Extremophiles (2008) 12:15–25
DOI 10.1007/s00792-006-0059-5

REVIEW

# Prokaryotic lifestyles in deep sea habitats

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Received: 1 November 2006 / Accepted: 10 December 2006 / Published online: 17 January 2007
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**Abstract** Gradients of physicochemical factors influence the growth and survival of life in deep-sea environments. Insights into the characteristics of deep marine prokaryotes has greatly benefited from recent progress in whole genome and metagenome sequence analyses. Here we review the current state-of-the-art of deep-sea microbial genomics. Ongoing and future genome-enabled studies will allow for a better understanding of deep-sea evolution, physiology, biochemistry, community structure and nutrient cycling.

**Keywords** Psychrophiles · Ecology · Comparative genomics · Piezophysiology · Piezophiles · High pressure biosciences · Biodiversity · Genomics

## Introduction

The largest fraction of the ocean is at depths >200 m and is believed to contain $6.5 \times 10^{28}$ cells $ml^{-1}$, accounting for 55% of all the prokaryotes found in aquatic habitats (Whitman et al. 1998), but very little is known about the adaptations allowing for growth and survival of microbes in the deep sea. In general abyssal (3,000–6,000 m) and hadal (>6,000 m) environments are characterized by low temperature, high hydrostatic pressure and the absence of solar radiation (Bartlett 1992). This physically uniform environment is believed to be populated by a high diversity but low abundance of organisms (Fuhrman et al. 1992; Sogin et al. 2006) and is occasionally interrupted by outbursts of activity in the locations of hydrothermal vents (Prieur et al. 1995), whale falls (Smith and Baco 2003) and cold seeps (Elvert et al. 2000). Here chemoautotrophic metabolisms dominate, fuelled by the presence of sources of reduced inorganic compounds.

Outside of these deep-sea oases, microbial life was, until recently, believed to be largely heterotrophic and supported by sporadic influxes of nutrients from the overlying productive layers of the water column (Witte et al. 2003). However, recent studies about the unique nature of deep-sea organic carbon (Aluwihare et al. 2002) and nitrogen (Aluwihare et al. 2005; Dell'Anno and Danovaro 2005) combined with the fact that the dominant group of archaea (Karner et al. 2001) in the deep sea might be chemoautotrophic (Francis et al. 2005; Konneke et al. 2005; Treusch et al. 2005) could change this view.

In the dark cold abyss, the most unique physical parameter is hydrostatic pressure. It can be more than three orders of magnitude higher than on the ocean's surface, reaching a maximum at ~110 MPa at the bottom of Challenger Deep in the Mariana Trench. Vertical zonation has often been explained by differential adaptation of some phylogenetic groups to this physical parameter. In particular the term "piezophile" [from the greek verb *piezo*, to press (Yayanos 1995)]

Communicated by D. A. Cowan.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00792-006-0059-5) contains supplementary material, which is available to authorized users.

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has been created to describe those microorganisms with optimal growth at pressures >0.1 MPa.

The field of piezomicrobiology was born more than 100 years ago (for a brief history see Simonato et al. 2006) but has suffered from the requirement for specialized and expensive collection vehicles such as ROVs and free vehicles, in addition to high-pressure culturing equipment. For this reason the number of scientists and labs involved in high-pressure microbiology studies is still limited.

Piezophilic microorganisms have been isolated from many regions of the world, displaying pressure optima for growth that span the entire range of pressures existing in the ocean biosphere (Yayanos et al. 1979; Nogi et al. 2002). Most of these isolates belong to the genera *Colwellia*, *Moritella*, *Photobacterium*, *Pyschromonas*, and *Shewanella* within the gamma proteobacteria (Kato et al. 1995; DeLong et al. 1997; Kato et al. 1998; Nogi et al. 1998a, b, c, 2002, 2004; Nogi and Kato 1999; Xu et al. 2003a, b) with the notable exception of two sulphate reducing isolates of the genus *Desulfovibrio* (Bale et al. 1997; Alazard et al. 2003) and one Gram-positive member of genus *Carnobacterium* (Lauro et al. 2006). It is believed that these isolates represent only a small fraction of the phylogenetic and physiological diversity present in hadal and abyssal environments. All of these ''confirmed'' inhabitants of the cold deep sea form distinct clades within phyla of microbes from polar regions suggesting common ancestry and that adaptations to low temperature could be a pre-requisite for the initial acclimation to the deep sea (Lauro et al. 2006). However, these isolates probably only represent the copiothrophic opportunists (r-strategists) and new culturing approaches (Rappe et al. 2002; Zengler et al. 2002, 2005) will have to be developed in order to isolate other members of the community.

In recent years, advances in biodiversity, genomic technology, and the environmental biotechnology of secondary metabolites (Simonato et al. 2006), has revived the interest in this field of microbiology. This short review summarizes the state of the art of deep-sea microbial genomics and metagenomics.

## The molecular diversity of deep-sea microbial communities

When delving into the ecology of any environment, the first question that comes to mind is invariably ''who is there''. Less than 1% of the marine microbes can be cultured (Fuhrman et al. 1992; Rappe and Giovannoni 2003) and despite the fact that a considerable amount of effort has been put forth in recent years to ''culture the unculturable'' (Rappe et al. 2002; Konneke et al. 2005) most of our knowledge of deep-sea microbial diversity comes from culture independent studies.

The use of 16S rRNA in microbial ecology is well established (Olsen et al. 1986) and has been applied to ocean samples from surface waters for many years (Giovannoni et al. 1990; Delong 1992; Fuhrman et al. 1992). Similar studies using deep-sea samples were not initiated until later (DeLong et al. 1997; Kato et al. 1997; Nogi et al. 1998b). These investigations provided the first clues to the extreme complexity of low-temperature deep-sea microbial communities.

More recently DeLong and colleagues (DeLong et al. 2006) end-sequenced ~5,000 fosmid clones from metagenomic libraries generated at 7 different depths (10, 70, 130, 200, 500, 770, 4,000 m) from station ALOHA (Karl and Lukas 1996) in the North Pacific Subtropical Gyre. This study provided the first snapshot of the vertical zonation of genes and pathways in the oligotrophic open ocean.

When the sequences obtained were classified by their best TBLASTX high scoring sequence pair match (HSPs), the photic zone (10, 70, 130 m) samples were enriched in *Prochlorococcus*-like sequences while the deepwater (500, 770, 4,000 m) samples were enriched in $\delta$-proteobacteria-like, *Actinobacteria*-like and *Planctomycete*-like sequences.

Sequences of putative $\alpha$-proteobacterial origin were highly abundant at all depths, while those of putative $\gamma$-proteobacterial origin only constituted a small fraction of the total at all depths. Readers interested in more detailed analyses of this work are referred to Worden et al. (2006).

A more comprehensive survey of the vertical zonation of phylogenetic groups of microbes in an oligotrophic marine setting was performed by Sogin and colleagues in the North Atlantic Ocean (Sogin et al. 2006). To avoid PCR-generated biases and increase the number of samples analyzed only the V6 hypervariable region of the 16S rRNA was sequenced using 454 technology (Margulies et al. 2005). A previous study had shown that in most cases the sequence variation within the V6 region is sufficient to broadly characterize the phylogenetic lineage of an organism (Kysela et al. 2005).

Here we re-analyzed this dataset (Materials and methods are provided as supplementary material) to show the variation of community composition as a function of depth (Fig. 1) and temperature. Some of the same trends observed for the ALOHA station sample were found. In particular $\alpha$-proteobacterial tags were highly abundant in samples from all depths and

temperatures and the deepest low temperature sample (112R) showed an increase in the relative abundance of tags classified as δ-proteobacteria, *Actinobacteria* and *Planctomycete*.

However, in contrast to the ALOHA station results, sequence tags of γ-proteobacteria were highly abundant in all samples, with percentages increasing at higher depths in the cold-water samples (Fig. 1b). This increase with depth in the percentage of δ- and γ-proteobacteria at the expense of α-proteobacteria was also observed in a depth comparison of samples from the Mediterranean and the Greenland seas (Zaballos et al. 2006).

The discrepancy between the Delong et al. (2006) and Sogin et al. (2006) results may be explained in three ways: (1) a fundamental differences between the two sets of samples, (2) technical differences in the sampling and processing of the samples or (3) a bias in the V6-tag database.

16S-based quantitative assessments of microbial communities are inherently skewed by differences in copy number of ribosomal operons among different phyla (Venter et al. 2004). Members of the phylum α-proteobacteria, for example, usually contain only one copy, as opposed to some γ-proteobacteria that can contain as many as 15, leading to an over-estimation of γ-proteobacteria when compared to other genetic markers.

Another complicating factor in the culture-independent studies of deep-sea samples is the problem of discriminating against surface derived bacteria. Many microbes will become attached to sinking particles, making their way to sediments and waters of the deep sea where they can survive in a metabolically inactive state for indefinite periods of time. For example, spores of surface-derived *Clostridium* sp. have been recovered from Japan Trench sediments at a depth of 6.3–7.3 km (Lauro et al. 2004). Upon germination, the vegetative cells showed no pressure adaptation, but the spores were extremely pressure resistant. Non-sporeforming bacteria can also exhibit remarkable pressure tolerance when starved (Berlin et al. 1999). The role of these ''foreign'' species in nutrient and energy cycling might be only passive, because of their contribution to the pool of organic matter that is removed from photic layer. Therefore, the understanding of biochemical cycling in the ocean requires means of distinguishing between autochthonous and allochthonous members of the community.

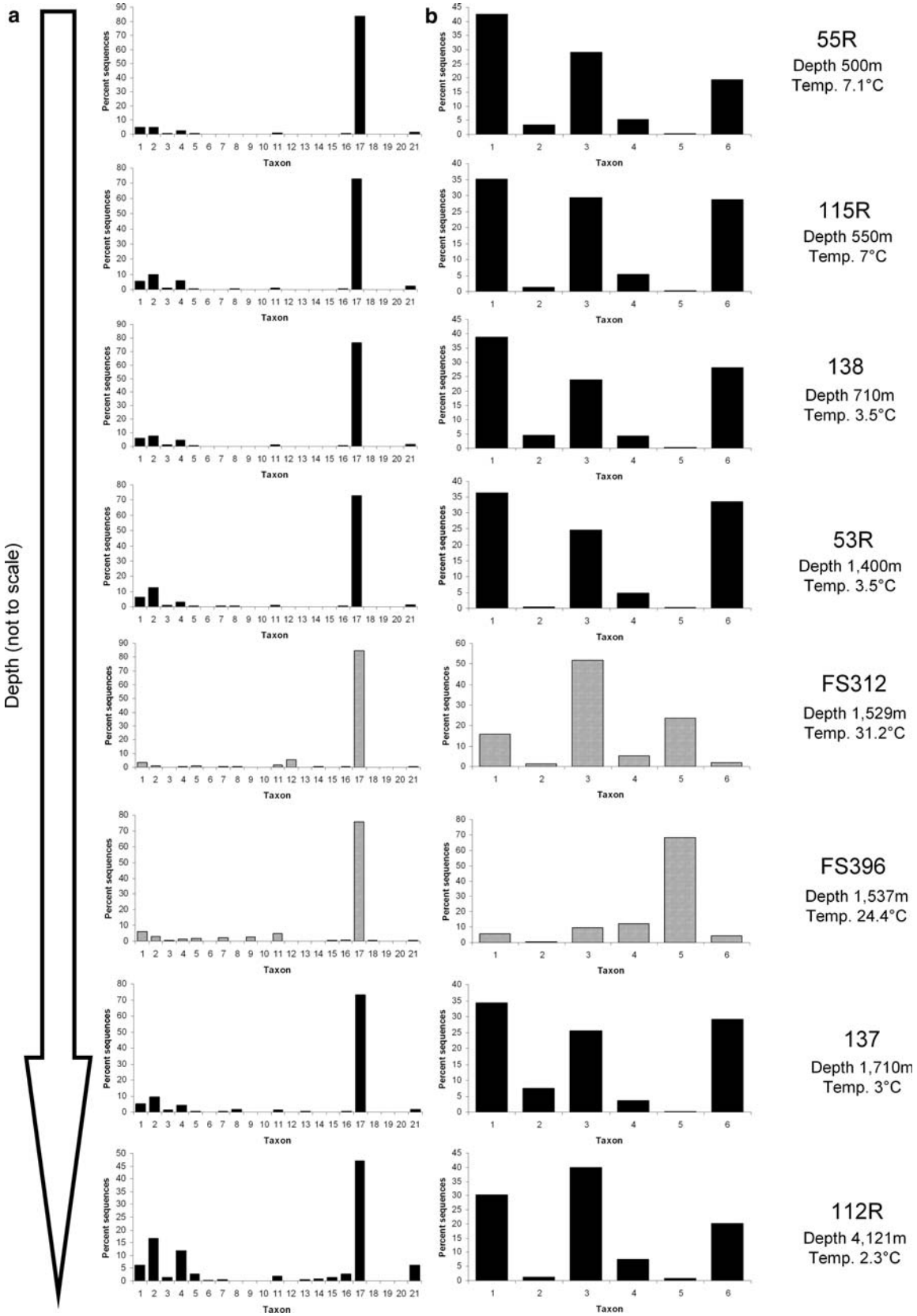
One solution to the problem is to pre-filter the collected water samples in order to remove surface-derived detritus (Worden et al. 2006), at the risk of losing those active members of deep-sea communities that colonize and degrade marine snow. Interestingly, one of the main differences in sample processing between the station ALOHA sample and the North Atlantic sample was that the first one was pre-filtered while the second one was not (Herndl and Sogin, personal communication). This difference between the two samples could have added or subtracted a large fraction of the population of γ-proteobacteria in the water column which live attached to particles as opposed to the predominantly free-living lifestyle of α-proteobacteria (Delong et al. 1993).

## High pressure, DNA damage and cell division

A number of reviews have been published on the physiological adaptations of microorganisms to the deep sea (Kato and Bartlett 1997; Bartlett 2002, 2006; Margesini and Nogi 2004; Bartlett et al. 2006; Simonato et al. 2006). These studies have traditionally focused on the effects of high hydrostatic pressure, a physical parameter whose increase will inhibit any process resulting in a positive volume change (Gross and Jaenicke 1994).

Deep-sea bacteria have been shown to possess piezo-specific adaptations in terms of membrane phospholipid fatty acid unsaturation (Delong and Yayanos 1985; Allen et al. 1999; Allen and Bartlett 2000) and cell division (Jannasch 1987; Yayanos and DeLong 1987; Bidle and Bartlett 1999; Ishii et al. 2004). Moreover, hydrostatic pressure has been shown in vitro to affect many protein–protein (Silva and Weber 1993) and protein–DNA (Chilukuri et al. 1997; Tang et al. 1998) interactions, and, in vivo to hinder essential cellular processes such as replication and translation (Yayanos and Pollard 1969; Welch et al. 1993; Ishii et al. 2005).

One interesting observation is that when a microorganism is grown above or below its optimal hydrostatic pressure, it tends to become filamentous (Jannasch 1987; Yayanos and DeLong 1987). In *E. coli* a partial explanation for this phenomenon has been provided: high pressure enhances the activity of the Mrr cryptic endonuclease (Aertsen and Michiels 2005) which produces double stranded DNA damage, triggering an SOS response (Aertsen et al. 2004). One aspect of this signaling pathway is the increase of the SulA regulatory protein which in turn inhibits FtsZ-mediated septal ring formation (Aertsen et al. 2004). This pressure-triggered SOS response can be exacerbated by mutation of the gene encoding the Lon protease, an enzyme which postranslationally limits SulA abundance. The only caveat in this otherwise elegant


a
b
Depth (not to scale)
Percent sequences
Taxon
55R
Depth 500m
Temp. 7.1°C
115R
Depth 550m
Temp. 7°C
138
Depth 710m
Temp. 3.5°C
53R
Depth 1,400m
Temp. 3.5°C
FS312
Depth 1,529m
Temp. 31.2°C
FS396
Depth 1,537m
Temp. 24.4°C
137
Depth 1,710m
Temp. 3°C
112R
Depth 4,121m
Temp. 2.3°C

◄ **Fig. 1** Classification of the V6-region rRNA tags produced by Sogin et al. (2006). **a** Left column: percentage composition the total bacterial community. Taxa: (1) No significant HSPs (2) only environmental HSPs (3) Acidobacteria (4) Actinobacteria (5) Bacteroidetes (6) Chlamydiae (7) Chloroflexi (8) Cyanobacteria (9) Deferribacteres (10) Deinococcus-Thermus (11) Firmicutes (12) Fusobacteria (13) Gemmatimonadetes (14) Lentisphaerae (15) Nitrospira (16) Planctomycetes (17) Proteobacteria (18) Spirochaetes (19) Thermodesulfobacteria (20) Thermotogae (21) Verrucomicrobia. **b** Right column: percentage composition among the proteobacteria. Taxa: (1) α-proteobacteria (2) β-proteobacteria (3) γ-proteobacteria (4) δ-proteobacteria (5) ε-proteobacteria (6) unclassified proteobacteria. *Solid black bars* are samples from the North Atlantic Deep Water: 53R and 55R from 58.300°N to 29.133°W; 112R and 115R from 50.400°N to 25.000°W; 137 and 138 from 60.900°N to 38.516°W. *Grey bars* (FS312 and FS396) are samples from hydrothermal vent fluids of the Axial Seamount in the Juan de Fuca Ridge, 45.916°N-129.983°W

story is the fact that filamentation still occurs in *sulA* mutants (Aertsen et al. 2004; Ishii et al. 2004; Kawarai et al. 2004), suggesting that an alternative filamentation pathway must also exist. The second pathway could arise from high hydrostatic pressure inhibition of the polymerization of tubulin-like protein FtsZ (Ishii et al. 2004).

The DNA damage signal for pressure-induced double-stranded DNA breaks is not known at this time but could involve RecD, which together with RecB an RecC composes exonuclease V, an enzyme with multiple activities centered on the control of genetic recombination (Amundsen et al. 1986). *recD* mutants in the moderate piezophile *Photobacterium profundum* SS9 are pressure sensitive (Chi and Bartlett 1993; Bidle and Bartlett 1999) and the introduction of the *recD* gene from *P. profundum* into *E. coli* inhibits cell filamentation at high pressure (Bidle and Bartlett 1999).

Interestingly, the piezo-sensitive *recD* mutant of *P. profundum* SS9 was isolated in a screen performed at atmospheric pressure for mutants altered in the production of the outer membrane protein OmpH (Chi and Bartlett 1993) and only subsequently was found to be pressure sensitive. *ompH* gene expression is induced at low pressure under carbon and energy starvation (Bartlett and Welch 1995), a condition that is known to trigger an SOS response (Janion et al. 2002). Consistent with this possible SOS connection, microarray studies of *P. profundum* SS9 indicate the upregulation of many genes associated with DNA repair (including *uvrA* and *uvrD*) when the cells are grown at atmospheric pressure but not when grown at 28 or 45 MPa (Campanaro et al. 2005). And, as previously noted by Aertsen et al. (2004) outer membrane proteins in *E. coli* are also known to be controlled by the SOS response (Garvey et al. 1985). Understanding the pathway by which this non-traditional SOS response is pressure-triggered should be a priority for future studies.

## High pressure and protein synthesis

An essential cellular process inhibited by hydrostatic pressure is protein synthesis. This has been explained through the dissociation of the ribosomal subunits that has been detected both in vitro (Schulz et al. 1976; Gross and Jaenicke 1990; Gross et al. 1993) and in vivo (Niven et al. 1999; Alpas et al. 2003). Transcriptome and proteome studies have shown that high hydrostatic pressure induces the synthesis of a number of ribosomal and heat-shock proteins in *Lactobacillus sanfranciscensis* (Pavlovic et al. 2005; Hormann et al. 2006) and *E. coli* (Welch et al. 1993; Ishii et al. 2005). The triggering of a heat-shock response is particularly interesting as the pressures used in these studies (<100 MPa) are too low to cause pressure-denaturation of proteins (Silva and Weber 1993). Instead, it is compatible with the hypothesis of Hormann et al. (2006) that suggest it is due to a partial loss-of-function in the ribosomes that produce truncated and misfolded proteins. As was the case for the SOS response, in the deep-sea bacterium *P. profundum* SS9 the opposite effect is observed with heat-shock genes being over-expressed at atmospheric pressure (Vezzi et al. 2005).

16S ribosomal RNA comparisons between strains from different depths has identified structures that appear to be specific to the piezophiles (Lauro et al. 2006), suggesting functional constraints for ribosomal function in the deep sea. Additional work will be required to clarify the extent of ribosomal adaptation to high pressure.

## Genomic adaptations to the deep sea

Recently we have initiated with colleagues the analysis of the first five genomes of psychropiezophilic (cold- and pressure-loving) bacterial isolates: *P. profundum* SS9 (Vezzi et al. 2005), *Shewanella* sp. KT99 (Lauro et al. 2006), *Moritella* sp. PE36 (DeLong et al. 1997), *Psychromonas* sp. CNPT3 (DeLong et al. 1997), *Carnobacterium* sp. AT7 (Lauro et al. 2006). Each of these strains has a number of phylogentically closely related species/strains that are adapted to surface water conditions (Table 1).

To aid in comparing related species differing in adaptation to deep-sea environments we have adopted the term ‘‘bathytype’’ (from the greek word *bathos*,

**Table 1** Deep bathytype microbial species and corresponding comparison species from shallow waters

| Strain | $P_{opt}$ | $T_{opt}$ | PUFA | Source | Phylogeny | Comparison strain |
|---|---|---|---|---|---|---|
| *Photobacterium profundum* SS9 (Vezzi et al. 2005) | 0.1 | 16 | EPA | Sulu Sea | Proteobacteria Gammaproteobacteria Vibrionales | *P. profundum* 3TCK *P.* sp. SKA34 |
| *Shewanella* sp. KT99 | ~98 | ~2 | EPA[a] | Kermadec Trench | Proteobacteria Gammaproteobacteria Alteromonadales | *Shewanella frigidimarina* NCIMB400 *Shewanella violacea* DSS12 |
| *Psychromonas* sp. CNPT3 | 52 | 12 | EPA[a] | Central North Pacific | Proteobacteria Gammaproteobacteria Alteromonadales | *Psychromonas ingrahamii* |
| *Moritella* sp. PE36 | 41 | 10 | DHA | Patton Escarpment | Proteobacteria Gammaproteobacteria Alteromonadales | *Moritella marina* |
| *Carnobacterium* sp. AT7 | 20 | 20 | ND | Aleutian Trench | Firmicutes Bacilli Lactobacillales | *Enterococcus faecalis* |

The analysis of fatty acid methyl esters was performed by gas chromotography-mass spectrometry

*PUFA* polyunsaturated fatty acid, *EPA* eicosapentaenoic acid (20:5n-3), *DHA* docosahexaenoic acid (22:6n3)

[a] Traces of DHA

depth) to identify depth-specific ecotypes, defined as "a population of a species genetically adapted to a certain depth in the water column". The very existence of closely related microbes differing in their bathytypes implies that relatively little genetic change is required to evolve and adapt to the deep sea. In fact, genomic comparison between strain SS9 (Nogi et al. 1998c) and strain 3TCK (Campanaro et al. 2005), the two bathytypes of *P. profundum* whose genome are completely sequenced, has revealed a high degree of synteny.

## What are the genomic features that restrict the depth of each bathytype?

All the deep bathytypes possess a high ratio of rRNA operon copies/genome size (Fig. 2) and larger-than-average intergenic regions (Fig. 3). The number of ribosomal operons in a genome has been correlated with the ecological strategy of bacteria (Klappenbach et al. 2000): the highest numbers of operons are present in bacteria, which respond most rapidly to environmental changes. Similarly, small intergenic regions have been correlated with an equilibrium strategy (K-strategy) (Macarthur 1960) as observed in the cosmopolitan marine clade SAR-11 (Giovannoni et al. 2005). Taken together these observations suggest that most of the deep bathytypes in culture have an opportunistic (r-strategy) lifestyle with a high degree of gene regulation. Future studies will help clarifying if this strategy is selected by deep-sea conditions or simply reflects a bias in piezophile isolation and cultivation. Interestingly, the analysis of two 16S-containing $\delta$-proteobacterial cosmid clones from a metagenomic library

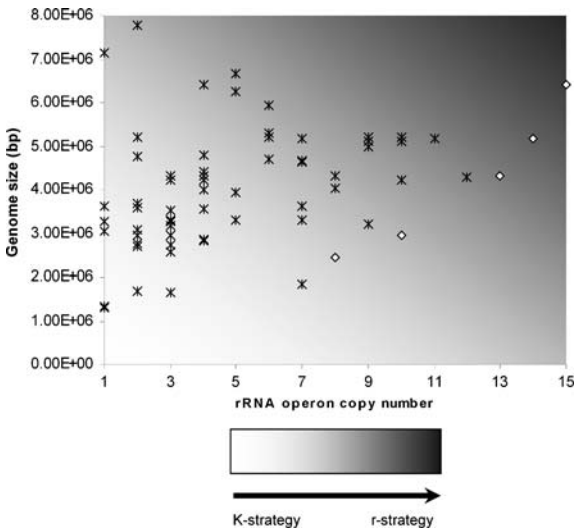



**Fig. 2** Relationship between genome size and rRNA copy number for 66 bacterial genomes. The *open diamonds* are the deep bathytypes

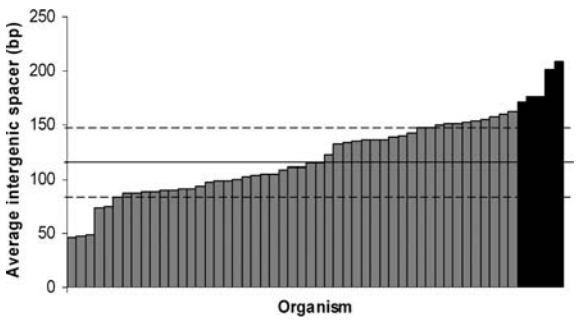



**Fig. 3** Average intergenic spacer for 54 bacterial genomes. The *dark bars* are the deep bathytypes. *Solid line* represents the mean, *dashed lines* the standard deviation

constructed from a 500 m deep Antarctic water sample showed that the average intergenic size varied from 163 to 858 bp (Moreira et al. 2006), suggesting that both strategies might coexist at intermediate depths.

Other hallmarks of all the current deep bathytype genomes are the presence of a large number of genes involved in membrane unsaturation, such as Δ-9 desaturases and polyketide synthase gene clusters (Allen et al. 1999; Allen and Bartlett 2002) for the synthesis of polyunsaturated fatty acids (Table 1), and the absence of photolyase (*phr*) genes. The *phr* gene product (Yasui and McCready 1998) uses blue-light energy in order to repair cyclobutane pyrimidine dimers in UV irradiated DNA. It is therefore expected to be absent from the genomes of any organisms that are never exposed to light. The previously mentioned metagenomic analysis of the vertical distribution of genes in the central Pacific Ocean (DeLong et al. 2006) showed that *phr*-like genes are substantially under-represented below the photic zone.

This bias in *phr* distribution is not universal. Indeed, photolyase-like genes are present in the genomes of several bacterial isolates obtained from the deep sea. These are *Idiomarina loihiensis* (Hou et al. 2004), *Oceanobacillus iheyensis* (Takami et al. 2002) and *Alteromonas macleodii* (draft sequence). However, it should be noted that while these organisms were derived from deep-sea environments, there is no data available on their pressure adaptation. They could be allochthonous to the deep-sea environment or recently evolved piezophiles in which surface derived genes have not yet been purged by natural selection.

Additional genetic modifications are expected for coping with long periods of starvation (Rice and Oliver 1992) and for searching for nutrients in patchy oligotrophic environments (Witte et al. 2003). In fact, studies conducted with the moderate piezophile *Psychromonas* sp. CNPT3 have shown interesting behavioral responses. Upon shifting to low-nutrient conditions, the cells decreased their biovolume and membrane unsaturated fatty acid content and increased their ability to attach to a glass substratum, particularly during incubations at high pressure. This latter attribute could reflect an adaptation for localization to nutrient-rich particles (Rice and Oliver 1992). How long can piezophiles survive with little or no food? In one long-term experiment, a batch culture of *Psychromonas* HS11 (Delong and Yayanos 1986) was maintained at high pressure for over 20 years (Chastain and Yayanos, personal communication). Upon decompression and dilution into new media, the culture displayed exponential growth within 2 weeks.

Motility is another important adaptation for marine bacteria, in particular to avoid grazing and for the continuous quest for nutrients (Grossart et al. 2001). In hadal and abyssal environments, the hunt for dissolved (Blackburn et al. 1998) and particulate (Azam and Long 2001; Kiorboe and Jackson 2001) organic matter might explain the large number of methyl-accepting chemotaxis (MCP) proteins present in the genomes of all the deep bathytypes. MCPs are signal transducing proteins that respond to gradients of chemicals in the environment, relaying a signal for directional swimming to the flagellar motor. These sensory systems must be able to detect miniscule changes in the surrounding chemistry in order to enable the cells to maximize their productivity and growth in environments of small amounts of spatially and temporally distributed food supplies (Wirsen and Molyneaux 1999).

Flagellar assembly and motor function is also worth studying in greater detail as a model for a high pressure-adapted system. Motility is arguably the most pressure-sensitive cellular process in surface-water prokaryotes (Meganathan and Marquis 1973; Bartlett 2002) and gene clusters for motility and chemotaxis are among the most divergent between bathytypes (Campanaro et al. 2005). In fact, microarray-based genome comparison between three strains of *P. profundum* has shown that the deep bathytypes [SS9 and DSJ4 (Nogi et al. 1998c)] have an additional gene cluster that is lacking in the shallow bathytype (3TCK) which resembles genes for the production of lateral flagella (Campanaro et al. 2005). The function of this additional cluster is unknown, but preliminary results have provided evidence that the deep bathytype SS9 swims well under high pressure, but very poorly at atmospheric pressure, while the opposite phenotype has been observed in the shallow bathytype 3TCK (Eloe et al., unpublished results).

## Future directions

Genomic analyses of piezophilic microorganisms is only starting to crack the codes for deep-sea adaptations. Continued sequencing of key deep-sea bathytypes within all three domains of life will be essential along with reinvigorated attempts to culture a more representative fraction of the deep-sea microbial community. With advances in computer modeling it will be possible to detect more subtle global adaptations such as specific protein motifs that allow enzymes to work under high pressure and low temperature and to identify novel sensory, regulatory and metabolic

pathways that allow growth under conditions of low and shifting nutrient conditions.

Another frontier will be the use of large-scale community sequencing to more fully understand deep-sea microbes at the community and ecosystem level (Azam and Worden 2004). Metagenomics is still in its infancy because of the amount of sequencing and cyber-infrastructure required to extensively sample complex environments (Venter et al. 2004). However it has been successfully applied to relatively simple systems such as acid mine drainage (Tyson et al. 2004) and anaerobic methane oxidizing microbial communities (Hallam et al. 2004).

With new technologies (Margulies et al. 2005) and approaches (Goldberg et al. 2006) it is hoped that it will be possible in the near future to characterize the bulk of the microbial community even in the case of environments of high diversity and complexity such as the deep sea. Certainly the work of DeLong et al. (2006), Ferrer et al. (2005) and Grzymski et al. (2006) are ground-breaking steps in this direction. In the meantime, insights into the function, the metabolic potential and the role of individual phylotypes in microbial populations could be gathered by using environmental microarrays (Zhou 2003). These come in various flavors: (1) Functional gene arrays (FGA) have probes designed to detect specific functional genes. They could be used to search for specific metabolic pathways that are expected to be over-represented in the deep sea (Wu et al. 2001, 2006) and to analyze changes in the expression of those pathways in response to spatio-temporal changes in the physico-chemical parameters (Gao et al. 2006). (2) SSU rRNA-based phylogenetic oligonucleotide arrays (POAs) could be used to analyze phylogenetic diversity and microbial community shifts in response to the same environmental fluctuations (Loy et al. 2002; Wilson et al. 2002; El Fantroussi et al. 2003). (3) Arrays based on the whole complement of open reading frames of a reference genome could be used to rapidly identify genomic variations among a large number of closely related isolates. Understanding the role of strain-specific genes in deep bathytypes (Campanaro et al. 2005) and the significance of horizontal gene transfer in producing genome variation (Murray et al. 2001; Earl et al. 2006) would provide valuable insights into the evolution of deep-sea genomes.

However these technologies must proceed in parallel with the development of new genetic tools and culturing approaches in order to test hypotheses stemming from the analyses of molecular data.

For example, it will be interesting to physiologically characterize deep-sea members of the group I Crenarchaea, whose numbers become dominant with depth (Karner et al. 2001). Based on the molecular and culturing data of a shallow-water representative from this group it appears that these microbes are chemoautothrophs which gain energy through the oxidation of ammonia (Francis et al. 2005; Konneke et al. 2005; Treusch et al. 2005). If true for the group in general, current models for carbon and nitrogen cycling in the deep ocean will need to be revised (Aluwihare et al. 2005; Ingalls et al. 2006).

**Acknowledgments** We are grateful to the National Science Foundation for their support (MCB02-37059, MCB04-009 and MCB05-44524) and to Xavier Mayali for insightful discussion.

## References

Aertsen A, Michiels CW (2005) Mrr instigates the SOS response after high pressure stress in *Escherichia coli*. Mol Microbiol 58:1381–1391

Aertsen A, Van Houdt R, Vanoirbeek K, Michiels CW (2004) An SOS response induced by high pressure in *Escherichia coli*. J Bacteriol 186:6133–6141

Alazard D, Dukan S, Urios A, Verhe F, Bouabida N, Morel F, Thomas P, Garcia JL, Ollivier B (2003) *Desulfovibrio hydrothermalis* sp nov., a novel sulfate-reducing bacterium isolated from hydrothermal vents. Int J Syst Evol Microbiol 53:173–178

Allen EE, Bartlett DH (2000) FabF is required for piezoregulation of *cis*-vaccenic acid levels and piezophilic growth of the deep-sea bacterium *Photobacterium profundum* strain SS9. J Bacteriol 182:1264–1271

Allen EE, Bartlett DH (2002) Structure and regulation of the omega-3 polyunsaturated fatty acid synthase genes from the deep-sea bacterium *Photobacterium profundum* strain SS9. Microbiology 148:1903–1913

Allen EE, Facciotti D, Bartlett DH (1999) Monounsaturated but not polyunsaturated fatty acids are required for growth of the deep-sea bacterium *Photobacterium profundum* SS9 at high pressure and low temperature. Appl Environ Microbiol 65:1710–1720

Alpas H, Lee J, Bozoglu F, Kaletunc G (2003) Evaluation of high hydrostatic pressure sensitivity of *Staphylococcus aureus* and *Escherichia coli* O157: H7 by differential scanning calorimetry. Int J Food Microbiol 87:229–237

Aluwihare LI, Repeta DJ, Chen RF (2002) Chemical composition and cycling of dissolved organic matter in the Mid-Atlantic Bight. Deep-Sea Res Part II Top Stud Oceanogr 49:4421–4437

Aluwihare LI, Repeta DJ, Pantoja S, Johnson CG (2005) Two chemically distinct pools of organic nitrogen accumulate in the ocean. Science 308:1007–1010

Amundsen SK, Taylor AF, Chaudhury AM, Smith GR (1986) recD: the gene for an essential 3Rd subunit of exonuclease V. Proc Natl Acad Sci USA 83:5558–5562

Azam F, Long RA (2001) Oceanography—sea snow microcosms. Nature 414:495–498

Azam F, Worden AZ (2004) Microbes, molecules, and marine ecosystems. Science 303:1622–1624

Bale SJ, Goodman K, Rochelle PA, Marchesi JR, Fry JC, Weightman AJ, Parkes RJ (1997) *Desulfovibrio profundus*

sp nov, a novel barophilic sulfate-reducing bacterium from deep sediment layers in the Japan Sea. Int J Syst Bacteriol 47:515–521
Bartlett DH (1992) Microbial life at high-pressures. Sci Prog 76:479–496
Bartlett DH (2002) Pressure effects on in vivo microbial processes. Biochim Biophys Acta 1595:367–381
Bartlett DH (2006) Extremophilic vibrionaceae. In: Thompson FL, Austin B, Swings J (eds) The biology of vibrios. American Society for Microbiology Press, Washington D.C., pp 156–171
Bartlett DH, Welch TJ (1995) ompH gene-expression is regulated by multiple environmental cues in addition to high-pressure in the deep-sea bacterium *Photobacterium* species strain SS9. J Bacteriol 177:1008–1016
Bartlett DH, Lauro FM, Eloe EA (2006) Microbial adaptation to high pressure. In: Gerday C, Glansdorff N (eds) Physiology and biochemistry of extremophiles. American Society for Microbiology Press, Washington D.C. (in press)
Berlin DL, Herson DS, Hicks DT, Hoover DG (1999) Response of pathogenic Vibrio species to high hydrostatic pressure. Appl Environ Microbiol 65:2776–2780
Bidle KA, Bartlett DH (1999) RecD function is required for high-pressure growth of a deep-sea bacterium. J Bacteriol 181:2330–2337
Blackburn N, Fenchel T, Mitchell J (1998) Microscale nutrient patches in planktonic habitats shown by chemotactic bacteria. Science 282:2254–2256
Campanaro S, Vezzi A, Vitulo N, Lauro FM, D'Angelo M, Simonato F, Cestaro A, Malacrida G, Bertoloni G, Valle G et al (2005) Laterally transferred elements and high pressure adaptation in *Photobacterium profundum* strains. BMC Genomics 6:122
Chi E, Bartlett DH (1993) Use of a reporter gene to follow high-pressure signal-transduction in the deep-sea bacterium *Photobacterium* sp strain SS9. J Bacteriol 175:7533–7540
Chilukuri LN, Fortes PAG, Bartlett DH (1997) High pressure modulation of *Escherichia coli* DNA gyrase activity. Biochem Biophys Res Commun 239:552–556
Dell'Anno A, Danovaro R (2005) Extracellular DNA plays a key role in deep-sea ecosystem functioning. Science 309:2179–2179
Delong EF (1992) Archaea in coastal marine environments. Proc Natl Acad Sci USA 89:5685–5689
Delong EF, Yayanos AA (1985) Adaptation of the membrane-lipids of a deep-sea bacterium to changes in hydrostatic-pressure. Science 228:1101–1102
Delong EF, Yayanos AA (1986) Biochemical function and ecological significance of novel bacterial lipids in deep-sea prokaryotes. Appl Environ Microbiol 51:730–737
Delong EF, Franks DG, Alldredge AL (1993) Phylogenetic diversity of aggregate-attached vs free-living marine bacterial assemblages. Limnol Oceanogr 38:924–934
DeLong EF, Franks DG, Yayanos AA (1997) Evolutionary relationships of cultivated psychrophilic and barophilic deep-sea bacteria. Appl Environ Microbiol 63:2105–2108
DeLong EF, Preston CM, Mincer T, Rich V, Hallam SJ, Frigaard NU, Martinez A, Sullivan MB, Edwards R, Brito BR et al (2006) Community genomics among stratified microbial assemblages in the ocean's interior. Science 311:496–503
Earl AM, Losick R, Kolter R (2006) *Bacillus subtilis* genome diversity. J Bacteriol. doi:10.1128/JB.01343-06
El Fantroussi S, Urakawa H, Bernhard AE, Kelly JJ, Noble PA, Smidt H, Yershov GM, Stahl DA (2003) Direct profiling of environmental microbial populations by thermal dissociation analysis of native rRNAs hybridized to oligonucleotide microarrays. Appl Environ Microbiol 69:2377–2382
Elvert M, Suess E, Greinert J, Whiticar MJ (2000) Archaea mediating anaerobic methane oxidation in deep-sea sediments at cold seeps of the eastern Aleutian subduction zone. Org Geochem 31:1175–1187
Ferrer M, Golyshina OV, Chernikova TN, Khachane AN, dos Santos VAPM, Yakimov MM, Timmis KN, Golyshin PN (2005) Microbial enzymes mined from the Urania deep-sea hypersaline anoxic basin. Chem Biol 12:895–904
Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB (2005) Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proc Natl Acad Sci USA 102:14683–14688
Fuhrman JA, Mccallum K, Davis AA (1992) Novel major archaebacterial group from marine plankton. Nature 356:148–149
Gao H, Yang ZK, Gentry TJ, Wu L, Schadt CW, Zhou J (2006) Microarray-based analysis of microbial community RNAs by whole community RNA amplification (WCRA). Appl Environ Microbiol. doi:10.1128/AEM.01771-06
Garvey N, Stjohn AC, Witkin EM (1985) Evidence for recA protein association with the cell membrane and for changes in the levels of major outer membrane proteins in SOS-induced *Escherichia coli* cells. J Bacteriol 163:870–876
Giovannoni SJ, Britschgi TB, Moyer CL, Field KG (1990) Genetic diversity in Sargasso sea bacterioplankton. Nature 345:60–63
Giovannoni SJ, Tripp HJ, Givan S, Podar M, Vergin KL, Baptista D, Bibbs L, Eads J, Richardson TH, Noordewier M et al (2005) Genome streamlining in a cosmopolitan oceanic bacterium. Science 309:1242–1245
Goldberg SMD, Johnson J, Busam D, Feldblyum T, Ferriera S, Friedman R, Halpern A, Khouri H, Kravitz SA, Lauro FM et al (2006) A Sanger/pyrosequencing hybrid approach for the generation of high-quality draft assemblies of marine microbial genomes. Proc Natl Acad Sci USA 103:11240–11245
Gross M, Jaenicke R (1990) Pressure-induced dissociation of tight couple ribosomes. FEBS Lett 267:239–241
Gross M, Jaenicke R (1994) Proteins under pressure. The influence of high hydrostatic pressure on structure, function and assembly of proteins and protein complexes. Eur J Biochem 221:617–630
Gross M, Lehle K, Jaenicke R, Nierhaus KH (1993) Pressure-induced dissociation of ribosomes and elongation cycle intermediates. Stabilizing conditions and identification of the most sensitive functional state. Eur J Biochem 218:463–468
Grossart HP, Riemann L, Azam F (2001) Bacterial motility in the sea and its ecological implications. Aquat Microb Ecol 25:247–258
Grzymski JJ, Carter BJ, DeLong EF, Feldman RA, Ghadiri A, Murray AE (2006) Comparative genomics of DNA fragments from six antarctic marine planktonic bacteria. Appl Environ Microbiol 72:1532–1541
Hallam SJ, Putnam N, Preston CM, Detter JC, Rokhsar D, Richardson PM, DeLong EF (2004) Reverse methanogenesis: testing the hypothesis with environmental genomics. Science 305:1457–1462
Hormann S, Scheyhing C, Behr J, Pavlovic M, Ehrmann M, Vogel RF (2006) Comparative proteome approach to characterize the high-pressure stress response of *Lactobacillus sanfranciscensis* DSM 20451. Proteomics 6:1878–1885
Hou SB, Saw JH, Lee KS, Freitas TA, Belisle C, Kawarabayasi Y, Donachie SP, Pikina A, Galperin MY, Koonin EV et al

(2004) Genome sequence of the deep-sea gamma-proteobacterium *Idiomarina loihiensis* reveals amino acid fermentation as a source of carbon and energy. Proc Natl Acad Sci USA 101:18036–18041

Ingalls AE, Shah SR, Hansman RL, Aluwihare LI, Santos GM, Druffel ERM, Pearson A (2006) Quantifying archaeal community autotrophy in the mesopelagic ocean using natural radiocarbon. Proc Natl Acad Sci USA 103:6442–6447

Ishii A, Sato T, Wachi M, Nagai K, Kato C (2004) Effects of high hydrostatic pressure on bacterial cytoskeleton FtsZ polymers in vivo and in vitro. Microbiology 150:1965–1972

Ishii A, Oshima T, Sato T, Nakasone K, Mori H, Kato C (2005) Analysis of hydrostatic pressure effects on transcription in *Escherichia coli* by DNA microarray procedure. Extremophiles 9:65–73

Janion C, Sikora A, Nowosielska A, Grzesiuk E (2002) Induction of the SOS response in starved *Escherichia coli*. Environ Mol Mutagen 40:129–133

Jannasch HW (1987) Effects of hydrostatic pressure on growth of marine bacteria. In: Jannasch HW, Marquis RE, Zimmerman AM (eds) Current prespectives in high pressure biology. Academic, Toronto, pp 1–15

Karl DM, Lukas R (1996) The Hawaii ocean time-series (HOT) program: background, rationale and field implementation. Deep Sea Res Part II Top Stud Oceanogr 43:129–156

Karner MB, DeLong EF, Karl DM (2001) Archaeal dominance in the mesopelagic zone of the Pacific Ocean. Nature 409:507–510

Kato C, Bartlett DH (1997) The molecular biology of barophilic bacteria. Extremophiles 1:111–116

Kato C, Sato T, Horikoshi K (1995) Isolation and properties of barophilic and barotolerant bacteria from deep-sea mud samples. Biodivers Conserv 4:1–9

Kato C, Li LN, Tamaoka J, Horikoshi K (1997) Molecular analyses of the sediment of the 11000-m deep Mariana Trench. Extremophiles 1:117–123

Kato C, Li L, Nogi Y, Nakamura Y, Tamaoka J, Horikoshi K (1998) Extremely barophilic bacteria isolated from the Mariana Trench, Challenger Deep, at a depth of 11,000 - meters. Appl Environ Microbiol 64:1510–1513

Kawarai T, Wachi M, Ogino H, Furukawa S, Suzuki K, Ogihara H, Yamasaki M (2004) SulA-independent filamentation of *Escherichia coli* during growth after release from high hydrostatic pressure treatment. Appl Microbiol Biotechnol 64:255–262

Kiorboe T, Jackson GA (2001) Marine snow, organic solute plumes, and optimal chemosensory behavior of bacteria. Limnol Oceanogr 46:1309–1318

Klappenbach JA, Dunbar JM, Schmidt TM (2000) rRNA operon copy number reflects ecological strategies of bacteria. Appl Environ Microbiol 66:1328–1333

Konneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, Stahl DA (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437:543–546

Kysela DT, Palacios C, Sogin ML (2005) Serial analysis of V6 ribosomal sequence tags (SARST-V6): a method for efficient, high-throughput analysis of microbial community composition. Environ Microbiol 7:356–364

Lauro FM, Bertoloni G, Obraztsova A, Kato C, Tebo BM, Bartlett DH (2004) Pressure effects on *Clostridium* strains isolated from a cold deep-sea environment. Extremophiles 8:169–173

Lauro FM, Chastain RA, Blankenship LE, Yayanos AA, Bartlett DH (2006) The unique 16S rRNA genes of piezophiles reflect both phylogeny and adaptation. Appl Environ Microbiol doi:10.1128/AEM.01726-06

Loy A, Lehner A, Lee N, Adamczyk J, Meier H, Ernst J, Schleifer KH, Wagner M (2002) Oligonucleotide microarray for 16S rRNA gene-based detection of all recognized lineages of sulfate-reducing prokaryotes in the environment. Appl Environ Microbiol 68:5064–5081

Macarthur R (1960) On the relative abundance of species. Am Nat 94:25–36

Margesini R, Nogi Y (2004) Psychropiezophilic microorganisms. Cell Mol Biol 50:429–436

Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen ZT et al (2005) Genome sequencing in microfabricated high-density picolitre reactors. Nature 437:376–380

Meganathan R, Marquis RE (1973) Loss of bacterial motility under pressure. Nature 246:525–527

Moreira D, Rodriguez-Valera F, Lopez-Garcia P (2006) Metagenomic analysis of mesopelagic Antarctic plankton reveals a novel deltaproteobacterial group. Microbiology 152:505–517

Murray AE, Lies D, Li G, Nealson K, Zhou J, Tiedje JM (2001) DNA/DNA hybridization to microarrays reveals gene-specific differences between closely related microbial genomes. Proc Natl Acad Sci USA 98:9853–9858

Niven GW, Miles CA, Mackey BM (1999) The effects of hydrostatic pressure on ribosome conformation in *Escherichia coli*: an in vivo study using differential scanning calorimetry. Microbiology 145:419–425

Nogi Y, Kato C (1999) Taxonomic studies of extremely barophilic bacteria isolated from the Mariana Trench and description of *Moritella yayanosii* sp. nov., a new barophilic bacterial isolate. Extremophiles 3:71–77

Nogi Y, Kato C, Horikoshi K (1998a) *Moritella japonica* sp. nov., a novel barophilic bacterium isolated from a Japan Trench sediment. J Gen Appl Microbiol 44:289–295

Nogi Y, Kato C, Horikoshi K (1998b) Taxonomic studies of deep-sea barophilic *Shewanella* strains and description of *Shewanella violacea* sp. nov. Arch Microbiol 170:331–338

Nogi Y, Masui N, Kato C (1998c) *Photobacterium profundum* sp. nov., a new, moderately barophilic bacterial species isolated from a deep-sea sediment. Extremophiles 2:1–7

Nogi Y, Kato C, Horikoshi K (2002) *Psychromonas kaikoae* sp nov., a novel piezophilic bacterium from the deepest cold-seep sediments in the Japan Trench. Int J Syst Evol Microbiol 52:1527–1532

Nogi Y, Hosoya S, Kato C, Horikoshi K (2004) *Colwellia piezophila* sp nov., a novel piezophilic species from deep-sea sediments of the Japan Trench. Int J Syst Evol Microbiol 54:1627–1631

Olsen GJ, Lane DJ, Giovannoni SJ, Pace NR, Stahl DA (1986) Microbial ecology and evolution: a ribosomal RNA approach. Annu Rev Microbiol 40:337–365

Pavlovic M, Hormann S, Vogel RF, Ehrmann MA (2005) Transcriptional response reveals translation machinery as target for high pressure in *Lactobacillus sanfranciscensis*. Arch Microbiol 184:11–17

Prieur D, Erauso G, Jeanthon C (1995) Hyperthermophilic life at deep-sea hydrothermal vents. Planet Space Sci 43:115–122

Rappe MS, Giovannoni SJ (2003) The uncultured microbial majority. Annu Rev Microbiol 57:369–394

Rappe MS, Connon SA, Vergin KL, Giovannoni SJ (2002) Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. Nature 418:630–633

Rice SA, Oliver JD (1992) Starvation response of the marine barophile Cnpt-3. Appl Environ Microbiol 58:2432–2437

Schulz E, Ludemann HD, Jaenicke R (1976) High-pressure equilibrium studies on dissociation association of *Escherichia coli* ribosomes. FEBS Lett 64:40–43

Silva JL, Weber G (1993) Pressure stability of proteins. Annu Rev Phys Chem 44:89–113

Simonato F, Campanaro S, Lauro FM, Vezzi A, D'Angelo M, Vitulo N, Valle G, Bartlett DH (2006) Piezophilic adaptation: a genomic point of view. J Biotechnol 126:11–25

Smith CR, Baco AR (2003) Ecology of whale falls at the deep-sea floor. Oceanography and Marine Biology, vol 41. pp 311–354

Sogin ML, Morrison HG, Huber JA, Welch DM, Huse SM, Neal PR, Arrieta JM, Herndl GJ (2006) Microbial diversity in the deep sea and the underexplored ''rare biosphere''. Proc Natl Acad Sci USA 103:12115–12120

Takami H, Takaki Y, Uchiyama I (2002) Genome sequence of *Oceanobacillus iheyensis* isolated from the Iheya Ridge and its unexpected adaptive capabilities to extreme environments. Nucleic Acids Res 30:3927–3935

Tang GQ, Tanaka N, Kunugi S (1998) In vitro increases in plasmid DNA supercoiling by hydrostatic pressure. Biochim Biophys Acta 1443:364–368

Treusch AH, Leininger S, Kletzin A, Schuster SC, Klenk HP, Schleper C (2005) Novel genes for nitrite reductase and Amo-related proteins indicate a role of uncultivated mesophilic crenarchaeota in nitrogen cycling. Environ Microbiol 7:1985–1995

Tyson GW, Chapman J, Hugenholtz P, Allen EE, Ram RJ, Richardson PM, Solovyev VV, Rubin EM, Rokhsar DS, Banfield JF (2004) Community structure and metabolism through reconstruction of microbial genomes from the environment. Nature 428:37–43

Venter JC, Remington K, Heidelberg JF, Halpern AL, Rusch D, Eisen JA, Wu DY, Paulsen I, Nelson KE, Nelson W et al (2004) Environmental genome shotgun sequencing of the Sargasso Sea. Science 304:66–74

Vezzi A, Campanaro S, D'Angelo M, Simonato F, Vitulo N, Lauro FM, Cestaro A, Malacrida G, Simionati B, Cannata N et al (2005) Life at depth: *Photobacterium profundum* genome sequence and expression analysis. Science 307:1459–1461

Welch TJ, Farewell A, Neidhardt FC, Bartlett DH (1993) Stress response of *Escherichia coli* to elevated hydrostatic pressure. J Bacteriol 175:7170–7177

Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: the unseen majority. Proc Natl Acad Sci USA 95:6578–6583

Wilson KH, Wilson WJ, Radosevich JL, DeSantis TZ, Viswanathan VS, Kuczmarski TA, Andersen GL (2002) High-density microarray of small-subunit ribosomal DNA probes. Appl Environ Microbiol 68:2535–2541

Wirsen CO, Molyneaux SJ (1999) A study of deep-sea natural microbial populations and barophilic pure cultures using a high-pressure chemostat. Appl Environ Microbiol 65:5314–5321

Witte U, Wenzhofer F, Sommer S, Boetius A, Heinz P, Aberle N, Sand M, Cremer A, Abraham WR, Jorgensen BB et al (2003) In situ experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor. Nature 424:763–766

Worden AZ, Cuvelier ML, Bartlett DH (2006) In-depth analyses of marine microbial community genomics. Trends Microbiol 14:331–336

Wu LY, Liu X, Schadt CW, Zhou JZ (2006) Microarray-based analysis of subnanogram quantities of microbial community DNAs by using whole-community genome amplification. Appl Environ Microbiol 72:4931–4941

Wu LY, Thompson DK, Li GS, Hurt RA, Tiedje JM, Zhou JZ (2001) Development and evaluation of functional gene arrays for detection of selected genes in the environment. Appl Environ Microbiol 67:5780–5790

Xu Y, Nogi Y, Kato C, Liang ZY, Ruger HJ, De Kegel D, Glansdorff N (2003a) *Moritella profunda* sp nov and *Moritella abyssi* sp nov., two psychropiezophilic organisms isolated from deep Atlantic sediments. Int J Syst Evol Microbiol 53:533–538

Xu Y, Nogi Y, Kato C, Liang ZY, Ruger HJ, De Kegel D, Glansdorff N (2003b) *Psychromonas profunda* sp nov., a psychropiezophilic bacterium from deep Atlantic sediments. Int J Syst Evol Microbiol 53:527–532

Yasui A, McCready SJ (1998) Alternative repair pathways for UV-induced DNA damage. Bioessays 20:291–297

Yayanos AA (1995) Microbiology to 10,500 meters in the deep-sea. Annu Rev Microbiol 49:777–805

Yayanos AA, Pollard EC (1969) A study of effects of hydrostatic pressure on macromolecular synthesis in *Escherichia coli*. Biophys J 9:1464–1482

Yayanos AA, DeLong EF (1987) Deep-sea bacterial fitness to environmental temperatures and pressure. In: Jannasch HW, Marquis RE, Zimmerman AM (eds) Current perspectives in high pressure biology. Academic, Toronto, pp 17–32

Yayanos AA, Dietz AS, Vanboxtel R (1979) Isolation of a deep-sea barophilic bacterium and some of its growth characteristics. Science 205:808–810

Zaballos M, Lopez-Lopez A, Ovreas L, Bartual SG, D'Auria G, Alba JC, Legault B, Pushker R, Daae FL, Rodriguez-Valera F (2006) Comparison of prokaryotic diversity at offshore oceanic locations reveals a different microbiota in the Mediterranean Sea. FEMS Microbiol Ecol 56:389–405

Zengler K, Toledo G, Rappe M, Elkins J, Mathur EJ, Short JM, Keller M (2002) Cultivating the uncultured. Proc Natl Acad Sci USA 99:15681–15686

Zengler K, Walcher M, Clark G, Haller I, Toledo G, Holland T, Mathur EJ, Woodnutt G, Short JM, Keller M (2005) High-throughput cultivation of microorganisms using microcapsules. Methods Enzymol 397:124–130

Zhou JH (2003) Microarrays for bacterial detection and microbial community analysis. Curr Opin Microbiol 6:288–294