Microbial Ecology (2021) 81:617–629
https://doi.org/10.1007/s00248-020-01609-3

ENVIRONMENTAL MICROBIOLOGY

Transcriptomic Analysis Reveals Common Adaptation Mechanisms Under Different Stresses for Moderately Piezophilic Bacteria

Han Wang[1] · Yu Zhang[1,2,3] · Douglas H. Bartlett[4] · Xiang Xiao[1,3,5]

Received: 26 May 2020 / Accepted: 21 September 2020 / Published online: 30 September 2020

Abstract

Piezophiles, by the commonly accepted definition, grow faster under high hydrostatic pressure (HHP) than under ambient pressure and are believed to exist only in pressurized environments where life has adapted to HHP during evolution. However, recent findings suggest that piezophiles have developed a common adaptation strategy to cope with multiple types of stresses including HHP. These results raise a question on the ecological niches of piezophiles: are piezophiles restricted to habitats with HHP? In this study, we observed that the bacterial strains *Sporosarcina psychrophila* DSM 6497 and *Lysinibacillus sphaericus* LMG 22257, which were isolated from surface environments and then transferred under ambient pressure for half a century, possess moderately piezophilic characteristics with optimal growth pressures of 7 and 20 MPa, respectively. Their tolerance to HHP was further enhanced by $MgCl_2$ supplementation under the highest tested pressure of 50 MPa. Transcriptomic analysis was performed to compare gene expression with and without $MgCl_2$ supplementation under 50 MPa for *S. psychrophila* DSM 6497. Among 4390 genes or transcripts obtained, 915 differentially expressed genes (DEGs) were identified. These DEGs are primarily associated with the antioxidant defense system, intracellular compatible solute accumulation, and membrane lipid biosynthesis, which have been reported to be essential for cells to cope with HHP. These findings indicate no in situ pressure barrier for piezophile isolation, and cells may adopt a common adaptation strategy to cope with different stresses.

Keywords Common adaptation · Transcriptome · $MgCl_2$ · Piezophile · High pressure · Stress

Introduction

Piezophiles ("pressure-loving," previously known as barophiles), by the commonly accepted definition, grow faster under high hydrostatic pressure (HHP) than under ambient pressure (0.1 MPa) [1]. In 1979, Yayanos and coworkers succeeded in isolating piezophilic microorganisms from amphipods recovered from a depth of 5782 m in the Philippine Trench, which was the first report on the isolation of a piezophilic microbe [2]. Since then, many attempts have been made to isolate piezophilic microorganisms. However, only 78 piezophilic isolates have been reported to date, of which 51 strains are psychrophilic Gram-negative bacterial species that belong to the Gammaproteobacteria class, while 2 strains belong to the Alphaproteobacteria class (see Table S1). All of the piezophilic archaea that have been isolated thus far are thermophilic and fall within the Euryarchaeota and Thermotogae phyla, including the obligate piezophilic anaerobic hyperthermophilic archaeon *Pyrococcus yayanosii* CH1 [3]. Although these piezophiles are almost all from deep-sea sediments, marine animals, and hydrothermal vents, few

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00248-020-01609-3) contains supplementary material, which is available to authorized users.

✉ Yu Zhang
zhang.yusjtu@sjtu.edu.cn

[1] State Key Laboratory of Microbial Metabolism, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai, China

[2] School of Oceanography, Shanghai Jiao Tong University, Shanghai, China

[3] State Key Laboratory of Ocean Engineering, School of Naval Architecture, Ocean and Civil Engineering, Shanghai Jiao Tong University, Shanghai, China

[4] Center for Marine Biotechnology and Biomedicine, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA, USA

[5] Laboratory for Marine Biology and Biotechnology, Pilot National Laboratory for Marine Science and Technology (Qingdao), Qingdao, China

strains are isolated from the shallow depth of the sea below 2000 m. The optimal pressure ranges of these piezophiles are from 7 to 120 MPa. In addition, one archaeal strain (*Methanococcus thermolithotrophicus* DSM2095) was isolated approximately 0.5 m deep from a sandy, geothermally heated seafloor 2 m offshore of Italy, and the optimal growth pressure of this strain is 50 MPa [4, 5]. One bacterial strain (*Clostridium paradoxum* DSM7308) isolated from a sewage plant in Athens was recently defined as moderately piezophilic with an optimal pressure of 22 MPa [6, 7].

It has been challenging to illustrate the mechanisms by which microorganisms cope with high pressure. To date, the understanding of HHP adaptation in deep-sea piezophiles has been primarily based on studies of two model psychrophilic, piezophilic bacterial strains, *Photobacterium profundum* SS9 [8] and *Shewanella piezotolerans* WP3 [9]. For example, HHP stabilizes DNA hydrogen bonds and stacking interactions [10]; regulates the respiratory chains [11]; influences the structure and function of proteins [12]; regulates flagellar motility, affecting microbial predation, chemotaxis, and the formation of biofilms [13, 14]; and increases the proportions of mono- and polyunsaturated fatty acids in the membrane to prevent the loss of fluidity [15]. Therefore, HHP regulates the metabolism, growth, and evolution of microbes by influencing the stability of macromolecules and the catalytic activity of proteins.

To our knowledge, no gene/pathway has been identified to be unique for HHP adaptation, and recent findings suggest that piezophiles employ a common adaptation strategy to cope with multiple types of stresses, such as starvation, acid, salt, temperature, oxidation, and osmosis [16]. For example, piezophiles accumulate low molecular weight osmolytes, primarily organic solutes, which have been reported to be responsible for the responses to extreme cold, heat, salinity, and pH [17]. Bacteria have a strong antioxidative capability to promote adaptation to HHP and low temperature [18]. Previously, magnesium and calcium ions were shown to enhance barotolerance in at least two members of the bacterial genus *Streptococcus* [19]. To approach the in vivo rates of protein synthesis in vitro, supplementation with magnesium and sodium ions was necessary for cell extracts of *Pseudomonas bathycetes*, a deep-sea isolate obtained from the Mariana Trench [20]. Both ribosome stability and DNA gyrase–mediated DNA supercoiling in *Escherichia coli* are sensitive to magnesium ion concentrations at different pressures such that the increasing magnesium concentration also renders the two reactions pressure insensitive [21]. However, the combined effect of high concentrations of $MgCl_2$ and HHP and whether microorganisms adapted to high concentrations of $MgCl_2$ are piezophiles have not been fully investigated.

In this study, we addressed the questions above by investigating the piezophily of two strains isolated from ambient environments with high tolerance to $MgCl_2$. *Sporosarcina psychrophila* DSM 6497 and *Lysinibacillus sphaericus* LMG 22257 were obtained from DSMZ in Germany and BCCM/LMG in Belgium, respectively. *S. psychrophila* DSM 6497 was isolated from soil, mud, and water [22], while *L. sphaericus* LMG 22257 was isolated from calcareous sludge from a biocatalytic ureolytic calcification reactor [23]. Both of these strains have shown high tolerance to high concentrations of $MgCl_2$ and $CaCl_2$ and therefore have been used to induce carbonate precipitation for industrial applications [24, 25]. Thus, we chose these two strains as candidate microorganisms to study the combined effect of $MgCl_2$ and HHP to further explore the possibility that microorganisms adapted to high concentrations of $MgCl_2$ are piezophiles.

Materials and Methods

Strains and Culture Conditions

The bacterial strains used in the study are *S. psychrophila* DSM 6497 and *L. sphaericus* LMG 22257. *S. psychrophila* DSM 6497 was grown in a medium containing (per liter) 13 g of nutrient broth medium, and the pH was adjusted to 7.0. *L. sphaericus* LMG 22257 was grown in a medium containing (per liter) 13 g of nutrient broth medium and 10 g of NH_4Cl, and the pH was adjusted to 8.8. In addition, 5 g/L of $NaNO_3$ was added to the medium for the growth of the two strains at different pressures under static cultivation. Triplicate cultures were incubated for the physiological characterization of the two strains. To identify the optimal temperature, pH, and salinity for growth, the two strains were cultured in shake flasks under aerobic conditions at 0.1 MPa. Optimal temperature conditions were determined at the range of 4–37 °C and 4–50 °C for *S. psychrophila* DSM 6497 and *L. sphaericus* LMG 22257, respectively. Optimal salinities were determined at the range of 0.36–7% NaCl, and optimal pH conditions were determined at the range of 5–10 for the two strains. For analysis of the growth of the strains at HHP, each culture was grown to the mid-log phase in shake flasks at 0.1 MPa and at the abovementioned optimal temperature, pH, and salinity. The log-phase cultures were diluted in the same medium, and aliquots of the diluted cultures were withdrawn into 50-ml disposable plastic syringes with needles stuck into rubber stoppers. The syringes were then incubated at different hydrostatic pressures in stainless steel pressure vessels. High pressure was achieved and controlled by adding water through the hand-operated pump which was fitted with a pressure gauge as described in previous works [26, 27]. The growth of the strains at atmospheric pressure was also measured in the syringes under static cultivation as controls. A range of pressures (0.1, 7, 20, 30, 40, and 50 MPa) were tested for *S. psychrophila* DSM 6497, and a different pressure range

(0.1, 10, 20, 30, 40, and 50 MPa) was tested for *L. sphaericus* LMG 22257. The optimal growth temperature, salinity, and pH were used to test the pressure range under anoxic condition for the two strains. The high-pressure cultivation equipment was developed at Shanghai Jiao Tong University, China. Cell growth was measured by optical density (OD600) analysis. Growth rates were calculated by fitting the exponential-phase growth data with a simple linear regression. If necessary, different concentrations of $MgCl_2$ (0.1, 0.2, 0.25, 0.3, and 0.4 M) were added to the medium.

Phylogenetic Analysis and Alignment

The 16S rRNA gene sequences were aligned with the same region of the closest relatives. Sequences were obtained with NCBI BLAST. The sequences were aligned using Clustal X 2.0 [28]. A phylogenetic tree was constructed using the neighbor-joining method [29] with the model Kimura 2-parameter, and a bootstrap analysis with 1000 replicates was performed to assess the robustness of the tree. Finally, the tree was plotted using MEGA version 6.0 [30].

RNA Extraction and Sequencing

Mid-log phase cells of *S. psychrophila* DSM 6497 at 50 MPa with and without 0.25 M $MgCl_2$ were harvested to extract total RNA using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Genomic DNA was removed with DNase I, and ribosomal RNA (rRNA) was removed with a Ribo-off rRNA Depletion Kit (bacteria) (Vazyme Biotech Co., Ltd., Nanjing, China). A complementary DNA (cDNA) library was generated using a VAHTS™ Stranded mRNA-seq Library Prep Kit for Illumina (Vazyme Biotech Co., Ltd.). Paired-end reads were sequenced on the Illumina HiSeq X Ten (Illumina, San Diego, CA, USA) platform at Sangon Biotech (Shanghai Co., Ltd., China). Three independent biological replicates were prepared under each condition.

Bioinformatics Analysis

The sequencing quality controls of the raw reads produced by Illumina HiSeq X Ten were assessed using FastQC [31], and all samples passed. The clean data were trimmed from raw reads with Trimmomatic [32] for further analysis by removing adapter sequences, low-quality reads (Q value of < 20), ambiguous "*N*" nucleotides, and fragments of < 35 bp. Unique sequences were mapped to the *S. psychrophila* DSM 6497 genome with Bowtie 2 [33], and the relevant mapping information was summarized. The uniquely mapped reads were collected and analyzed with the DESeq2 package based on the Poisson distribution to identify the differentially expressed genes (DEGs) (estimation of gene expression levels based on the transcript per million (TPM) values [34]). The DEGs were selected by a threshold of false discovery rate (FDR) ≤ 0.05 and an absolute log2 ratio value ≥ 1 among the three biological replicates. Furthermore, the sequences of the DEGs were compared with those in the NCBI nonredundant (Nr), Gene Ontology (GO), Clusters of Orthologous Groups of proteins (COG), and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases to identify and annotate the obtained DEGs using the BLAST software. An FDR of ≤ 0.05 was used to determine the significance of the GO, COG, and KEGG enrichment analyses. The GO enrichment analysis was performed using the topGO package [35].

Quantitative Real-time PCR

To validate the RNA-Seq data, the expression levels of 10 randomly selected genes were quantified using quantitative real-time PCR (qRT-PCR). The primers were designed using Primer Express (Applied Biosystems, Foster City, CA, USA) and are listed in Table S2. According to the manufacturer's instructions for PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, Foster City, CA, USA), qRT-PCR was performed on a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using the following program: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. All qRT-PCR analyses were performed with three biological and three technical replicates. The RNA-Seq fold changes were plotted against the qRT-PCR fold changes, and the correlation coefficients (R^2) between these two data sets were calculated.

Results

The Two Bacterial Strains from Atmospheric Environments Exhibited Moderately Piezophilic Characteristics

The temperature, salinity, and pH ranges for *S. psychrophila* DSM 6497 and *L. sphaericus* LMG 22257 were first determined. For *S. psychrophila* DSM 6497, apparent growth was recorded at temperatures between 4 and 30 °C (optimum 25 °C), at pH 5–10 (optimum pH 7–9), and with 0.36–5% NaCl (optimum at 0.36%). For *L. sphaericus* LMG 22257, apparent growth was observed at temperatures between 4 and 45 °C (optimum 30 °C), at pH 6.3–10 (optimum pH 8.8) and with 0.36–7% NaCl (optimum at 0.36%) (Fig. S1). The growth pressures of *S. psychrophila* DSM 6497 and *L. sphaericus* LMG 22257 at the optimal temperature, pH, and NaCl were then measured. The pressure ranges of *S. psychrophila* DSM 6497 and *L. sphaericus* LMG 22257 were both 0.1 to 50 MPa, and they exhibited optimal growth at 7 and 20 MPa, respectively (Fig. 1a, b). In a previous study,

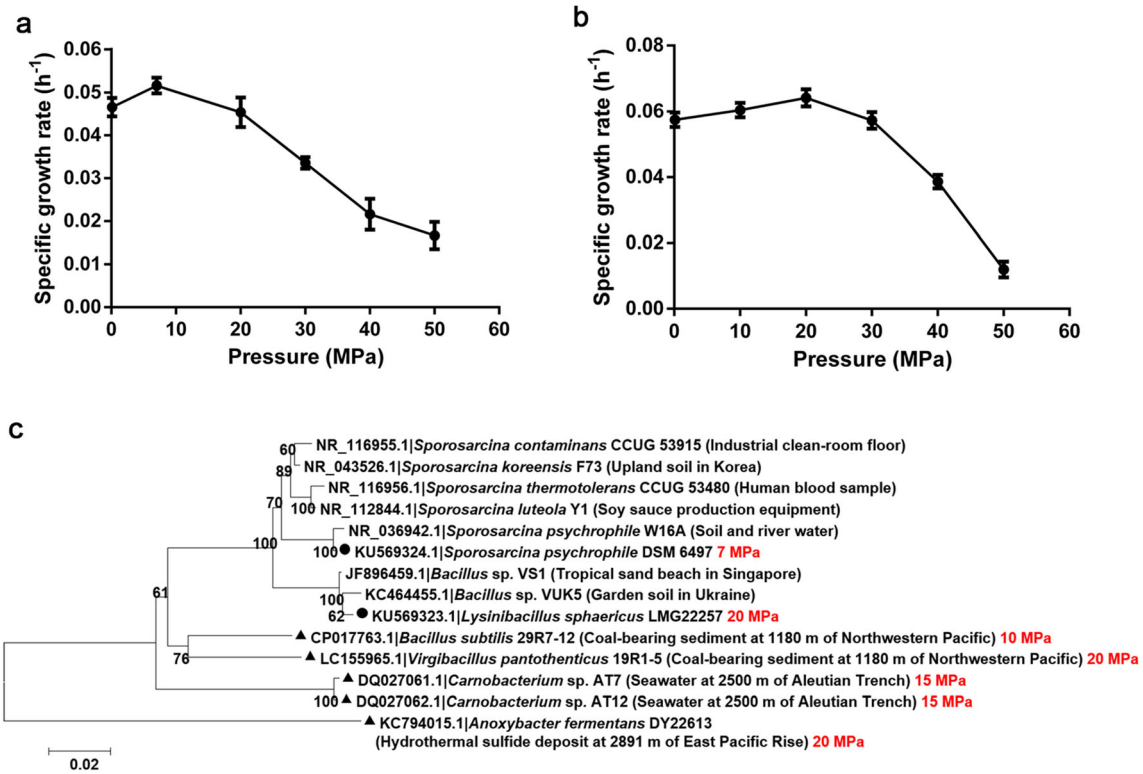

Fig. 1 Specific growth rates of **a** *S. psychrophila* DSM 6497 and **b** *L. sphaericus* LMG 22257 under different pressures. **c** Phylogenetic tree of the two strains based on 16S rRNA sequences. The related sequences and the isolation environment are shown. The piezophiles in this study are marked with black dots, and the piezophiles isolated from the deep sea are marked with black triangles. The red font indicates the optimal pressure of the piezophile. The tree was constructed with MEGA 6.0 using the neighbor-joining method with 1000 replicates of bootstrapping. Bar 0.02 substitutions per nucleotide position

moderately piezophilic bacteria are defined as bacteria displaying optimal growth at a pressure less than 40 MPa, and which are able to grow well at atmospheric pressure [11]. Therefore, the two strains are moderate piezophiles. Based on the phylogenetic tree of 16S rRNA genes, these strains clustered with strains isolated from ambient environments and were distinguished from other piezophiles isolated from environments under HHP (Fig. 1c).

Effects of $MgCl_2$ on the Growth of *S. psychrophila* DSM 6497 and *L. sphaericus* LMG 22257

Different concentrations of $MgCl_2$ (0, 0.1, 0.2, 0.25, 0.3, and 0.4 M) were supplemented into the culture medium to study its effect on the growth of *S. psychrophila* DSM 6497 and *L. sphaericus* LMG 22257. The growth of *S. psychrophila* DSM 6497 was inhibited to a certain extent at ambient pressure (0.1 MPa) in a medium supplemented with 0.1 to 0.4 M $MgCl_2$ (Fig. 2a, d). The growth of *S. psychrophila* DSM 6497 was also inhibited at optimal pressure (7 MPa) when the medium was supplemented with $MgCl_2$ (Fig. 2b, d). Furthermore, when cultured at the extreme HHP (50 MPa), the growth of this strain was stimulated in the medium supplemented with $MgCl_2$ at a concentration below 0.25 M (Fig. 2c, d). *L. sphaericus* LMG 22257 showed a similar inhibited phenomenon in its growth at 0.1 and 20 MPa supplemented with $MgCl_2$, and its growth at 50 MPa was also stimulated by the addition of $MgCl_2$ (Fig. S2). Therefore, the growth of these two moderately piezophilic strains tested under extreme pressure conditions was enhanced by supplementing the medium with $MgCl_2$.

Global Transcriptome Analysis

S. psychrophila DSM 6497 cells at the mid-logarithmic growth phase at 50 MPa in the medium supplemented with or without 0.25 M $MgCl_2$ were collected to extract RNA for sequencing. As shown in Fig. 2d, 0.25 M is the maximum concentration of $MgCl_2$ that has apparent stimulating effect on the bacterial growth under 50 MPa. Using the HiSeq X Ten platform, we generated an average of approximately 4.18 Gb from the $MgCl_2$-treated samples (ML) and 4.02 Gb clean reads from the control samples (CL). In this study, the average mapping ratios with the reference genome were 99.43% (ML) and 91.76% (CL), the average mapping ratios for genes were 96.70% (ML) and 91.28% (CL), and 4390 genes were detected for both samples (Table S3). An absolute |fold change| ≥ 2 and a q value ≤ 0.05 were the thresholds applied to assess the

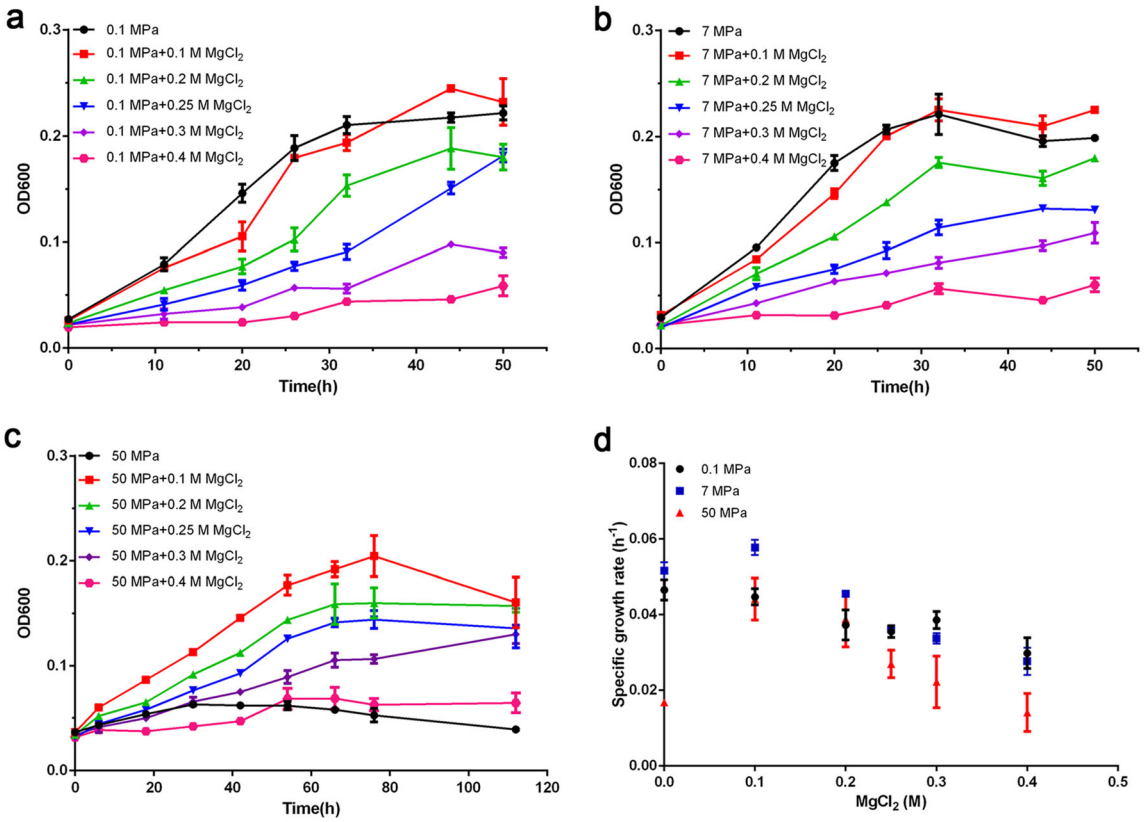

Fig. 2 Growth curves of *S. psychrophila* DSM 6497 under **a** 0.1 MPa, **b** 7 MPa, and **c** 50 MPa supplemented with different concentrations of $MgCl_2$, and **d** specific growth rates calculated based on the above data

significance of the differences in gene expression. A total of 915 DEGs were detected, where 449 DEGs were upregulated and 466 DEGs were downregulated between the ML and CL samples (Table S4). Subsequently, 10 selected genes were selected to validate the RNA-Seq results by qRT-PCR (R^2 = 0.9602) (Fig. S3), and the results confirmed the reliability of the transcriptome data.

GO and COG Analysis of DEGs

The functions of all the DEGs identified in this study were classified by GO assignments [36]. Of the total of 915 DEGs, 747 were annotated according to the GO database and are classified as "biological process," "cellular component," and "molecular function." The results covered 46 important and functional groups including 21 for biological process, 14 for cellular components, and 11 for molecular function (Fig. S4). The two largest subcategories observed in the three GO categories were as follows: "cellular process" and "metabolic process" in the "biological process" category, "cell part" in the "cellular component" category, and "catalytic activity" and "binding" in the "molecular function" category. GO terms enriched in all of the DEGs were identified using a threshold of q value ≤ 0.05. The results revealed that 25 GO terms were significantly enriched, all of which were distributed in the "biological processes" categories, and the functions of the GO terms were primarily involved in ion homeostasis and transport, thiamine metabolism, and IMP biosynthetic process. The analysis of the enriched DEGs in GO terms showed that most of the DEGs were upregulated genes.

The functions of the DEGs identified in this study were also classified by COG assignments. Based on a q value $\leq$ 0.05, the COGs enriched in all of the DEGs were primarily distributed in inorganic ion transport and metabolism and coenzyme transport and metabolism (Fig. 3).

Identification of Important DEGs Between ML and CL

Based on the results of the GO and COG analysis, DEGs between ML and CL were primarily related to inorganic ion transport and metabolism (ion homeostasis) and coenzyme transport and metabolism (thiamine metabolism). Therefore, the DEGs involved in these functions were further described in detail (Table 1).

Ion Transport and Metabolism

Most of the genes involved in iron transport and metabolism, including the iron dicitrate ABC transporter, iron siderophore transporter, ferrichrome ABC transporter, and heme ABC

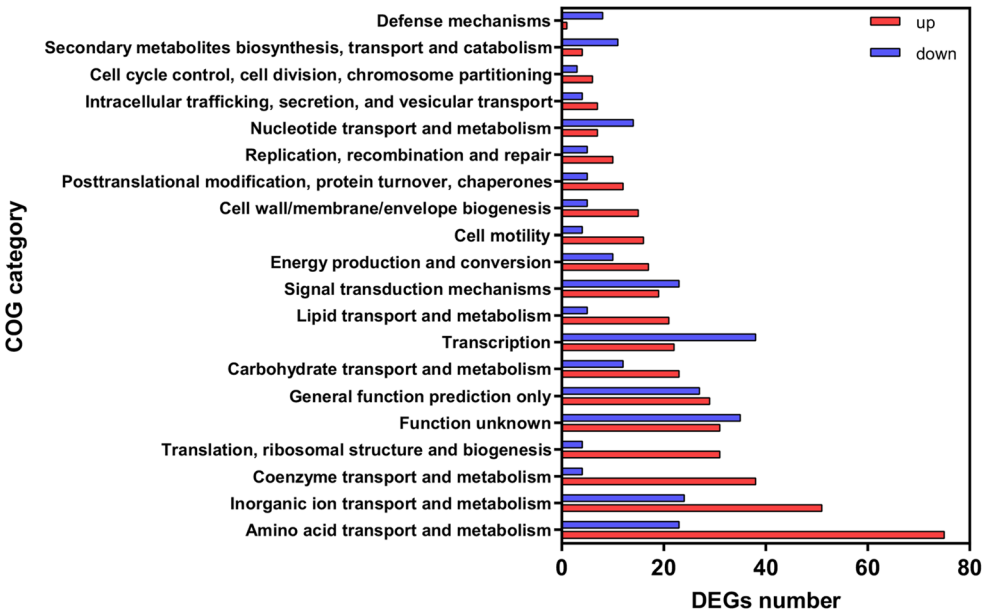

Fig. 3 COG categories of DEGs for *S. psychrophila* DSM 6497 between the 50-MPa incubation samples with 0.25 M $MgCl_2$ (ML) and the control samples without $MgCl_2$ (CL). The magenta histogram indicates upregulated genes of ML compared with those of CL; the blue histogram indicates downregulated genes of ML compared with those of CL

transporter, were upregulated by the addition of $MgCl_2$. The ferric uptake regulatory protein (*fur*, AZE41_13160) showed a 2.2-fold higher expression level in ML than in CL. Fur is typically considered a repressor of ferric uptake, and the promoters of iron uptake genes are negatively regulated by Fur [37]. In addition, genes related to cysteine, including cysteine synthase (AZE41_06755), cysteine ABC transporter permease (AZE41_06825), and cysteine ABC transporter permease (AZE41_04940), were observed to be upregulated. Furthermore, three genes annotated as Fe-S cluster assembly proteins (AZE41_04950, AZE41_04945, and AZE41_04935) were all upregulated. In terms of manganese ion transport-related DEGs, AZE41_07265, AZE41_07270, and AZE41_07260 were significantly upregulated. Moreover, the manganese regulatory gene AZE41_02440 (*mntR*) was downregulated. MntR is generally responsible for manganese ion homeostasis and belongs to the DtxR protein superfamily. This protein represses the expression of manganese ion transport proteins [38]. These results showed that the addition of $MgCl_2$ enhanced manganese ion transport.

Oxidative Stress Defense

For oxidative stress defense, the gene encoding catalase (AZE41_05625) was upregulated by 2.76-fold. Catalase primarily converts H_2O_2 to H_2O and O_2 to reduce damage to organisms and plays a crucial role at high concentrations of H_2O_2. The thioredoxin reductase genes (AZE41_14520 and AZE41_04900) were upregulated by 55.44- and 2.56-fold, respectively, and another thioredoxin gene (AZE41_03300) was downregulated 2.04-fold. Thioredoxin reductase, as an important antioxidant, could constitute a thioredoxin system with thioredoxin and NADPH; thus, oxidized thioredoxin would be reduced to reduced thioredoxin. The addition of $MgCl_2$ resulted in increased generation of reduced thioredoxin to defend against oxidative stress.

Compatible Solute Accumulation

The genes associated with glycine betaine synthesis and transport were upregulated and included the betaine-aldehyde dehydrogenase gene (AZE41_14080) and glycine betaine transport genes (AZE41_06795, AZE41_06790, and AZE41_06785), of which betaine-aldehyde dehydrogenase is the key enzyme in the synthesis of glycine betaine. In addition, the genes encoding proteins involved in the transport of ectoine and hydroxyectoine (AZE41_06830 and AZE41_10390) were upregulated. The upregulated genes also included those encoding glutamate synthase (AZE41_08330) and the glutamate/protein symporter (AZE41_18560), whereas the genes encoding the Na^+/glutamate symporter were downregulated. These results suggested that supplementation with $MgCl_2$ promoted the accumulation of compatible solutes in the cells.

Amino Acid Biosynthesis

The genes involved in valine, leucine, and isoleucine biosynthesis (AZE41_17870, AZE41_17875, AZE41_17900, AZE41_17905, AZE41_17895, AZE41_17880, AZE41_17885, AZE41_17890, and AZE41_12410) were all upregulated (Fig. S5). The branched chain amino acids valine, leucine, and isoleucine form the hydrophobic core of proteins and are precursors of branched chain fatty acid biosynthesis. The membrane lipid fatty acids of most *Bacillus* species are branched chain fatty acids that can maintain the fluidity of the

Table 1 Selected significant differentially expressed genes (DEGs) for *S. psychrophila* DSM 6497 between samples at 50 MPa with (ML) and without (CL) 0.25 M $MgCl_2$

Functions	Gene	Gene description	Mean TPM (ML)	Mean TPM (CL)	Log2 (FoldChange)	*q* value
Iron transport and metabolism	AZE41_02985	Iron ABC transporter	34.97	1.88	4.22	8.5E−12
	AZE41_08375	Iron ABC transporter	130.31	8.61	3.92	7.09E−28
	AZE41_17920	Iron ABC transporter ATP-binding protein	145.52	14.37	3.34	4.68E−17
	AZE41_20900	Iron ABC transporter ATP-binding protein	33.45	2.61	3.68	4.02E−17
	AZE41_02255	Iron ABC transporter ATP-binding protein	266.68	16.44	4.02	3E−22
	AZE41_02245	Iron ABC transporter permease	253.97	21.56	3.56	4.33E−13
	AZE41_20905	Iron ABC transporter permease	15.88	1.88	3.08	1.35E−06
	AZE41_02250	Iron ABC transporter permease	200.04	12.48	4.00	1.38E−27
	AZE41_08385	Iron ABC transporter permease	65.52	5.43	3.59	2.08E−10
	AZE41_02980	Iron ABC transporter permease	130.65	6.11	4.42	1.69E−49
	AZE41_08370	ATP-binding protein	150.32	18.72	3.01	7.45E−21
	AZE41_02990	Iron siderophore-binding protein	30.33	1.95	3.96	4.51E−23
	AZE41_14560	Ferrichrome ABC transporter permease	80.38	2.06	5.29	1.13E−26
	AZE41_17930	Heme ABC transporter substrate-binding protein IsdE	31.22	1.83	4.09	0.015
	AZE41_13160	Fe^{2+}/Zn^{2+} uptake regulation proteins fur	99.80	45.20	1.14	0.0097
	AZE41_02260	ABC transporter	62.62	5.10	3.62	1.2E−26
	AZE41_18740	ABC transporter	53.04	1.87	4.82	3.6E−24
	AZE41_21700	ABC transporter	161.66	22.53	2.84	2.5E−21
	AZE41_19685	ABC transporter ATP-binding protein	61.69	19.50	1.66	7.12E−08
	AZE41_17925	ABC transporter permease	48.88	5.46	3.16	6.82E−17
	AZE41_14970	ABC transporter permease	7.35	3.12	1.24	0.044
	AZE41_14550	ABC transporter substrate-binding protein	68.23	1.84	5.22	1.16E−55
	AZE41_14530	ABC transporter substrate-binding protein	62.05	3.23	4.26	4.35E−19
	AZE41_18845	ABC transporter substrate-binding protein	50.86	1.48	5.10	1.68E−29
	AZE41_06755	Cysteine synthase	117.34	12.68	3.21	5.52E−09
	AZE41_06825	Cysteine ABC transporter permease	221.45	86.62	1.35	0.0044
	AZE41_04940	Cysteine desulfurase	1982.91	918.54	1.11	0.00092
	AZE41_04950	Fe-S cluster assembly protein SufB	1237.89	561.64	1.14	0.00039
	AZE41_04945	Fe-S cluster assembly scaffold protein NifU	1323.75	581.17	1.19	0.00016
	AZE41_04935	Fe-S cluster assembly protein SufD	1369.07	637.52	1.10	0.0013
Manganese transport	AZE41_07265	Manganese ABC transporter permease	166.57	6.50	4.68	3.46E−55
	AZE41_07270	Manganese ABC transporter substrate-binding protein	164.25	7.53	4.45	1.27E−28
	AZE41_07260	Manganese transporter	34.86	1.32	4.72	0.0051
	AZE41_02440	Manganese transport transcriptional regulator	8.27	40.05	− 2.28	1.79E−05
Antioxidant activity	AZE41_07325	Catalase	16.05	5.76	1.47	0.020
	AZE41_14520	Thioredoxin reductase	160.59	2.90	5.80	4.85E−60
	AZE41_04900	Thioredoxin reductase	25.99	10.15	1.36	0.0090
	AZE41_03300	Thioredoxin	178.12	363.48	− 1.03	2.13E−05
	AZE41_00005	Glutathione peroxidase	18.06	40.78	− 1.17	0.0042
Compatible solutes	AZE41_14080	Betaine-aldehyde dehydrogenase	7.85	1.87	2.08	4.91E−06
	AZE41_06795	ABC-type proline/glycine betaine transport systems, periplasmic components	33.11	4.72	2.81	2.69E−09
	AZE41_06790	ABC-type proline/glycine betaine transport system, permease component	96.56	17.38	2.47	0.00016
	AZE41_06785	ABC-type proline/glycine betaine transport system, ATPase component	65.34	14.75	2.15	4.94E−06
	AZE41_06830	Ectoine/hydroxyectoine ABC transporter ATP-binding protein EhuA	46.79	18.1	1.37	0.020
	AZE41_10390		6.78	1.42	2.26	0.0061

Table 1 (continued)

Functions	Gene	Gene description	Mean TPM (ML)	Mean TPM (CL)	Log2 (FoldChange)	*q* value
		Ectoine/hydroxyectoine ABC transporter ATP-binding protein EhuA				
	AZE41_08330	Glutamate synthase	9.74	3.21	1.60	0.0076
	AZE41_18560	Glutamate:protein symporter	25.18	3.33	2.92	1.8E−07
	AZE41_20125	Na^+/glutamate symporter	4.54	43.10	− 3.25	1.43E−16
	AZE41_17845	Na^+/glutamate symporter	10.95	23.24	− 1.09	0.00031
Membrane lipids	AZE41_17870	Serine/threonine dehydratase	111.10	48.95	1.18	0.0059
	AZE41_17875	3-Isopropylmalate dehydratase small subunit	150.12	69.77	1.11	0.0078
	AZE41_17900	Acetolactate synthase small subunit	71.78	31.95	1.17	0.0014
	AZE41_17905	Acetolactate synthase catalytic subunit	82.97	27.76	1.58	4.38E−07
	AZE41_17895	Ketol-acid reductoisomerase	46.61	12.89	1.85	4.58E−07
	AZE41_17880	Isopropylmalate isomerase	192.01	65.09	1.56	2.52E−06
	AZE41_17885	3-Isopropylmalate dehydrogenase	170.50	60.14	1.50	2.15E−06
	AZE41_17890	2-Isopropylmalate synthase	139.85	65.04	1.10	0.0021
	AZE41_12410	Leucine dehydrogenase	301.64	127.63	1.24	8.51E−07
	AZE41_07075	β-Ketoacyl-ACP synthase II	18.28	6.44	1.50	0.0010
Thiamine metabolism	AZE41_01095	Hydroxymethylpyrimidine/phosphomethylpyrimidine kinase	71.56	25.92	1.46	2.43E−05
	AZE41_01100	Thiamine phosphate synthase	72.92	21.52	1.76	3.31E−05
	AZE41_02005	Thiamine biosynthesis protein MoeB	461.32	96.96	2.25	4.74E−06
	AZE41_02010	Thiazole synthase	348.39	72.49	2.26	2.78E−07
	AZE41_02015	Thiamine biosynthesis protein ThiS	69.95	18.45	1.92	0.00052
	AZE41_02020	Hypothetical protein	572.67	191.91	1.58	0.00084
	AZE41_02025	Hypothetical protein	717.68	200.42	1.84	0.00067
	AZE41_05955	Acetylornithine deacetylase	5.94	1.40	2.09	0.0047
	AZE41_07275	ABC transporter substrate-binding protein	11.80	2.06	2.52	0.010
	AZE41_07285	ABC transporter ATP-binding protein	18.66	3.44	2.44	7.37E−06
	AZE41_07290	Thiaminase II	18.02	2.28	2.98	1.62E−05
	AZE41_20215	Thiamine biosynthesis protein ThiF	101.31	49.1	1.04	0.045

membrane [39]. Furthermore, the genes encoding β-ketoacyl-ACP synthase II and enzymes involved in thiamine pyrophosphate (TPP) biosynthesis were upregulated. β-Ketoacyl-ACP synthase II can catalyze cis-9-octadecenoic acid biosynthesis, and TPP, as a cofactor of the enzyme, plays a significant role in the decarboxylic reaction of α-keto acids.

Discussion

Moderate Piezophiles from the Shallow Sea

In this study, the two piezophiles isolated from ambient environments showed moderately piezophilic characteristics. In 1949, Zobell and Johnson began investigating the effects of hydrostatic pressure on microbial activities. They first used the term "barophilic" to define organisms whose optimal growth occurred at pressures higher than 0.1 MPa or exhibited a requirement for increased pressure for growth [40]. The term "piezophilic" has been proposed to replace "barophilic," as the prefixes "baro" and "piezo," derived from Greek, mean "weight" and "pressure," respectively [41]. Thus, the word "piezophilic" is more suitable than "barophilic" to describe bacteria that grow better at high pressure than at atmospheric pressure. For the two strains assessed in our study, the two isolates grew better at high pressure than at atmospheric pressure (Fig. 1a, b). Therefore, we believe that the two strains are moderately piezophilic microorganisms. Currently, only 78 piezophiles have been isolated, excluding the two piezophiles in this study. Detailed information on these piezophiles is summarized in Table S1. After calculating the ratio of the specific growth rate under optimal and atmospheric pressures, we also identified 7 strains that could be defined as moderate piezophiles (Table S5). Previous research supported barophily

as a ubiquitous characteristic of bacteria from the cold, deep sea and from depths between 1957 and 10,476 m, and bacteria from the shallower depths of deep seas should not be barophilic by extrapolation [42]. However, with the increase in isolated piezophiles, 9 piezophiles have been isolated from depths of less than 2000 m: *Desulfovibrio profundus* 500-1^T, *Desulfovibrio profundus* 80-55^T, *Desulfovibrio piezophilus* C1TLV30^T, *Bacillus subtilis* 29R7-12, *Virgibacillus pantothenticus* 19R1-5, *Thermosipho japonicus* IHB1^T, *Methanococcus thermolithotrophicus* DSM2095, *Palaeococcus ferrophilus* DMJ^T, and *Thermococcus peptonophilus* OG-1^T (Table S1). This finding suggests that piezophiles also exist in the shallow depths of seas. In addition, a correlation between the isolation depth and optimal growth pressure was established (Fig. 4). The isolated piezophilic archaea are primarily mesophilic and thermophilic, and their optimal growth pressures are higher than the in situ pressure of their isolating environments; meanwhile, the deeper and hot biosphere is assumed to be their ultimate source. The isolated bacteria are primarily psychrophilic and mesophilic, and their optimal growth pressures are lower than the in situ pressure of their natural habitats. In this study, we reported *S. psychrophila* DSM 6497 and *L. sphaericus* LMG 22257 as moderately piezophilic bacteria originating from the surface environment, as are the other two isolated strains *Methanococcus thermolithotrophicus* DSM2095 [4, 5] and *Clostridium paradoxum* DSM7308 [6, 7]. This discovery of the piezophiles above from the surface environments provides an alternative strategy for exploring piezophilic microbial resources.

Transcriptomic Response to $MgCl_2$ Supplementation Under HHP

Surprisingly, Mg^{2+} transporter genes were not among the DEGs detected, indicating tight control of the intracellular Mg^{2+} concentration, which was unfortunately not monitored in this study. Organisms must maintain physiological levels of Mg^{2+}, which is crucial for the stabilization of membranes and ribosomes and the neutralization of nucleic acids, and it is used as a cofactor in various enzymatic reactions [43]. Furthermore, the supplementation of Mg^{2+} triggers various cellular responses, such as ion transport and biomolecular synthesis.

Based on the RNA-Seq results, ion transport and utilization are important for *S. psychrophila* DSM 6497 to adapt to $MgCl_2$ supplementation under HHP. Iron is essential for microorganisms as a cofactor of proteins involved in oxidative phosphorylation, photosynthesis, nitrogen fixation, and DNA synthesis, and it exists at suboptimal concentrations owing to its low solubility under aerobic neutral or alkaline environments. Microorganisms have a high-affinity siderophore transport system that can discharge iron ions into the environment and uptake iron chelators [44, 45]. Moreover, excess iron is toxic to microorganisms. Under oxygen-rich conditions resulting from the Fenton reaction, iron is a source of dangerous radicals [46]. Therefore, the intracellular iron level needs to be carefully adjusted. The key protein regulating the balance of intracellular iron ions is Fur, a repressor protein. Under iron-rich conditions, Fur inhibits transcription from virtually all the genes and operons repressed by the metal. In contrast, when iron is scarce, the equilibrium is shifted toward the release of Fe^{2+}, RNA polymerase accesses cognate promoters, and the genes for the biosynthesis of siderophores and other iron-related functions are expressed [44]. In a previous study, high-salinity-induced iron limitation of *B. subtilis* cells not only caused a derepression of the Fur-controlled dhb operon but also affected other genes involved in iron homeostasis, including *fhuD*, *fhuB*, *feuA*, *yfiY*, and *yfmC* [47]. Subsequent studies have shown that not all iron limitation-induced genes are regulated by Fur, and this also reflects the multiple effects of iron limitation defects in *B. subtilis* [48]. In our study, the addition of $MgCl_2$ enhanced the transport of iron ions as well as the transcriptional level of Fur, indicating the co-occurrence of iron limitation and toxicity. Furthermore, the uptake of manganese was stimulated, which helps to prevent protein damage from the oxidation stress generated from the Fenton reaction [49]. Antioxidative genes/proteins are commonly

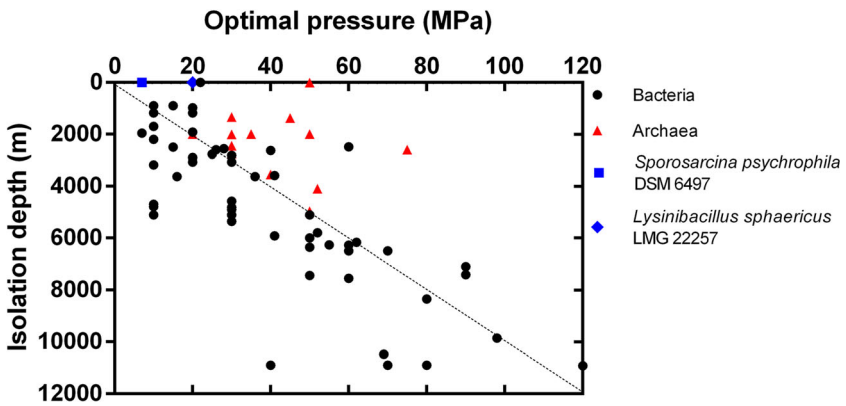

Fig. 4 The optimal pressures of piezophiles isolated thus far in relation to the isolation depth of the in situ environment. The black dots represent bacterial piezophiles, and the red triangles represent archaeal piezophiles. The blue square is *S. psychrophila* DSM 6497, and the blue diamond is *L. sphaericus* LMG 22257

present in piezophiles. In our study, the antioxidant defense systems, including the catalase and thioredoxin systems, were both enhanced to defend against oxidative stress in the cells at extreme pressure with $MgCl_2$ supplementation. In addition, several genes related to cysteine were upregulated. Cysteine plays an important role in iron transport, because a high level of cysteine is an essential component for siderophore biosynthesis in iron transport [50]. In bacteria, cysteine is primarily derived from the transformation of other amino acids and directly obtained extracellular cysteine. Furthermore, cysteine desulfurase can provide sulfur, which is subsequently incorporated during in vivo Fe-S cluster synthesis [51]. The genes encoding proteins involved in Fe-S cluster assembly were also upregulated, suggesting an enhancement of electron transport to promote ATP generation and aid in the growth of *S. psychrophila* DSM 6497 at extreme pressure. Preliminary research focusing on *Streptococcus faecalis* revealed that growth inefficiency under pressure could be due to a substantially reduced ATP supply [52].

The genes associated with glycine betaine transport and synthesis, ectoine/hydroxyectoine transport, and glutamate biosynthesis were all upregulated, suggesting the accumulation of intracellular compatible solutes with the addition of $MgCl_2$. Compatible solutes have protective properties, such as protecting cell metabolism by serving as antioxidants that scavenge free radicals and reactive oxygen species generated under stress treatments and stabilizing the structure of biological macromolecules (proteins and membranes) [53]. Glycine betaine is one of the most widespread compatible solutes in bacteria, which generally take it up from the complex medium. The accumulation of glycine betaine in cells typically occurs when microorganisms are subjected to hyperosmotic conditions [54]. Glycine betaine amassed in *S. psychrophila* DSM 6497 may protect against osmotic stress after the addition of $MgCl_2$. Piezophiles tend to accumulate compatible solutes in cells at high pressure. In *P. profundum* SS9, cells preferentially accumulate β-hydroxybutyrate and β-hydroxybutyrate oligomers at optimal growth pressure (28 MPa) [55]. In another study, intracellular glutamate concentration increased as the hydrostatic pressure increased, confirming the role of glutamate as a piezolyte for the piezophiles *Desulfovibrio hydrothermalis* AM13 [56] and *Desulfovibrio piezophilus* C1TLV30^T [57]. Previously, the function of ectoine as a compatible solute in bacterial cells was primarily believed to maintain osmotic homeostasis in a high-salt environment [58]. Recently, ectoine was demonstrated to react with hydroxyl radicals and showed antioxidant features as a hydroxyl radical scavenger [59]. Cells under HHP suffer from an imbalance in oxidation and reduction, and the excessive reactive oxygen species (ROS) generated from this imbalance can cause cellular damage [16]. Consequently, an increase of $MgCl_2$ concentration can cause the accumulation of compatible solutes, such as ectoine/hydroxyectoine and glutamate, in cells that are resistant to cell damage induced by extreme pressure.

The genes related to membrane lipid biosynthesis, including branched chain amino acid biosynthesis, TPP biosynthesis, and β-ketoacyl-ACP synthase II, were all upregulated after the addition of $MgCl_2$. Branched fatty acids are important components that maintain the fluidity of the membrane. TPP is a cofactor of acetylated acid synthase, the key enzyme in branched amino acid biosynthesis [60]. The *fabF* gene encodes the enzyme β-ketoacyl-ACP synthase II, which catalyzes the production of cis-vaccenic acid [61]. Chemical mutants of *P. profundum* SS9 producing diminished amounts of monounsaturated fatty acids are pressure sensitive [62], as is a strain with a *fabF* mutation [63]. The increases in branched and monounsaturated fatty acid production promote the growth of *S. psychrophila* DSM 6497 under extreme pressure with $MgCl_2$ treatment. Moreover, eicosapentaenoic acid (EPA) has been demonstrated as essential polyunsaturated fatty acids for *Shewanella piezotolerans* WP3 to grow under HHP [64]. However, the genes related to polyunsaturated fatty acids were not among the DEGs in *S. psychrophila* DSM 6497, suggesting their insignificant role to cope with the high concentration of $MgCl_2$ under elevated pressure.

Common Adaptation Strategy for Piezophiles

Summarizing the transcriptomic results, we propose a schematic diagram to explain the common adaptation mechanism of piezophiles at extreme pressure treated with $MgCl_2$ (Fig. 5). Based on the hypothesis of a common adaptation strategy, microorganisms simultaneously adapt to HHP along with multiple other stresses [16]. In this study, we focused on the correlated effects of HHP and elevated concentrations of $MgCl_2$. Supplementation with $MgCl_2$ upregulated the expression of genes involved in the antioxidant defense system, compatible solute accumulation, and membrane lipid biosynthesis, and the apparent stimulation of growth under HHP was observed. These stimulated pathways have been shown to be beneficial for cells to cope with HHP. Antioxidant defense is commonly observed in bacterial responses to environmental stresses, including cold, acid, heat, heavy metal, high salt concentrations, and UV. For example, antioxidation is essentially helpful for the growth of the deep-sea bacterial strain *S. piezotolerans* WP3 under HHP and low temperature [18]. Piezophiles have also been confirmed to accumulate compatible solutes, primarily organic solutes, which are responsible for the responses to extreme cold, heat, salinity, and pH [17]. Deep-sea microbes are thought to maintain membrane fluidity and preserve membrane fluidity at HHP and low temperature by increasing the proportion of unsaturated fatty acids in their lipids, because low temperature and HHP both have related and synergistic effects on biological membranes [65]. Our data support the idea that piezophiles have developed a

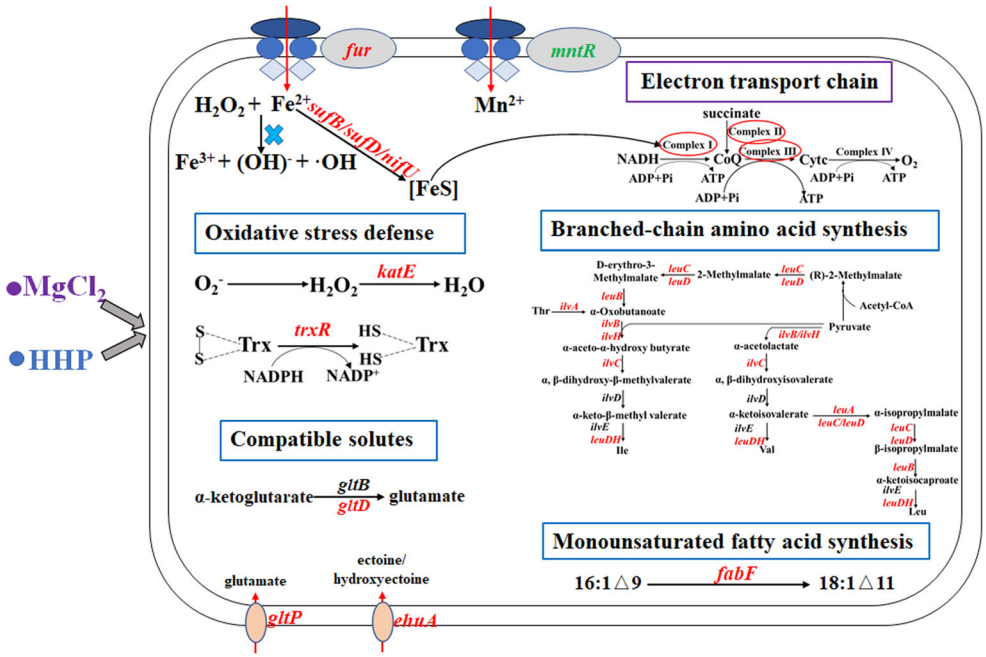

Fig. 5 Schematic diagram of DEGs for *S. psychrophila* DSM 6497 between the 50 MPa incubation samples with 0.25 M $MgCl_2$ (ML) and the control samples without $MgCl_2$ (CL). The upregulated genes are shown in red, and the downregulated genes are shown in green. The functions or pathways in the blue box represent the strategies of microorganisms in coping with the stresses of HHP and $MgCl_2$, whereas those in the purple box represent the strategy of microbes under high concentrations of $MgCl_2$. HHP, high hydrostatic pressure; *fur*, gene encoding ferric uptake regulation proteins; *mntR*, gene encoding manganese transport transcriptional regulator; *katE*, gene encoding catalase; *trxR*, gene encoding thioredoxin reductase; *gltB/gltD*, genes encoding glutamate synthase; *sufB/sufD/nifD*, genes encoding Fe-S cluster assembly protein; *gltP*, gene encoding glutamate:protein symporter; *ehuA*, gene encoding ectoine/hydroxyectoine ABC transporter ATP-binding protein; *fabF*, gene encoding β-ketoacyl-ACP synthase II; *ilvA*, gene encoding threonine dehydratase; *ilvB*, gene encoding acetolactate synthase catalytic subunit; *ilvH*, gene encoding acetolactate synthase small subunit; *ilvC*, gene encoding ketol-acid reductoisomerase; *ilvD*, gene encoding dihydroxy-acid dehydratase; *leuA*, gene encoding 2-isopropylmalate synthase; *leuB*, gene encoding 3-isopropylmalate dehydrogenase; *leuC*, gene encoding isopropylmalate isomerase; *leuD*, gene encoding 3-isopropylmalate dehydratase small subunit; *leuDH*, gene encoding 3-isopropylmalate dehydratase small subunit; [FeS], Fe-S cluster; ·OH, hydroxyl radical; CoQ, coenzyme Q; Cytc, cytochrome c; Trx, thioredoxin; $TrxS_2$, oxidized thioredoxin; $Trx(SH)_2$, reduced thioredoxin; TrxR, thioredoxin reductase; 16:1Δ9, cis-hexadecenoic acid; 18:1Δ11, cis-octadecenoic acid

common adaptation strategy to cope with HHP, which also helps them cope with other types of environmental stresses. This phenomenon may also be true for all extremophiles.

Conclusion

The rediscovery of two moderately piezophilic bacteria from ambient environments extends the window of isolation depth of known piezophiles. The growth of the two strains was also enhanced under the extreme pressure of 50 MPa after the addition of $MgCl_2$ to the medium. By comparing the transcriptome data of the sample with or without $MgCl_2$ at 50 MPa for *S. psychrophila* DSM 6497, we identified 915 DEGs that were primarily involved in the antioxidant defense system, intracellular compatible solute accumulation, and branched chain and monounsaturated fatty acid biosynthesis, all of which have been reported to be essential for cells to cope with HHP. These findings support the hypothesis that piezophiles possess a common adaptation strategy to cope with various environmental stresses.

Author Contributions HW, YZ, and XX were involved in experimental design. HW conducted the experimental procedure and analyzed the data. HW, YZ, and XX wrote the manuscript. DB made comments and suggestions to improve the manuscript. All authors read and approved the manuscript.

Funding This study was financially supported by the National Key Research and Development Program of China (No. 2018YFC0309800) and the National Natural Science Foundation of China (grant nos. 41776173, 41530967, 91951117, and 41921006).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Statement This article does not contain any studies with human participants or animals performed by any of the authors.

Data Accessibility All sequence reads have been deposited in the NCBI Sequence Read Archive (SRA) database (https://www.ncbi.nlm.nih.gov/sra) and are accessible through Bioproject accession number PRJNA577046.

References

1. Kato C, Nogi Y, Arakawa S (2008) Isolation, cultivation, and diversity of deep-sea piezophiles. In: Michiels C, Bartlett DH, Aersten A (eds) High-pressure microbiology. ASM, Washington, pp 203–217
2. 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. https://doi.org/10.1126/science.205.4408.808
3. Zeng X, Birrien J-L, Fouquet Y, Cherkashov G, Jebbar M, Querellou J, Oger P, Cambon-Bonavita M-A, Xiao X, Prieur D (2009) *Pyrococcus* CH1, an obligate piezophilic hyperthermophile: extending the upper pressure-temperature limits for life. ISME J 3:873–876. https://doi.org/10.1038/ismej.2009.21
4. Huber H, Thomm M, König H, Thies G, Stetter K (1982) *Methanococcus thermolithotrophicus*, a novel thermophilic lithotrophic methanogen. Arch Microbiol 132:47–50. https://doi.org/10.1007/bf00690816
5. Bernhardt G, Jaenicke R, Lüdemann HD, König H, Stetter KO (1988) High pressure enhances the growth rate of the thermophilic archaebacterium *Methanococcus thermolithotrophicus* without extending its temperature range. Appl Environ Microbiol 54:1258–1261. https://doi.org/10.1128/aem.54.5.1258-1261.1988
6. Li Y, Mandelco L, Wiegel J (1993) Isolation and characterization of a moderately thermophilic anaerobic alkaliphile, Clostridium paradoxum sp. nov. Int J Syst Evol Microbiol 43:450–460. https://doi.org/10.1099/00207713-43-3-450
7. Scoma A, Garrido-Amador P, Nielsen SD, Røy H, Kjeldsen KU (2019) The polyextremophilic acterium Clostridium paradoxum attains piezophilic traits by modulating its energy metabolism and cell membrane composition. Appl Environ Microbiol 85:e00802–e00819. https://doi.org/10.1128/aem.00802-19
8. Lauro FM, Eloe EA, Liverani N, Bertoloni G, Bartlett DH (2005) Conjugal vectors for cloning, expression, and insertional mutagenesis in Gram-negative bacteria. BioTechniques 38:708–712. https://doi.org/10.2144/05385bm06
9. Chen Y, Wang F, Xu J, Mehmood MA, Xiao X (2011) Physiological and evolutionary studies of NAP systems in Shewanella piezotolerans WP3. ISME J 5:843–855. https://doi.org/10.1038/ismej.2010.182
10. Macgregor RB (2002) The interactions of nucleic acids at elevated hydrostatic pressure. BBA- Protein Struct M 1595:266–276. https://doi.org/10.1016/s0167-4838(01)00349-1
11. Kato C, Qureshi MH, Horikoshi K (1999) Pressure response in deep-sea piezophilic bacteria. J Mol Microbiol Biotechnol 1:87–92
12. Gross M, Jaenicke R (1994) Proteins under pressure. Eur J Biochem 221:617–630. https://doi.org/10.1111/j.1432-1033.1994.tb18774.x
13. Campanaro S, Vezzi A, Vitulo N, Lauro FM, D'Angelo M, Simonato F, Cestaro A, Malacrida G, Bertoloni G, Valle G, Bartlett DH (2005) Laterally transferred elements and high pressure adaptation in Photobacterium profundum strains. BMC Genomics 6:122. https://doi.org/10.1186/1471-2164-6-122
14. Wang F, Wang J, Jian H, Zhang B, Li S, Wang F, Zeng X, Gao L, Bartlett DH, Yu J, Hu S, Xiao X (2008) Environmental adaptation: genomic analysis of the piezotolerant and psychrotolerant deep-sea iron reducing bacterium Shewanella piezotolerans WP3. PLoS One 3:e1937. https://doi.org/10.1371/journal.pone.0001937
15. Winter R, Jeworrek C (2009) Effect of pressure on membranes. Soft Matter 5:3157–3173. https://doi.org/10.1039/B901690B
16. Zhang Y, Li X, Bartlett DH, Xiao X (2015) Current developments in marine microbiology: high-pressure biotechnology and the genetic engineering of piezophiles. Curr Opin Biotechnol 33:157–164. https://doi.org/10.1016/j.copbio.2015.02.013
17. Ambily Nath IV, Loka Bharathi PA (2011) Diversity in transcripts and translational pattern of stress proteins in marine extremophiles. Extremophiles 15:129–153. https://doi.org/10.1007/s00792-010-0348-x
18. Xie Z, Jian H, Jin Z, Xiao X (2018) Enhancing the adaptability of the deep-sea bacterium Shewanella piezotolerans WP3 to high pressure and low temperature by experimental evolution under H2O2 stress. Appl Environ Microbiol 84:e02342–e02317. https://doi.org/10.1128/aem.02342-17
19. Marquis RE, ZoBell CE (1971) Magnesium and calcium ions enhance barotolerance of Streptococci. Arch Microbiol 79:80–92. https://doi.org/10.1007/BF00412043
20. Landau J, Pope D (1980) Recent advances in the area of barotolerant protein synthesis in bacteria and implications concerning barotolerant and barophilic growth. Adv Aquat Microbiol 2:49–76
21. Chilukuri LN, Fortes PG, Bartlett DH (1997) High pressure modulation of Escherichia coli DNA gyrase activity. Biochem Bioph Res Co 239:552–556
22. Larkin J, Stokes J (1966) Isolation of psychrophilic species of Bacillus. J Bacteriol 91:1667–1671
23. Dick J, De Windt W, De Graef B, Saveyn H, Van der Meeren P, De Belie N, Verstraete W (2006) Bio-deposition of a calcium carbonate layer on degraded limestone by Bacillus species. Biodegradation 17:357–367
24. Larkin JM, Stokes JL (1967) Taxonomy of psychrophilic strains of Bacillus. J Bacteriol 94:889–895. https://doi.org/10.1128/jb.94.4.889-895.1967
25. Micallef R, Vella D, Sinagra E, Zammit G (2016) Biocalcifying Bacillus subtilis cells effectively consolidate deteriorated Globigerina limestone. J Ind Microbiol Biotechnol 43:941–952. https://doi.org/10.1007/s10295-016-1768-0
26. Yayanos AA, Boxtel RV (1982) Coupling device for quick high-pressure connections to 100 MPa. Rev Sci Instrum 53:704–705. https://doi.org/10.1063/1.1137011
27. Li S, Xiao X, Li J, Luo J, Wang F (2006) Identification of genes regulated by changing salinity in the deep-sea bacterium Shewanella sp. WP3 using RNA arbitrarily primed PCR. Extremophiles 10:97–104. https://doi.org/10.1007/s00792-005-0476-x
28. Larkin MA, Blackshields G, Brown N, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948. https://doi.org/10.1093/bioinformatics/btm404
29. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454
30. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729. https://doi.org/10.1093/molbev/mst197
31. Andrews S (2010) FastQC: a quality control tool for high throughput sequence data
32. Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170
33. Langmead B, Salzberg SL (2012) Fast gapped-read alignment with bowtie 2. Nat Methods 9:357–359
34. Wagner GP (2012) Measurement of mRNA abundance using RNA-seq data RPKM measure is inconsistent among samples. Theory Biosci 131:281–285. https://doi.org/10.1007/s12064-012-0162-3
35. Alexa A, Rahnenfuhrer J (2016) topGO: enrichment analysis for gene ontology. R package version 2.30.1
36. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP,

Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G (2000) Gene ontology: tool for the unification of biology. Nat Genet 25:25–29. https://doi.org/10.1038/75556

37. Bagg A, Neilands JB (1985) Mapping of a mutation affecting regulation of iron uptake systems in Escherichia coli K-12. J Bacteriol 161:450–453. https://doi.org/10.3182/20120829-3-MX-2028.00122
38. Que Q, Helmann JD (2000) Manganese homeostasis in Bacillus subtilis is regulated by MntR, a bifunctional regulator related to the diphtheria toxin repressor family of proteins. Mol Microbiol 35:1454–1468
39. Kaneda T (1991) Iso-and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. Microbiol Mol Biol R 55: 288–302. https://doi.org/10.1128/mmbr.55.2.288-302.1991
40. 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. https://doi.org/10.1128/jb.57.2.179-189.1949
41. Yayanos AA (1995) Microbiology to 10,500 meters in the deep sea. Annu Rev Microbiol 49:777–805. https://doi.org/10.1146/annurev.mi.49.100195.004021
42. 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. https://doi.org/10.1128/aem.44.6.1356-1361.1982
43. Groisman EA, Hollands K, Kriner MA, Lee E-J, Park S-Y, Pontes MH (2013) Bacterial Mg2+ homeostasis, transport, and virulence. Annu Rev Genet 47:625–646. https://doi.org/10.1146/annurev-genet-051313-051025
44. Escolar L, Pérez-Martín J, De Lorenzo V (1999) Opening the iron box: transcriptional metalloregulation by the Fur protein. J Bacteriol 181:6223–6229. https://doi.org/10.1128/jb.181.20.6223-6229.1999
45. Hantke K (2001) Iron and metal regulation in bacteria. Curr Opin Microbiol 4:172–177. https://doi.org/10.1016/s1369-5274(00)00184-3
46. Touati D (2000) Iron and oxidative stress in bacteria. Arch Biochem Biophys 373:1–6. https://doi.org/10.1006/abbi.1999.1518
47. Hoffmann T, Schütz A, Brosius M, Völker A, Völker U, Bremer E (2002) High-salinity-induced iron limitation in Bacillus subtilis. J Bacteriol 184:718–727. https://doi.org/10.1128/jb.184.3.718-727.2002
48. Steil L, Hoffmann T, Budde I, Völker U, Bremer E (2003) Genome-wide transcriptional profiling analysis of adaptation of Bacillus subtilis to high salinity. J Bacteriol 185:6358–6370. https://doi.org/10.1128/jb.185.21.6358-6370.2003
49. Anjem A, Varghese S, Imlay JA (2009) Manganese import is a key element of the OxyR response to hydrogen peroxide in Escherichia coli. Mol Microbiol 72:844–858. https://doi.org/10.1111/j.1365-2958.2009.06699.x
50. Crosa JH, Walsh CT (2002) Genetics and assembly line enzymology of siderophore biosynthesis in Bacteria. Microbiol Mol Biol R 66:223–249. https://doi.org/10.1128/mmbr.66.2.223-249.2002
51. Schwartz CJ, Djaman O, Imlay JA, Kiley PJ (2000) The cysteine desulfurase, IscS, has a major role in in vivo Fe-S cluster formation in Escherichia coli. P Natl Acad Sci USA 97:9009–9014. https://doi.org/10.1073/pnas.160261497
52. Matsumura P, Marquis RE (1977) Energetics of streptococcal growth inhibition by hydrostatic pressure. Appl Environ Microbiol 33:885–892. https://doi.org/10.1128/aem.33.4.885-892.1977
53. Yancey PH (2005) Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. J Exp Biol 208:2819–2830
54. Kempf B, Bremer E (1998) Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. Arch Microbiol 170:319–330. https://doi.org/10.1007/s002030050649
55. Martin D, Bartlett DH, Roberts MF (2002) Solute accumulation in the deep-sea bacterium Photobacterium profundum. Extremophiles 6:507–514
56. Amrani A, Bergon A, Holota H, Tamburini C, Garel M, Ollivier B, Imbert J, Dolla A, Pradel N (2014) Transcriptomics reveal several gene expression patterns in the piezophile Desulfovibrio hydrothermalis in response to hydrostatic pressure. PLoS One 9: e106831-e106831. https://doi.org/10.1371/journal.pone.0106831
57. Amrani A, van Helden J, Bergon A, Aouane A, Ben Hania W, Tamburini C, Loriod B, Imbert J, Ollivier B, Pradel N, Dolla A (2016) Deciphering the adaptation strategies of Desulfovibrio piezophilus to hydrostatic pressure through metabolic and transcriptional analyses. Environ Microbiol Rep 8:520–526. https://doi.org/10.1111/1758-2229.12427
58. Kuhlmann AU, Bremer E (2002) Osmotically regulated synthesis of the compatible solute ectoine in Bacillus pasteurii and related Bacillus spp. Appl Environ Microbiol 68:772–783. https://doi.org/10.1128/aem.68.2.772-783.2002
59. Brands S, Schein P, Castro-Ochoa KF, Galinski EA (2019) Hydroxyl radical scavenging of the compatible solute ectoine generates two N-acetimides. Arch Biochem Biophys 674:108097. https://doi.org/10.1016/j.abb.2019.108097
60. Singh BK, Shaner DL (1995) Biosynthesis of branched chain amino acids: from test tube to field. Plant Cell 7:935–944. https://doi.org/10.1105/tpc.7.7.935
61. De Mendoza D, Ulrich AK, Cronan JE (1983) Thermal regulation of membrane fluidity in Escherichia coli. Effects of overproduction of beta-ketoacyl-acyl carrier protein synthase I. J Biol Chem 258: 2098–2101
62. 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. https://doi.org/10.1128/aem.65.4.1710-1720.1999
63. 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
64. Wang F, Xiao X, Ou H-Y, Gai Y, Wang F (2009) The role and regulation of fatty acid biosynthesis in Shewanella piezotolerans WP3 response to different temperatures and pressures. J Bacteriol 191:2574–2584. https://doi.org/10.1128/jb.00498-08
65. Oger PM, Jebbar M (2010) The many ways of coping with pressure. Res Microbiol 161:799–809. https://doi.org/10.1016/j.resmic.2010.09.017