contamination. In 1960, Jacques Piccard and D. Walsh dived to the world's deepest point

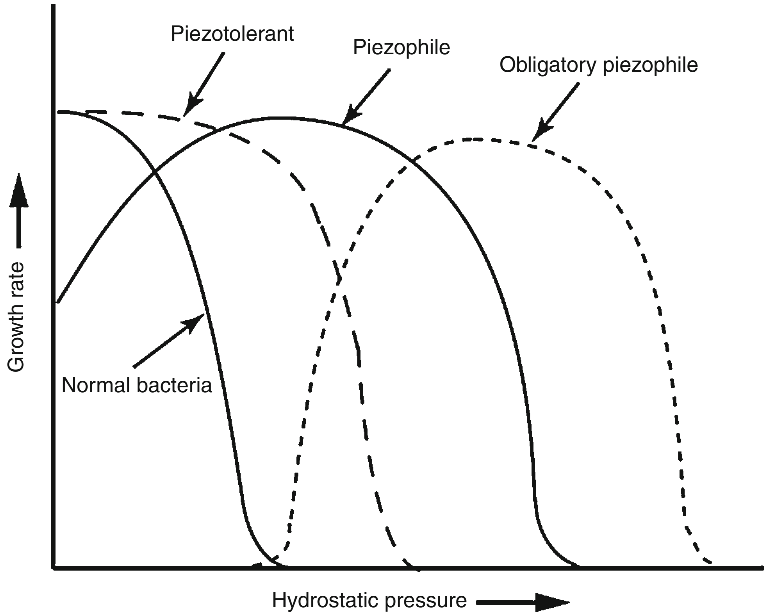

Fig. 5.1.1
Definition of piezophilic growth properties

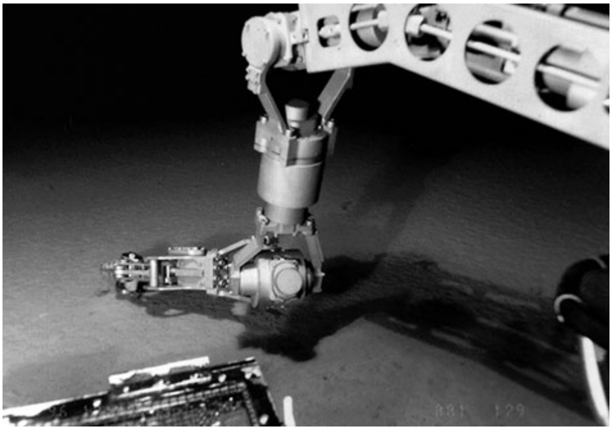

Fig. 5.1.2
Sediment sampling at the Mariana Trench Challenger Deep by means of the unmanned submersible, "KAIKO" system, Dive 10K#21 in 1996

aboard the manned submersible *TRIESTE*. They reported seeing several deep-sea animals and fishes there (Piccard and Dietz 1961). Unfortunately, that dive was not for scientific purposes and no film pictures were made or samples collected, so their story could not be confirmed scientifically.

The Mariana Trench is characterized by cold, dark, remoteness and the highest pressure environment in the ocean bottom. Relatively little is known about the natural bacterial communities in the sediment there because of the need to use uncommon instruments to keep deep-sea organisms at high-pressure and to develop new methods to maintain and cultivate these organisms in the laboratory. There are two isolates, *Pseudomonas bathycetes* and an obligatory barophilic bacterium strain MT41 that have been isolated from the Mariana Trench (Morita 1976; Yayanos et al. 1981). Apparently, *P. bathycetes* is not an organism adapted to the deep-sea environment, as its generation time is 33-days under such conditions (2°C, 100 MPa) (Schwarz and Colwell 1975). This bacterium grows at atmospheric pressure and at temperatures as high as 25°C (Pope et al. 1975). Bacterium strain MT41 was isolated from a deep-sea amphipod (*Hirondellea gigas*) obtained from the Mariana Trench using an insulated trap (Yayanos et al. 1981). This bacterium could grow only under pressure conditions greater than 50 MPa, and is closely related to the genus *Colwellia* (Yayanos et al. 1981; DeLong et al. 1997). The obligatory piezophiles, *M. yayanosii* DB21MT-5 and *Shewanella benthica* DB21MT-2 were isolated from the sediment of the Mariana Trench (Kato et al. 1998; Nogi and Kato 1999). In order to understand the piezophily and natural bacterial communities in the sediment of the Mariana Trench, several future studies have been progressing.

Distribution and Diversity of the Piezophiles

Most *Shewanella* spp. are isolated from ocean environments and some are psychrophilic or psychrotrophic bacteria. The piezophilic *Shewanella* species *S. benthica* and *S. violacea* are also categorized as psychrophilic at atmospheric pressure (Nogi et al. 1998b). *S. gelidimarina* and *S. frigidimarina* isolated from Antarctic ice (Bowmam et al. 1997) and *S. hanedai* isolated from the Arctic Ocean (Jensen et al. 1980) are cold-adapted psychrotrophic bacteria that grow well at low temperature. A phylogenetic tree of these *Shewanella* species within the gamma-Proteobacteria subgroup constructed based on 16S rRNA gene sequences is shown in *Fig. 5.1.3*.

In this tree, two major branches are recognizable in the genus *Shewanella*, indicated by *Shewanella* group 1 and *Shewanella* group 2. Deep-sea *Shewanella* forming the *Shewanella* piezophilic branch are categorized as members of group 1. Interestingly, most *Shewanella* species shown to be psychrophilic or psychrotrophic also belong to group 1. The other species in group 1, *S. pealeana* and *S. woodyi*, isolated from ocean squid and detritus, respectively, grow optimally at 25°C (Leonardo et al. 1999; Makemson et al. 1997) and thus these strains might also be included in the group of cold-adapted bacteria. Most *Shewanella* species in group 2 are not cold-adapted bacteria. They grow well under mesophilic conditions at 25–35°C. *S. frigidimarina*, which can grow optimally below 25°C, is the only exception in this category, although this species belongs to group 2 (Kato and Nogi 2001).

The growth of some of these *Shewanella* species under high-pressure conditions indicates that the members of *Shewanella* group 1 show piezophilic (*S. benthica* and *S. violacea*) or piezotolerant (*S. gelidimarina* and *S. hanedai*) growth properties, although the members of *Shewanella* group 2 generally show piezosensitive growth, that is, no growth at a pressure of 50 MPa (Kato and Nogi 2001). Only a limited number of experiments have been performed examining the growth of these bacteria under high-pressure conditions, but generally members of *Shewanella* group 1 are characterized as cold adapted and pressure tolerant, whereas the

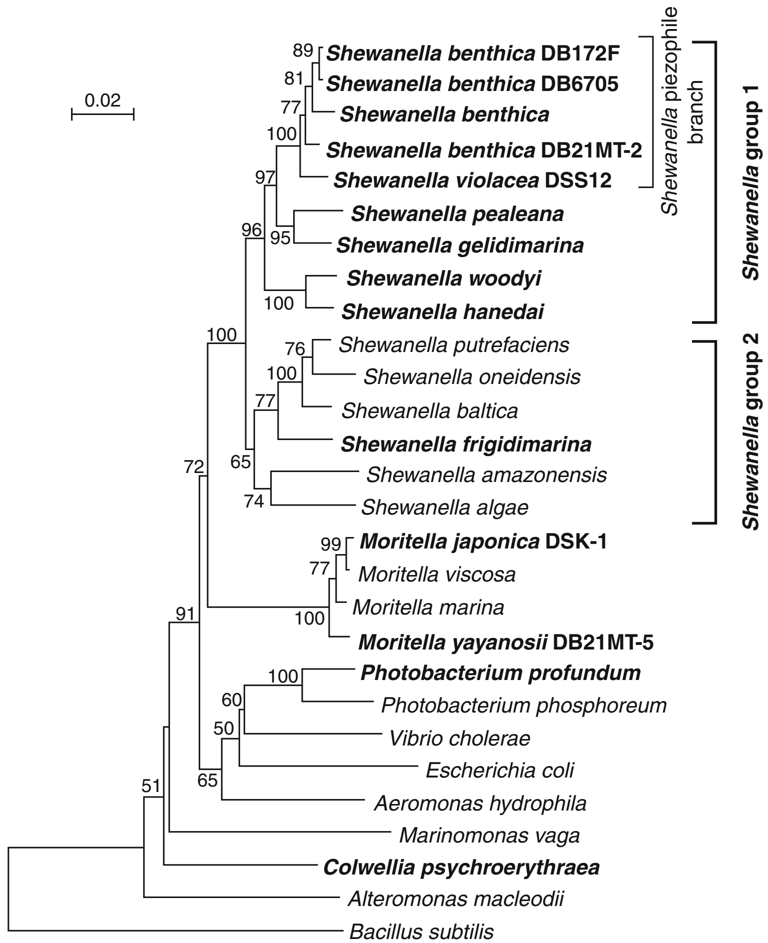

Fig. 5.1.3

Phylogenetic tree showing the relationships of the *Shewanella* species within the gamma-Proteobacteria subgroup constructed based on 16S rRNA gene sequences with the neighbor-joining method. The scale represents the average number of nucleotide substitutions per site. Bootstrap values (%) were calculated from 1,000 trees. Psychrophilic and/or piezophilic bacteria are shown in *bold*

members of *Shewanella* group 2 are mostly mesophilic and pressure sensitive. Some *Shewanella* species are known to produce polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA). It is clear that the members of *Shewanella* group 1 produce substantial amounts of EPA (11–16% of total fatty acids), whereas members of *Shewanella* group 2 produce no EPA or only limited amounts. In terms of other fatty acids, the membrane lipid profiles of members of the genus *Shewanella* are basically similar. This observation also supports the view described above (Kato and Nogi 2001).

On the basis of the properties of *Shewanella* species, we would like to propose that two major branches of the genus *Shewanella* should be recognized taxonomically, *Shewanella* group 1 and group 2 (*Fig. 5.1.3*). The two subgenus branches of *Shewanella* would be as follows: *Shewanella* group 1 is characterized as a group of high-pressure, cold-adapted species that produce substantial amounts of EPA and *Shewanella* group 2 is characterized as a group of mostly mesophilic and pressure-sensitive species.

The deep-sea bottom and other cold-temperature environments are probably similar in terms of microbial diversity. Members of *Shewanella* group 1 live in such environments, and most of them show piezophilic or piezotolerant growth properties. In this regard, it is interesting to consider the influence of the ocean circulation as deep ocean water is derived from polar ice (in the Arctic and/or Antarctic regions) that sinks to the deep-sea bottom (Schmitz 1995), probably along with microbes. It was reported that *Psychrobacter pacificensis* isolated from seawater of the Japan Trench at a depth of 5,000–6,000 m was taxonomically similar to the Antarctic isolates *P. immobilis*, *P. gracincola*, and *P. fridigicola* (Maruyama et al. 2000). The occurrence of *Psychrobacter* in cold seawater deep in the Japan Trench and at the surface of the Antarctic sea suggests that bacterial habitation of the deep-sea and their evolution have been influenced by the global deep ocean circulation linked to the sinking of cooled seawater in polar regions. Thus, it is possible that the ocean circulation may be one of the major factors influencing microbial diversity on our planet.

Taxonomy of the Piezophiles

Bacteria living in the deep-sea have several unusual features, which allow them to thrive in their extreme environments. We have isolated and characterized several piezophilic and piezotolerant bacteria from cold deep-sea sediments at depths ranging from 2,500 to 11,000 m using sterilized sediment samplers by means of the submersibles *SHINKAI 6500* and *KAIKO*, systems (Kato 1999; Kato et al. 1995, 2004). Most isolated strains are not only piezophilic but also psychrophilic and cannot be cultured at temperatures higher than 20°C.

The isolated deep-sea piezophilic bacterial strains have been characterized in an effort to understand the interaction between the deep-sea environment and its microbial inhabitants (Kato et al. 1998; Margesin and Nogi 2004; Yayanos et al. 1979). Thus far, all piezophilic bacterial isolates fall into the gamma subgroup of the Proteobacteria according to phylogenetic classifications based on 5S and 16S ribosomal RNA gene sequence information (DeLong et al. 1997; Kato 1999; Margesin and Nogi 2004). The only deep-sea piezophilic bacterial species of these genera were named to be *S. benthica* (Deming et al. 1984; MacDonell and Colwell 1985) in the genus *Shewanella*, and *Colwellia hadaliensis* (Deming et al. 1988) in the genus *Colwellia*, prior to the reports by the JAMSTEC group. We have identified several novel piezophilic species within these genera based on the results of chromosomal DNA–DNA hybridization studies and several other taxonomic properties. Both previously described and novel species of bacteria have been identified among the piezophilic bacterial isolates. Based upon these studies we have indicated that cultivated psychrophilic and piezophilic deep-sea bacteria could be affiliated with one of the five genera within the gamma-Proteobacteria subgroup: *Shewanella*, *Photobacterium*, *Colwellia*, *Moritella*, and *Psychromonas*. *Figure 5.1.4* shows the phylogenetic relations between the taxonomically identified piezophilic species (shown in bold) and other bacteria within the gamma-Proteobacteria subgroup. The taxonomic features of the piezophilic genera were determined as described below.

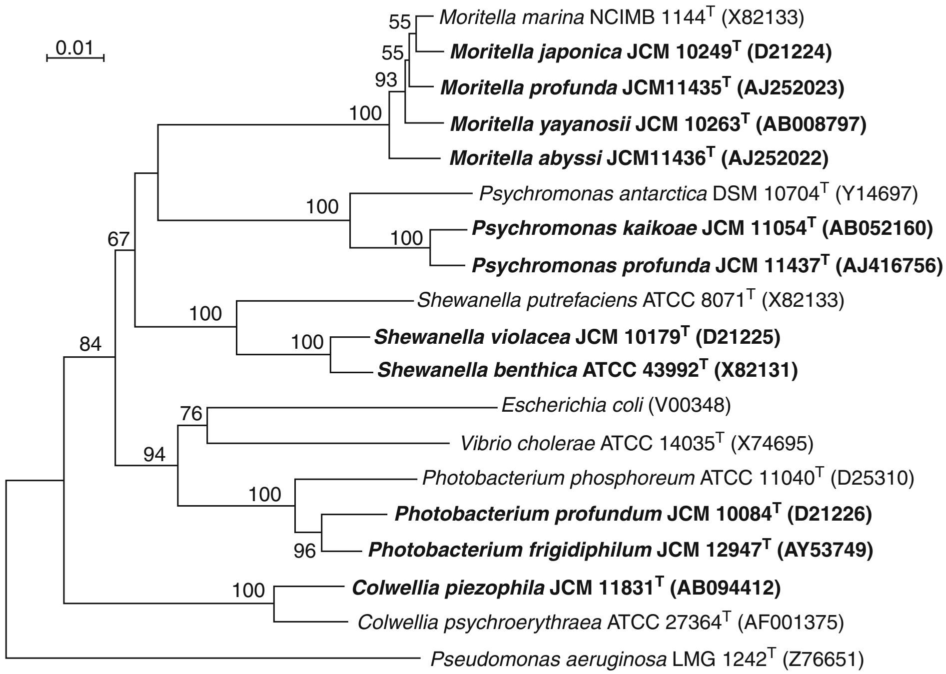

Fig. 5.1.4

Phylogenetic tree showing the relationships between isolated deep-sea piezophilic bacteria (in *bold*) within the gamma-Proteobacteria subgroup determined by comparing 16S rRNA gene sequences using the neighbor-joining method (references for species description are indicated in the text). The scale represents the average number of nucleotide substitutions per site. Bootstrap values (%) are shown for frequencies above the threshold of 50%

The Genus *Shewnella*

Members of the genus *Shewanella* are not unique to marine environments of Gram-negative, aerobic and facultatively anaerobic gamma-Proteobacteria (MacDonell and Colwell 1985). The type strain of this genus is *S. putrefaciens*, which is a bacterium formerly known as *Pseudomonas putrefaciens* (MacDonell and Colwell 1985; Owen et al. 1978). Recently, however, several novel marine *Shewanella* species have been isolated and described. These isolates are not piezophilic species and thus prior to the present report *S. benthica* and *S. violacea* were the only known members of the genus *Shewanella* showing piezophilic growth properties (Nogi et al. 1998b). *Shewanella* piezophilic strains, PT-99, DB5501, DB6101, DB6705, and DB6906, DB172F, DB172R, and DB21MT-2 were all identified as members of the same species, *S. benthica* (Nogi et al. 1998b; Kato and Nogi 2001). The psychrophilic and piezophilic *Shewanella* strains, including *S. violacea* and *S. benthica*, produce eicosapentaenoic acid (EPA) and thus the production of such long-chain polyunsaturated fatty acid (PUFA) is a property shared by many deep-sea bacteria to maintain the cell-membrane fluidity under conditions of extreme cold and high hydrostatic pressure environments (Fang et al. 2003).

S. violacea strain DSS12 has been studied extensively, particularly with respect to its molecular mechanisms of adaptation to high-pressure (Kato et al. 2000; Nakasone et al. 1998, 2002). This strain is moderately piezophilic, with a fairly constant doubling time at pressures between 0.1 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. Therefore, the genome analysis on strain DSS12 has been performed as a model deep-sea piezophilic bacterium (Aono et al. 2010).

The Genus *Photobacterium*

The genus *Photobacterium* was one of the earliest known bacterial taxa and was first proposed by Beijerinck in 1889 (Beijerinck 1889). Phylogenetic analysis based on 16S rRNA gene sequences has shown that the genus *Photobacterium* falls within the gamma-Proteobacteria and, in particular, is closely related to the genus *Vibrio* (Nogi et al. 1998c). *Photobacterium profundum*, a novel species, was identified through studies of the moderately piezophilic strains DSJ4 and SS9 (Nogi et al. 1998c). *P. profundum* strain SS9 has been extensively studied with regard to the molecular mechanisms of pressure regulation (Bartlett 1999) and subsequently genome sequencing and expression analysis (Vezzi et al. 2005). Recently, *P. frigidiphilum* was reported to be slightly piezophily: its optimal pressure for growth is 10 MPa (Seo et al. 2005). Thus, *P. profundum* and *P. frigidiphilum* are the only species within the genus *Photobacterium* known to display piezophily and the only two known to produce the long-chain polyunsaturated fatty acid (PUFA), eicosapentaenoic acid (EPA). No other known species of *Photobacterium* produces EPA (Nogi et al. 1998c).

The Genus *Colwellia*

Species of the genus *Colwellia* are defined as facultative anaerobic and psychrophilic bacteria (Deming et al. 1988), which belong to the gamma-Proteobacteria. In the genus *Colwellia*, the only deep-sea piezophilic species reported was *C. hadaliensis* strain BNL-1 (Deming et al. 1988), although no public culture collections maintain this species and/or its 16S rRNA gene sequence information. Bowman et al. (1998) reported that *Colwellia* species produce the long-chain PUFA, docosahexaenoic acid (DHA). We have recently isolated the obligately piezophilic strain Y223G^{T} from the sediment at the bottom of the deep-sea fissure of the Japan Trench, which was identified as *C. piezophila* (Nogi et al. 2004). Regarding fatty acids, this strain did not produce EPA or DHA in the membrane layer, whereas high levels of unsaturated fatty acids (16:1 fatty acids) were produced. This observation suggested that the possession of long-chain PUFA should not be a requirement for classification as a piezophilic bacterium; however, the production of unsaturated fatty acids could be a common property of piezophiles.

The Genus *Moritella*

The type strain of the genus *Moritella* is *M. marina,* previously known as *Vibrio marinus* (Colwell and Morita 1964), which is one of the most common psychrophilic organisms isolated from marine environments. However, *V. marinus* has been reclassified as *M. marina* gen. nov. comb. nov. (Urakawa et al. 1998). *M. marina* is closely related to the genus *Shewanella* on the basis of 16S rRNA gene sequence data and is not a piezophilic bacterium. Strain DSK1, a moderately piezophilic bacterium isolated from the Japan Trench, was identified as *M. japonica* (Nogi et al. 1998a). This was the first piezophilic species identified in the genus *Moritella*. Production of the long-chain PUFA, DHA, is a characteristic property of the genus *Moritella*. The extremely piezophilic bacterial strain DB21MT-5 isolated from the world's deepest sea bottom, the Mariana Trench Challenger Deep at a depth of 10,898 m, was also identified as a *Moritella* species and designated *M. yayanosii* (Nogi and Kato 1999). 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 pressures as high as 100 MPa (Kato et al. 1998). The fatty acid composition of piezophilic strains changes as a function of pressure and, in general, greater amounts of PUFAs are synthesized at higher growth pressures. Approximately 70% of the membrane lipids in *M. yayanosii* are unsaturated fatty acids, which is a finding consistent with its adaptation to very high pressures (Nogi and Kato 1999; Fang et al. 2000). Two other species of the genus *Moritella*, *M. abyssi* and *M. profunda*, were isolated from a depth of 2,815 m off the West African coast (Xu et al. 2003a); they are moderately piezophilic and the growth properties are similar to *M. japonica*.

The Genus *Psychromonas*

The genus *Psychromonas* described psychrophilic bacterium, which also belongs to the gamma-Proteobacteria, is closely related to the genera *Shewanella* and *Moritella* on the basis of 16S rRNA gene sequence data. The type species of the genus *Psychromonas*, *P. antarctica*, was isolated as an aerotolerant anaerobic bacterium from a high-salinity pond on the McMurdo ice-shelf in Antarctica (Mountfort et al. 1998). This strain did not display piezophilic properties. *P. kaikoae*, isolated from sediment collected from the deepest cold-seep environment with chemosynthesis-based animal communities within the Japan Trench at a depth of 7,434 m, is a novel obligatory piezophilic bacterium (Nogi et al. 2002). The optimal temperature and pressure for growth of *P. kaikoae* are 10°C and 50 MPa, respectively, and both PUFAs, EPA and DHA, are produced in the membrane layer. *P. antarctica* does not produce either EPA or DHA in its membrane layer. DeLong and coworkers stated that strain CNPT-3 belonged to an unidentified genus of piezophiles (DeLong et al. 1997) and this strain proved to be closely related to *P. kaikoae*. Thus, the genus *Psychromonas* is the fifth genus reported to contain piezophilic species within the gamma-Proteobacteria. In addition, *P. profunda* is a moderately piezophilic bacterium isolated from deep Atlantic sediments at a depth of 2,770 m (Xu et al. 2003b). This strain is similar to the piezosensitive strain *P. marina*, which also produces small amounts of DHA. Only *P. kaikoae* produces both EPA and DHA in the genus *Psychromonas*.

Fatty Acid Composition of the Piezophiles

The piezophilic and psychrophilic *Shewanella* and *Photobacterium* strains produce EPA (Nogi et al. 1998b, c), *Moritella* strains produce DHA (Nogi et al. 1998a; Nogi and Kato 1999), and *P. kaikoae* produces both EPA and DHA (Nogi et al. 2002) but *C. piezophila* does not produce such PUFAs (Nogi et al. 2004). The fatty acid composition of piezophilic strains changes as a function of pressure, and in general greater amounts of PUFAs are synthesized under higher-pressure conditions for their growth (DeLong and Yayanos 1985, 1986). Psychrophilic and piezophilic bacteria were believed to produce one of the long-chain PUFAs, either EPA or DHA, but this does not appear to be obligatory. For example, Allen et al. (1999) reported that monounsaturated fatty acids, but not PUFAs, are required for the growth of the piezophilic bacterium *P. profundum* SS9 based on the analysis of pressure-sensitive mutants. In their mutant experiment, 18:1 fatty acids proved to be necessary for growth under low-temperature and/or high-pressure conditions. In the case of *C. piezophila*, this specie had no 18:1 fatty acids but produced a large amount of 16:1 fatty acids in its cell membrane layer. All piezophilic and psychrophilic bacteria analyzed so far have 16:1 fatty acids (*Table 5.1.1*); thus, this fatty acid would appear to be one of the important components required for high-pressure growth.

Table 5.1.1

Whole-cell fatty acid composition (%) of the piezophilic isolates (type strains)

Fatty acid	Sh	Ph	Co	Mo	Ps
12:0	2	2	1		1
14:0	13	3	3	15	6
15:0		1	3	1	1
16:0	14	9	31	13	15
17:0					
18:0		1			
iso-13:0	5	2			
iso-14:0		4			
iso-15:0	11	2			
iso-16:0		15			
14:1		3	9	6	10
15:1			2		
16:1	31	31	50	53	55
17:1					
18:1	2	9		1	2
【EPA】20:5	16	13			2
【DHA】22:6				11	2
3OH-12:0	1	5	1		2
3OH-iso-13:0	5				
3OH-14:0					4
Unsaturated (%)	49	56	61	71	71
Saturated (%)	51	44	39	29	29
Ratio (U/S)	0.96	1.27	1.56	2.45	2.45

Sh Shewanella benthica ATCC 43992^T, *Ph Photobacterium profundum* JCM 10084^T, *Co Colwellia piezophila* Y223G^T, *Mo Moritella yayanosii* JCM 10263^T, *Ps Psychromonas kaikoae* JCM 11054^T.

The fatty acid compositions of those piezophilic strains are distinct depending on their genus and commonly high amounts of unsaturated fatty acids (49–71%) are involved in their membrane layer as shown in *Table 5.1.1*.

Conclusion

Cultured deep-sea piezophilic and psychrophilic bacteria are affiliated with one of the five genera within the gamma-Proteobacteria subgroup: *Shewanella, Photobacterium, Colwellia, Moritella,* and *Psychromonas.* These piezophiles are characterized to contain unsaturated fatty acids in their cell membrane layers but PUFAs, like EPA and DHA, are not obligatory necessary for the high-pressure growth. The diversity of the piezophilic bacteria are closely related with the global deep-sea ocean circulation. These observations indicate that the piezophilic bacteria could be adapted to any of deep-sea cold and high-pressure environments.

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Cross-References

5.2 High Pressure and Prokaryotes
5.5 Contributions of Large-Scale DNA Sequencing Efforts to the Understanding of Low-Temperature Piezophiles
5.6 Cultivation Methods for Piezophiles
6.2 Taxonomy

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