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Comparative genomic analysis of obligately piezophilic *Moritella yayanosii* DB21MT-5 reveals bacterial adaptation to the Challenger Deep, Mariana Trench

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Abstract

Hadal trenches are the deepest but underexplored ecosystems on the Earth. Inhabiting the trench bottom is a group of microorganisms termed obligate piezophiles that grow exclusively under high hydrostatic pressures (HHP). To reveal the genetic and physiological characteristics of their peculiar lifestyles and microbial adaptation to extreme high pressures, we sequenced the complete genome of the obligately piezophilic bacterium *Moritella yayanosii* DB21MT-5 isolated from the deepest oceanic sediment at the Challenger Deep, Mariana Trench. Through comparative analysis against pressure sensitive and deep-sea piezophilic *Moritella* strains, we identified over a hundred genes that present exclusively in hadal strain DB21MT-5. The hadal strain encodes fewer signal transduction proteins and secreted polysaccharases, but has more abundant metal ion transporters and the potential to utilize plant-derived saccharides. Instead of producing osmolyte betaine from choline as other *Moritella* strains, strain DB21MT-5 ferments on choline within a dedicated bacterial microcompartment organelle. Furthermore, the defence systems possessed by DB21MT-5 are distinct from other *Moritella* strains but resemble those in obligate piezophiles obtained from the same geographical setting. Collectively, the intensive comparative genomic analysis of an obligately piezophilic strain *Moritella yayanosii* DB21MT-5 demonstrates a depth-dependent distribution of energy metabolic pathways, compartmentalization of important metabolism and use of distinct defence systems, which likely contribute to microbial adaptation to the bottom of hadal trench.

DATA SUMMARY

The genome of *Moritella yayanosii* DB21MT-5 has been deposited at the EMBL-EBI (https://www.ebi.ac.uk/) under the accession number of ERA3569304. In addition, this genome is also available at the National Center for Biotechnology Information (NCBI) genome database (https://www.ncbi.nlm.nih.gov/) under the accession number of NZ_LS483250.1, at MicroScope (https://mage.genoscope.cns.

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Keywords: obligate piezophile; hadal trench; Moritella; high hydrostatic pressure (HHP); bacterial microcompartment (BMC); defense system.

Abbreviations: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenases; ANI, average nucleotide identities; BMC, bacterial microcompartment; BREX, bacteriophage exclusion; CDSs, coding DNA sequences; COGs, clusters of orthologous groups of proteins; CRISPR, clustered regularly interspaced short palindromic repeats; Cty, cytochrome; FDH, formate dehydrogenase; FRD, fumarate reductase; GalNAc, N-acetyl-galactosamine; GIs, genomic islands; GlcNAc, N-acetyl-glucosamine; G3P, 3-phosphate; HHP, high hydrostatic pressures; KDG, 2-keto-3-deoxygluconate; KDI, 5-keto-4-deoxyuronate; KEGG, kyoto encyclopedia of genes and genomes; MCP, Methyl-accepting chemotaxis protein; MGEs, mobile genetic elements; NAP, periplasmic nitrate reductase; NAR, transmembrane nitrate reductase; NIR, nitrite reductase; ONT, Oxford Nanopore technologies; PTAC, phosphotransacetylase; PTS, phosphotransferase system; R-M, restriction-modification; SDH, succinate dehydrogenase; T-A, toxin-antitoxin; TBDTs, TonB-dependent transporters; TMA, trimethylamine; TOR, TMAO reductase; T5SS, type-V secretion systems; UQ, ubiquinone.

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Data statement: All supporting data, code and protocols have been provided within the article or through supplementary data files. Eight supplementary tables and two supplementary figures are available with the online version of this article.

fr/microscope/home/index.php?) and at Integrated Microbial Genomes and Microbiomes (IMG/M) system (https://img.jgi.doe.gov/). The GenBank accession numbers of all genomes used for the comparative analysis are provided in Table 1.

The authors confirm all supporting data, code and protocols have been provided within the article or through supplementary data files.

Impact Statement

Hadal trenches with depth greater than 6000 m are featured with extreme HHP that exerts severe effects on micro-organisms. Obligate piezophiles are a group of micro-organisms that grow exclusively under extreme HHP, they are mostly found at the deepest bottom of hadal trenches. Due to the difficulties in sample collection and microbial cultivation, the physiologic and genetic features of this peculiar group remain largely unexplored. In this study, we present the complete genome of the obligate piezophile *Moritella yayanosii* DB21MT-5, which was isolated from the deepest oceanic site, the Challenger Deep. Comparison against pressure sensitive and piezophilic strains revealed unique genomic features in the obligately piezophilic strain DB21MT-5, which are probably involved in the microbial adaptation to the extreme HHP. In particular, certain features can be observed in obligate piezophiles from distantly related taxa, indicating prevailing strategies are adopted for bacterial survival at the bottom of the hadal trench. Taken together, our results provide novel insight into understanding bacterial acclimation to the extreme environment at the hadal trench and the peculiar life-style of obligate piezophiles.

INTRODUCTION

The hadopelagic zone, which lays beneath 6000 m, constitutes the deepest 45% vertical depth of ocean on the Earth. It is featured with low temperature, darkness, as well as extremely high hydrostatic pressure (HHP) and frequent subduction-zone earthquakes. Hadal environments resemble adjacent abyssal plains regarding several physical and chemical characters, such as temperature, salinity, pH and dissolved oxygen level, but exhibit higher microbial cell abundance and microbial carbon turnover rates [1–3]. Increasing evidence shows that fresh and labile organic matters are accumulated at the hadal bottom, possibly attributed to the funnel effect of trench geomorphology and higher sedimentation rates, and supports the bacterial community dominated by heterotrophic Gamma-proteobacteria and Bacteroidetes [2, 4].

As a physical stress confronted by all the deep-sea and trench organisms, HHP affects the conformation of macromolecules, cellular metabolic activity and eventually viability [5, 6]. To cope with high pressures, deep-sea microbes developed regulation systems responsive to pressures that are broadly involved in the regulation of transporters, respiration systems and metabolic reactions [7–9]. They synthesize membrane lipids with increased proportion of unsaturated fatty acids, small molecules called piezolytes that stabilize proteins against HHP, electron transfer chain and metabolic enzymes with better pressure tolerance under low-temperature and high-pressure conditions [10–12]. Additionally, to be more competitive in the oligotrophic environments, deep-sea bacteria produce more secreted carbohydrate enzymes that enable them to utilize recalcitrant organic materials [13].

The current understandings of microbial adaptation to HHP are mainly derived from the studies of facultative piezophilic deep-sea strains that are capable of growing at both atmospheric and high pressures. Inhabiting the deepest oceanic areas is a group of micro-organisms termed obligate piezophiles. They grow exclusively at HHP conditions and have optimal growth pressures over 50 MPa. Incubation at atmospheric pressure condition damages their cellular ultrastructure and leads to death [14]. Thus far, 18 obligate piezophiles have been reported. All of them, except for a single hyperthermophilic archaeon isolated from deep-sea hydrothermal vent [15], are psychrophilic bacteria isolated from hadopelagic environments [16–18]. The majority of them belong to the heterotrophic and copiotrophic genera of *Colwellia* [17, 19, 20], *Shewanella* [21, 22], *Psychromonas* [23, 24] and *Moritella* [21]. Physiological study of this distinctive group is obstructed by the stringent requirement in their isolation and cultivation. In an effort to illustrate their peculiar lifestyles and adaptation to the extreme HHP environments, a number of metagenomic and comparative genomic studies have been carried out [25–27]. Eloe *et al.* reported an enrichment of genes related to signal transduction and polysaccharide degradation in the metagenomes from the Puerto Rico Trench compared to shallow water samples [28]. Additionally, abundant genes involved in inorganic ion transport, heavy metal resistance and stress response are detected at the bottom of Yap Trench as well as the Puerto Rico Trench, suggesting a common characteristic of hadal micro-organisms [25, 28]. Consistent with previous findings, it is recently confirmed experimentally that three obligate piezophiles from the genus of *Colwellia* exhibited higher resistance to copper compared to their pressure sensitive counterparts [26].

The genus *Moritella* is commonly found in trench sediments and is one of the dominant genera in enrichment cultures simulating *in situ* conditions [29, 30]. It consists of seven described species, the fish pathogen *M. viscosa* [31], the *M. dasanensis* first isolated from the Arctic Ocean [32], and five species from deep-sea environments including the *M. marina* [33], the piezophilic *M. abyssi* [34], *M. japonica* [35] and *M. profunda* [34], and the obligate piezophile *M. yayanosii*, which was discovered following enrichment from Challenger Deep sediments [21]. Other unclassified strains affiliated to *Moritella*, i.e. *Moritella* sp. JT01 [36] and *Moritella* sp. PE36 [37] have also been reported. Despite the significant differences in their pressure tolerance, all members of *Moritella*

Table 1. General information of genomes available from the genus of *Moritella*

	M. yayanosii DB21MT-5*	*Moritella* sp. JT01	*Moritella* sp. PE36	*M. marina* MP-1		*M. dasanensis* ArB0140	*M. viscosa* MVIS1		
Assembly level	Complete genome	Scaffold	Contig	Complete genome		Scaffold	Complete genome		
Replicon	–	–	–	Chr	Unnamed plasmid	–	Chr	pMVIS39	pMVIS41
Size (Mbp)	4.43	4.84	5.22	4.73	0.026	4.89	5.09	0.0038	0.0041
G+C content (mol%)	41.20	40	41	40.71	38.11	40.8	39.39	36.4	31.2
Average size of CDS (bp)	888.07	904.63	933.12	959.51	577.84	968.78	910.44	1285.33	431.57
No. of scaffolds	1	42	131	1	1	61	1	1	1
No. of contigs	1	68	131	1	1	61	1	1	1
No. of predicted CDSs	4311	4193	4422	4206	31	4154	4988	3	6
No. of rRNAs	50	3	32	53	–	2	35	–	–
No. of tRNAs	114	93	117	141	–	90	131	–	–
No. of hypothetical proteins (no database match)	501	361	224	549	19	311	262	1	1
GenBank genome accession number	NZ_LS483250.1	NZ_LOCN01000007.1	NZ_ABCQ01000001.1	NZ_CP044399.1	NZ_CP044398.1	AKXQ00000000.1	NZ_LN554852.1	NZ_LN554854.1	NZ_LN554853.1

are psychrophilic and presumably share genetic and physiologic features for cold adaptation, which makes them ideal objects for the study of bacterial adaptation to HHP through comparative genomic analysis.

The obligately piezophilic strain *M. yayanosii* DB21MT-5 was isolated from surface sediment collected at the Challenger Deep, Mariana Trench at the depth of 10898 m. It grows under HHP conditions ranging from 60 to 100 MPa, with optimal temperature and pressure for growth at 10 °C and 80 MPa, respectively [21]. In this study, we sequenced the complete genome of strain DB21MT-5 and performed comparative genomic analysis along with five publicly available genomes from the genus *Moritella*. Identification of genes possessed specifically by strain DB21MT-5 allows exploration of bacterial adaptation to the deepest oceanic zone.

METHODS

Genome sequencing

The cultivation and DNA extraction of strain *M. yayanosii* DB21MT-5 was carried out as described previously [21]. In brief, cells are inoculated into 2216 marine broth medium and transferred into sterile culture bags. The bags are heat sealed and placed in a high-pressure vessel pre-chilled at 10 °C. The hydrostatic pressure was applied with a water pump to 80 MPa. Cells were lysed by SDS and proteinase K before chromosomal DNAs were extracted with phenol:chloroform, and precipitated with isopropanol. The genome sequencing of *Moritella yayanosii* DB21MT-5 was performed at Genoscope mixing Illumina and Oxford Nanopore (ONT) technologies. First, a multiplexed overlapping paired-end library with 276 bp insert size was constructed and loaded on an Illumina MiSeq instrument (2×150 bp). In parallel, long reads were generated by loading a R9-Long Read 1D library on a MinION R9.4 flow cell. An in-house filtering tool 'Filtlong' (https://github.com/rrwick/Filtlong) was applied to ONT data to keep only the longest reading for a genomic coverage of 20-fold. This subset and the Illumina reads (around 300-fold coverage) were assembled using SPAdes version 3.6.0 (http://cab.spbu.ru/software/spades/). Automatic functional annotation was performed using the MicroScope platform (http://www.genoscope.cns.fr/agc/microscope) [38].

Genomic annotation

The expert gene annotation was performed at the MicroScope following the instructions (https://microscope.readthedocs.io/en/3.14.3/), by integrating the results of similarity searches against the databases of UniProt (http://www.uniprot.org/) [39], clusters of orthologous groups of proteins (COGs) predicted by COGnitor (http://www.ncbi.nlm.nih.gov/COG/) [40] and functional prediction by InterPro (http://www.ebi.ac.uk/interpro/) [41]. Protein localization was predicted by PsortB (http://www.psort.org/) [42], SignalP 4.1 [43] and TMhmm 2.0 c [44]. The metabolic pathways were examined using a KEGG Automatic Annotation Server (KAAS, http://www.genome.jp/tools/kaas/). Methyl-accepting chemotaxis proteins are recognized by searching for MCP signalling domain (PF00015) in Interpro database.

Phylogenetic analysis

A set of 120 ubiquitous single-copy bacterial proteins were used as marker genes to build the phylogenetic tree as previously reported [45]. The homologous sequences were aligned with MUSCLE software and then concatenated in the same order using Sequence-Matrix [46]. Phylogenetic tree was generated via maximum-likelihood analysis with TreeBeST (http://treesoft.sourceforge.net/treebest.shtml), and 1000 bootstrap replicates were included in a heuristic search with a random tree and the tree bisection-reconnection branch-swapping algorithm. Average nucleotide identities (ANI) values were computed for pairwise genome comparison using fastANI [47].

Comparative genomics

The genome sequence of *Moritella* sp. JT01 (NZ_LOCN01000007.1), *Moritella* sp. PE36 (NZ_ABCQ01000001.1), *M. dasanensis* ArB0140 (AKXQ00000000.1), *M. marina* MP-1 (NZ_CP044399.1 and NZ_CP044398.1), *M. viscosa* (NZ_LN554852.1, NZ_LN554854.1 and NZ_LN554853.1), *S. benthica* DB21MT-2 (NZ_LS483452.1), *S. benthica* KT99 (ABIC00000000.1), *Colwellia* sp. MT41 (NZ_CP013145.1), *C. piezophila* Y223G (NZ_ARKQ00000000.1) and *C. marinimaniae* MTCD1 (NZ_BDQM00000000.1) were downloaded from NCBI for comparative genomic analysis. The *M. yayanosii* DB21MT-5 genome and comparative results were visualized with the Circular Genome Viewer [48]. Specific genes are identified using the Phyloprofile Exploration function at MaGe platform with a cut-off value for a homologue set as 50% amino acid identity and 80% coverage [38]. Genomic island prediction is performed using four different island prediction methods: SIGI-HMM, IslandPath-DIMONB, IslandPick and Islander at Islandviewer 4 (www.pathogenomics.sfu.ca/islandviewer) [49].

RESULTS AND DISCUSSION

General genomic features of *M. yayanosii* DB21MT-5

The complete genome of hadal strain DB21MT-5 consists of a single chromosome with the size of 4433651 bp and GC content of 41.20 mol% (Table 1, Fig. 1a). We identified 114 tRNA and 50 rRNA genes, including 16 complete rRNA operons and 2 single 5S rRNAs. DB21MT-5 genome encodes 4311 coding DNA sequences (CDSs), approximately one-tenth of them (501 CDSs, 11.6%) have no hit in both SwissProt and TrEMBL databases. Twenty-three genomic islands (GIs) ranging from 4 to 25 kb in length are identified in the genome and in total 362 genes are encoded within these regions (Fig. 1a, Table S1, available in the online version of this article).

By the time this study was carried out, 22 genomes from the genus of *Moritella* were available at NCBI, including three genomes of strain *M. marina* MP-1, one of strain *M. dasanensis* ArB0140, thirteen of *M. viscosa* isolates, three of uncharacterized

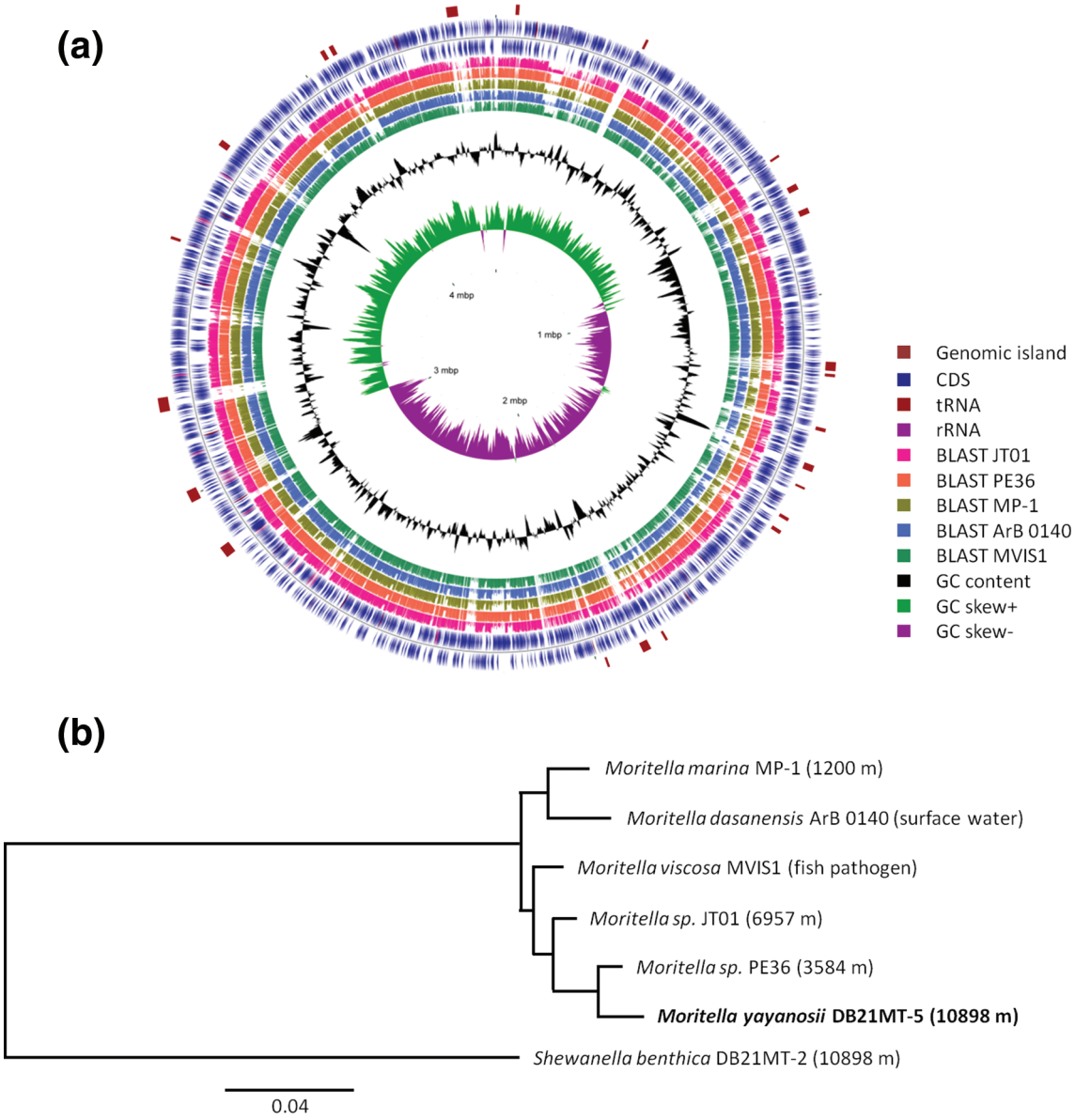

Fig. 1. Graphical circular map of *Moritella yayanosii* DB21MT-5 genome and phylogenetic tree of *Moritella* genomes. (a) Graphical circular map of *M. yayanosii* DB21MT-5 genome. From outside to centre, ring 1 shows genomic islands; rings 2 and 3 show genes (CDS in blue, tRNA in maroon and rRNA in purple) oriented in the forward and reverse directions, respectively; rings 4 to 8 show BLAST of genes in other *Moritella* genomes in the order of JT01, PE36, MP-1, ArB0140 and MVIS1, each bar represents a gene and the length shows the identity; ring 9 shows G+C mol% content plot (black) and ring 10 shows GC skews, where green indicates positive values and purple indicates negative values. (b) Phylogenetic tree of *Moritella* genomes constructed using a concatenated alignment of 120 conserved marker genes.

isolates and five assemblies from metagenome data. Considering the status of genome assembly and the isolation source, five genomes are included in this study for comparative analysis with strain DB21MT-5: complete genomes of fish pathogen *M. viscosa* (hereafter called MVIS1) and strain *M. marina* MP-1 (hereafter called MP-1), and draft genomes of strain *Moritella* sp. JT01 (hereafter called JT01), strain *Moritella* sp. PE36 (hereafter called PE36) and strain *M. dasanensis* ArB0140 (hereafter called ArB0140) (Table 1). Among them, strain JT01 isolated from the depth of 6957 m and strain PE36 isolated from the depth of 3584 m are psychro-piezophiles. Their optimal growth pressures are 30 and 41.4 MPa, and optimal growth temperature are 5–8 and 13 °C, respectively [50, 51]. Strain MP-1 was isolated from a seawater sample collected at 1200 m depth, it is sensitive to elevated pressure when cultured at its optimal growth temperature of 15 °C [35]. *M. dasanensis* ArB0140 and *M. viscosa* 06/09/139 were isolated from seawater close to glacier and Atlantic salmon in Norway. They are both psyhrophiles

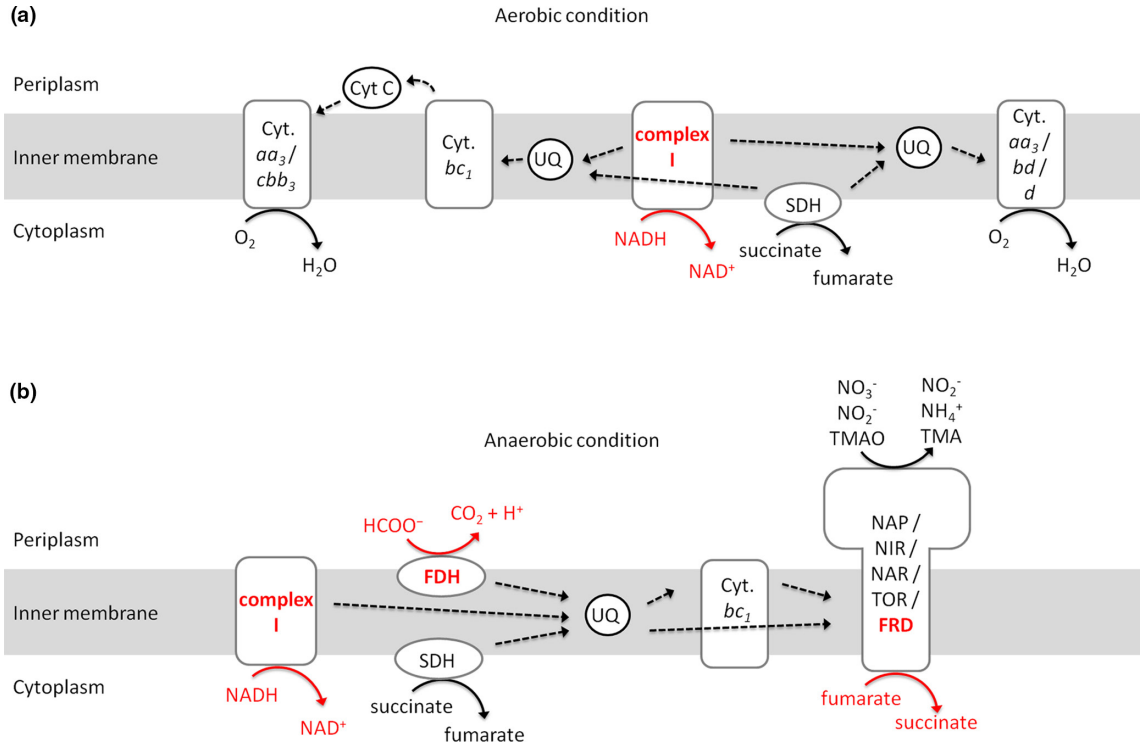

Fig. 2. Electron transfer chains predicted in DB21MT-5 and other *Moritella* strains. (a) Electron transfer chains at aerobic conditions. (b) Electron transfer chains at anaerobic conditions. Cty, cytochrome; UQ, ubiquinone; SDH, succinate dehydrogenase; FDH, fumarate dehydrogenase; NAP, periplasmic nitrate reductase; NAR, transmembrane nitrate reductase; NIR, nitrite reductase; TOR, TMAO reductase; FRD, fumarate reductase. Components in black are conserved in all *Moritella* strains, and components in red indicate the processes are differently possessed by shallow water, deep-sea and trench strains.

with optimal growth temperatures of 9 and 15 °C, respectively, and the pressure tolerance of the two strains have not been examined [32, 52] (Table S2). The ANI analysis suggested a closer relationship between strains DB21MT-5 and PE36 (Table S3). Consistently, the phylogenetic tree constructed using 120 conserved orthologues showed that strain DB21MT-5 is most closely related to deep-sea piezophilic strain PE36. They form a cluster with piezophilic strains JT01 and the fish pathogenic strain, while the two strains from shallower water zones cluster together (Fig. 1b).

In total 3256 CDSs from DB21MT-5 are classified into at least one Cluster of Orthologous Group of Proteins (COGs). Compared to other *Moritella* strains, strain DB21MT-5 has greater proportion of genes involved in energy production and conversion (COG category C) (5.92% vs 4.63–5.71%), coenzyme transport and metabolism (H) (3.90% vs 3.40–3.70%), and cell-cycle control, cell division, chromosome partitioning (D) (1.28% vs 0.96–1.02%), but considerably fewer genes for signal transduction mechanisms (T) (5.10% vs 5.49–6.94%) (Table S4).

Genes specific to obligately piezophilic and piezophilic strains

In order to better understand the obligately piezophilic lifestyle at the bottom of the Mariana Trench and microbial adaptation to HHP environments, we looked for genes exclusively present in DB21MT-5 but absent from the other *Moritella* genomes (termed obligate-specific genes), and those shared by DB21MT-5 and at least one of the piezophilic strains (JT01 and PE36) but excluded from any of the others (ArB0140, MP-1 and MVIS1) (termed piezo-specific genes). Using 50% amino acid identity and 80% coverage as the cut-off value for the presence of orthologues, we identified 763 obligate-specific genes and 257 piezo-specific genes (Tables S5 and S6). Unlike piezo-specific genes that are scattered over the genome, most obligate-specific genes are in succession and locate within predicted genomic islands (GI) (Table S5). We identified one GI composing ten bacteriophage-related genes that are specific to the strain DB21MT-5, and 14 GIs containing obligate-specific genes with mobile genetic elements (MGEs) such as transposases, integrases and recombinases located adjacent. It should be noted that the presence of MGEs near genes specific to piezophilic strains has also been observed within the genus of *Colwellia* and *Shewanella*, and it has been proposed that the MGEs may enhance acquisition of genes and acclimation to HHP environment [26].

The genes not exceeding 150 bp are likely to be gene remnants and excluded from further analysis. Among the remaining 608 obligate-specific genes and 225 piezo-specific genes, half and one-third code for protein of unknown function, respectively.

Genes involved in cell-cycle control, cell division, chromosome partitioning (COG Category D), defence mechanisms (V) and secondary metabolites biosynthesis, transport and catabolism (Q) are enriched in obligate-specific genes. The piezo-specific genes are mostly involved in energy production and conversion (C) and inorganic ion transport and metabolism (P) (Fig. S1). Genes exclusively encoded by obligately piezophilic and piezophilic strains may confer special traits required for the adaptation to corresponding environments and their functions are discussed in detail below.

Energy metabolism

The electron transfer chain in strain DB21MT-5 includes NADH:ubiquinone oxidoreductase (complex I), succinate dehydrogenase (complex II) and cytochrome bc1 complex. We also identified aa3-type and cbb3-type cytochrome c oxidases and cytochrome d ubiquinol oxidase for aerobic respiration, terminal reductases for nitrate, nitrite and TMAO anaerobic respiration and multiple fermentation pathways. The majority of them are highly conserved among all *Moritella* strains, but exceptions are observed as well (Fig. 2, Table S7).

The *nuo* gene cluster coding for complex I is identified in the hadal and deep-sea strains (DB21MT-5, JT-01 and PE36), but absent from the three non-piezophiles. Complex I transfers two electrons from NADH to the quinol pool while translocating four protons across the cytoplasmic membrane into the periplasm. The presence of the *nuo* gene cluster enables the piezophilic *Moritella* strains to acquire electrons from NADH, while the non-piezophilic strains might rely on other forms of reductant for energy generation. The same trait has been observed in strains from the genus of *Colwellia*, in which the *nuo* genes are uniquely present in three hadal piezophiles [26]. It is speculated that the generation and consumption of NADH are effected by high pressures, and the additional *nuo* gene cluster may facilitate the energy generation and protect cells from reductive stress caused by the excess of NADH. Although the major NADH generation pathways, such as glycolysis and the TCA cycle, are well conserved within the genus of *Moritella*, we cannot exclude the possibility that their activities, or that of other bioprocesses involving NADH/NAD^+ are influenced differently by high pressures amongst piezophiles and non-piezophiles.

Formate dehydrogenase (FDH) is the only component of the electron transfer chain that is conserved in all *Moritella* strains except for DB21MT-5. FDH catalyses the oxidation of formate to CO_2 and contributes two electrons to the membrane quinol pool. Many prokaryotes use formate produced from pyruvate respiration as a major electron donor under anaerobic conditions [53]. Lack of FDH suggested that DB21MT-5 is incapable of obtaining electrons from formate, even though the pyruvate formate lyase, which catalyses the conversion of pyruvate into formate is encoded within its genome.

The membrane bound fumarate reductase (FRD) catalyses the final step of fumarate anaerobic respiration, the reduction of fumarate into succinate. The FRD systems locate in a highly conserved region in all *Moritella* strains. However, unlike FRD in most Gamma-proteobacteria, including the other five *Moritella* strains, that is composed of four components (FrdABCD), the FRD in strain DB21MT-5 has three components (FrdABC), which resemble those found in Delta- and Epsilon-proteobacteria [54, 55]. Further sequence analysis demonstrated that, compared to membrane subunits from other *Moritella* strains (FrdCs are 129AA in length), FrdC from DB21MT-5 is about twice the length and has two more trans-membrane segments (222AA in length) and one more haem-binding site (Fig. S2). One hypothesis is while FrdCD subunits together are required to anchor the Frd complex onto the cytoplasmic membrane and accomplish the electron transfer in most *Moritella* strains, the same function can be fulfilled by FrdC alone in strain DB21MT-5. A more concise membrane imbedded component might be better suited to the extreme HHP niche where structure and composition of cytoplasmic membrane are most affected.

Collectively, apart from the succinate dehydrogenase that is conserved in all *Moritella* strains analysed, the distribution of enzyme complex that delivers electron to the quinol pool exhibits a depth-dependent profile, i.e. non-piezophilic strains could generate electrons from formate using FDH, the obligately piezophilic strain dwelling in the trench bottom may acquire electrons from NADH through complex I, and deep-sea piezophilic strains bear both pathways. The terminal oxido-reductases that receive electrons from the quinol pool are rather conserved, with an only exception of fumarate reductase. The FRD in obligately piezophilic strain DB21MT-5 is distinct from all the other *Moritella* strains and most Gamma-proteobacteria.

Energy metabolism is one of the biological processes most susceptible to HHP. Possessing multiple cognate respiratory terminal oxido-reductases with different pressure tolerance and pressure-regulated electron transfer chain have been reported in diverse deep-sea microbes [56–58]. For example, in piezophilic *Shewanella* strain DB172F, the electron transfer chain utilized under atmospheric pressure is composed of bc1 complex, cytochrome c-551 and c-552 and cytochrome c oxidase, whereas the one under high pressure condition consists of cytochrome c-551 and quinol oxidase [59]. The hypothesis is a more compact electron transfer chain might be favourable for energy generation under high pressures, and it may also be applied to explain the distinctive FRD complex observed in hadal strain DB21MT-5. We also noticed that the adaptation strategy of energy metabolism differs amongst genera. For example, NADH ubiquinone oxidoreductase is characteristic of obligate piezophiles of *Moritella* and *Colwellia*, but not confined to hadal obligate piezophiles in the genus of *Shewanella* where the bb3-type cytochrome c oxidase is a specific marker of obligate piezophiles from hadal environments [60].

Choline fermentation in bacterial microcompartment

We identified 22 obligate-specific genes residing in a 15 kb genomic island that are responsible for choline

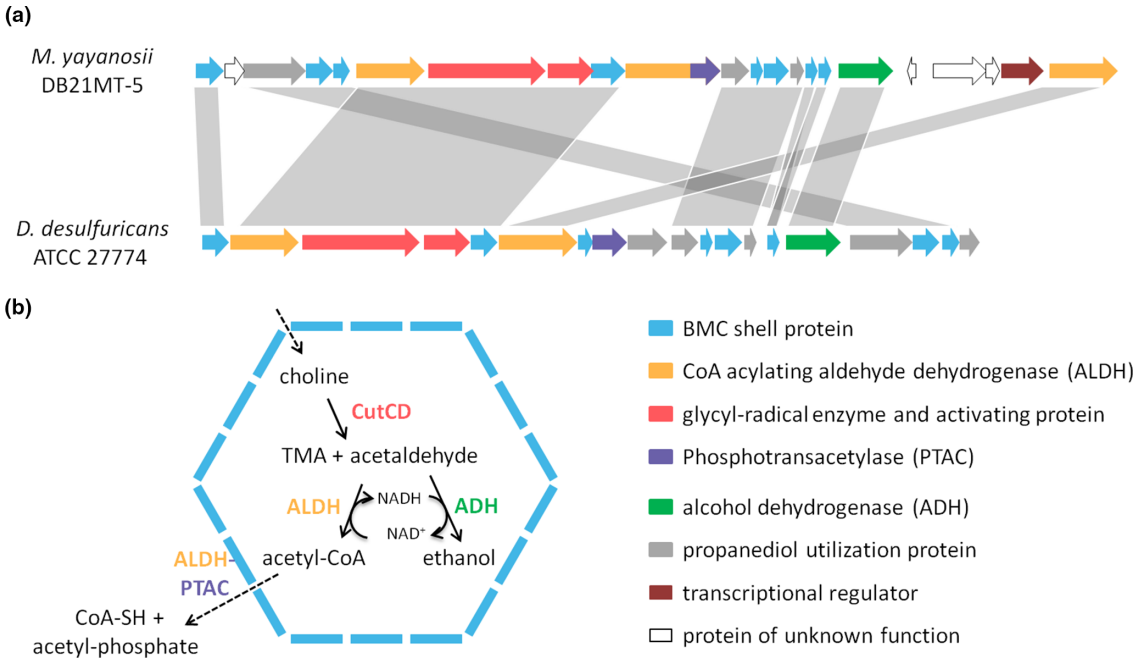

Fig. 3. Organization of gene cluster (a) and schematic of metabolic reactions (b) of BMC in strain *M. yayanosii* DB21MT-5. Colour of gene and corresponding product indicates their function.

fermentation and bacterial micro-compartment (BMC) synthesis (Fig. 3). BMC is a polyhedral bacterial organelle with a semi-permeable protein shell. To date, over 20 types of BMCs have been identified in 23 bacterial phyla, and diverse metabolic processes can take place within a BMC, including propanediol, ethanolamine and choline utilization [61]. The BMC gene locus in DB21MT-5 encodes glycyl-radical enzyme CutC and glycyl-radical enzyme activating protein CutD, which cleave the C-N bond in choline and produce trimethylamine (TMA) and acetaldehyde. The acetaldehyde could be further converted into ethanol and acetyl-CoA by alcohol dehydrogenase (ADH) and aldehyde dehydrogenases (ALDH), respectively, with concomitant circulation of NADH and NAD^+. Within the BMC locus there is a gene coding for a fusion protein consisting of the ALDH domain at its N-terminus and phosphotransacetylase (PTAC) domain at its C-terminus. Based on the study in *Desulfovibrio desulfuricans* ATCC 27774, the PTAC catalyses the conversion of acetyl-CoA into acetyl-phosphate and CoA-SH. The former could be a substrate for the generation of ATP in cytoplasm, and the latter will be reused for the production of acetyl-CoA [62]. To summarize, DB21MT-5 encodes all necessary functional elements to convert choline to TMA, ethanol and acetyl-CoA within the BMC, and is probably capable of generating CoA-SH and acetyl-phosphate from acetyl-CoA for ATP production.

It is noticeable that DB21MT-5 is the only strain capable of choline fermentation within the genus. All the other five strains oxidizes choline into betaine, which is known as an osmolyte and a potential piezolyte that protects biomolecules against osmotic pressure and HHP stresses [63, 64]. Both enzymes involved in betaine synthesis (choline dehydrogenase and betaine aldehyde dehydrogenase) are absent from the genome of DB21MT-5, suggesting generation of betaine from choline oxidation is not favoured by this strain, for a currently unknown reason, and fermentation of choline is relatively more important for the growth of strain DB21MT-5 at the bottom of the Mariana Trench.

Secluding catabolism reaction within a BMC is believed to protect the cell from toxic intermediate metabolic products and better control the reactions. However, the assembly of BMC is a sophisticated procedure. It requires highly coordinated interaction amongst shell components and metabolic enzymes, possibly consuming a great expense of energy [61]. Previous studies suggested that catabolic BMC provides a competitive advantage in specific environmental niches [61]. The choline fermentative BMCs are widespread in human gut microbiomes and related to colonization and virulence of some pathogenic bacteria [65, 66]. Although the contribution of choline fermentation to microbes at hadal trenches remains obscure, the DB21MT-5 cell possibly benefits more from using choline as an alternative fermentative substrate for energy generation than as a precursor for betaine synthesis, which seems a plausible explanation for the presence of choline fermentation BMC, an energy-consuming bioprocess in deep ocean where energy is believed to be limited in general [67].

Table 2. Number of polysaccharide degradation enzymes identified in the genomes from the genus of *Moritella*

	DB21MT-5	JT01	PE36	MP-1	ArB0140	MVIS1
Amylase	–	3	1	–	2	4
Isoamylase	1	–	1	1	1	–
Chitinase	–	7	3	–	1	4
Galactosidase	–	–	1	2	1	–
Glucosidase	2	2	3	2	3	1
Glycosidase	1	–	–	2	1	–
Pullulanase	–	1	1	–	–	–
Xylanase	1	–	–	–	–	–

Catabolism

Previous studies showed that deep-sea microbes code for more extracellular polysaccharases and peptidases compared to species inhabiting upper oceanic layers to enhance their capability for organic matter acquisition and utilization [2, 13, 68–70]. Unlike piezophilic strains JT01 and PE36 that encode amylase, glucosidase, pullulanase and abundant chitinases [36], a smaller number and fewer types of polysaccharases are identified in the genome of DB21MT-5 (Table 2). On the other hand, strain DB21MT-5 is the only type strain in this genus utilizing xylose, one of the major components of hemicellulose, as a carbon source [32]. Consistently, a secreted endoxylanase for linear hemicellulose degradation is uniquely identified in the genome of DB21MT-5.

Twenty-five obligate-specific genes are assigned to the functional category of carbonhydrate transport and metabolism. Among them, we identified a gene cluster related to N-acetylgalactosamine (GalNAc) and 5-keto-4-deoxyuronate (KDI) metabolism. The GalNAc catabolic pathway includes a phosphotransferase system (PTS) responsible for the transport and phosphorylation of GalNAc, and enzymes catalyse the sequential conversion of GalNAc-6-P into GalN-6-P, Tag-6-P and Tag-1,6-PP. It should be noted that Tag-1,6-PP aldolase AgaY, which catalyses the cleavage of Tag-1,6-PP into glyceraldehyde 3-phosphate (G3P) and glycerone phosphate, is not detected in this gene cluster. But the loss of AgaY can be compensated by a non-committed aldolase outside the BMC locus, e.g. fructose-bisphosphate aldolase, as proposed in *Shewanella* [71]. Downstream of GalNAc metabolic genes locates the four genes that catalyse conversion of DKI into 2-keto-3-deoxygluconate (KDG) and subsequently pyruvate and 3-phosphoglyceraldehyde. GalNAc and DKI could be generated from bacterial degradation of animal- and plant-originated polysaccharides. Generally, polysaccharides are broken down into oligosaccharides and passively transported through outer-membrane porins into the periplasm, where they are further degraded into monosaccharides such as GalNAc and DKI during degradation of chondroitin sulphate, or DKI, glucuronate and galacturonate in the case of pectin degradation [72–74]. Within this gene cluster, we identified genes encoding for an unsaturated glucuronyl hydrolase that converts disaccharides into monosaccharides, and an outer-membrane porin that is probably involved in the transportation of oligosaccharides, but not the enzyme responsible for the breakdown of polysaccharides. Within the genome, two strain-specific type-V secretion systems (T5SS) are recognized. Passenger proteins of both T5SSs contain a conserved domain of pectin lyase fold/virulence factor (IPR011050) at their N-terminus, suggesting their potential function in the first step of eukaryotic polysaccharide degradation (Fig. 4).

A *pel* operon is present exclusively in piezophilic strains DB21MT-5 and PE36 but not shallow water non-piezophilic counterparts. The *pel* genes are responsible for the synthesis and translocation of the Pel polysaccharides, which is a critical component for the formation and maintaining of the bacterial biofilm matrix [75, 76]. Apart from its well-known functions in cell interaction and pathogenicity, the biofilm matrix also helps to retain secreted enzymes, increasing the efficiency of metabolizing external nutrient, and even serving as a reservoir of organic carbon itself [77]. Pel is composed of N-acetyl-glucosamine (GlcNAc) and GalNAc and their deacetylated derivatives. It is potentially a supplementary organic source for DB21MT-5 during periodic depletion of nutrients at the trench bottom considering the strain-specific GalNAc utilization pathway and a ubiquitous GlcNAc utilization pathway detected in the genome of DB21MT-5.

The special V-shape topography makes the hadal trench a trap of organic matters sinking from the euphotic zone and lateral transported from abyssal plains. Increased organic matter quality, biomass and microbial activity supported the hypothesis that there are more labile and fresher organic matter deposited into the deepest bottom of the trenches than adjacent abyssal plains [3, 67]. Compared to the other deep-sea piezophilic *Moritella* strains, strain DB21MT-5 has distinct potential for metabolizing hemicelluloses, xylose, GalNAc and DKI, which might compensate the short of chitinase, amylase and pullulanase in nutrient acquisition. Aside from hadal prokaryotes, enzymes targeting at wooden debris have been detected in amphipod *Hirondellea gigas* collected at 10000 m depth and they are proven functional under *in situ* low-temperature and high-pressure

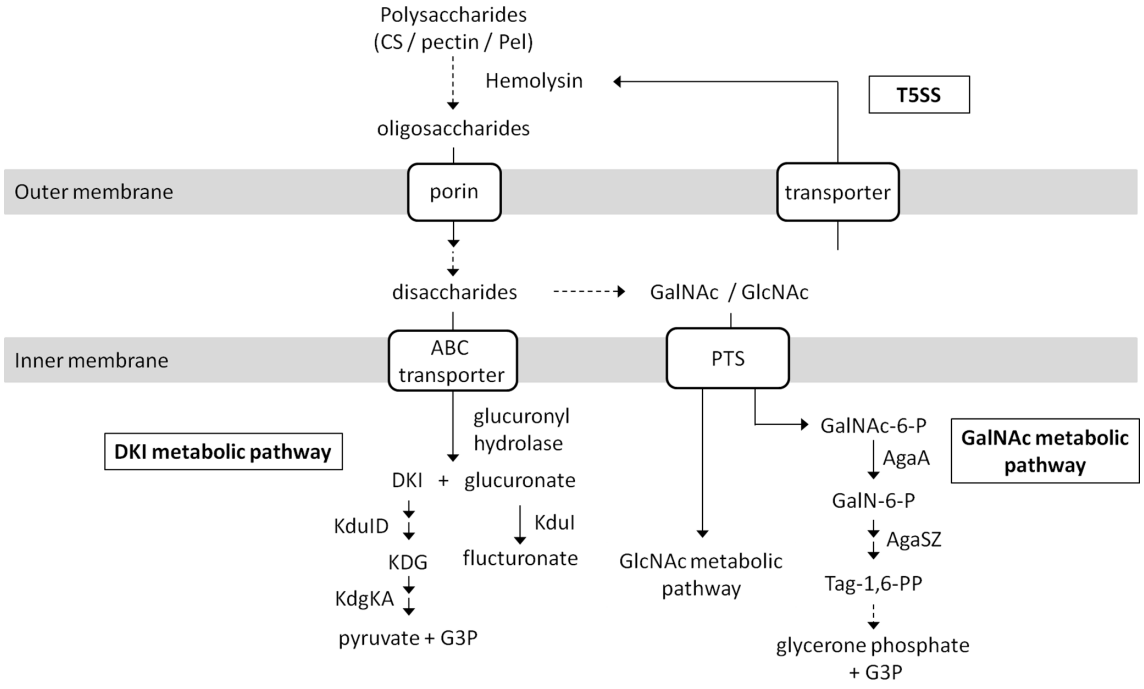

Fig. 4. Hadal-specific carbohydrate metabolic pathways identified in strain *M. yayanosii* DB21MT-5. Solid lines indicate proteins carrying out the reaction have been identified in the genome, dashed lines indicate reactions or processes hypothesized. CS, chondroitin sulphate; GalNAc, N-acetyl-galactosamine; GlcNAc, N-acetyl-glucosamine; KDI, 5-keto-4-deoxyuronate; PTS, phosphotransferase system; G3P, glyceraldehyde 3-phosphate.

conditions [78, 79]. The presence of abundant digestive enzymes targeting at plant-derived polysaccharide suggests that plant debris could be an important source of organic matter for life at the bottom of hadal trench.

Defense systems

Prokaryotes developed diverse immune systems to defend against viral infection, such as the innate immunity carried out by restriction-modification (R-M) system, and the acquired immunity provided by CRISPR (clustered regularly interspaced short palindromic repeats)-Cas system [80, 81]. Strain DB21MT-5 encodes two R-M systems and one bacteriophage exclusion (BREX) system, all of which are specific to the hadal strain. We also identified ten sets of T-A systems, including one locus (TA-V) constituting one toxin and two antitoxins. Among them, TA-II, TA-IV and TA-X are highly conserved in strains MP-1 and PE36, respectively (over 90% amino acid identities), while all the remaining seven T-A systems (TA-I, TA-III and TA-V to TA-IX) are specific to strain DB21MT-5 (Fig. 5). Like most *Moritella* strains, CRISPR-Cas locus was not found in the genome of DB21MT-5.

There are estimated 10^7 to 10^8 viral particles/g in the surface sediment of hadal trenches, and the viral production is much higher compared to adjacent abyssal sites [82]. Highly active and dynamic hadal viruses may direct frequent DNA exchanges and release considerable amounts of labile organic materials, but also pose a threat to benthic organisms. It is generally believed that possessing multiple innate immune systems that differ in the nature of defensive action keeps the host resistant to viruses, even those evolved to escape from one defending system [83, 84]. Both R-M and BREX systems distinguish phage DNA from host DNA by the state of methylation. The former recognizes inherited palindromic sequences and degrades foreign DNA directly, while the latter recognizes asymmetric sequence and blocks phage replication in a more complex manner [83, 84]. T-A systems block phage propagation in a population by inducing abortive infection programmed cell death upon phage infection [85]. The abundant and diverse innate immune systems may provide DB21MT-5 more effective and comprehensive protection from phage infection and be beneficial for bacterial survival within the hadal benthos.

The defence systems are frequently associated with transposable elements and form 'defence island', which enables them to be transferred among microbes inhabiting the same niche [85]. In the genome of DB21MT-5, the BREX system is surrounded by putative transposases and integrases in GI-14, and the T-A systems highly conserved in obligate piezophiles (TA-II, TA-III, TA-IV and TA-V) locate in GI-6 (Table S1). We further characterized the occurrence of these defence systems in four other obligate piezophiles, including *Colwellia* sp. MT41, *C. marinimaniae* MTCD1 and *Shewanella benthica* DB21MT-2 isolated from the Mariana Trench, and *S. benthica* KT99 from the Kermadec Trench. The two R-M systems have no orthologues found in

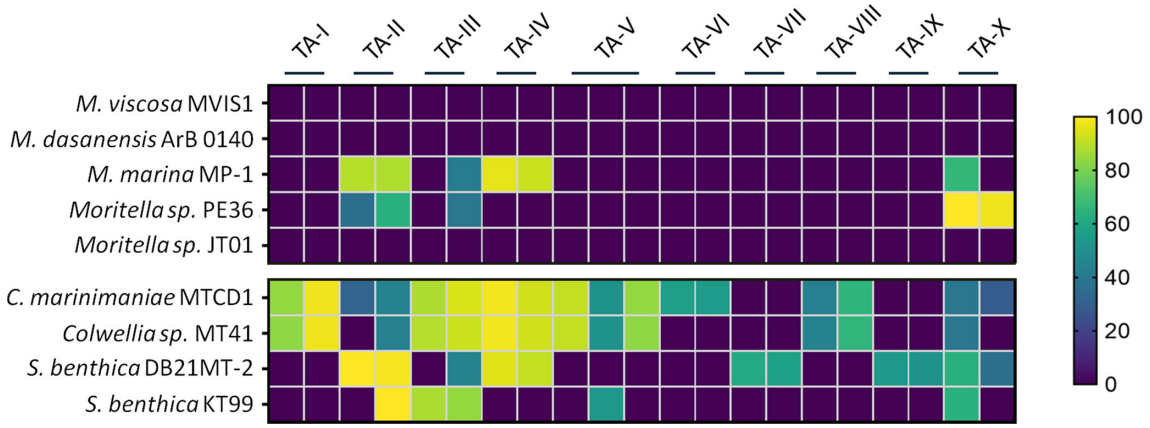

Fig. 5. Comparison of Toxin-antitoxin systems identified in *Moritella* and four obligate piezophiles. The upper part shows similarities of T-A systems in strain DB21MT-5 to those in genomes from the genus of *Moritella*. The lower part shows the similarities compared to four obligate piezophiles from the genus of *Colwellia* and *Shewanella*.

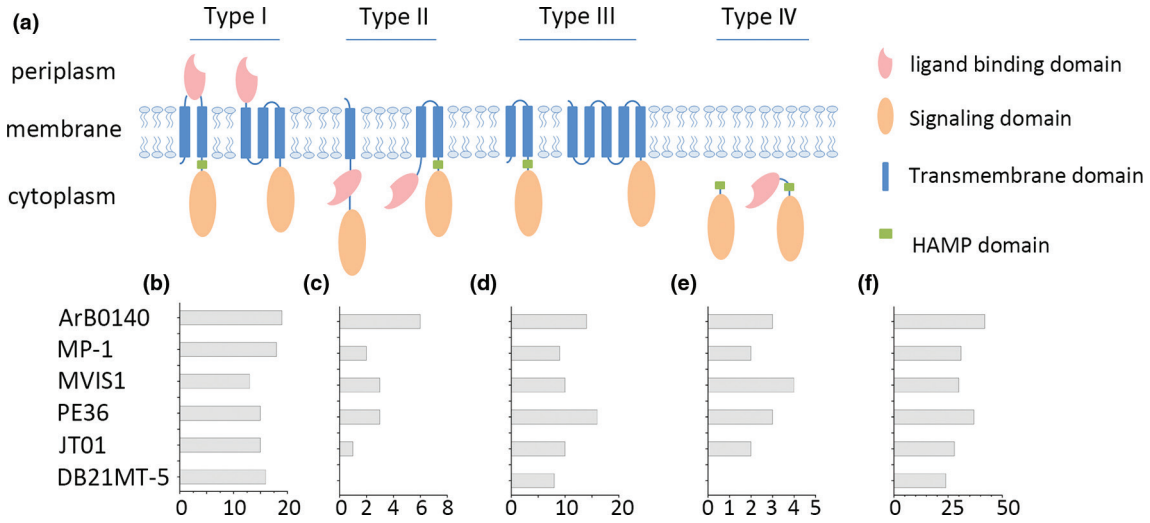

Fig. 6. Diagram of four types of MCPs and the MCPs identified in genomes from the genus of *Moritella*. (a) Diagram of four types of MCPs. (b)– (f) shows the number of each type of MCP and the total number of MCPs identified in the five genomes of *Moritella*.

any obligate piezophiles while a BREX system with over 90% identities of amino acid sequences is identified in *S. benthica* DB21MT-2, which was isolated from the same sediment sample as strain DB21MT-5 [21]. Most T-A systems identified in DB21MT-5 are well conserved in all three obligately piezophilic bacteria from the Mariana Trench. The strains MTCD1, MT41 and DB21MT-2 possess eight, seven and six T-A systems closely related to those found in DB21MT-5, respectively. Meanwhile, *S. benthica* KT99, which is phylogenetically closely related to *S. benthica* DB21MT-2 but isolated from Kermadec Trench, shares only four T-A systems with strain DB21MT-5 (Fig. 5). This observation shows that T-A systems reflect more of the geology relatedness than taxonomic affiliation of the strains, and their potential as indicatives of geologically isolated hadal trenches and functions in HHP and hadal trench acclimation are worthy of further examinations.

Iron acquisition and heavy metal homeostasis

Iron is essential for all life forms, but the low solubility of ferric iron prohibits its effective absorption from oxidized marine environment. Iron uptake in marine bacteria can be achieved through diverse pathways, including siderophore-based iron acquisition systems, inorganic ferric iron transporters and ferrous transporters [86–88]. In the genome of DB21MT-5, we identified non-ribosomal peptide synthetase possibly involved in the biosynthesis of siderophore, eight TonB-dependent transporters (TBDTs) and seven sets of ABC transporters that are predicted to be involved in inorganic ferric or iron-siderophore uptake (Table S8). Remarkably, the siderophore synthetase and three TBDTs (MORIYA_v4_1160, 2139 and 4278) are specific to DB21MT-5, while another TBDT (MORIYA_v4_2144) and two ABC transporters (MORIYA_v4_2094 to 2096 and 2146

to 2149) are uniquely found in piezophilic strains. Additionally, acquisition of ferrous iron in most *Moritella* strains is achieved through the well conserved ferrous iron transport system FeoAB, except for strain DB21MT-5, in which an obligate-specific high-affinity Fe^{2+}/Pb^{2+} transporter is predicted to take part in this process.

Five genes related to copper oxidation, sequestration and regulation and two cobalt transporter subunits are highly conserved and exclusively present in DB21MT-5 and two piezophilic strains. This is in agreement with previous reports that piezophilic *Colwellia* strains from the Mariana Trench are more resistant to copper than piezo-sensitive counterparts [26] and with the observation of abundant heavy metal resistance genes at the bottom of the Yap Trench [25]. Considering the enriched of trace metals, including Cu, Mn and Ni in surface sediment at hadal trenches [89, 90], and the discovery that HHP increases copper toxicity to *Palaemon varians* [91], micro-organisms inhabiting abyssopelagic and hadopelagic zones are more likely to experience heavy metal stress. All the evidences indicate that competing for essential metal elements and maintaining their homeostasis are common challenges for bacteria inhabiting hadal environments. Elucidating the biological and ecological significance of the metal ion uptake, efflux and regulation systems in deep-sea and hadal micro-organisms would be beneficial to understanding the microbial adaptation as well as the nature of the deep oceanic environments.

Signal sensing and transduction

Methyl-accepting chemotaxis proteins (MCPs) are the most common receptors in micro-organisms. They recognize environmental and intracellular signals and transmit the signals to histidine kinase to regulate multiple cellular behaviours such as motility, colonization and biofilm formation [92, 93]. Fewer MCPs are identified in the genome of DB21MT-5 compared to other strains in general. According to the classification of MCPs based on subcellular localization of ligand binding domain [92], two-thirds of them belong to type-I MPCs that sense external signals, and the left are type-III MCPs with multiple transmembrane segments but no obvious ligand binding domain, while type-II and type-IV MCPs detecting internal signals are absent (Fig. 6). Among them, two type-I MCPs (MORIYA_v4_0483 and 2468) are uniquely found in DB21MT-5, and a type-III MCP (MORIYA_v4_1386) has orthologue in PE36 but not other strains, indicating novel functions or special signals they may sense in the HHP environments. It is generally believed that bacteria harbour a large number and more diverse signal transduction gene to cope with complex environment such as soil and aquatic niches, whereas those inhabiting a relatively stable environment require less [94]. Limited number and type of MCPs and fewer genes affiliated to the COG category of signal transduction in strain DB21MT-5 suggests that compared to shallow water and sediment, the bottom of the hadal trench might be less dynamic with respect to chemical and physical changes.

CONCLUSION

Through comparative genomic analyses, we identified some unique genomic features of the obligately piezophilic bacterium *M. yayanosii* DB21MT-5 inhabiting the surface sediment at the Challenger Deep, Mariana Trench. Unlike phylogenetically close-related deep-sea strains, DB21MT-5 encodes fewer secreted polysaccharases but has the potential to utilize plant-derived polysaccharides. The depth-dependent distribution of respiratory pathways and the cellular organelle specified for choline fermentation reflected special requirement for energy metabolism under extreme HHP environments. More importantly, several genomic properties are shared by obligately piezophilic strains from different genera, such as the presence of heavy metal resistant genes, abundant mobile genomic elements and defence systems, which probably play a vital role in the peculiar life-style of obligate piezophiles as well as bacterial survival at the bottom of hadal trenches. It would be of great interest to discover their biological functions and the mechanism they participate in the bacterial adaptation to the deepest oceanic zone on Earth.

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Author contributions
W.J.Z., J.Y. and L.F.W. designed the experiments. S.M., S.F. and T.G. carried out the genome sequencing. W.J.Z., C.Z., S.Z., X.G.L., X.Q.Q. conducted the comparative genomic analysis. The manuscript was written by W.J.Z., D.H.B. and L.F.W. All authors read and accepted the final manuscript.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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