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Genomic evidence on the distribution and ecological function of *Pseudomonas* in hadal zone

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Abstract

The hadal zone is the deepest region on Earth. It serves as a depositional zone for the sinking matter from surface ocean and continental margin, aided by its unique V-shaped structure. Due to extreme depth (over 6000 m), normally only organic matter with low degradability typically reaches the bottom of the trench. Concurrently, reports have indicated highly active carbon turnover and dense bacterial cells in the Mariana Trench. There remains a cognitive gap in understanding the connection between this phenomenon and the microbial taxa along with their metabolic activities. Here, we surveyed the *Pseudomonas*, one of the most widely distributed bacterial genera on Earth. The result revealed widespread distribution of *Pseudomonas* in the hadal zones. We obtained 21 metagenome-assembled genomes (MAGs) from seawater and sediment samples of the Mariana Trench, including three novel species. Comparative genomic analysis showed that hadal *Pseudomonas* possess more unique ortholog groups of genes related to energy generation and substances transport, distinct from those in other marine zones. These bacteria exhibit the ability to utilize diverse electron acceptors and accumulate compatible solutes, indicating two key strategies for adaptation for high hydrostatic pressure conditions. Furthermore, predicted genomic capabilities suggest that *Pseudomonas* could decompose various components of organic matter, particularly aromatics, as supported by metatranscriptomic datasets. These findings significantly enhance our understanding of *Pseudomonas* diversity and metabolic potential, providing valuable insights into the carbon and nitrogen cycles in hadal trench ecosystems.

Keywords Hadal Trench, *Pseudomonas*, Metagenome-assembled genomes, Hydrostatic pressure adaptation, Metabolic functions

Introduction

Hadal zones, defined as the ocean zone with a water depth exceeding 6000 m, are characterized by high hydrostatic pressure, low temperature, and low carbon availability [1, 2]. Despite the extreme conditions, microbial populations and their activities remain remarkably high [3, 4]. Previous geochemical studies have revealed unexpectedly high rates of microbial carbon turnover in hadal sediments, comparable to those in shallower and more productive oceanic regions [3]. Additionally, microbial cell abundance in the hadal sediments compared exhibit higher biomass, particularly in deeper layers, compared to abyssal sediments [5–7]. These findings

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suggest that the hadal zone, the deepest part of the ocean, may play a more significant role in carbon cycling than previously estimated [3, 8].

In hadal zones, the dominant microbial phyla include Thaumarchaeota, Proteobacteria, Planctomycetes, Chloroflexi, and Bacteroidetes. Besides hydrostatic pressure, organic matter and redox gradients are additional factors influencing the microbial composition in these extreme environments [6, 9]. *Pseudomonas*, a genus with the Proteobacteria, is one of the most prevalent bacterial groups in hadal water and sediments [10, 12, 13]. Amplicon and metagenomic datasets have shown that *Pseudomonas* are the major taxon in both the Kuril Kamchatka Trench [14] and the Mariana Trench [15]. *Pseudomonas* is one of the most diverse and ecologically significant group of bacteria on the planet [16]. Its members are widely distributed in all major natural environments (terrestrial, freshwater, and marine) and also form intimate associations with plants and animals [16]. This universal distribution highlights a remarkable physiological and genetic adaptability. *Pseudomonas* can utilize and produce various carbon and nitrogen compounds through divergent metabolic pathways. For example, *Pseudomonas* sp. strain MT-1, isolated and characterized from mud recovered from a depth of 11,000 m in the Mariana Trench, owns the ability for denitrification [17]. *Pseudomonas* was adapted to utilize both labile and ring-structured refractive substrates, as demonstrated by a 7-months chemostat experiment [18]. Therefore, understanding the role of *Pseudomonas* will provide essential insights into the carbon and nitrogen dynamics in the deep ocean.

The unique and extreme environmental conditions of the hadal zone result in metabolic potential differences between its microbial inhabitants and their relatives in shallower marine regions. For example, single-cell genomes of Atribacterota JS1 in the Japan Trench possess more genes related to organic matter degradation compared to closely related clades from other environments [19]. The deep-ocean Chloroflexi employs a "feast-or-famine" metabolic strategy to endure fluctuating and diverse inputs of organic matter in the hadal trench [20]. Comparative genomic analysis based on single amplified genomes has shown that genes involved in cell wall/membrane/envelope biogenesis, transcriptional regulation, and metal transport are crucial for the adaptation of hadal *Roseobacter* and *Alteromonas* lineages [21]. Therefore, using closely related species from shallower marine regions as references is not ideal for analyzing the metabolic potential of microorganisms in the hadal zone. Instead, studies based on MAGs derived from in situ environments offer a more accurate perspective. Moreover, certain microorganisms in the hadal zone may exhibit low or even no transcriptional activity despite their presence [22]. To accurately assess their contributions to elemental cycling, in situ metatranscriptomic data are essential for providing more reliable evidence.

In this study, we retrieved 21 *Pseudomonas* MAGs and reveal their taxonomic diversity, metabolic potential, and distribution based on amplicon and metagenomic datasets. Comparative genomic analysis revealed that *Pseudomonas* in the hadal zone possess unique ortholog groups (OGs) of genes associated with energy generation and substance transport, distinguishing them from their counterparts in other marine zones. Metatranscriptomic results further demonstrated that *Pseudomonas* actively contributes to nitrogen and carbon cycling in the hadal environment.

Results

Wide distribution of the genus *Pseudomonas* in the hadal trench environment

To determine the distribution pattern of *Pseudomonas* in the hadal trench environment, 81 amplicon datasets from the Mariana Trench were analyzed (Fig. 1A, Table S1). The genus *Pseudomonas* was detected in 58 datasets, with relative abundance ranging from 0.02% to 7.00% (average 0.94%) in the bacterial community (Fig. 1B). For 24 datasets from hadal zones, the genus *Pseudomonas* was detected in 20 datasets, with relative abundance ranging from 0.05% to 7.00% (average1.08%) in the bacterial community. We also estimated the relative abundance of *Pseudomonas* based on 120 metagenomic datasets by directly classifying reads (Fig. 2A, Table S1). All the metagenomic datasets possessed reads belonging to *Pseudomonas*, ranging from 0.40% to 10.59% (average 2.30% for 27 seawater samples and 2.74% for 93 sediment samples) of the prokaryotic community (Fig. 2B). As for the datasets in hadal zones, the values became change to values were observed, 2.57% (seawater samples, $n=17$) and 2.75% (sediment samples, $n=88$).

Genomic feature and the distribution of *Pseudomonas* in the hadal zones

We reconstructed 21 *Pseudomonas* metagenome-assembled genomes (MAGs) with completeness > 50% and contamination < 10% from the 120 metagenomic datasets, covering different sediment layers and different depths in the Mariana Trench (Table S1). The result of GTDB-Tk showed that the obtained MAGs belonged to 4 genera, *Pseudomonas* ($n=1$), *Pseudomonas_A* ($n=9$), *Pseudomonas_D* ($n=6$), and *Pseudomonas_E* ($n=5$) (Fig. 3). These MAGs were further dereplicated at 95% average nucleotide identity (ANI) yielding 12 representatives MAGs with average completeness of 83.55% (62.23% to 100.00%) and contamination of 3.18% (0.00% to 5.69%), among which seven were high-quality

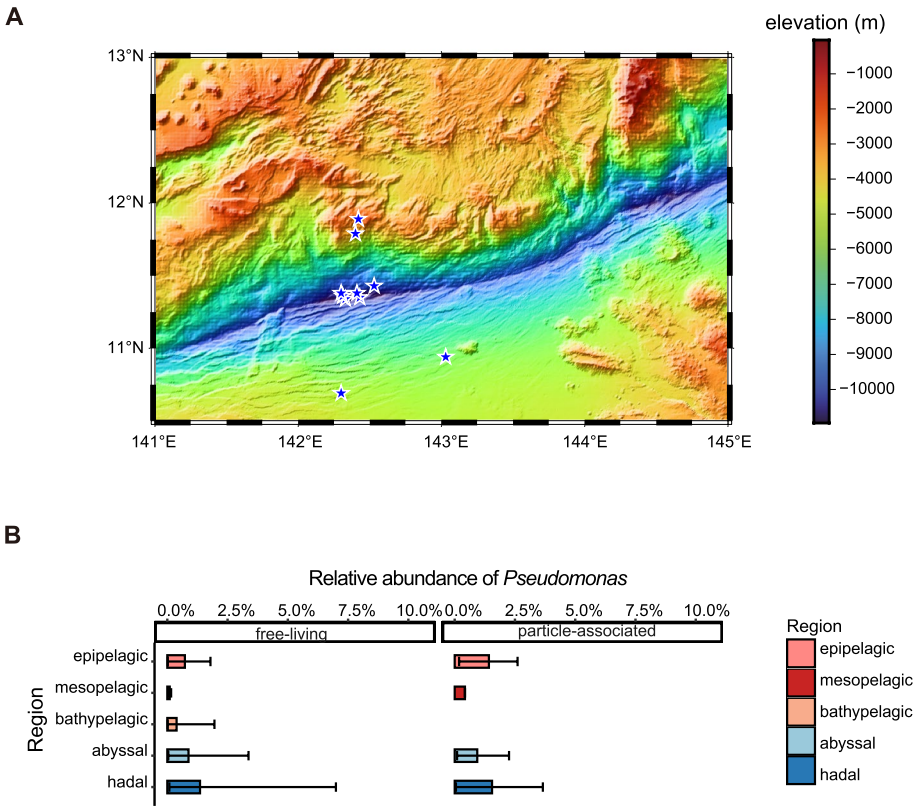

Fig. 1 Relative abundance of *Pseudomonas* in different marine zones based on amplicon datasets. The water depth for regions is: epipelagic, 0 ~ 200 m; mesopelagic, 200 ~ 1000 m; bathypelagic, 1000 ~ 4000 m; abyssal, 4000 ~ 6000 m; hadal, more than 6000 m. **A** The sampling sites of the amplicon datasets. **B** Relative abundance of *Pseudomonas*. The samples were categorized into free-living and particle-associated groups

MAGs (> 90% completeness and < 5% contamination). The genome sizes ranged between 2.48 and 6.85 Mbp, and GC contents were between 60.42% and 66.89% (Table S2). The result of GTDB-Tk and average nucleotide identity with reference genomes revealed that there were 3 representative MAGs belonging to novel species, with no reference genomes available (Sed6, SW9, and SW8) (Table 1 and Table S3). The distribution of the reconstructed MAGs was evaluated by read recruitments against 120 metagenomic datasets derived from Mariana Trench, including seawater from different elevations and sediments of different depths (Fig. 2C). Consistent with the result of metagenomic reads classification, *Pseudomonas* MAGs were identified from all samples (Fig. S1). The novel species occupied 0.09% to 91.69% (average 18.66%) of all *Pseudomonas* in 112 datasets, 0.01% to 90.26% (average 13.52%) for Sed6, 0.01% to 32.52% (average 2.22%) for SW8, and 0.03% to 56.14% (average 5.11%) for SW9. Although *Pseudomonas* were widely distributed, no significant correlation was observed between water depth and RPKM values for most MAGs (Fig. S2). Exceptions were MAG SW8 ($R^2 = 0.57$, $p = 0.0032$) and MAG Sed10 ($R^2 = 0.57$, $p = 0.0023$) in sediment samples, both of which showed higher abundance in deeper sediments.

Metabolic potentials of *Pseudomonas* in hadal zones

To investigate the metabolic potentials of *Pseudomonas* in the hadal trench, eight representative MAGs with completeness > 80% and contamination < 5% were further analyzed (Fig. 4, Table S5). All MAGs contain the complete or nearly complete TCA cycle (M00009), gluconeogenesis (M00003) and assimilatory sulfate reduction (M00176). Except for Sed6, all the other seven MAGs have all the genes for sulfate transportation. For nitrogen metabolism, complete dissimilatory nitrate reduction (M00530) was identified in 6 MAGs, and denitrification (M00529) was identified in five MAGs. Genes related to nitrate/nitrite transporter were detected in five MAGs, with all but the MAG Sed10 containing genes associated with nitrate reduction. Four types of complex IV of the electron transport chain were detected (*bd* type, *bo* type, caa_3-tpye and cbb_3-type), with caa_3-tpye and cbb_3-type being found in all MAGs.

The metabolic reconstruction of the recovered MAGs revealed the potential of hadal *Pseudomonas* for the

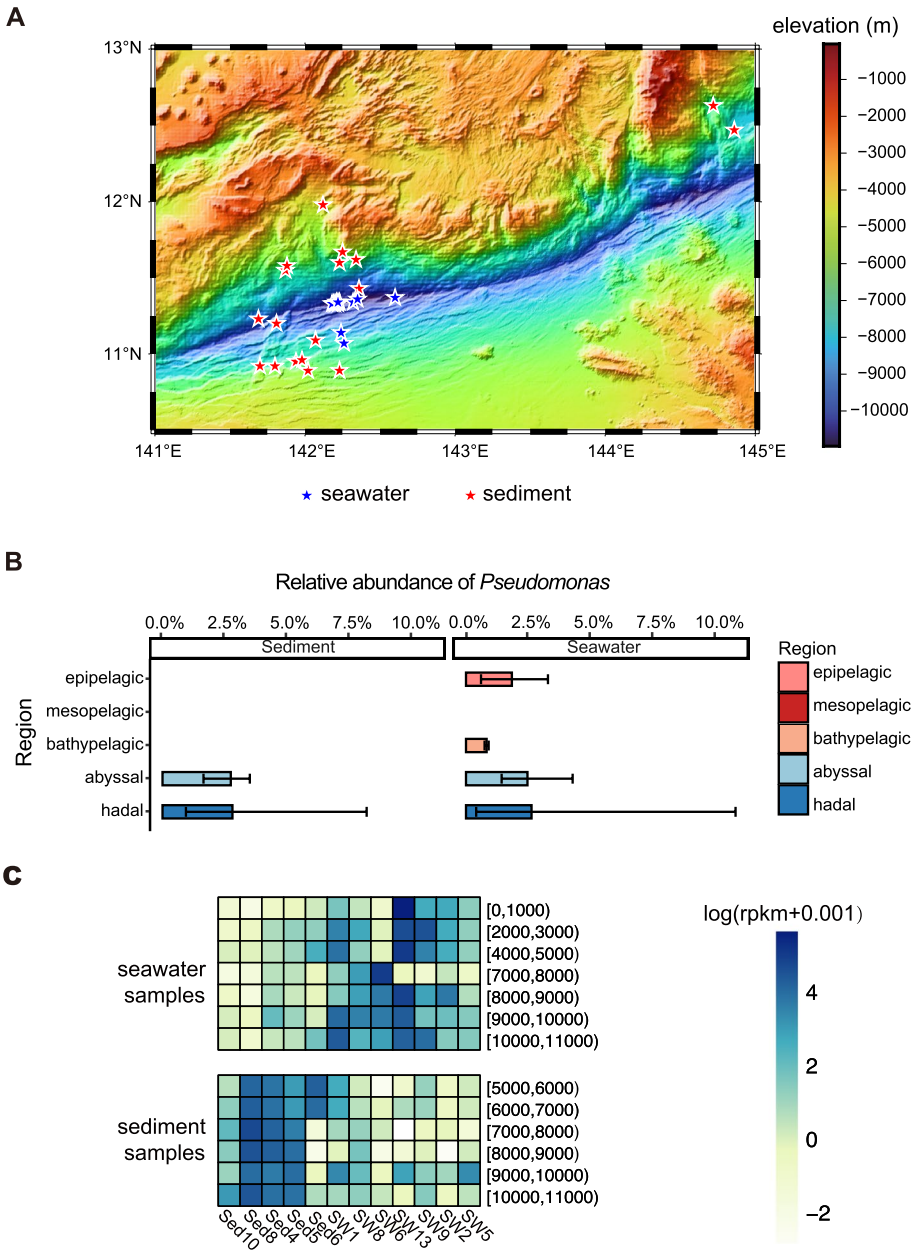

Fig. 2 Relative abundance of *Pseudomonas* in different marine zones based on metagenomic datasets. The water depth for regions is: epipelagic, 0 ~ 200 m; mesopelagic, 200 ~ 1000 m; bathypelagic, 1000 ~ 4000 m; abyssal, 4000 ~ 6000 m; hadal, more than 6000 m. The samples were categorized into seawater and sediment groups. **A** The sampling sites of the metagenomic datasets. **B** Relative abundance of *Pseudomonas* based on reads classification. **C** Relative abundance of *Pseudomonas* representative MAGs

absorption and synthesis of compatible solutes. Firstly, the modules of the synthesis of ectoine (M00033) and trehalose (M00565) were detected in five and six MAGs respectively. Only MAG SW2 completely lacked both modules. Secondly, genes for the transporters of various compatible solutes were found, such as those for inorganic matters (nitrate, and nitrite) and small organic matters (ribose, branched amino acid, putrescine, and so on) (Table S5).

Transporters for complex organic matters (such as gamma-hexachlorocyclohexane and benzoate) were found in almost all representative MAGs. To verify this, we investigated the presence of genes associated with the degradation of various complex organic matters

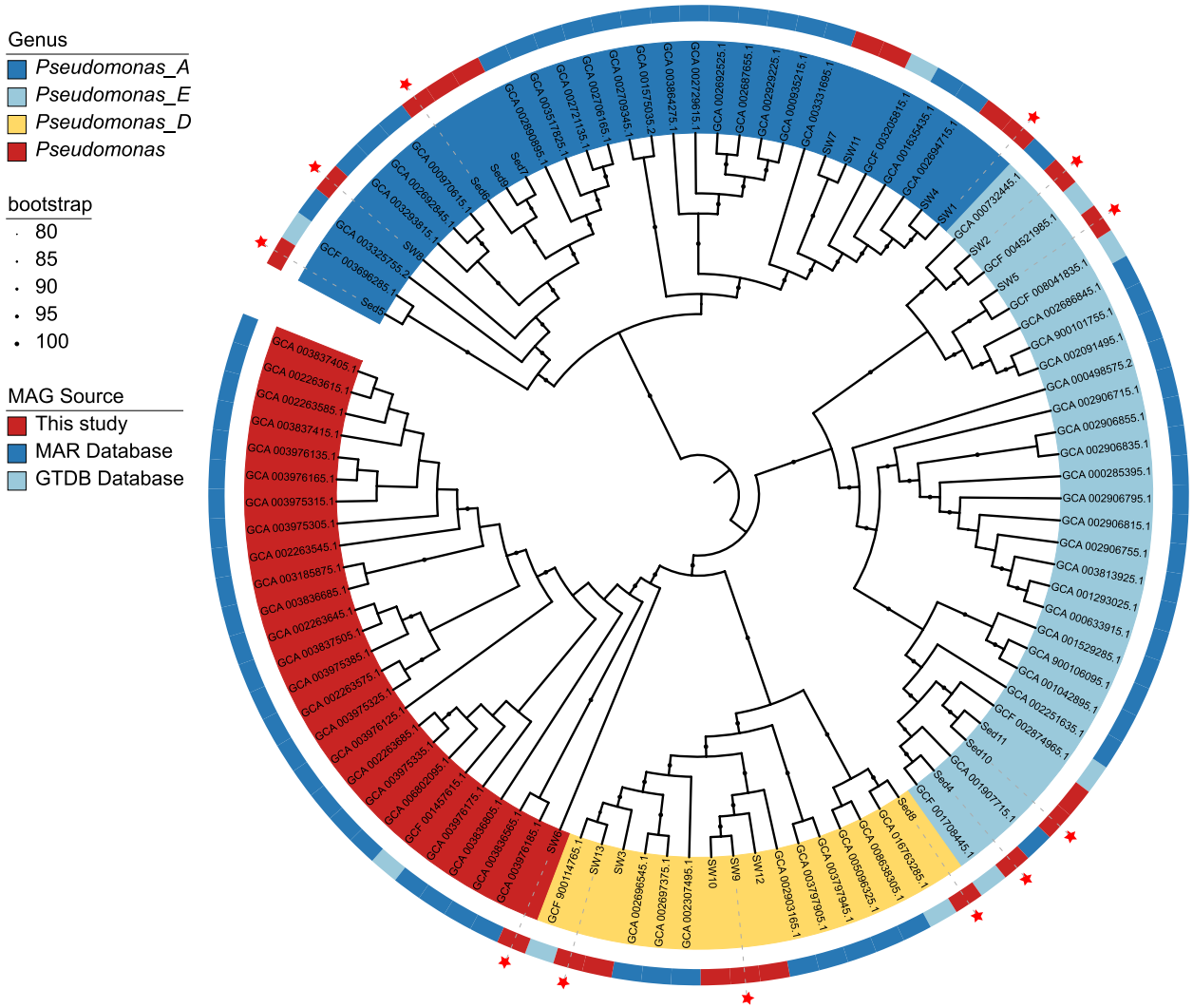

Fig. 3 *Pseudomonas* MAGs from marine metagenomic datasets. The four genera are marked in different background colors. The source of MAGs is shown with the colors of strip. The red asterisk indicates the representative *Pseudomonas* MAGs from hadal zone

Table 1 Summary of the three representative MAGs for putative novel species

MAGs	% Completeness	% Contamination	# contigs	Total length (Mbp)	GC (%)	N50
Sed6	98.02	4.53	257	4.59027	60.63	27,599
SW8	62.23	3.65	831	3.03881	60.42	4,064
Sw9	99.59	2.78	32	3.732608	61.40	166,959

reported in a previous study [20] (Fig. S3A). The result showed that genes of chitinase were found in three MAGs. Additionally, a nearly complete pathway of dichloroethane degradation was found in four MAGs. We also evaluated the completeness of modules for aromatics degradation. Five MAGs were found to have the capacity to degrade benzoate, anthranilate, catechol, and phenylacetate, producing simpler organic matter (Fig. S3A).

We also found 43 types of extracellular carbohydrate-active enzymes (Table S4). Among them, AA1, CBM50, and GH23 were the most widely distributed. AA1 enzymes are multicopper oxidases that use diphenols and related substances as donors with oxygen as

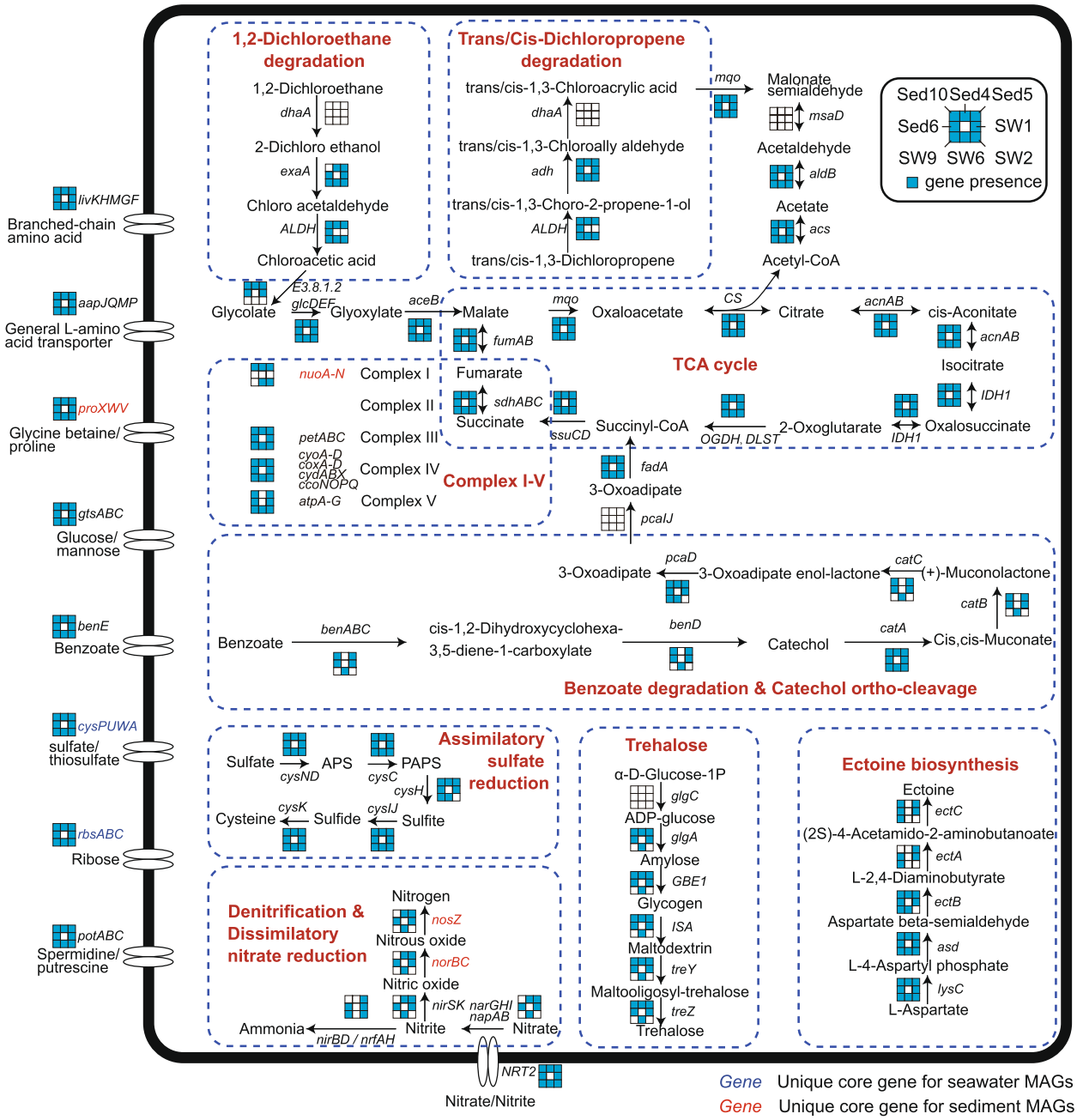

Fig. 4 Overview of the metabolic potentials in the assembled *Pseudomonas* MAGs. Colored names indicate the genes only exist in the core genome of MAGs from hadal trench and the colors represent the source of MAGs, blue for seawater and ref for sediment. Colored squares indicate the related genes are present

the acceptor. CBM50 were enzymes targeting the peptidoglycan such as peptidases and amidases, the related genes in hadal *Pseudomonas* MAGs were annotated as N-acetylmuramoyl-L-alanine amidase (K01448), secretion pathway protein D (K02453), murein DD-endopeptidase (K19304), and lipoprotein NlpD (K06194). GH23 was related to lytic transglycosylases, with the related genes being mainly responsible for membrane-bound lytic murein transglycosylase except the transporters. Additionally, there were also 2 peptidases found in almost all MAGs (Fig. S3B, Table S4), such as zinc protease (K07263), and metalloendopeptidase (K23010), hydrolyzing peptide bonds located in the center of proteins [23].

Three metatranscriptomic datasets from Mariana Trench were also collected to analyze the transcript activity for 12 representative MAGs. The result showed that all MAGs were detected with transcriptomic activity (Fig. S4). For these MAGs, expressed genes covered a complete TCA cycle, complex I-V for electron transfer, and transporters for branched-chain amino acid and benzoate (Fig. S4C). Genes in the biosynthesis of trehalose (K00700, K00703 and K06004) and ectoine (K00133, K00836 and K00928) were expressed in at least one MAG. Genes related to aromatics degradation were expressed, such as anthranlilate degradation (M00637), catechol orgho-cleavage (M00568) and phenylacetate degradation (M00878, only genes for K02615 were not transcribed) (Fig. S4). *Pseudomonas* is estimated to contribute between 3.62% and 19.99% of the transcripts related to three aromatic compounds, based on transcripts per million (TPM). Additionally, it contributes between 3.40% and 21.14% to carbohydrate-active enzymes, specifically AA1, CBM50, and GH23 (Fig. S4A). Besides, genes in trans/cis-dichloropropene degradation (K00001, K13954, K22474), DMSP/DMS degradation (K17228), 1,2-dichloropropene degradation (K00128, K01560, K01561) were also detected with expression (Table S4).

Pseudomonas MAGs from non-hadal zones (marine samples with water depth smaller than 6000 m) were collected as reference group for comparative genomic analysis based on core genome (Table S2). For MAGs from seawater samples, 100 and 173 OGs were unique in MAGs in reference group and hadal group, respectively. And for MAGs from sediment samples, 92 and 202 OGs were unique in MAGs in reference group and hadal group, respectively (Fig. S5A). The functional category of unique OGs was similar for MAGs from seawater and sediment samples. For example, OGs unique in reference group were mostly related to "translation, ribosomal structure and biogenesis" (category J). While OGs unique in hadal group were related to "function unknown" (category S), "energy production and conversion" (category C), and "amino acid transport and metabolism" (category E), "inorganic ion transport and metabolism" (category P), and "carbohydrate transport and metabolism" (category G). The number of OGs for these categories in hadal group were also larger than that in reference group (Fig. S5B). For hadal MAGs from seawater samples, unique OGs covered the transport of sulfate/thiosulfate and ribose and for hadal MAGs from sediment samples, unique OGs covered the transport of glycine betaine/proline and partial denitrification (Fig. 4).

Discussion

Pseudomonas are widely distributed in the hadal zones

Pseudomonas was detected in more than 80% of amplicon datasets and almost all metagenomic datasets from hadal zones (Figs. 1 and 2, Fig, S1). For instance, amplicon sequence variants of *Pseudomonas* have been detected in sediment and seawater samples from the Marian Trench, the Japan Trench, and the Kuril Kamchatka Trench [12, 14, 24]. *Pseudomonas* also dominate the microbial communities in the Mariana Trench and the Kermadec Trench [10–13]. Our results also showed that *Pseudomonas* accounted for 1.08% of the bacterial community and about 2.5% of the prokaryotic community based on the amplicon and metagenomic datasets, respectively. Our results were based on the 19 amplicon and 105 metagenomic datasets from 32 sampling sites in the hadal zone, covering the Mariana Trench from the water depth from 6014 to 10911m, making the conclusion more representative [25]. Besides, 24 strains of *Pseudomonas* were isolated from Mariana Trench sediment samples [26] and there were two piezophilic *Pseudomonas* isolated from Mariana Trench [27] and Japan Trench [28]. Furthermore, the near-ubiquitous distribution of most MAGs across various metagenomic datasets, obtained from different cruises and laboratories, indicates that these *Pseudomonas* are not contaminants. The metatranscriptomic analysis results indicated that *Pseudomonas* MAGs were transcriptionally active (Fig. S4B). We acknowledge the possibility that some microorganisms may have sunk from shallower depths and are not specific to the hadal zone. However, given the transcriptional activity of most MAGs, it can be inferred that they actively participate in carbon and nitrogen cycles within the hadal zone (Fig. S4C). These findings collectively suggest that hadal *Pseudomonas* are well-adapted to the extreme conditions of the hadal environments.

Genomic analysis of *Pseudomonas* reveals their living strategy adapting to the hadal trench environment

By reconstructing MAGs, we obtained 12 representative *Pseudomonas* MAGs and revealed its taxonomy compositions. Compared with reference genomes in database and isolates from deep sea, three MAGs were putative novel species, extending the knowledge of the *Pseudomonas* diversity (Fig. 3, Table S3). Genomic evidence highlights two key adaptation strategies. The first strategy involves multiple energy generation pathways, including the ability to utilize multiple types of electron acceptors. In extreme environments, particularly in the deep sea under high hydrostatic pressure, cells faces inadequate energy supply [29], and tend to upregulate genes for energy metabolism [30–34]. All representative MAGs in

this study possess both caa_3-tpye and cbb_3-type oxidases, allowing adaptation to varying oxygen concentration [35, 36]. Moreover, seven MAGs in this study could use nitrate or nitrite as an electron acceptor through denitrification or dissimilatory nitrate reduction (Fig. 4), ensuring effective energy generation and growth in extreme environments. This may explain the existence of *Pseudomonas* in different layers of sediment, even in metagenomic dataset "Mariana_T1B10-23" (46 cm below seafloor), and their dominance in the 12 to 18 cm samples from the Mariana Trench [15]. The presence of nitrite and nitrate may be attributed to ammonia-oxidizing archaea (AOA) and nitrite-oxidizing bacteria, with AOA being a dominant group in hadal zone [37, 38]. The second strategy is the absorption and synthesis of compatible solutes to maintain osmotic pressure balance across cells, commonly seen in piezophiles. The reported compatible solutes in hadal trench organisms were DMSP [39], trimethylamine n-oxide (TMAO) [40] and glycine betaine [41]. Trehalose and ectoine were also compatible solutes to cope with high hydrostatic pressure [42, 43]. All *Pseudomonas* MAGs in this study could produce at least one of them. Metatranscriptomic analysis indicated the transcript activity of gene *GBE1*, *treY* and *ectB*, involved in the biosynthesis of trehalose and ectoine (Fig. S4C). In addition to synthesis, *Pseudomonas* can also uptake small organic compounds like glycine betaine, spermidine, putrescine, and amino acids from the environment (Table S5) [44]. Hadal *Pseudomonas* also encode at least two types of peptidases, breaking down long protein chains into short peptides, which may also act as compatible solutes (Fig. S3B). Another abundant phylum in hadal zones, SAR406, shows similar genomic features, with hadal-depth MAGs enriched in amino acid metabolism genes [45]. Comparative genomic analysis reveals that the core genome of hadal *Pseudomonas* MAGs had more unique OGs related to energy production and conversion, and the transport and metabolism of amino acid and inorganic iron (Fig. S5), consistent with the two strategies mentioned above.

Pseudomonas plays an important role in the degradation of organic matter

Genes for chitinases were found in three MAGs, Sed10, SW6, and Sed4 (Fig. S3A). Sed4 is one of the most abundant bacterial groups in hadal trench sediments (Fig. 2B). There would be abundant amphipods serving as the source of chitin in Mariana Trench [46]. Both metagenomics and cultivated isolations from hadal zones indicated prokaryotes could degrade chitin as a carbon and nitrogen source [47–49]. Hadal *Pseudomonas* MAGs also have the potential to degrade aromatic compounds, by the cleavage of benzene rings (catechol ortho-cleavage, M00568; phenylacetate degradation, M00878) or the degradation of complex compounds (benzoate degradation, M00551; anthranilate degradation, M00637) (Fig. S3A). Benzoate transporters are present in almost all MAGs, as well as 3-phenylpropionic acid (K05820) (Table S5), enabling the uptake of exocellular aromatics. Metatranscriptomic analysis showed the transcriptional activity of four modules for degradation of aromatic compound in MAG Sed4 and Sed10 (Table S4). Hadal *Pseudomonas* MAGs also possess genes coding for extracellular enzymes, targeting the peptidoglycan (CBM50) and murein (GH23, GH103). Extracellular enzyme secretion is a common method for *Pseudomonas* to obtain resources from complex chemical substrates [50–52]. The percentage of TPM of the expressed genes showed that *Pseudomonas* play a vital role in the aromatics and carbohydrate-active enzymes (Fig. 4A). Additionally, genes for the degradation of halogenated compounds, such as 1,2-Dichloroethane, were identified in four MAGs (Fig. S3A). Recent studies have documented the accumulation of halogenated organic pollutants in the Mariana Trench and the presence of microorganisms capable of degrading these pollutants [6, 20, 53]. Furthermore, numerous genes the transport of ribose, oligopeptides, and branched-chain amino acids align with previous findings that *Pseudomonas* play vital roles in amino acid and sugar turnover [54–56]. These findings confirm the key roles of *Pseudomonas* in the cycling of organic compounds in hadal trenches.

In summary, the result of amplicon and metagenomic datasets indicated the wide distribution of *Pseudomonas* in hadal zones. MAGs reconstruction revealed three novel species of *Pseudomonas*, enhancing our understanding of *Pseudomonas* diversity. Genomic potential and comparative genomic analysis highlight the strategies for *Pseudomonas* adapting to high hydrostatic pressure, including utilizing multiple types of electron acceptors for energy generation and accumulating compatible solutes through both biosynthesis and transport. Combined with metatranscriptomic results, *Pseudomonas* in hadal zones were determined to have the ability to degrade complex organic matters as substrate for energy generation. Metatranscriptomic datasets from various sampling sites are also needed to explore the expressed pattern of *Pseudomonas* in the hadal zone. Overall, *Pseudomonas* plays a vital ecological role in driving biogeochemical carbon and nitrogen cycling in the hadal zone.

Materials and methods

Collection of public datasets and amplicon sequencing analysis on 16S rRNA

In this study, 81 amplicon datasets, 120 metagenomic datasets and 14 metatranscriptomic from previous studies for Mariana Trench were collected [20, 26, 38, 57–64].

The sampling site were visualized by GMT [65]. The publications (except SRR7974510, SRR6057436), accession numbers and information of these datasets are shown in Table S1. The analysis of amplicon datasets was performed by using the pipeline QIIME2 (version 2022.2) [66]. First, the partial region was extracted with the corresponding primer set from the sequences in the SILVA database (version 138) [67] and used to train a classifier by using the "feature-classifier" plugin. Then, all the datasets were grouped by primer. After trimming by the corresponding primer, the sequencing quality of the raw reads was manually checked to determine a proper truncated position to filter the regions with low quality. Then, the paired-end reads were merged and dereplicated by using the "dada2" plugin. Taxonomy of obtained amplicon sequence variants (ASVs) was determined by the classifier trained above. Unassigned, archaeal, or eukaryotic ASVs were removed. Then rare ASVs, with relative abundance < 0.001 and prevalence < 0.001 were discarded.

Sample collection, DNA extraction and sequencing

One hundred twenty metagenomic datasets from 31 sites were analyzed. For 33 samples present in this study (labeled as "This study" in Table S2), sediment push core samples were collected from the Mariana Trench during cruises by DY37II in 2016. Each core was cut into 4-cm-wide or 2.5-cm-wide sections and immersed in RNAlater (Thermo Fisher Scientific, Waltham, USA). The sediment sample stored in RNAlater was collected by centrifugation at 8 000 × g for 10 min and washed with PBS buffer. Two grams of RNAlater-free sediment sample were mixed with half weight of 0.1 mm and 0.5 mm glass beads mixture and 6 mL of extraction buffer by low-speed vortexing for 5 min. The mixture was homogenized with MP FastPrep-24 homogenizer (MP Biomedicals, CA, USA) at 4.0 m/s for 20 s with a 2 min interval for two cycles. Then, 60 µl lysozyme (100 mg/ml) and 60 µl proteinase-K (20 mg/ml) were added, and the mixture was incubated for 30 min at 37 °C. Then, 420 µl 20% sodium dodecyl sulfate was added and incubated at 65 °C for 2 h. The supernatant was collected by centrifugation at 10 000 × g for 10 min at room temperature. The extraction was repeated once with residual pellets. The supernatants from the two rounds of extraction were combined and mixed with equal volumes of phenol:chloroform:isoamyl alcohol (24:25:1 [v/v], pH 8.0). The aqueous phase was recovered by centrifugation and then precipitated overnight at 4 °C with a 0.6 volume of isopropyl alcohol and 0.1 volume of 3 M sodium acetate (pH 5.2). The nucleic acid pellet was collected by centrifugation at 15 000 × g for 20 min at 4 °C. The supernatant was removed, and the nucleic acid pellet was washed twice with precooled 70% ethanol and then dissolved in TE buffer. DNA was further purified with the synchronous coefficient of drag alteration (SCODA) method, performed with the Boreal Genomics' Aurora Nucleic Acid Extraction System (Boreal Genomics, Vancouver, BC), and quantified by a Qubit 3.0 fluorometer with a DNA HS Assay Kit (Thermo Fisher Scientific, MA, USA). In total, 50 ~ 300 ng purified DNA samples were sent to the Beijing Genomic Institute (BGI, Shenzhen, China) for paired-end sequencing by the HiSeq 2500 platform.

Metagenome-assembled genome creation

Firstly, raw reads were trimmed with Trimmomatic [68]. Clean reads were classified by Kraken2 [69] with its pre-build "PlusPFP" database. The relative abundance of *Pseudomonas* was estimated by Bracken [69]. Reads classified as Eukaryota and Viruses were ignored when determined the relative abundance. Then, the assembly was performed by using either Megahit (version 1.2.9) [70] with "–presets meta-sensitive". Contigs shorter than 1000 bp were discarded. Reads mapping and binning were performed with MetaWRAP (version 1.3) [71]. The quality of each MAG was evaluated using CheckM (version 1.1.3) and MAG classification was performed with GTDB-Tk tools (version 2.2.3) with the GTDB database (version207v2) [72–74]. Gene was predicted using Prodigal (version 2.6.3) [75], and open reading frames shorter than 33 amino acids were trimmed. MAGs classified as *Pseudomonas* were dereplicated with dRep (version 3.2.2) [76]. For identification of novel species, we collected the *Pseudomonas* genomes isolated from deep sea [77–80], MAGs belonging to the same genus in the GTDB database and from MAR database (Table S2), then performed an ANI analysis with fastANI [81]. 16S rRNA genes were identified using Barrnap (version 0.9, https://github.com/tseemann/barrnap) and genes shorther than 900 bp were discarded. Gene annotation was primarily performed by using KofamKOALA [82]. Unannotated genes would be aligned with Uniprot [83] database by DIAMOND [84], with identity > 40% and coverage of query > 40%. OGs were determined by eggnog-mapper, with identity > 40% and coverage of query > 40% [85]. Enzyme locations were predicted using SignalP 6.0 with fast mode [86]. Potential carbohydrate-active enzymes were identified using dbcan [87]. The metabolic potential of each MAG was evaluated based on the completeness of metabolic modules, determined by the presence/absence of KO terms with a custom Python script. Reads recruitments were performed using BBmap, with qualified hits used to compute the RPKM (Reads Per Kilobase of transcript, per Million mapped reads) values by coverM (https://github.com/wwood/CoverM), which reflect a normalized abundance allowing the comparison across different genomes and metagenomes.

Comparative genomic analysis based on OGs

A total of 71 *Pseudomonas* MAGs sourced from shallower marine zones (water depth less than 6000 m) were collected from MAR database [88]. According to the metadata from MAR database, 17 MAGs were from sediment samples and 54 MAGs were from seawater samples. The methods for processing of these 71 MAGs were the same as the description above. 33 representative MAGs were obtained. Representative MAGs with completeness > 80% and contamination < 5% were chosen for comparative genomic analysis. MAGs were categorized into four groups: reference MAGs from sediment samples ($n = 12$), reference MAGs from seawater samples ($n = 17$), MAGs from hadal sediment samples ($n = 4$) and MAGs from hadal seawater samples ($n = 4$). OG of gene were determined by eggnog-mapper. If OG was present in at least 75% of MAGs in each group, the corresponding gene would be defined as core genes.

Phylogenomic analysis

Genomes of *Pseudomonas* from MAR database and GTDB database were chosen as the reference for the tree of MAGs. The bacterial single copy geneset was identified by using GTDB-Tk tools. Then, for each marker gene set, aligning and trimming with the same parameter were performed separately by using MAFFT (version 7.490) [89] and trimAL (version 1.4) [90] with the automated1 model. Then, all the alignments were concatenated for each genome. IQTREE (version 2.2.0.3) [91] was used to construct the phylogenetic tree.

Processing of metatranscriptomic datasets

Raw reads were first tirmmed by Trimmomatic for poor-quality base and then filtered by Ribodetector [92] for remove rRNA reads. MAGs from all metagenomic datasets were dereplicated with dRep. The unaligned mRNA reads were mapped to the representative MAGs by BBmap. Finally, featureCounts was used to count the read number of each gene [93]. Genes with reads > 50 in any dataset were regarded as expressed genes. TPM was calculated with a custom Python script.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-03834-7.

Supplementary Material 1.

Supplementary Material 2.

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Authors' contributions

Y. L., and Y. Z. designed the research. Y.L., X.W. and Y.Z. performed the research and analyzed the data. Y.L., L.Z., and Y.Z. drafted the manuscript.

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Data availability

The metagenomic sequencing data have been deposited in the GSA database with the accession CRA021595 and CRA021596 (https://ngdc.cncb.ac.cn/gsa/browse/CRA021595 and https://ngdc.cncb.ac.cn/gsa/browse/CRA021596) [94, 95]. All metagenome-assembled genomes have been deposited in Figshare (https://doi.org/10.6084/m9.figshare.25131239.v1).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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