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REVIEW

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The molecular biology of barophilic bacteria

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Abstract Many microorganisms from the deep-sea display high-pressure-adapted – also described as barophilic or piezophilic – growth characteristics. Phylogenetic studies have revealed that a large proportion of the barophilic bacteria currently in culture collections belong to a distinct subgroup of the genus *Shewanella*, referred to as the "barophile branch." Many of the basic properties of barophiles that enable their survival at extremes of pressure remain to be elucidated. However, several genes whose expression is regulated by pressure, or which appear to be critical to baroadaptation, have been uncovered. One such operon, whose presence appears to be restricted to the "barophile branch," has been identified in DNA samples obtained from sediments recovered in the deepest ocean trench. In the case of another set of pressure-regulated genes, regulatory elements required for pressure signaling have been uncovered. The nature and regulation of these genes is discussed.

Key words Barophile branch · Barophilic bacteria · Deep-sea · High pressure · *Photobacterium* sp. · Pressure-regulated gene · Pressure sensing · *Shewanalla* sp. · *toxR/S*

Introduction

The deep-sea is a unique environment typified by high pressure, low temperature, and reduced and altered nutrient availability. In this article we would like to focus on the influence of pressure in particular as an environmental variable in the oceans, and its effects on the genetic architecture of deep-sea bacteria. The physical basis of pressure effects is well documented to arise from the inhibitory effects of increased pressure on biochemical processes which are accompanied by a positive volume change (Bartlett 1992).

ZoBell and Johnson (1949) first coined the term barophile, and ZoBell and Morita (1957) obtained the first evidence for barophilic growth in mixed microbial cultures obtained from deep-sea sediments. The first pure culture isolates of barophilic bacteria were reported in 1979 (Yayanos et al. 1979). Since that time several studies have suggested that increased unsaturation of membrane fatty acids is critical to the maintenance of optimal membrane function at high pressure in barophiles (DeLong and Yayanos 1985, 1986; Wirsen et al. 1987; Kamimura et al. 1993). However, the evolution of barophilicity has surely required the modification of many other cell structures and processes. Molecular and genetic studies are providing clues to the identification and characterization of such cellular components, many of which appear to localize to the membrane.

Isolation and characterization of pressure-adapted microorganisms from deep-sea environments

Several species of high-pressure-adapted bacteria have been isolated from deep-sea sediment samples obtained at depths of 2500–6500 m by means of the manned submersible *Shinkai 6500,* operated by the Japan Marine Science and Technology Center (JAMSTEC) (Kato et al. 1995a,c, 1996a). A list of these isolates together with other bacteria to be described is shown in Table 1. Most of the deep-sea bacteria isolated are either barophilic or barotolerant, and also psychrophilic, i.e., unable to grow at temperatures above 20°C. Examples of typical growth profiles of these bacteria at several pressures and temperatures were shown in a previous report (Kato et al. 1995a). The barophilic

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Table 1. Bacterial strains described in this study, and some of their properties

Bacterial strain	Properties	Source	Reference
Barophilic bacteria[a]			
DB5501	Optimal growth at 50 MPa, 10°C	Suruga Bay, 2485 m depth	Kato et al. 1995a
DB6101	Optimal growth at 50 MPa, 10°C	Ryukyu Trench, 5110 m depth	Kato et al. 1995a
DB6705	Optimal growth at 50 MPa, 10°C, no growth at 0.1 MPa	Japan Trench, 6356 m depth	Kato et al. 1995a
DB6906	Optimal growth at 50 MPa, 10°C, no growth at 0.1 MPa	Japan Trench, 6269 m depth	Kato et al. 1995a
DB172F	Optimal growth at 70 MPa, 10°C, no growth at 0.1 MPa	Izu-Bonin Tench, 6499 m depth	Kato et al. 1996b
PT99	Optimal growth at 69 MPa, 10°C, no growth at 0.1 MPa	Philippine Trench, 8600 m depth	DeLong and Yayanos 1986
Moderately barophilic bacteria[b]			
DSS12	Optimal growth at 30 MPa, 8°C	Ryukyu Trench, 5110 m depth	Kato et al. 1995a
S. benthica[c]	Optimal growth at 30 MPa, 4°C	Walvis Ridge, 4575 m depth	MacDonell and Colwell 1985
SC2A	Optimal growth at 20 MPa, 20°C	San Clemente Basin, 1957 m depth	Yayanos et al. 1982
SS9	Optimal growth at 20 MPa, 18°C	Sulu Trough, 2551 m depth	DeLong 1986
DSJ4	Optimal growth at 10 MPa, 10°C	Ryukyu Trench, 5110 m depth	Kato et al. 1995a
Barotolerant bacteria			
DSK1	Optimal growth at 0.1 MPa, 10°C	Japan Trench, 6356 m depth	Kato et al. 1995a
DSK25	Optimal growth at 0.1 MPa, 35°C	Japan Trench, 6500 m depth	Kato et al. 1995c
S. hanedai[c]	Optimal growth at 0.1 MPa, 14°C	–	MacDonell and Colwell 1985
Barosensitive bacteria			
E. coli W3110	Optimal growth at 0.1 MPa, 37°C	–	–
S. alga[c]	Optimal growth at 0.1 MPa, 30°C	–	Rossell-Mora et al. 1994
S. putrefaciens[c]	Optimal growth at 0.1 MPa, 26°C	–	MacDonell and Colwell 1985

[a] Barophilic bacteria are defined as those whose optimal growth pressure is more than 40 MPa.
[b] Moderately barophilic bacteria are defined as those whose optimal growth pressure is above atmospheric pressure but less than 40 MPa, and which are capable of growth at atmospheric pressure.
[c] Species numbers of the *Shewanella* strains are ATCC43992 (*S. benthica*), IAM12641 (*S. hanedai*), IAM14159 (*S. alga*), and IAM12079 (*S. putrefaciens*).

strains DB6705 and DB6906 are capable of growth at atmospheric pressure at 4°C but not at temperatures at or above 10°C. In contrast, under high pressure conditions above 50 MPa (approximately equal to a depth of 5 km) these strains grow better at 10°C than at 4°C. Growth of the barotolerant strain DSK1 is also better at a relatively high temperature (15°C) than at a relatively low temperature (10°C) under high-pressure conditions above 50 MPa. The specific growth rate profiles of these deep-sea bacteria indicate that they exhibit optimal high-pressure growth near their upper temperature limit for growth. This appears to be a general tendency of deep-sea barophilic strains (Yayanos 1986; Kato et al. 1995a, 1996b).

Comparisons of 16S rRNA sequences obtained from numerous barophilic and barotolerant deep-sea bacteria to those sequences held in the GenBank and EMBL databases indicates that many barophilic and barotolerant strains belong to the Proteobacteria γ-subgroup (Kato et al. 1996a). All of the strictly barophilic strains (DB5501, DB6101, DB6705, DB6906, DB172F, and *Shewanella* sp. PT99) and some of the moderately barophilic strains (DSS12 and *Shewanella benthica*) group together in the same subbranch of the genus *Shewanella*, which has been designated the "barophile branch" (Fig. 1). Other moderately barophilic strains (*Shewanella* sp. SC2A, *Photobacterium* sp. SS9, and DSJ4) and barotolerant strains (*S. hanedai* and DSK1) are widely distributed throughout the Proteobacteria γ-subgroup, but outside of the barophile branch, and the Gram-positive barotolerant strain DSK25 (Kato et al. 1995c) falls into the genus *Bacillus*. It is striking that all of the strictly barophilic microbes reported thus far group within a particular branch of a single genus.

Identification of pressure-regulated genes from deep-sea *Shewanella*

In a sense all organisms respond to pressure changes. In the case of terrestrial organisms (which experience little pressure variation in their natural environment), this probably reflects a stress response to the adverse effects of increased pressure (Bartlett et al. 1995). On the other hand, aquatic organisms from shallow as well as deep environments have evolved mechanisms for sensing and coping with pressure changes. Examples from higher organisms include fish swim bladders and crab statocysts, which help maintain the organisms' vertical position within the water column (Pelster and Scheid 1992; Fraser and Macdonald 1994). Marine microoganisms may also experience large pressure changes as a result of physical or biological transport processes (Bartlett 1991).

Pressure-sensing has been analyzed among the barophile branch bacteria by searching for genes activated by high pressure. This was accomplished by screening for promoter DNA from the barophilic bacterium DB6705 which was

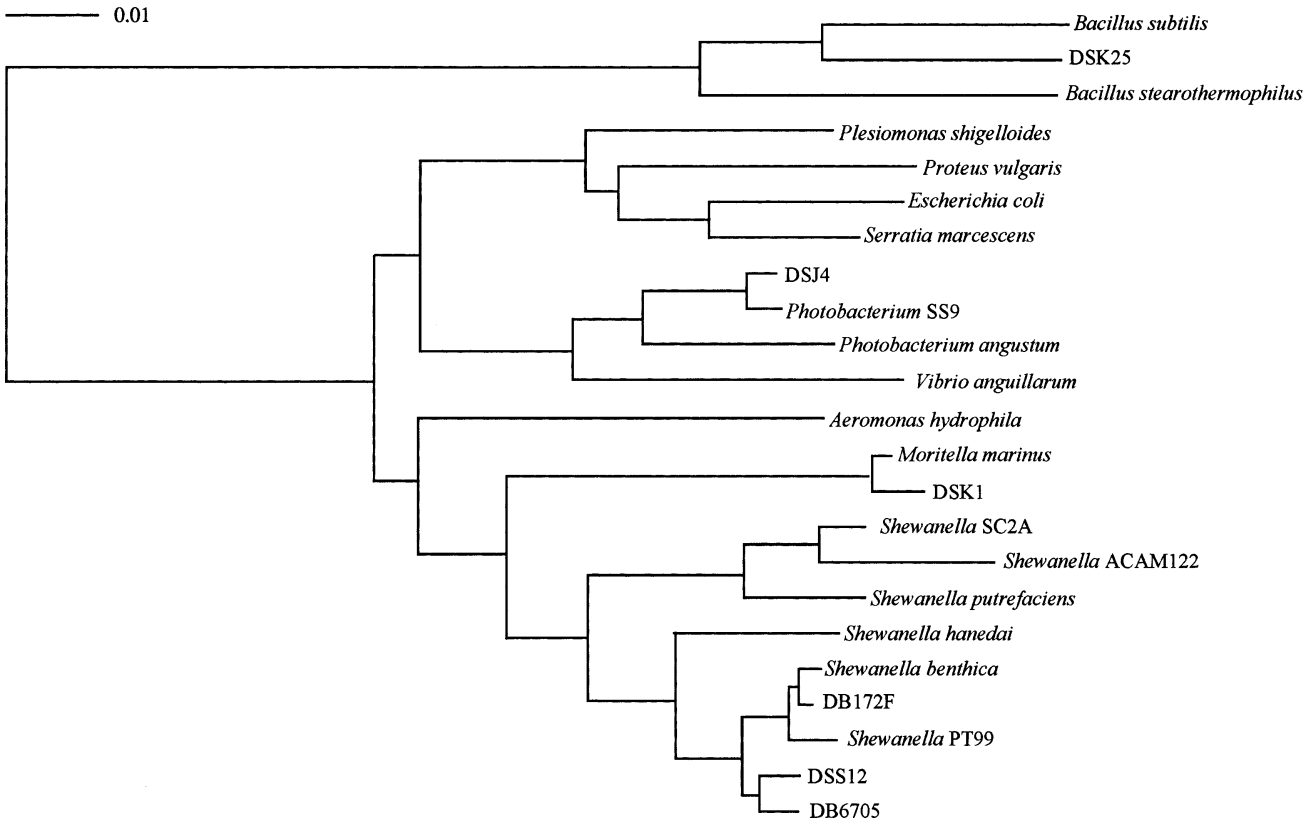

Fig. 1. Phylogenetic tree showing the relationships between isolated deep-sea-adapted bacteria within the γ-subgroup of the Proteobacteria and genus *Bacillus* as determined by comparing 16S ribosomal DNA sequences using the neighbor-joining method (Saitou and Nei 1987). The *scale* represents the average number of nucleotide substitutions per site

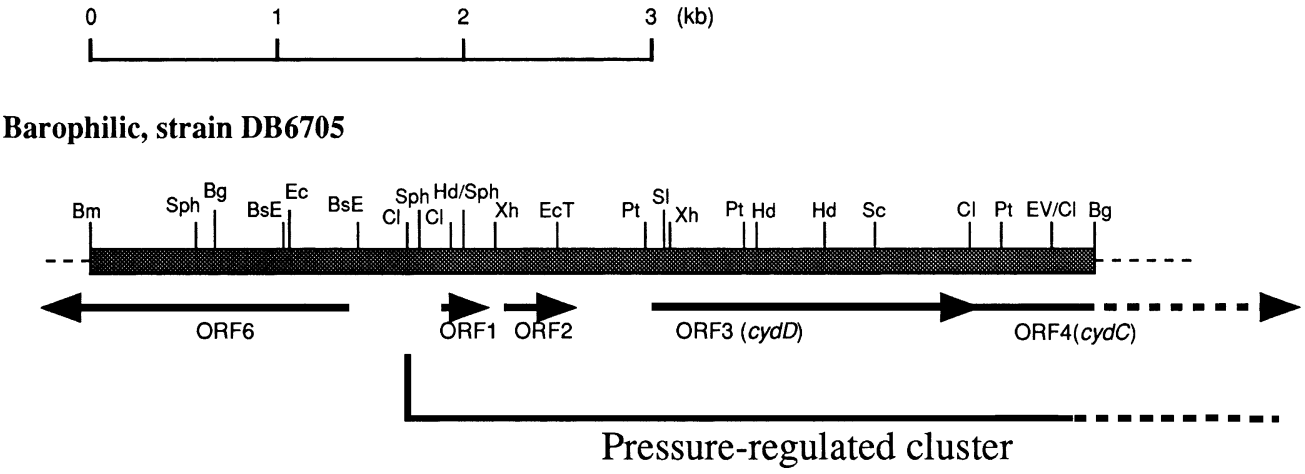

Fig. 2. Restriction map of the DNA fragment containing the two pressure-regulated operons and flanking open reading frames (*ORF*s) from the barophilic strain DB6705, and the structure of the genes. *Arrows* indicate open reading frames. Restriction endonucleases are indicated as follows: Bg, *BglII*; Bm, *Bam*HI; BsE, *Bst*EII; Cl, *Cla*I; Ec, *Eco*RI; EcT, *Eco*T22; EV, *Eco*RV; Hd, *Hin*dIII; Pt, *Pst*I; Sc, *Sac*I; Sl, *Sal*I; Sph, *Sph*I; and Xh, *Xho*I. The accession number of this DNA sequence from strain DB6705 is D88688 in the DDBJ, EMBL, and GenBank DNA databases

capable of controlling chloramphenicol acetyltransferase gene expression in *Escherichia coli* at moderate pressure (Kato et al. 1995b). A promoter fragment isolated in this manner was subsequently found by primer extension and Northern blot analysis to direct high-pressure-inducible transcription in both the barophilic strain DB6705 and in *E. coli* transformants harboring plasmids containing this promoter. Downstream from this promoter, two small unidentified open reading frames (ORF1 and ORF2) were found which together comprise a pressure-regulated operon (Kato

et al. 1996c), as shown in Fig. 2. The highest transcript levels of ORFs 1 and 2 mRNA in DB6705 were observed at 70 MPa (Kato et al. 1996c). The structure of the promoter sequence located upstream of ORFs 1 and 2 is similar (14/20 bp match within and upstream of −10 region) to that of a pressure-inducible promoter discovered in another unrelated deep-sea bacterium: the *ompH* promoter from *Photobacterium* sp. SS9 (Bartlett et al. 1989; Bartlett and Welch 1995; see later). A similar pressure-regulated operon to that present in DB6705 was cloned from the moderately barophilic bacterium strain DSS12 and sequenced (Kato et al. 1997a). Its sequence is almost identical to that of the operon from DB6705, and its expression is also induced by elevated pressure.

Downstream from this operon, another pressure-regulated operon exists whose first ORF is designated ORF3. Expression of ORF3 is also induced by high pressure (Kato et al. 1997a). Unlike the situation with ORFs 1 and 2, it has been possible to identify the function of ORF3. Based upon its amino acid sequence and the results of heterologous complementation studies in *E. coli*, ORF3 appears to encode the CydD protein (Kato et al. 1996d). In *E. coli*, CydD is required for the assembly of the cytochrome *bd* complex, one of the components of the aerobic respiratory chain (Poole et al. 1994). *E. coli cydD* mutants display increased sensitivity to high pressure, but display wild-type levels of high pressure sensitivity when bearing the DSS12 ORF3 gene on a plasmid (Kato et al. 1996d). Thus, it is likely that cytochrome *bd* localization and assembly within the inner membrane is critical to growth at high pressure in strain DSS12 and related bacteria. Studies with other deep-sea bacteria also indicate the importance of certain membrane proteins to pressure adaptation (DeLong and Yayanos 1987; Chi and Bartlett 1993; Bidle and Bartlett unpublished data).

PCR amplification of ORFs 1 and 2 or an internal portion of ORF3 have been attempted from many different bacteria. Using primers specific for ORF1 and ORF3, amplification was positive for all of the strictly barophilic strains (DB5501, DB6101, DB6705, DB6906, DB172F, and *Shewanella* sp. PT99) and some of moderately barophilic strains (DSS12 and *S. benthica*), but not for the other *Shewanella* strains (*S. hanedai*, *S. alga*, *S. putrefaciens*, and *Shewanella* sp. SC2A), other deep-sea bacterial strains, (*Photobacterium* sp. SS9, DSK1, DSJ4, and DSK25), or *E. coli*. All of the strains which yielded positive PCR results belong in the barophile branch (Fig. 1). Thus, the ability to amplify ORFs 1 and 2 and part of ORF3 from certain deep-sea bacterial strains appears to reflect the close taxonomic relationships of these bacteria to DB6705 and DSS12 (Li et al. unpublished data).

To investigate the distribution of ORF1, 2, and 3 within deep-sea microbial assemblages, DNA fragments encoding portions of these genes were amplified by PCR from DNA isolated from the world's deepest ocean basin, the Mariana Trench, at a depth of approximately 11 000 m using the new unmanned submersible *Kaiko* operated by JAMSTEC. DNA fragments corresponding to the pressure-regulated gene clusters were clearly amplified by PCR. DNA obtained from PCR was cloned and sequenced, and the results of ORF1-3 sequence comparisons are shown in Table 2. The sequences corresponding to ORFs1-3 found in DNA from the Mariana sediment were more similar to those of the strictly barophilic strain DB6705 than those of the moderately barophilic strain DSS12 (Table 2a,b). These results suggest that those benthic bacteria inhabiting the sediments within the Mariana Trench which possess the pressure-regulated operons are strictly barophilic organisms (Kato et al. 1997b). In fact, it has been possible to isolate obligately barophilic strains from the Mariana

Table 2. Similarity (%) of DNA sequences of **a** the pressure-regulated operon (ORF1, 2) and **b** ORF3, amplified from the Mariana sediment DNA

a

	DB6705	DSS12	#9	#11	#13	#24	#55
DB6705	100%	93.4%	97.9%	98.6%	98.3%	98.3%	98.2%
DSS12		100	92.2	92.4	92.2	92.7	92.5
#9			100	99.3	99.0	99.0	99.2
#11				100	99.8	99.8	99.7
#13					100	99.5	99.5
#24						100	99.5
#55							100

b

	DB6705	DSS12	#26	#28	#30	#63	#66
DB6705	100%	87.3%	95.8%	99.8%	95.6%	95.0%	95.3%
DSS12		100	88.3	87.2	88.2	87.8	88.1
#26			100	95.6	99.8	99.6	99.8
#28				100	95.4	94.8	95.1
#30					100	99.4	99.6
#63						100	99.6
#66							100

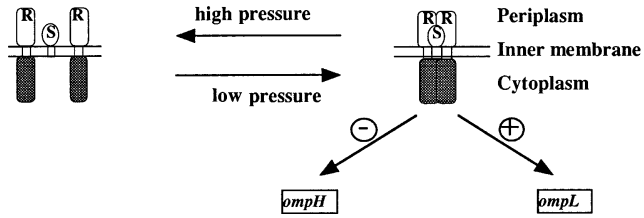

Fig. 3. Model of ToxR/S function in the regulation of *omp* gene expression in SS9. The associated ToxR/S protein complex functions as both a repressor and activator of gene expression

sediment by maintaining culture conditions of 100 MPa and 4°C, and some of these bacteria have been found to possess ORFs1-3 in their chromosomal DNA (Kato et al. unpublished data).

Pressure-regulated gene expression in the deep-sea bacterium *Photobacterium* sp. SS9

Another example of pressure sensing in a deep-sea bacterial isolate concerns the inverse pressure regulation of *ompH* and *ompL* gene expression in the genetically manipulatable moderate barophile *Photobacterium* sp. SS9. These genes encode outer membrane proteins. The OmpH protein is maximally abundant when SS9 is grown at its pressure optimum, 28 MPa, whereas the OmpL protein is produced in greatest quantity when SS9 is grown at 0.1 MPa (Bartlett et al. 1989; Chi and Bartlett 1993; Welch and Bartlett 1996). Although *ompH* mutants are not high-pressure-sensitive and *ompL* mutants are not low-pressure-sensitive, physiological experiments with various mutants suggest that OmpH may enable the uptake of a greater range of nutrients than OmpL (Bartlett and Chi 1994), a trait which could be important in the deep sea where nutrients are frequently limiting.

Mutants stuck in the high pressure mode of OmpH/L regulation have been isolated, first by transposon mutagenesis and later following gene replacement mutagenesis (Welch and Bartlett unpublished data). These mutants bear disruptions in a homolog of the *toxRS* operon from *Vibrio cholerae* (Miller at al. 1987; DiRita and Mekalanos 1991). In *V. cholerae,* ToxR and ToxS localize to the inner membrane where they act as environmental sensors, regulating the expression of a number of virulence genes in response to changes in temperature, osmolarity, pH, and the levels of certain extracellular amino acids. ToxR is a dimeric transmembrane protein whose cytoplasmic face binds those genes under its direct control. ToxS appears to modulate ToxR activity. Evidence indicates that SS9 ToxR pressure-sensing depends on the physical state of the cytoplasmic membrane. Local anesthetics at concentrations known to increase bacterial membrane fluidity result in low-pressure ToxR signaling, even when the cells are grown at high pressure. One possible explanation for this result is that while membrane fatty acyl chains are highly sensitive to lateral compaction at increasing pressure (Braganza and Worcester 1986), this effect may be counterbalanced by membrane fluidizing agents such as anesthetics (Fig. 3). The anesthetic result is particularly interesting in view of the fact that opposing effects of pressure and anesthetics on a variety of neurological activities in higher organisms have long been known (Johnson and Miller 1970). It will be interesting in the future to learn in greater detail how ToxR and ToxS sense changes in pressure, what additional genes are under the control of ToxR at low and high pressure, and if the activities of these genes influence SS9 adaptation to pressure changes.

Conclusion

The study of barophilic bacteria promises to provide information on the requirements for life in the largest portion of the biosphere, the cold, dark, low-nutrient, high-pressure conditions prevailing in the deep sea. In addition to the value inherent in studying a major category of extremophiles whose adaptational mechanisms are only beginning to be understood, barophiles may also contribute to biotechnology in a broad range of areas including deep-sea waste disposal, the production of novel natural products and catabolic activities, and the provision of enzymes for high-pressure bioreactors. There is value in the exploration of both the deep-sea and the genomes of its denizens.

References

Bartlett DH (1991) Pressure sensing in deep-sea bacteria. Res Microbiol 142:923–925

Bartlett DH (1992) Microbial life at high pressures. Sci Prog 76:479–496

Bartlett DH, Chi E (1994) Genetic characterization of *ompH* mutants in the deep-sea bacterium *Photobacterium* species strain SS9. Arch Microbiol 162:323–328

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, Kato C, Horikoshi K (1995) High pressure influences on gene and protein expression. Res Microbiol 146:697–706

Bartlett D, Wright M, Yayanos AA, Silverman M (1989) Isolation of a gene regulated by hydrostatic pressure in a deep-sea bacterium. Nature 342:572–574

Braganza LF, Worcester DL (1986) Structural changes in lipid bilayers and biological membranes caused by hydrostatic pressure. Biochemistry 25:7484–7488

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

DeLong EF (1986) Adaptations of deep-sea bacteria to the abyssal environment. PhD thesis, University of California, San Diego

DeLong EF, Yayanos AA (1985) Adaptation of the membrane lipids of a deep-sea bacterium to changes in hydrostatic pressure. Science 228:1101–1103

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, Yayanos AA (1987) Properties of the glucose transport system in some deep-sea bacteria. Appl Environ Microbiol 53:527–532

DiRita VJ, Mekalanos JJ (1991) Periplasmic interaction between two membrane regulatory proteins, ToxR and ToxS, results in signal transduction and transcriptional activation. Cell 64:29–37

Fraser PJ, Macdonald AG (1994) Crab hydrostatic pressure sensors. Nature 371:383–384

Johnson SM, Miller KW (1970) Antagonism of pressure and anaesthesia. Nature 228:75–76

Kamimura K, Fuse H, Takimura O, Yamaoka Y (1993) Effects of growth pressure and temperature on fatty acid composition of a barotolerant deep-sea bacterium. Appl Environ Microbiol 59:924–926

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

Kato C, Smorawinska M, Sato T, Horikoshi K (1995b) Cloning and expression in *Escherichia coli* of a pressure-regulated promoter region from a barophilic bacterium, strain DB6705. J Mar Biotechnol 2:125–129

Kato C, Suzuki S, Hata S, Ito T, Horikoshi K (1995c) The properties of a protease activated by high pressure from *Sporosarcina* sp. strain DSK25 isolated from deep-sea sediment. JAMSTEC Res 32:7–13

Kato C, Inoue A, Horikoshi K (1996a) Isolating and characterizing deep-sea marine microorganisms. Trends Biotechnol 14:6–12

Kato C, Masui N, Horikoshi K (1996b) Properties of obligately barophilic bacteria isolated from a sample of deep-sea sediment from the Izu-Bonin trench. J Mar Biotechnol 4:96–99

Kato C, Smorawinska M, Sato T, Horikoshi K (1996c) Analysis of a pressure-regulated operon from the barophilic bacterium strain DB6705. Biosci Biotech Biochem 60:166–168

Kato C, Tamegai H, Ikegami A, Usami R, Horikoshi K (1996d) Open reading frame 3 of the barotolerant bacterium strain DSS12 is complementary with *cydD* in *Escherichia coli*: *cydD* functions are required for cell stability at high pressure. J Biochem 120:301–305

Kato C, Ikegami A, Smorawinska M, Usami R, Horikoshi K (1997a) Structure of genes in a pressure-regulated operon and adjacent regions from a barotolerant bacterium strain DSS12. J Mar Biotechnol (in press)

Kato C, Li L, Tamaoka J, Horikoshi K (1997b) Molecular analyses of the sediment of the 11000m-deep Mariana Trench. Extremophiles 1:117–124

MacDonell MT, Colwell RR (1985) Phylogeny of the Vibrionaceae, and recommendation for two new genera, *Listonella* and *Shewanella*. Syst Appl Microbiol 6:171–182

Miller VL, Taylor RK, Mekalanos JJ (1987) Cholera toxin transcriptional activator ToxR is a transmembrane DNA binding protein. Cell 48:271–279

Pelster B, Scheid P (1992) Countercurrent concentration and gas secretion in the fish swim bladder. Physiol Zool 65:1–16

Poole RK, Gibson F, Wu G (1994) The *cydD* gene product, component of a heterodimeric ABC transporter, is required for assembly of periplasmic cytochrome *c* and of cytochrome *bd* in *Escherichia coli*. FEMS Microbiol Lett 117:217–224

Rossello-Mora RA, Caccavo F Jr, Osterlehner K, Springer N, Spring S, Schuler D, Ludwig W, Amann R, Vanncanneyt M, Schleifer KH (1994) Isolation and taxonomic characterization of a halotolerant, facultatively iron-reducing bacterium. Syst Appl Microbiol 17:569–573

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Welch TJ, Bartlett DH (1996) Isolation and characterization of the structural gene for OmpL, a pressure-regulated porin-like protein from the deep-sea bacterium *Photobacterium* species strain SS9. J Bacteriol 178:5027–5031

Wirsen CO, Jannasch HW, Wakeham SG, Canuel EA (1987) Membrane lipids of a psychrophilic deep-sea bacterium. Curr Microbiol 14:319–322

Yayanos AA (1986) Evolutional and ecological implications of the properties of deep-sea barophilic bacteria. Proc Natl Acad Sci USA 83:9542–9546

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

Yayanos AA, Dietz AS, Van Boxtel R (1982) Dependence of reproduction rate on pressure as a hallmark of deep-sea bacteria. Appl Environ Microbiol 44:1356–1361

ZoBell CE, Johnson FH (1949) The influence of hydrostatic pressure on the growth and viability of terrestrial and marine bacteria. J Bacteriol 57:179–189

Zobell CE, Morita RY (1957) Barophilic bacteria in some deep-sea sediments. J Bacteriol 73:563–568