Chapter 7
Microbial Life in the Deep Sea: Psychropiezophiles

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Abstract Psychropiezophiles are microorganisms specialized for living in the deep-sea environment. Piezophiles display maximum growth at high pressure. Some can also grow at atmospheric pressure; those that cannot are referred to as obligatory piezophiles. A temperature change affects deep-sea psychropiezophiles more than a pressure change. Therefore, sample collection and cultivation temperature should be kept low. The preferred method for the long-term preservation and storage of psychropiezophiles is freezing in the vapor phase of liquid nitrogen. Initially, cultures of deep-sea psychropiezophilic bacteria were only species affiliated with one of five genera within the *Gammaproteobacteria* subgroup: *Shewanella*, *Photobacterium*, *Colwellia*, *Moritella*, and *Psychromonas*. However, more recently, species classified as *Alphaproteobacteria* and *Firmicutes* have also been found. The genome of several of these bacteria has been analyzed, which revealed characteristic features of these microorganisms. Psychropiezophiles contain unsaturated fatty acids in their cell membrane layers, but the presence of polyunsaturated fatty acids, like eicosapentaenoic acid and docosahexaenoic acid, is not obligatory for growth under high pressure. In the future, along with the development of culture methods and isolation techniques, a variety of other psychropiezophilic species will be discovered and the relationship between pressure and growth of psychropiezophiles will be clarified.

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R. Margesin (ed.), *Psychrophiles: From Biodiversity to Biotechnology*,
DOI 10.1007/978-3-319-57057-0_7

7.1 Introduction

Despite the fact that up to 60% of the Earth's surface is covered by seas with depths exceeding 1000 m, the study of microorganisms in the deep sea is very incomplete. The deep sea is regarded as an extreme environment with high hydrostatic pressures (because pressure in the ocean increases by about 0.1 megapascal [MPa, 1 Pa =1 kg/(m·s^2) = 9.87 × 10^{-6} atm] for each 10 meters in depth up to 110 MPa), predominantly low temperatures (2–4 °C) (Fig. 7.1) but with occasional regions of extremely high temperatures (up to 370 °C) at hydrothermal vents, darkness, and low nutrient availability, although with sufficient dissolved oxygen (Fig. 7.1).

Until the exploratory voyage of the *Challenger* in the late nineteenth century, the deep sea was considered devoid of life. In the early twentieth century, only inconsequential organisms were considered to inhabit the deep sea. In 1949, research started on the effects of hydrostatic pressure on microbial activities (ZoBell and Johnson 1949). The word "barophilic" was first used by ZoBell and Johnson (1949), and it is defined today as optimal growth at pressure greater than 0.1 MPa or by a requirement for increased pressure for growth. The term "piezophile" was proposed as a replacement for "barophile" as the Greek translations of the prefixes "baro" and "piezo" mean "weight" and "pressure," respectively (Yayanos 1995). Thus, the word piezophile is more suitable than barophile to describe bacteria that grow better at high pressure than at atmospheric pressure. Therefore, researchers have opted to use the term "piezophilic bacteria" meaning high-pressure-loving bacteria. The growth patterns of piezotolerant and piezophilic bacteria are shown in Fig. 7.2. Piezophiles display maximum growth at high pressure. Some can also grow at atmospheric pressure; those that cannot are referred to as obligatory piezophiles. Piezotolerant bacteria grow best at atmospheric pressure but can sustain growth at high pressure.

7.2 Deep-Sea Psychropiezophiles

Bacteria living in the deep sea have several unusual characteristics that allow them to grow in their extreme environment. In 1979, the first pure culture isolate of a piezophilic bacterium was reported (Yayanos et al. 1979). The spirillum-like bacterial strain CNPT-3 had a rapid doubling rate at 50 MPa but did not grow at atmospheric pressure. However, no public culture collections are maintained and no

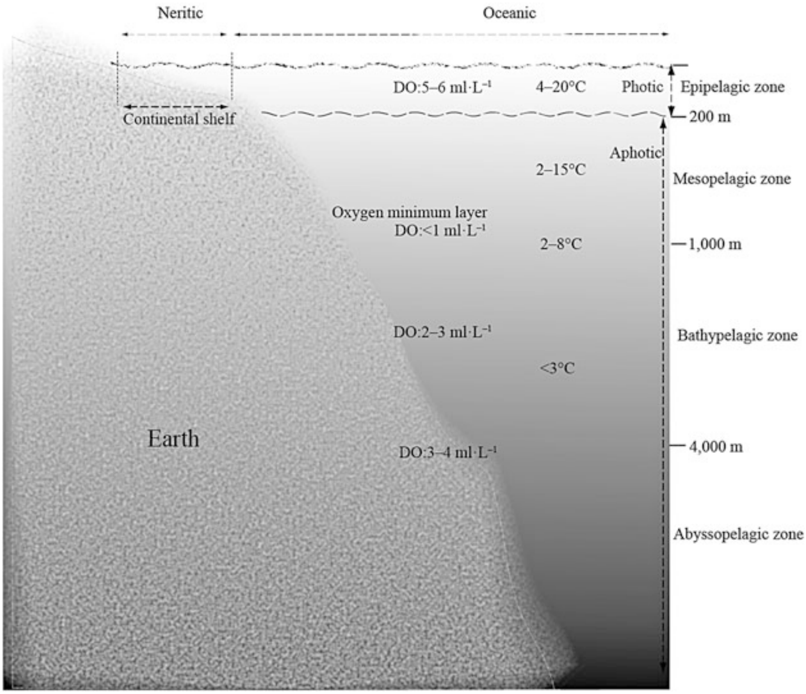

Fig. 7.1 Layers of the ocean. The oceans are divided into two broad realms, the pelagic and the benthic. These layers, known as "zones," extend from the surface to the most extreme depths where light can no longer penetrate. Biologists divide the pelagic zone into the epipelagic (less than 200 m, where photosynthesis can occur), the mesopelagic (200–1000 m, the "twilight" zone with faint sunlight but no photosynthesis), the bathypelagic (1000–4000 m), the abyssopelagic (4000–6000 m), and the deepest zone, the hadopelagic (trenches more than 6000 m deep). Thermoclines vary in thickness from a few hundred meters to nearly 1000 m. The temperature of the epipelagic zone in the tropics is usually higher than 20 °C. From the base of the epipelagic, the temperature drops to 2–8 °C at 1000 m. It continues to decrease to the bottom, but at a much slower rate. Below 1000 m, the water is isothermal between 2 °C and 4 °C. The cold water stems from the sinking of heavy surface water in the polar regions. *DO*, dissolved oxygen

name has been added to strain CNPT-3. The first psychropiezophile to be named was *Shewanella benthica* (Table 7.1). *S. benthica* strain W I45 was isolated from the holothurian at a depth of 4575 m in the South Atlantic Ocean (Deming et al. 1984). Thereafter, we isolated and characterized numerous piezophilic and piezotolerant bacteria from cold deep-sea sediments at depths ranging from 2500 m to 11,000 m using sterilized sediment samplers (Fig. 7.3) on the submersibles SHINKAI 6500 and KAIKO systems operated by the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) (Kato et al. 1995; Nogi and Kato 1999; Nogi et al. 2007). Groups at the Scripps Research Institute collected deep-sea organisms such as amphipods using traps and isolated psychropiezophiles among them (Lauro et al. 2007; Cao et al. 2014). Most isolated strains are not only

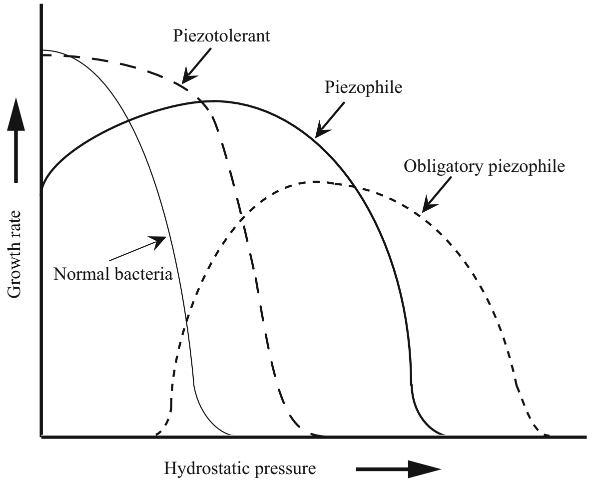

Fig. 7.2 Definition of piezophilic growth properties. This figure is a conceptual chart, and the *x*-axis value does not indicate specific pressure. Piezophiles display maximum growth at high pressure. They may or may not grow at atmospheric pressure, and in the latter case are obligatory piezophiles. Piezotolerant bacteria grow optimally at atmospheric pressure, but can sustain growth at high pressure (about 30–50 MPa), whereas normal bacteria (piezosensitive) stop growing at about 30–50 MPa

piezophilic but also psychrophilic (psychropiezophilic) and cannot be cultured at temperatures higher than 20 °C.

7.3 Isolation and Preservation of Deep-Sea Psychropiezophiles

The isolation of deep-sea psychropiezophiles requires the maintenance of samples at low temperature (0–4 °C). Furthermore, the culture medium used for isolation must also be cooled in advance. In the polar zone where the temperature of surface seawater is low, deep-sea samples can be collected relatively easily at the optimum temperature. However, in higher surface seawater temperature areas, special samplers are required. The cell walls of bacteria allow easy entry and exit of water, which do not destroy the cell if an instantaneous pressure change does not occur. A temperature change affects deep-sea psychropiezophiles more than a pressure change. JAMSTEC researchers use the sampler shown in Fig. 7.3 to collect deep-sea microbes (Ikemoto and Kyo 1993; Kato et al. 1995), which ensures only a minimal change in the sample temperature. It is possible to culture many psychropiezophiles other than obligatory piezophiles on agar plates as well as in a liquid medium under atmospheric pressure.

Table 7.1 Growth properties and genetic information of cultivated psychropiezophilic bacteria

Strain	Culture collection no.	Opt. temp. (°C)	Opt. pressure (MPa)	Isolation source (depth [m])	16S rRNA Genbank accession no.	Complete genome	Reference
Gammaproteobacteria							
Colwelliaceae							
Colwellia hadaliensis BNL-1^T		10	90	Puerto Rico Trench (7410)			Deming et al. (1988)
Colwellia piezophila Y223G^T	JCM 11831^T	10	60	Japan Trench, sediment (6278)	NR 024805	NZ KB905161	Nogi et al. (2004)
Colwellia sp. strain MT41		8	103	Mariana Trench, decaying amphipod (10,476)	DQ027051	NZ CP013145	Yayanos et al. (1981)
Moritellaceae							
Moritella abyssi 2693^T	JCM 11436^T	10	30	Atlantic Ocean, sediment (2815)	AJ252022		Xu et al. (2003b)
Moritella japonica DSK1^T	JCM 10249^T	15	50	Japan Trench, sediment (6356)	D21224		Nogi et al. (1998a)
Moritella profunda 2674^T	JCM 11435^T	6	30	Atlantic Ocean, sediment (2815)	AJ252023		Xu et al. (2003b)
Moritella yayanosii DB21MT-5^T	JCM 10263^T	10	80	Mariana Trench, sediment (10,898)	AB008797		Nogi and Kato (1999)
Moritella sp. strain PE36	ATCC BAA-1251	10	41	North Pacific Ocean, amphipod trap water (3584)	DQ027053		Yayanos (1986)
Vibrionaceae							
Photobacterium profundum DSJ4^T	JCM 10084^T	10	10	Ryukyu Trench, sediment (5110)	D21226		Nogi et al. (1998c)
Photobacterium profundum SS9	ATCC BAA-2589	15	28	Sulu Trough, amphipod homogenate (2551)	AB003191	NC 006370.1	DeLong et al. (1997)
Oceanospirillaceae							
"*Profundimonas piezophila*" YC-1^T	ATCC BAA-2591	8	50	Puerto Rico Trench, water column (6000)	HQ230045		Cao et al. (2014)

(continued)

Table 7.1 (continued)

Strain	Culture collection no.	Opt. temp. (°C)	Opt. pressure (MPa)	Isolation source (depth [m])	16S rRNA Genbank accession no.	Complete genome	Reference
Psychromonadaceae							
Psychromonas hadalis K41G^T	JCM 11830^T	6	60	Japan Trench, sediment (7542)	AB094413	NZ_ATUO01000000	Nogi et al. (2007)
Psychromonas kaikoae JT7304^T	JCM 11054^T	10	50	Japan Trench, cold-seep sediment (7434)	AB052160		Nogi et al. (2002)
Psychromonas profunda 2825^T	JCM 11437^T	10	25	Atlantic Ocean sediment (2770)	AJ416756		Xu et al. (2003a)
Psychromonas sp. strain CNPT3		12	52	Central North Pacific, decaying amphipod (5800)	DQ027056	NC 020802.1	Yayanos et al. (1979)
Shewanellaceae							
Shewanella benthica W145^T	DSM 8812^T	10–15	50	Intestine, holothurian, South Atlantic Ocean (4575)	X82131		Deming et al. (1984)
Shewanella benthica KT99	ATCC BAA-2590	~2	~98	Kermadec Trench, amphipod homogenate (9856)	DQ027058	NZ_ABIC01000000	Lauro et al. (2007)
Shewanella piezotolerans WP3^T	JCM 13877^T	15–20	20	West Pacific, deep-sea sediment (1914)	AJ551090	NC 011566.1	Xiao et al. (2007)
Shewanella psychrophila WP2^T	JCM 13876^T	10–15	20	West Pacific, deep-sea sediment (1914)	AJ551089		Xiao et al. (2007)
Shewanella violacea DSS12^T	JCM 10179^T	10	30	Ryukyu Trench, sediment (5110)	D21225	NC 014012.1	Nogi et al. (1998b)
Alphaproteobacteria							
Rhodobacterales bacterium PRT1		10	80	Puerto Rico Trench, seawater (8350)	JF303756		Eloe et al. (2011)
Firmicutes							
Carnobacterium sp. AT7		20	20	Aleutian Trench, water column (2500)	DQ027061	NZ_ABHH00000000.1	Lauro et al. (2007)

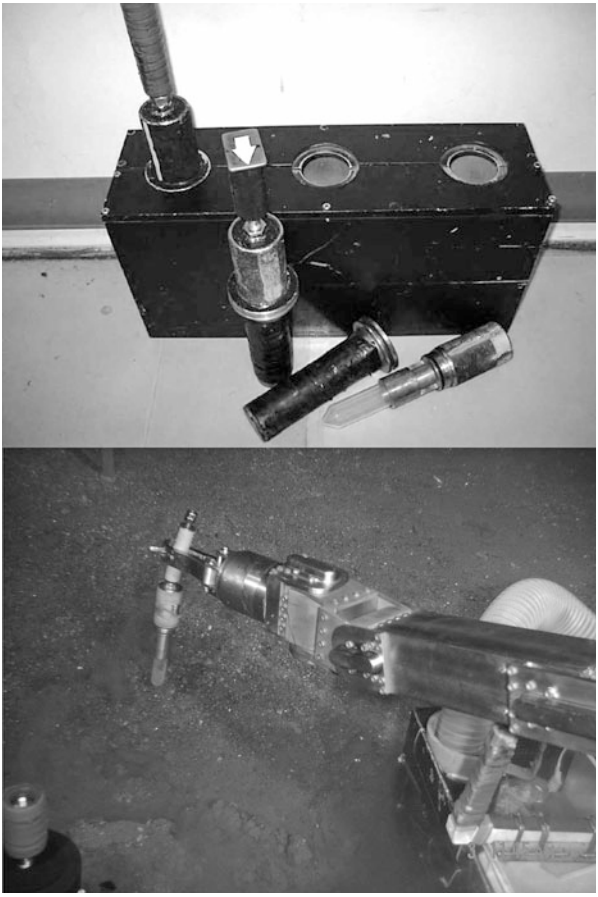

Fig. 7.3 Sterilized sediment sampler (*upper photograph*) and sediment sampling (*lower photograph*)

The preferred method for the long-term preservation and storage of psychropiezophiles is freezing in the vapor phase of liquid nitrogen (–130 °C and lower). Long survival of more than 20 years and good recovery rates can be achieved with this method. Cultures to be stored in liquid nitrogen are usually grown to the late-log growth phase and mixed with a cryopreservative (10% glycerol or 5% DMSO). Sample cultures can also be preserved at –70 to –80 °C. However, since the survival and recovery rates decrease with this method, it is necessary to use a preservative for dense suspensions of cells. Since almost all psychropiezophilic strains die after freeze-drying, this method cannot be used.

7.4 Taxonomy of the Psychropiezophiles

Numerous deep-sea piezophilic bacterial strains have been isolated and characterized in an effort to understand the interaction between the deep-sea environment and its microbial inhabitants (Yayanos et al. 1979; Kato et al. 1998; Margesin and Nogi 2004; Lauro et al. 2007; Eloe et al. 2011). Approximately 30 years after psychropiezophiles were first isolated from the deep sea, they were assigned to the gamma-subgroup of the *Proteobacteria* according to phylogenetic classifications based on 5S and 16S ribosomal RNA (rRNA) sequence information (DeLong et al. 1997; Kato 1999; Margesin and Nogi 2004; Nogi et al. 2007). Prior to the reports by the JAMSTEC group, only two deep-sea piezophilic bacterial species had been described; they were named *Shewanella benthica* (Deming et al. 1984; MacDonell and Colwell 1985) and *Colwellia hadaliensis* (Deming et al. 1988). We 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 were identified among the piezophilic bacterial isolates. Nogi et al. (2002) reported that cultivated psychropiezophilic deep-sea bacteria are represented by the genera *Colwellia*, *Moritella*, *Psychromonas*, and *Shewanella* within the *Alteromonadaceae* family of the *Gammaproteobacteria*, and that the genus *Photobacterium* is assigned to the *Vibrionaceae* family within the *Gammaproteobacteria* (Table 7.1). Subsequently, Lauro et al. (2007) reported that a psychropiezophile isolated from seawater of the Aleutian Trench was classified as the genus *Carnobacterium* of *Firmicutes* based on 16S rRNA analysis. In addition, Eloe et al. (2011) isolated a psychropiezophile from seawater of the Puerto Rico Trench which was classified from 16S rRNA analysis as a clade of the *Rhodobacterales* of the *Alphaproteobacteria* subgroup (Fig. 7.4). However, the complete identification of these two psychropiezophiles was not performed, and they were not deposited in a culture collection. More recently, Cao et al. (2014) isolated psychropiezophiles from seawater of the Puerto Rico Trench which were classified from 16S rRNA analysis as the genus *Profundimonas* in the *Oceanospirillales* clade of the *Gammaproteobacteria* subgroup (Table 7.1). In the future, it is expected that a wide variety of other psychropiezophilic genera will be isolated and identified.

7.4.1 The Genus Colwellia

Species of the genus *Colwellia* are defined as facultatively anaerobic and psychrophilic bacteria, and the type species of this genus is *Colwellia psychroerythrus* (Deming et al. 1988). This genus belongs to the *Gammaproteobacteria*. At the time of writing, the genus comprised 15 species with validly published names. *Colwellia hadaliensis*, *Colwellia piezophila*, and *Colwellia* sp. strain MT41 are the only known members of the genus *Colwellia* showing psychropiezophilic growth

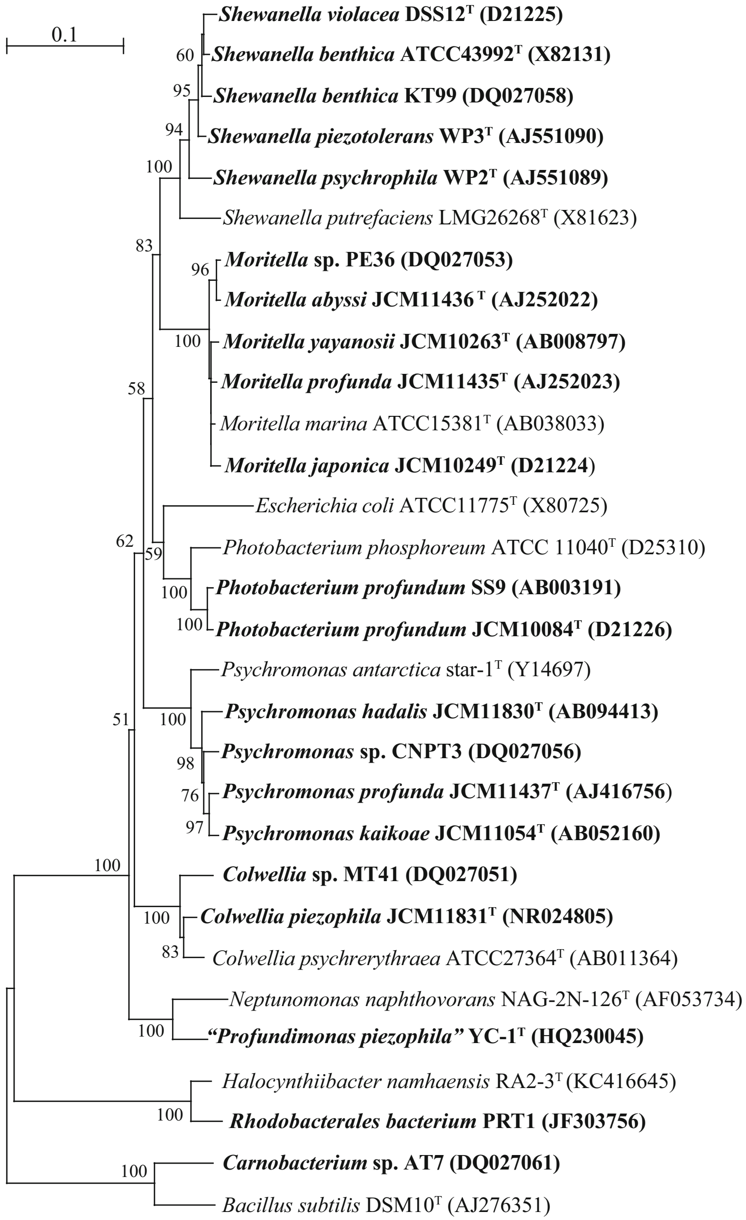

Fig. 7.4 Phylogenetic tree showing the relationships between isolated deep-sea piezophilic bacteria (in *bold*) determined by comparing 16S rDNA sequences using the neighbor-joining method (references for species description are indicated in the *text*). The scale represents the average number of nucleotide substitutions per site. Bootstrap values (%) are calculated from 1000 trees and shown for frequencies above the threshold of 50%

properties (Deming et al. 1988; Nogi et al. 2004). The taxonomic data for *C. hadaliensis* have been published, although the 16S rRNA gene sequence information has not, and it has not been deposited in public culture collections. Furthermore, the taxonomic data for *Colwellia* sp. strain MT41 have not been published and only the 16S rRNA gene sequence information has been registered. The other species, *C. piezophila*, has been isolated as an obligatory psychropiezophilic strain from the sediment of the deep-sea fissure of the Japan Trench (Nogi et al. 2004). Bowman et al. (1998) reported that *Colwellia* species produce the long-chain polyunsaturated fatty acid (PUFA) docosahexaenoic acid (DHA). However, *C. piezophila* does not produce eicosapentaenoic acid (EPA) or DHA in the membrane layer, although high levels of unsaturated fatty acids ($C_{16:1}$) are produced. This suggests that the production of long-chain PUFAs should not be a requirement for classification as a piezophilic bacterium, although the production of unsaturated fatty acids could be a common property of psychropiezophiles. In addition, genome analysis has been performed for *C. psychroerythrus* 34H^T, *C. piezophila* Y223G^T, and *Colwellia* sp. strain MT41 (Methé et al. 2005; Stelling et al. 2014). As a result, further progress in the analysis of psychropiezophiles is also expected.

7.4.2 The Genus **Moritella**

The type strain of the genus *Moritella* is *Moritella marina* (Urakawa et al. 1998), previously known as *Vibrio marinus* (Colwell and Morita 1964), and is one of the most common psychrophilic organisms isolated from marine environments. At the time of writing, the genus *Moritella* consisted of seven species. Many species of the genus *Moritella* are psychropiezophilic, but *M. marina* is not a piezophilic bacterium.

Strain DSK1, a moderately psychropiezophilic bacterium isolated from the Japan Trench, was identified as *Moritella japonica* (Nogi et al. 1998a). It 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 obligatory psychropiezophilic 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 *Moritella 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 psychropiezophilic 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*, *Moritella abyssi* and *Moritella profunda,* were isolated from a depth of

2815 m off the West African coast (Xu et al. 2003b), and strain PE36 was isolated from a depth of 3584 m in the North Pacific Ocean (Yayanos 1986); they are moderately piezophilic and their growth properties are similar to those of *M. japonica*.

7.4.3 The Genus Photobacterium

The genus *Photobacterium* was one of the earliest known bacterial taxa (Beijerinck 1889), and the type species of this genus is *Photobacterium phosphoreum*. Phylogenetic analyses based on 16S rRNA gene sequences showed that this genus falls within the *Gammaproteobacteria*, is particularly closely related to the genus *Vibrio* (Nogi et al. 1998c), and is typical of marine bacterial genera.

Photobacterium profundum, a novel species, was identified through studies of the moderately psychropiezophilic strains DSJ4 and SS9 (Nogi et al. 1998c), and *Photobacterium frigidiphilum* was reported to be slightly piezophilic; its optimal pressure for growth is 10 MPa (Seo et al. 2005). About 25 *Photobacterium* species have been isolated, but *P. profundum* and *P. frigidiphilum* are the only species within this genus known to display piezophily and to produce the long-chain PUFA EPA. No other known species of *Photobacterium* produces EPA (Nogi et al. 1998c). *P. profundum* strain SS9 has been extensively studied and subjected to genome sequencing and expression analysis. The genome consists of a 4.1-Mbp circular chromosome, a 2.2-Mbp minor circular chromosome, and an 80-kbp circular plasmid (Vezzi et al. 2005). A study of strain SS9 showed that several stress response genes are upregulated in response to atmospheric pressure, including *htpG*, *dnaK*, *dnaJ*, and *groEL* (Vezzi et al. 2005). In addition, studies were conducted in relation to the pressure regulation of the outer membrane proteins OmpH and OmpL (Bartlett and Welch 1995).

7.4.4 The Genus Psychromonas

The genus *Psychromonas* is composed of psychrophilic bacteria; it also belongs to the *Gammaproteobacteria* and 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*, *Psychromonas antarctica*, was isolated as an aerotolerant anaerobic bacterium from a high-salinity pond in Antarctica (Mountfort et al. 1998). This strain does not display piezophilic properties. At the time of writing, the genus comprised 14 species with validly published names.

Psychromonas kaikoae and *Psychromonas hadalis* are novel obligatory psychropiezophilic bacteria (Nogi et al. 2002, 2007). *P. kaikoae* was isolated from sediment collected from the deepest cold-seep environment (sometimes called a cold vent, an area of the ocean floor where hydrogen sulfide, methane, and other hydrocarbon-rich fluid seepage occurs) in the Japan Trench at a depth of 7434 m, where chemoautotrophic animal communities were also found. The optimal temperature and pressure for the growth of *P. kaikoae* are 10 °C and 50 MPa, respectively, and both EPA and DHA are produced in the membrane layer. *P. hadalis* was isolated from sediment collected from the bottom of the Japan Trench at a depth of 7542 m. The optimal temperature and pressure for the growth of *P. hadalis* are 6 °C and 60 MPa, respectively, and DHA is produced in the membrane layer. *Psychromonas profunda* is a moderately piezophilic bacterium isolated from deep Atlantic sediments at a depth of 2770 m (Xu et al. 2003a). In contrast, *P. profunda* is similar to the piezosensitive strain *P. antarctica* and does not produce either EPA or DHA in its membrane layer. The piezosensitive strains *Psychromonas marina* and *Psychromonas ossibalaenae* (Miyazaki et al. 2008) also produce small amounts of DHA. In the genus *Psychromonas*, only *P. kaikoae* produces both EPA and DHA. *Psychromonas* strain CNPT-3 proved to be closely related to *Psychromonas* species based on 16S rRNA sequence information, and therefore it was assumed that strain CNPT-3 should be included in the genus *Psychromonas*.

7.4.5 The Genus Shewanella

The genus *Shewanella* comprises Gram-negative, aerobic, and facultatively anaerobic *Gammaproteobacteria* (MacDonell and Colwell 1985) and is typical of deep-sea bacterial genera (DeLong et al. 1997). The genus includes psychrophilic and mesophilic species that are widely distributed in marine environments. The type species of this genus is *Shewanella putrefaciens*, a bacterium formerly known as *Pseudomonas putrefaciens* (MacDonell and Colwell 1985; Owen et al. 1978). About 60 *Shewanella* species have been isolated and described.

Prior to the present report, *S. benthica, Shewanella piezotolerans, Shewanella psychrophila*, and *Shewanella violacea* were the only known members of the genus *Shewanella* showing psychropiezophilic growth properties (Nogi et al. 1998b; Xiao et al. 2007). The psychrophilic and piezophilic *Shewanella* strains, including *S. benthica*, *S. piezotolerans*, *S. psychrophila*, and *S. violacea*, produce EPA, and thus the production of such long-chain PUFAs is a property shared by many deep-sea bacteria to maintain cell-membrane fluidity under conditions of extreme cold and high hydrostatic pressure (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). As there are only a few differences in the growth characteristics of strain DSS12 under different pressure conditions, this strain is a very convenient deep-sea bacterium for the study of the mechanisms of adaptation to high-pressure environments. In terms of respiratory proteins (Tamegai et al. 2005), the RNA polymerase subunit (Kawano et al. 2009), dihydrofolate reductase (Ohmae et al. 2015), and isopropylmalate dehydrogenase (De Poorter et al. 2004), piezophilic proteins are unique for adaptation to high-pressure environments, and some are notably more stable and active under higher-pressure conditions. Therefore, genome analysis of strain DSS12 was performed as a model deep-sea psychropiezophilic bacterium. This strain contains 4.96 Mbp, a single chromosome, and no known plasmids. It has 4346 protein genes and 169 RNA genes (Aono et al. 2010).

7.4.6 The Genus Profundimonas

The genus *Profundimonas* is a Gram-negative, facultatively anaerobic heterotroph within the family *Oceanospirillaceae*, closely related to the uncultured symbiont of the deep-sea whale bone-eating worms of the genus *Osedax* (Cao et al. 2014). This taxonomic name has been effectively published but not validly published under the rules of the International Code of Nomenclature of Bacteria.

Profundimonas piezophila, the type species of the genus *Profundimonas*, was isolated from deep seawater collected from the Puerto Rico Trench at a depth of 6000 m as novel obligatory psychropiezophilic bacterium. The optimal temperature and pressure for the growth of *P. piezophila* are 8 °C and 50 MPa, respectively, and it does not produce either EPA or DHA in its membrane layer.

7.4.7 The Genus Carnobacterium

The genus *Carnobacterium* is a Gram-positive, facultatively anaerobic, heterofermentative, psychrotolerant, rod-shaped lactic acid bacteria that produces L-lactic acid from glucose, within the family *Leuconostocaceae* of *Firmicutes*. The type species of this genus is *Carnobacterium divergens*. It is found in vacuum-packed meat and is capable of growing in products stored at low temperatures, including refrigerated food (Collins et al. 1987). About 12 *Carnobacterium* species have been isolated and described. *Carnobacterium* sp. strains AT7 and AT12 were isolated from seawater collected from a depth of 2500 m in the Aleutian Trench and are novel psychropiezophilic bacteria. These strains are closely related to the recently isolated *Carnobacterium pleistocenium* (Lauro et al. 2007), but this is the first report of a piezophilic isolate of this species as well as the first Gram-positive piezophile ever identified. The pressure range for growth of strains AT7 and AT12 is 0.1–60 MPa, with the optimum at 15 MPa (Yayanos and DeLong 1987). Detailed data on this strain have not yet been reported. However, since genome analysis has been carried out, a detailed report is expected in the future.

7.4.8 *The Order* Rhodobacterales

Strain PRT1 was isolated from hadal seawater collected from the Puerto Rico Trench at a depth of 8350 m as a novel obligatory psychropiezophilic bacterium within the *Roseobacter* clade of the order *Rhodobacterales* within the *Alphaproteobacteria*. The optimal temperature and pressure for the growth of this strain are 10 °C and 80 MPa, respectively. This is the first report of a piezophilic isolate of *Alphaproteobacteria*. Strain PRT1 is the slowest growing (minimal doubling time, 36 h) and lowest cell density-producing (maximal densities of 5.0 $\times$ 10^6 cells ml^{-1}) (Eloe et al. 2011). Therefore, taxonomic studies appear to be difficult. However, it is expected that it will be proposed as a novel genus after further analysis.

7.5 Fatty Acid Composition of Psychropiezophiles

The psychropiezophilic *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 these psychropiezophilic strains is dependent on the taxonomic affiliation (genus); high levels of unsaturated fatty acids (about 40–70%), including EPA or DHA, are commonly found in their membrane layer. Generally, species included in these genera other than piezophilic bacteria tend to have a high ratio of unsaturated fatty acids (Table 7.2). However, the ratios of unsaturated fatty acids of obligatory psychropiezophilic bacteria are particularly high (60% or more). This indicates that it is important for psychropiezophilic bacteria to contain high ratios of unsaturated fatty acids.

The fatty acid composition of psychropiezophilic strains also changes as a function of pressure, and, in general, greater amounts of PUFAs are synthesized under high-pressure conditions for growth (DeLong and Yayanos 1985, 1986). All pychropiezophilic 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 psychropiezophilic bacterium *P. profundum* SS9 based on the analysis of pressure-sensitive mutants. In their mutant experiments, the $C_{18:1}$ fatty acid proved to be necessary for growth under low-temperature and/or high-pressure conditions. In the case of *C. piezophila* Y223G^T and *P. profunda* 2825^T, either EPA or DHA and the $C_{18:1}$ fatty acid are absent but the strain produces a large amount of the fatty acid $C_{16:1}$ in the cell membrane (Table 7.2). All psychropiezophilic bacteria analyzed so far have the $C_{16:1}$ fatty acid, and thus this fatty acid appears to be one of the important components required for high-pressure growth.

Table 7.2 Whole-cell fatty acid compositions of psychropiezophiles and related strains

Strain	Char.	Whole-cell fatty acid composition (%) of:																							UFA (%)
		$C_{11:0}$	$C_{12:0}$	$C_{13:0}$	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{17:0}$	$C_{18:0}$	iso-$C_{13:0}$	iso-$C_{14:0}$	iso-$C_{15:0}$	iso-$C_{16:0}$	$C_{14:1}$	$C_{15:1}$	$C_{16:1}$	$C_{17:1}$	$C_{18:1}$	$C_{20:5}$ ω3	$C_{22:6}$ ω3	$C_{10:0}$ 3OH	$C_{12:0}$ 3OH	$C_{14:0}$ 3OH	iso-$C_{13:0}$ 3OH	
C. psychroerythraea ATCC27364[T]	P				7	6	30	1	1					6		40	1	1	1	7					56
C. piezophila Y223G[T]	OPP		1		3	3	31							9	2	50						1			61
M. marina	P		1		16	1	21						1	1		45		2		12					60
M. abyssi 2693[T]	PP				22		11							10		46				6		6			62
M. japonica DSK1[T]	PP				18	1	21							2		50		2		6					60
M. profunda 2674[T]	PP				16		13							10		45				11		5			65
M. yayanosii DB21MT-5[T]	OPP				15	1	13							6		53		1		9					69
Pho. phosphoreum ATCC11040[T]	M		6		11		26		1					2		40		3				9	3		44
Pho. profundum DSJ4[T]	PP		2		3	1	9		1	2	4	2	15	3		32		9	13			5			57
Pho. profundum SS9	PP		4		10	1	22		2	1			6	5		30		7	7			6	1		48
"Pro. piezophila" YC-1[T]	OPP						7									36		46			7				81
Psy. antarctica DSM 10704[T]	P		1				24							8		58		3					6		69
Psy. hadalis K41G[T]	OPP		1		1		31							17		37				8			3		62
Psy. kaikoae JT7304[T]	OPP		1		6	1	15							10		54		2	2	2		2	4		76

(continued)

Table 7.2 (continued)

Strain	Char.	Whole-cell fatty acid composition (%) of:																							UFA (%)
		$C_{11:0}$	$C_{12:0}$	$C_{13:0}$	$C_{14:0}$	$C_{15:0}$	$C_{16:0}$	$C_{17:0}$	$C_{18:0}$	iso-$C_{13:0}$	iso-$C_{14:0}$	iso-$C_{15:0}$	iso-$C_{16:0}$	$C_{14:1}$	$C_{15:1}$	$C_{16:1}$	$C_{17:1}$	$C_{18:1}$	$C_{20:5}$ ω3	$C_{22:6}$ ω3	$C_{10:0}$ 3OH	$C_{12:0}$ 3OH	$C_{14:0}$ 3OH	iso-$C_{13:0}$ 3OH	
Psy. profunda 2825^T	PP						31							15		44							10		59
S. putrefaciens IAM12079^T	M		5	1	3	6	10	1		11		13		1	1	31	13	3							49
S. benthica ATCC43992^T	PP	2	5		17		15			11		5				37		1	8						46
S. benthica DB21-MT2	OPP	1	2		3	1	15			1		3				35		13	25						73
S. piezotolerans WP3^T	PP		3	1	3	3	10	2	2	8		10				24	6	12	13			1		1	54
S. psychrophila WP2^T	PP		6		8		13		2	7		4				38	2	9	7					2	56
S. violacea DSS12^T	PP	2	4		6	7	15	1		8		14		1		19	1	5	15						41

Char., strain characteristics: P, psychrophile; PP, psychropiezophile; OPP, obligatory psychropiezophile; M, mesophile, UFA, the proportion of unsaturated fatty acids

7.6 Conclusions

Initially, cultures of deep-sea psychropiezophilic bacteria were only species affiliated with one of five genera within the *Gammaproteobacteria* subgroup: *Shewanella*, *Photobacterium*, *Colwellia*, *Moritella*, and *Psychromonas*. However, more recently, species classified as *Alphaproteobacteria* and *Firmicutes* have also been found. In the future, a variety of other species will no doubt be discovered with the development of culture methods and isolation techniques. These psychropiezophiles are characterized by containing unsaturated fatty acids in their cell membrane layers but PUFAs, like EPA and DHA, are not obligatory for growth under high pressure. Subsequent fatty acid analysis of psychropiezophilic species of *Alphaproteobacteria* and *Firmicutes* will clarify the relationship between psychropiezophilic bacteria and unsaturated fatty acids. Progress in genome research will enable researchers to elucidate numerous details, such as the pressure response of psychropiezophilic bacteria.

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