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Cultivation of deep-sea bacteria from the Northwest Pacific Ocean and characterization of *Limnobacter profundi* sp. nov., a phenol-degrading bacterium

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Despite previous culture-independent studies highlighting the prevalence of the order *Burkholderiales* in deep-sea environments, the cultivation and characterization of deep-sea *Burkholderiales* have been infrequent. A total of 243 deep-sea bacterial strains were isolated from various depths in the Northwest Pacific Ocean, with 33 isolates (13.6%) from a depth of 4000 m classified into *Burkholderiales*. Herein, we report the isolation and genome characteristics of strain SAORIC-580^T, from a depth of 4000 m in the Northwest Pacific Ocean. The strain showed a close phylogenetic relationship with *Limnobacter thiooxidans* CS-K2^T, sharing 99.9% 16S rRNA gene sequence identity. The complete whole-genome sequence of strain SAORIC-580^T comprised 3.3 Mbp with a DNA G+C content of 52.5%. Comparative genomic analysis revealed average nucleotide identities between 79.4–85.7% and digital DNA-DNA hybridization values of 19.9–29.5% when compared to other *Limnobacter* genomes, indicating that the strain represents a novel species within the genus. Genomic analysis revealed unique adaptations to deep-sea conditions, including genes associated with phenol degradation, stress responses, cold adaptation, heavy metal resistance, signal transduction, and carbohydrate metabolism. The SAORIC-580^T genome was found to be more abundant in the deep sea than at the surface in the trenches of the Northwest Pacific Ocean, suggesting adaptations to the deep-sea environment. Phenotypic characterization highlighted distinct differences from other *Limnobacter* species, including variations in growth conditions, enzyme activities, and phenol degradation capabilities. Chemotaxonomic markers of the strain included ubiquinone-10, major fatty acids such as $C_{16:0}$, $C_{16:1}$, and $C_{18:1}$, and major polar lipids including phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. Based on the polyphasic taxonomic data, it is concluded that strain SAORIC-580^T (= KACC 21440^T = NBRC 114111^T) represents a novel species, for which the name *Limnobacter profundi* sp. nov. is proposed.

KEYWORDS

***Limnobacter profundi*, deep sea, *Burkholderiales*, genome, novel species, phenol degradation**

1 Introduction

The order *Burkholderiales* within the class *Betaproteobacteria* encompasses approximately 130 genera distributed across five families, according to validly published names as recorded on the List of Prokaryotic names with Standing in Nomenclature (LPSN) as of May 2024 (https://lpsn.dsmz.de). Members of this order display considerable phenotypic diversity, exemplified by variations in oxygen relationships (ranging from strict aerobes to facultative anaerobes), morphologies (including rods and cocci), temperature preferences (from psychrophiles to mesophiles), flagellar arrangements, and types of respiratory quinones (ubiquinone and methylmenaquinone) (De Ley et al., 1986; Garrity et al., 2005; Morotomi et al., 2011). Moreover, these bacteria exhibit a broad metabolic spectrum, capable of processes such as aerobic anoxygenic phototrophy, nitrogen fixation, oxidation of substances including hydrogen, iron, and thiosulfate, and degradation of plastic and aromatic compounds (Garrity et al., 2005; Imhoff et al., 2018; Syranidou et al., 2019). *Burkholderiales* bacteria are ubiquitously distributed across diverse ecological niches, including soil, freshwater, seawater, groundwater, activated sludge, coral reefs, and animal gastrointestinal tracts, with notably high abundance in certain deep-sea environments (Ginige et al., 2013; Moreira et al., 2015; Aguirre-Von-Wobeser et al., 2018; Meng et al., 2019; Li et al., 2019a).

Culture-independent analyses based on 16S rRNA gene sequences have revealed that *Burkholderiales* constitute up to 30–40% of bacterial communities in specific deep-sea samples, underscoring their significant presence and potential adaptations to the extreme conditions found below 200 m depth, characterized by high pressure, low temperature, and nutrient scarcity (Eloe et al., 2011a, b; Zhang et al., 2018; Sinha et al., 2019). Recent genomic studies have significantly advanced our understanding of the genetic features of deep-sea bacteria, including the enrichment of transposable elements, transcriptional regulators, toxin-antitoxin systems, and genes involved in the degradation of aromatic compounds (Martin-Cuadrado et al., 2007; Konstantinidis et al., 2009; Zhang et al., 2018, 2019). Despite these advances, the physiological and genetic diversity of deep-sea *Burkholderiales* remains largely unexplored due to the scarcity of isolated strains.

The genus *Limnobacter*, a member of the order *Burkholderiales*, was first proposed following the isolation and description of a thiosulfate-oxidizing bacterium from freshwater lake sediment (Spring et al., 2001). As of August 2024, the genus comprises 5 species with validly published names in LPSN. Members of the genus have been isolated from diverse habitats including freshwater lake sediment, volcanic deposits, humus soil, surface seawater, and marine algae (Lu et al., 2011; Vedler et al., 2013; Nguyen and Kim, 2017; Duan et al., 2020). This genus is characterized by the production of polyhydroxybutyrate (PHB) as a storage compound, being chemolithoheterotrophic bacteria that utilize thiosulfate as an energy source through biological oxidation, the presence of a single polar flagellum, and a rod-shaped cell morphology (Nguyen and Kim, 2017). *Limnobacter* genomes show a genome size ranging 3.3–3.4 Mbp, with an average G+C content of 52.5–53.2%, and include diverse metabolic pathways such as sulfur oxidation, citronellol degradation, and exopolysaccharide production (Chen et al., 2016; Duan et al., 2020; Naruki et al., 2024). Although species of the genus *Limnobacter* have been discovered in diverse environments, no deep-sea isolates of the genus have yet been validly published. However, in the literature survey conducted in this work, we found an abundance of 16S rRNA gene sequences associated with this genus in several deep-sea habitats, including the deep-sea water (6000 m) of the Puerto Rico Trench (Eloe et al., 2011b) and the sediment (3788 m) of the Indian Ocean (Khandeparker et al., 2014). Additionally, a significant number of metagenomic and metatranscriptomic reads retrieved from the deep waters of the Mariana Trench matched *Limnobacter*-related genomes (Dang et al., 2024). Therefore, the genus *Limnobacter* might be recognized as part of the deep-sea *Burkholderiales*.

In this study, we isolated a proteobacterial strain, designated SAORIC-580^T, from seawater at a depth of 4000 m in the Northwest Pacific Ocean. The complete genome sequence of SAORIC-580^T was obtained and comparatively analyzed against other *Limnobacter* species using Clusters of Orthologous Groups (COG) categories, Pfam domains, and Genomic Islands (GIs). SAORIC-580^T features metabolic pathways, including phenol degradation, that suggest adaptation to the deep-sea environment. To the best of our knowledge, this genome represents the first genome in the order *Burkholderiales* cultivated from the deep sea. Phylogenetic and phenotypic analysis indicated that the strain belongs to the genus *Limnobacter* and is considered to represent a novel species within this genus. Based on these findings, we propose the name *Limnobacter profundi* for the new type strain, SAORIC-580^T.

2 Materials and methods

2.1 Isolation of deep-sea bacteria and bacterial cultures

During the research cruise MR-11-05 aboard the RV 'Mirai' (Japan Agency for Marine-Earth Science and Technology [JAMSTEC]) as part of the K2S1 project (Honda et al., 2017), deep seawater samples were collected at depths of 1000, 2000, 3000, and 4000 m at S1 station (32°00' N, 145°00' E) in the Northwest Pacific Ocean in July 2011 and immediately transferred to the cleanroom of the RV. Total cells of each seawater sample were stained with 4',6-diamidino-2-phenylindole (DAPI) and counted via epifluorescence microscopy (Nikon 80i, Nikon, Japan). An aliquot of each water sample (100 µl per plate) was spread on agar plates with two different compositions: one-fifth strength Marine Agar 2216E (1/5 MA; BD Difco, USA) and one-tenth strength R2A (1/10 R2A; BD Difco, USA), both dissolved in 1 L of seawater that had been aged in the dark for 1 year. After incubation at 10°C for 4 weeks in darkness, all colonies grown on the agar plates were transferred to new plates for pure cultivation and further analyses.

Among the colonies isolated from a depth of 4000 m, a beige-colored colony, designated SAORIC-580^T, was maintained on R2A or in R2A broth at 30°C, with transfers every three days, and also preserved as glycerol suspensions (20%, v/v) at –80°C for long-term preservation. For phenotypic and chemotaxonomic comparisons, *L. thiooxidans* KACC 13837^T (the type species of the genus *Limnobacter*), *L. alexandrii* KCTC 72281^T, *L. litoralis* NBRC105857^T, and *L. humi* KACC 18574^T were obtained from the Korean Collection for Type Cultures (KCTC), the Korean Agricultural Culture Collection (KACC), and NITE Biological Resource Center (NBRC), respectively, and routinely cultured on R2A at 30°C.

2.2 Phylogenetic analyses of the deep-sea isolates

Bacterial cells were treated using InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions, yielding supernatants that can be used for PCR. The 16S rRNA genes of the isolates were amplified by PCR using bacterial universal primers 27F and 1492R (Weisburg et al., 1991). PCR amplifications were performed according to the following protocol: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 60 sec, extension at 72°C for 90 sec, and a final extension at 72°C for 5 min. The resultant PCR products were digested with *Hind*III (Takara, Japan) at 37°C for 8 hours, visualized by electrophoresis on a 3% agarose gel, grouped using *Hind*III-based restriction fragment length polymorphism (RFLP) analyses, and representative PCR products were sequenced using a Sanger sequencer. The 16S rRNA gene sequences were analyzed using the BLASTn search in the GenBank database and the 16S-based ID application in the EzBioCloud (Yoon et al., 2017).

To determine the phylogenetic position of strain SAORIC-580^T, the 16S rRNA gene sequences of the strain and its closely related species were aligned using the ClustalW program (Thompson et al., 1997). Phylogenetic trees were constructed from unambiguously aligned 1472 bp of the 16S rRNA gene sequences using the maximum-likelihood method (Felsenstein, 1981) with the GTR +G+I model, the neighbor-joining method (Saitou and Nei, 1987) with Jukes-Cantor correction, and the minimum evolution method (Rzhetsky and Nei, 1993) employing the Tree-Bisection-Reconnection (TBR) method, as implemented in MEGA X (Kumar et al., 2018). The topological integrity of the phylogenetic trees was confirmed through bootstrap analyses (Felsenstein, 1985) based on 1000 replications.

2.3 Whole genome sequencing and genome analyses

Genomic DNA of strain SAORIC-580^T was extracted using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Whole-genome sequencing of the strain was conducted at Macrogen (Seoul, Republic of Korea) using the Illumina MiSeq platform, employing a 2×300 paired-end run. Additionally, nanopore sequencing was performed. Genomic DNA was extracted with the MagAttract HMW DNA Kit (Qiagen, Hilden, Germany), and a sequencing library was prepared using the Ligation Sequencing Kit (SQK-LSK109, Oxford Nanopore Technologies, Oxford, UK) and Native Barcoding Expansion pack (EXP-NBD104, Oxford Nanopore Technologies, Oxford, UK), following the protocols provided by the manufacturer. Sequencing was then performed using an R9.4.1 flow cell on a MinION Mk1B device (Oxford Nanopore Technologies, Oxford, UK) and the resultant data were basecalled using Guppy (v3.4.5) in high accuracy mode. The quality of the long reads was checked using SeqKit (v2.8.0). After quality checking (Q20: 59.7%; Q30: 27.6%; N50: 11355 bp), all long-read sequences were used for assembly. A hybrid assembly combining both Illumina short reads and nanopore long reads was performed using Unicycler version 0.4.8 (Wick et al., 2017). The quality of the assembled genome was evaluated using CheckM2 version 1.0.2 (Chklovski et al., 2023). The CGView program was used to generate a circular genome map (Stothard and Wishart, 2005). The protein sequences were also used for comparative pan- and core-genome analyses using GET_HOMOLOGUES software with the orthoMCL algorithm (Contreras-Moreira and Vinuesa, 2013).

Genome annotation was performed using the Integrated Microbial Genomes Expert Review (IMG-ER) system (Markowitz et al., 2009) and the NCBI Prokaryotic Genome Annotation Pipeline (Tatusova et al., 2016). Comparative genomics involved genomes of *L. thiooxidans* DSM 13612^T (IMG genome ID; 2795386119), *L. alexandrii* LZ-4^T (2890930897), and *Limnobacter* sp. MED105 (640963026) against the SAORIC-580^T genome (2887797443). Genomic relatedness between the strains was estimated using average nucleotide identity (ANIb) and digital DNA-DNA hybridization (dDDH) values, calculated by the OrthoANI algorithm on the EzBioCloud web service (Yoon et al., 2017) and the genome-to-genome distance calculator (GGDC 3.0) (Meier-Kolthoff et al., 2022), respectively. To infer genome-based phylogenetic trees, 92 universal bacterial core genes were extracted using the up-to-date bacterial core gene set and pipeline (UBCG2) (Kim et al., 2021) and analyzed using RAxML (Stamatakis, 2014) based on amino acid alignment.

Metabolic pathways were reconstructed by additionally annotating each genome with Prokka (Seemann, 2014), which utilizes databases such as Prodigal, RNAmmer, Aragorn, SignalP, and Infernal with a default e-value cut-off of 1e-09. The resulting proteins were then queried in BlastKOALA (Kanehisa et al., 2016) and KofamKOALA (Aramaki et al., 2020). To analyze the Clusters of Orthologous Groups (COG) categories (Galperin et al., 2015) and Pfam domains (El-Gebali et al., 2019), protein sequences generated by Prokka were queried against the COG and Pfam databases using RPS-BLAST with an e-value cutoff of 0.01. Genomic islands within the SAORIC-580^T genome were identified using IslandViewer4, employing the IslandPath-DIMOB method (Bertelli et al., 2017).

2.4 Metagenome fragment recruitment analyses

To explore the distribution of deep-sea bacteria, 12 bacterial genomes retrieved from the Northwest Pacific Ocean, including the

SAORIC-580^T genome, were matched against metagenomes obtained from the Japan Trench (DRA005791), Kuril Trench (DRP003768), Mariana Trench (DRA005792) and Ogasawara Trench (DRA005790) of the Northwest Pacific Ocean. The genomes utilized in these recruitment analyses include 10 genomes from *Pseudomonadota*, one from *Bacteroidota*, and one from *Verrucomicrobiota*. Prior to analysis, RNA-coding genes were masked within the isolate genomes. Subsequently, the metagenomes were quality-trimmed and randomly subsampled to yield 1 million reads each. Recruitment analysis was conducted using BlastN, with the isolate genomes serving as queries and the metagenomes as subject databases. Metagenomic reads that exhibited at least 95% similarity and an alignment length of 150 bp or more with the isolate genomes were considered successfully recruited (Hyatt et al., 2012).

2.5 Morphological, physiological, and biochemical characterization

Cell morphology, cell size, and the presence of flagella were observed using transmission electron microscopy (TEM, CM200, Philips, Bend, OR, USA). Cells were placed on a carbon-coated copper grid and stained with 2.0% uranyl acetate. Gram staining was performed using a Gram staining kit (bioMérieux, Marcy-l'Etoile, France). Catalase and oxidase activities were determined by spraying 3% (v/v) hydrogen peroxide solution and 1% (w/v) Kovac's reagent (bioMérieux, Marcy-l'Etoile, France), respectively, to fresh colonies. Flagellum-based motility was evaluated in semi-solid marine agar containing 0.5% (w/v) agar, following incubation at 30°C for 3 days. Temperature range and optimum for growth were monitored at 4, 10, 15, 20, 25, 30, 37, 42, and 45°C, and pH range and optimum were assessed from pH 5.0 to 10.0 (at 1.0 pH intervals) using R2A broth. The pH was adjusted using the following buffering system: 1 M MES for pH 5.0–6.0, 1 M MOPS for pH 6.5–7.0, 1 M HEPES for pH 7.5–8.0, 1 M Tris for pH 8.5–9.0 and 0.5 M CHES for pH 9.5–10.0. NaCl requirement and tolerance tests were conducted with concentrations of 0–6% NaCl in 1% increments, using R2A broth as the basal medium. Growth under these conditions was monitored by measuring culture turbidity using a UV/Vis spectrophotometer (UV2600, Shimadzu, Kyoto, Japan). Anaerobic growth was monitored using an AnaeroPack system (Mitsubishi Gas Chemical, Tokyo, Japan) over a period of up to two weeks.

Phenol degradation was tested using a basal medium (8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4, 0.24 g KH_2PO_4 in 1 L deionized water) supplemented with NH_4Cl at a final concentration of 10 μM. Phenol was added as the sole carbon source into the basal medium with final concentrations ranging from 0 to 4.5 mM in 0.5 mM increments. Cellular growth was monitored every two days using an Easy-Cyte flow cytometer (Millipore, MA, USA) following 1 h staining of a 100 μl culture aliquot with 1:2000 (v/v) dilution of SYBR-Green I (Invitrogen, Carlsbad, CA, USA). Additional biochemical tests were conducted using API 20 NE, API ZYM strips (bioMérieux, Marcy-l'Etoile, France) and GEN III MicroPlate (Biolog, Hayward, CA, USA), with results recorded according to the manufacturer's guidelines after five days of incubation.

Analysis of cellular fatty acid profiles was carried out in accordance with the standard protocols provided by the MIDI/Hewlett Packard Microbial Identification system (Sasser, 1990). Fatty acid methyl esters (FAME) of strain SAORIC-580^T and other *Limnobacter* strains were extracted from colonies cultured on identical plate sectors and were identified using the TSBA 6 database. Polar lipids were analyzed following methods outlined by Minnikin et al. (1984). Total polar lipids separated by two-dimensional thin-layer chromatography were identified by spraying with molybdophosphoric acid, and lipids containing specific functional groups were further identified by spraying with ninhydrin, molybdenum blue, and alpha-naphthol solutions. Determination of respiratory quinones was performed using thin-layer chromatography as previously described (Collins and Jones, 1981).

2.6 Nucleotide sequence accession numbers

The 16S rRNA gene sequence and the complete whole-genome sequence of strain SAORIC-580^T has been deposited in the GenBank/EMBL/DDBJ database under the accession numbers OQ777717 and CP053084, respectively.

3 Results and discussion

3.1 Isolation of deep-sea bacteria in the Northwest Pacific Ocean

During a microbial isolation campaign from deep-sea environments, a total of 243 pure bacterial cultures (115 from 1/5MA and 127 from 1/10R2A) were successfully isolated from water samples collected in the Northwest Pacific Ocean in July 2011, as detailed in Supplementary Table S1. The quantity and taxonomic distribution of the isolates derived from each water sample are depicted in Figure 1 and Supplementary Tables S2–S5. Phylogenetic analysis based on 16S rRNA gene sequences revealed that these deep-sea bacterial isolates predominantly belonged to the phyla *Pseudomonadota* (formerly *Proteobacteria*, 223 strains, 91.8%), *Bacteroidota* (10 strains, 4.1%), and *Actinomycetota* (10 strains. 4.1%). The substantial prevalence of the phylum *Pseudomonadota* along with the frequent occurrence of the phyla *Bacteroidota* and *Actinomycetota* in the majority of deep-sea bacterial cultures, aligns with findings from previous studies (Kai et al., 2017; Sanz-Sáez et al., 2023). However, the distribution of cultured strains within the phylum *Pseudomonadota*, classified by class, and the prevalence of the most abundant species varied significantly with sampling depths. Isolates assigned to *Alphaproteobacteria* were dominant at depths of 1000 and 2000 m, *Gammaproteobacteria* at 3000 m, and *Betaproteobacteria* at 4000 m. Specifically, of the 82 and 64 isolates obtained from water samples at 1000 and 2000 m, respectively, the genus *Sulfitobacter* from the order *Rhodobacterales* of *Alphaproteobacteria* was the most

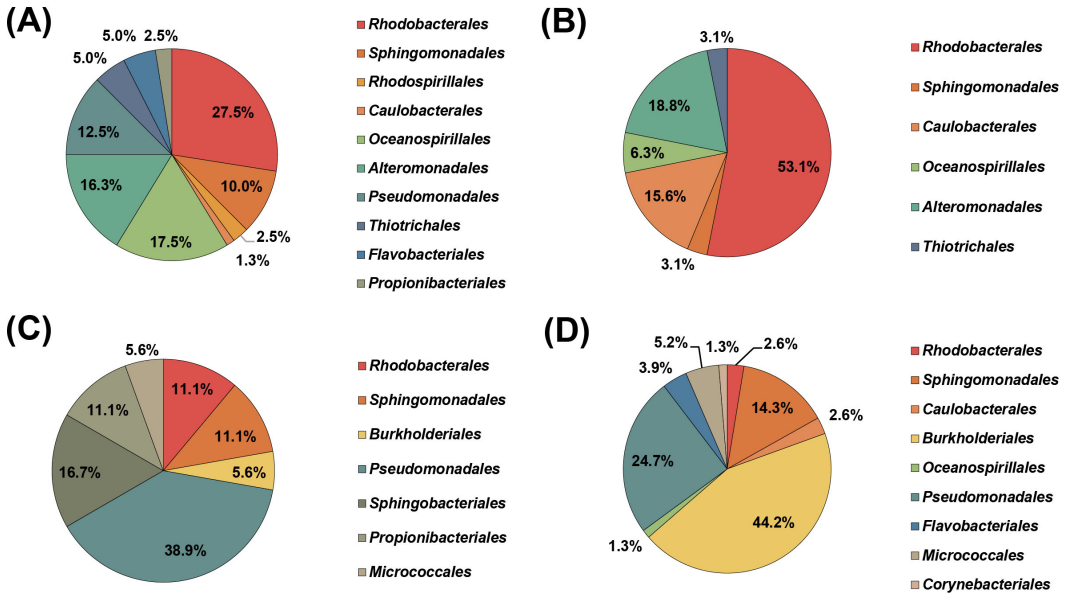

FIGURE 1
Order-level taxonomic distribution of bacterial isolates cultured from various depths at station S1 in the Northwest Pacific Ocean. Each pie chart represents results from different depths: 1000 m **(A)**, 2000 m **(B)**, 3000 m **(C)**, and 4000 m **(D)**.

abundant. From the 3000 m depth sample, the genus *Alkanindiges* within the order *Pseudomonadales* of *Gammaproteobacteria* was predominant. Notably, 42.9% of the isolates (33 out of 77 strains) cultured from the 4000 m sample were classified within the order *Burkholderiales* of the class *Betaproteobacteria*. The majority of these *Burkholderiales* isolates exhibited a close phylogenetic relationship to *L. thiooxidans* CS-K2^T, showing more than 99% 16S rRNA gene sequence identity. The notable presence of *Limnobacter* spp. in several 16S rRNA gene studies from deep-sea ecosystems (Eloe et al., 2011b; Khandeparker et al., 2014), despite their scarcity in culture, coupled with the finding that the predominant bacterial isolates from a depth of 4000 m in this study were members of the genus *Limnobacter*, has led to further taxonomic and genomic investigations of a representative strain of the *Limnobacter* isolates.

3.2 Molecular phylogeny of SAORIC-580^T, a representative of deep-sea *Limnobacter*

Among the 33 deep-sea *Limnobacter* strains that exhibited over 99% 16S rRNA gene sequence identity, a representative strain, designated SAORIC-580^T, was selected for genomic and taxonomic analyses. The 16S rRNA gene sequence (1472 bp) analysis of strain SAORIC-580^T showed that the strain is most closely related to *L. thiooxidans* CS-K2^T (99.9% sequence identity), followed by *L. alexandrii* LZ-4^T (99.2%), *L. litoralis* KP1-19^T (97.7%), and *L. humi* UCM-39^T (97.6%). In the phylogenetic tree constructed from the 16S rRNA gene sequences (Figure 2), strain SAORIC-580^T formed a robust cluster with the type strains of the genus *Limnobacter*, supported by 99–100% bootstrap values, suggesting that the strain is a member of this genus.

The whole genome sequence of strain SAORIC-580^T was obtained to analyze genome-based molecular phylogenetic relationships. The SAORIC-580^T genome shared 79.4% and 85.5% average nucleotide identity (ANI) with *L. thiooxidans* DSM 13612^T and *L. alexandrii* LZ-4^T genomes, respectively, and 21.8% and 29.5% digital DNA-DNA hybridization (dDDH) values with the two genomes, respectively (Supplementary Table S6). The ANI and dDDH values between SAORIC-580^T and other *Limnobacter* genomes are well below the thresholds proposed for bacterial species demarcation, which are 95–96% for ANI and 70% for dDDH (Richter and Rossello-Mora, 2009; Chun et al., 2018). In the phylogenomic tree of *Burkholderiales* genomes (Figure 3), strain SAORIC-580^T was clustered with the other *Limnobacter* genomes, suggesting that this novel strain represents a new species of the genus *Limnobacter*.

3.3 Genome characteristics and comparative genome analysis of SAORIC-580^T

The complete whole-genome sequence of strain SAORIC-580^T, comprising a single circular chromosome of 3,297,222 bp, was obtained (Supplementary Figure S1). The genome completeness of the strain was estimated to be 99.8%, with 0.02% contamination. The SAORIC-580^T genome encoded 3,077 protein-coding genes, 40 tRNA genes, and 6 rRNA genes (two rRNA operons) (Supplementary Table S6). Two copies of the 16S rRNA gene sequences (1,525 bp) on the genome exhibited 100% identity to each other and matched 100% with the Sanger sequencing counterpart amplified by PCR. The DNA G+C content of the

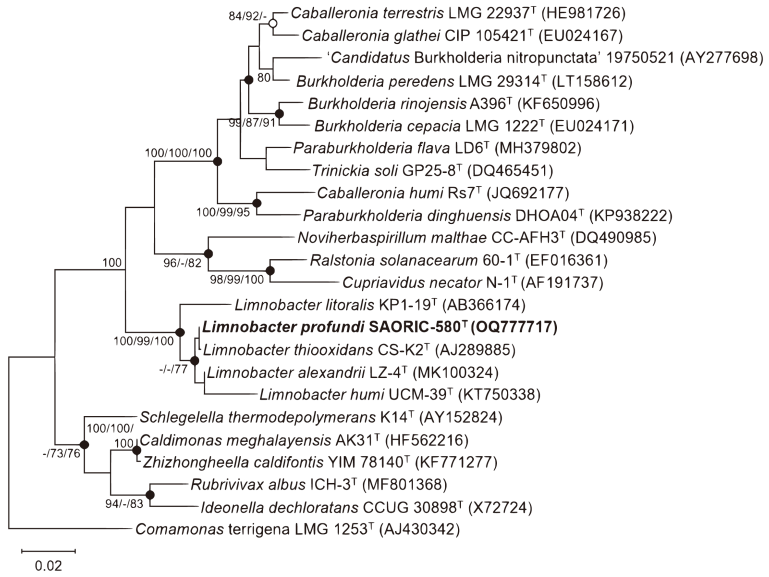

FIGURE 2
Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences showing the position of strain SAORIC-580^T. Numbers at nodes represent bootstrap percentages (above 70%) obtained from maximum-likelihood (ML), neighbor-joining (NJ), and minimum evolution (ME) methods, respectively (ML/NJ/ME; –, less than 70%). Filled circles indicate nodes recovered by all treeing methods; open circles indicate nodes recovered by any two of the three methods. *Comamonas terrigena* LMG 1253^T (AJ430342) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

SAORIC-580^T genome was 52.5%, similar to those of other *Limnobacter* genomes (Supplementary Table S6). For genomic comparison within the genus *Limnobacter*, three strains were selected: *L. thiooxidans* DSM 13612^T from freshwater lake sediment, *L. alexandrii* LZ-4^T from the phycosphere of an alga, and *Limnobacter* sp. MED105 from surface seawater. The proportions of shared CDSs to total ORFs in each genome (0.72–0.76) and hypothetical proteins to total ORFs (0.43–0.45) were very similar across the four genomes (Supplementary Table S6). The major COG categories included general function prediction only (10.1%), signal transduction mechanisms (7.0%), cell wall/membrane/envelope biogenesis (6.9%), transcription (6.8%), energy production and conversion (6.7%), and translation (6.7%) (Supplementary Table S7). These results revealed no significant structural differences among the four genomes. Therefore, we examined the unique gene set and core gene set of the genomes using Get_HOMOLOGOUS, which resulted in a Venn diagram (Supplementary Figure S2). This analysis showed that SAORIC-

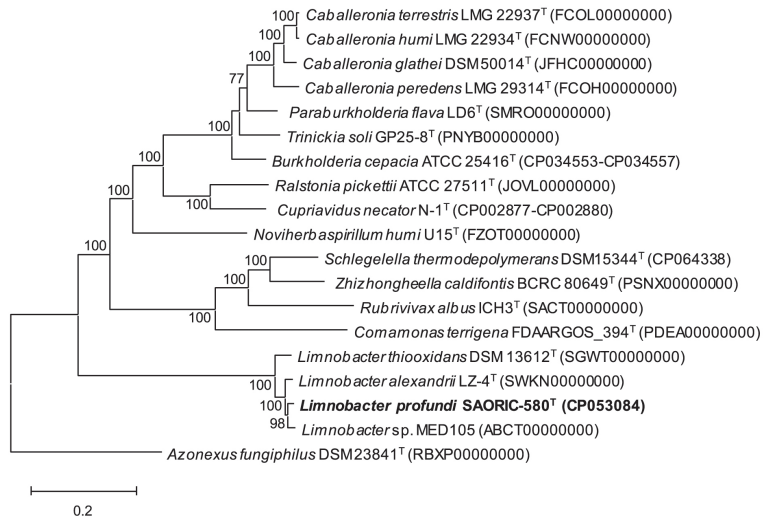

FIGURE 3
Phylogenomic tree generated using RAxML based on whole-genome sequences showing the position of strain SAORIC-580^T. Values at nodes represent bootstrap support (above 70%) by RAxML. *Azonexus fungiphilus* DSM 23841^T (RBXP00000000) was used as an outgroup. Bar, 0.2 substitutions per amino acid position.

580^T shared 2329 common genes with the other *Limnobacter* genomes, but also possessed 199 unique genes. This suggests that SAORIC-580^T not only has substantial gene content overlap with other *Limnobacter* strains but also harbors a specific set of genes not present in the other strains.

Given that genomic islands (GIs) can enhance bacterial adaptation to specific environments (Juhas et al., 2009), we investigated the presence of GIs in the SAORIC-580^T genome. Three GIs were identified in the SAORIC-580^T genome using IslandViewer4, with lengths of 5–36, 1599–1632, and 1836–1853 kb. Genetic maps of these GIs, designated GI1, GI2, and GI3, are illustrated in Supplementary Figure S3. Transposases or integrases, hallmark genes of mobile genetic elements (MGEs), were found in the GIs. MGEs play a role in enhancing genetic plasticity of microbes in stressful or extreme environments (Leduc and Ferroni, 1994; Nelson et al., 2011). The enrichment of transposases and integrases in deep-sea bacterial genomes and metagenomes is well documented (DeLong et al., 2006; Martin-Cuadrado et al., 2007; Lauro et al., 2008; Konstantinidis et al., 2009). Several transcriptional regulators, including the XRE family transcriptional regulators, terR, merR, araC, and tetR, were also found in the GIs. These regulators are known to be involved in stress response, oxidative stress resistance, multidrug resistance, and degradation of aromatics (Molina-Henares et al., 2006; Issa et al., 2018). Additionally, TonB-dependent receptors, DEAD/DEAH box helicase, and cytochrome P450 were found in GI1, GI2, and GI3, respectively. These proteins are known to be up-regulated under low temperature in marine bacteria (Ting et al., 2010) and play crucial roles in substrate transport and stress adaptation (Moeck and Coulton, 1998; Redder et al., 2015). Cytochromes P450 in several deep-sea bacteria exhibit features associated with adaption to high hydrostatic pressure (Davydov et al., 2013). Methyl-accepting chemotaxis sensory transducers and sel1 repeat proteins in GI2 may respond to environmental signals such as nutrient influxes and temperature or pressure fluctuations (Mittl and Schneider-Brachert, 2007). Genes related to heavy metal resistance, such as Co/Zn/Cd efflux systems, mercury transporters and reductases, copper resistance-related lipoproteins, and heavy metal response regulators, were also found in the GIs, consistent with the enrichment of heavy metal-resistant bacteria in the deep sea (Sayed et al., 2014; Gillard et al., 2019).

The various and simplified metabolic pathways predicted from BlastKOALA and KofamKOALA are summarized in Figure 4. The SAORIC-580^T and other *Limnobacter* genomes were all predicted to possess complete pathways for central carbon metabolism, including glycolysis, the tricarboxylic acid cycle (TCA), and the non-oxidative branch of the pentose phosphate pathway. Genes related to the SOX system for thiosulfate oxidation (*sox*ABCDXYZ) and polyhydroxybutyrate (PHB) biosynthesis (*pha*ABC) were identified in all four *Limnobacter* genomes (Supplementary Table S8). Although phenotypic tests were not conducted, the presence of these complete pathways suggests that SAORIC-580^T has the potential to utilize thiosulfate and produce PHB, both of which are characteristic features of the genus *Limnobacter* (Nguyen and Kim, 2017). Furthermore, genes for assimilatory sulfate reduction, flagella assembly, and ABC transporters for tungstate, nitrate/nitrite/cyanate, molybdate, iron (III), phosphate, phosphonate, glutamate/aspartate, and branched-

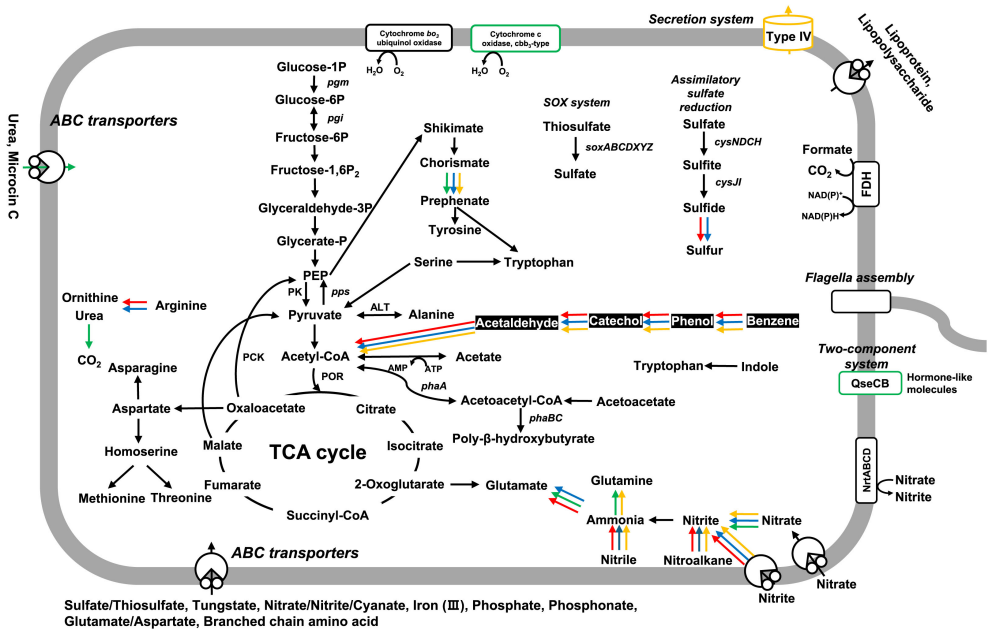

FIGURE 4
Schematic overview of predicted metabolic pathways in deep-sea *Limnobacter* genomes. The presence and absence of genes were predicted based on KEGG annotations. The color of the arrows indicates the presence of the corresponding gene in each *Limnobacter* genome as follows: Black arrow, all genomes; Red, SAORIC-580^T; Green, DSM 13612^T; Blue, LZ-4^T; Yellow, MED105. The complete pathway for phenol degradation in marine *Limnobacter* genomes is highlighted with white font on a black background.

chain amino acids were encoded in all four *Limnobacter* genomes. However, the presence or absence of certain genes differed among the *Limnobacter* genomes. Notably, the genomes of SAORIC-580^T, LZ-4^T, and MED105, derived from marine environments, exhibited differences from the DSM 13612^T genome, derived from lake sediment, in terms of the presence of the phenol degradation pathway (Figure 4). Phenol degradation by marine *Limnobacter* strains has been previously reported (Vedler et al., 2013). Additionally, the metabolic potential for the degradation of aromatic compounds has been well documented in deep-sea metagenomes (Zhang et al., 2018) and deep-sea bacterial genomes (Song et al., 2019a; Zhou et al., 2020). Conversely, only the DSM 13612^T genome contained cbb3-type cytochrome oxidase, which plays an important role in various cellular processes under low oxygen conditions (Arai, 2011; Hamada et al., 2014). The presence of this enzyme in strain DSM 13612^T may be related to the oxygen-deficient conditions of the lake sediment where the strain was isolated (Spring et al., 2001).

We further compared the SAORIC-580^T genome to other genomes to gain insights into the genomic features of deep-sea bacteria. Annotation comparisons based on the Pfam database revealed that numerous protein families (Pfam entries; hereafter referred to as proteins) are either unique to or more abundantly represented in the SAORIC-580^T genome. Specifically, the unique proteins and those with at least twice the number of copies in the SAORIC-580^T genome compared to other genomes are listed in Supplementary Table S9. To investigate the unique genes of *Limnobacter* genomes from different environments, we categorized the four analyzed genomes into two groups based on their habitats: freshwater and marine (deep-sea, algae, surface seawater). The unique genes for each habitat are presented in Table 1. In the deep-sea isolate strain SAORIC-580^T, five genes encoding haemolysin-type calcium binding protein-related domains and two genes encoding the C-terminal domain of ChrB were uniquely identified. Additionally, SAORIC-580^T and other marine isolates displayed a distinctive set of genes, including glutathione S-transferase, sodium neurotransmitter symporter family proteins, and methane/phenol/alkene hydroxylase related to phenol degradation pathway. The SAORIC-580^T genome showed an enrichment of PIN (PilT N-terminus) domain and tetratricopeptide repeat-like domain proteins (Supplementary Table S9), similar to another deep-sea bacterium (Song et al., 2019b). Tetratricopeptide repeat proteins are known to be up-regulated at low temperatures (Ting et al., 2010), suggesting their role in adapting to cold deep-sea conditions. The genome also contained an abundance of chromate transporter, chromate resistance protein, and Fe/Mn-superoxide dismutases (Fe/Mn-SOD), which aligns with the higher prevalence of heavy metal resistance genes in deep-sea bacteria (Simonato et al., 2006; Ivars-Martinez et al., 2008; Lauro et al., 2008). Chromium is commonly found in deep-sea environments (Goring-Harford et al., 2018), which may explain the enrichment of chromate-related genes. Heavy metals induce oxidative stress, and Fe/Mn-SOD helps mitigate this by converting superoxide to less harmful molecules (Li et al., 2019b). The genome also exhibited an enrichment of DnaB-like helicase, crucial for DNA replication, which could aid in coping with the pressures and low temperatures of deep-sea habitats where DNA replication is challenged (Campanaro et al., 2005; Goring-Harford et al., 2018). Additionally, polyphosphate kinase 2 (PPK2), essential for polyphosphate synthesis, was abundant, suggesting a role in adaptation to extreme environments (Seufferheld et al., 2008). Collectively, the SAORIC-580^T genome harbors unique or enriched genes, suggesting its adaptation to the deep-sea environment and its potential involvement in deep-sea biogeochemical processes. Additionally, it is noteworthy that the adaptation of strain SAORIC-580^T to the deep-sea may also be related to transcription, post translational modifications, and possibly proteins of unknown function.

Although present in minority, fragment recruitment analysis suggested that bacterial genomes retrieved from the Northwestern Pacific Ocean (Supplementary Table S10) matched metagenome fragments obtained from various depths of the four trenches in the Northwest Pacific Ocean (Figure 5). Most deep-sea genomes corresponded more closely with deep-sea metagenomes. The abundance of *Colwellia* and *Rubritalea* genomes in the Kuril Trench was comparable to other metagenomic data derived from the Kuril Trench (Gorrasi et al., 2023a, b). Additionally, the presence of *Sulfitobacter* genomes in the Mariana Trench corresponded to findings from a culture-based experiment (Zhao et al., 2020). Notably, the SAORIC-580^T genome was relatively enriched at depths of 10,899 m and 3,507 m in the Mariana Trench and Ogasawara Trench, respectively, compared to surface counterparts and other deep-sea bacterial genomes. The Mariana Trench is well known as the deepest of the world's deep-sea trenches. The abundance of *Limnobacter* members in the Mariana Trench has also been found in other metagenomic data (Dang et al., 2024). These results suggest that strain SAORIC-580^T thrives in the deep-sea environment of the Northwest Pacific Ocean and represents a member of deep-sea *Burkholderiales*.

Conclusively, the comparative genomic analysis of strain SAORIC-580^T revealed its adaptation to the deep-sea environment, highlighted by the presence of diverse metabolic pathways and specific protein families associated with cold adaptation, and heavy metal resistance, distinguishing it from other *Limnobacter* strains. The genome contained unique or differentially abundant genes involved in various functions including signal transduction and mobile genetic elements, when compared to other *Limnobacter* genomes. Many of these genes were relevant to survival strategies that may enhance the ecological fitness in the deep-sea, suggesting that strain SAORIC-580^T is well adapted to the deep-sea environment. To the best of our knowledge, the SAORIC-580^T genome represents the first deep-sea genome to be reported for the order *Burkholderiales* and provides insight into the adaptation strategies used by its members.

3.4 Phenotypic characterization and taxonomic conclusion

Transmission electron microscopy (TEM) images showed that cells of strain SAORIC-580^T are rod-shaped, measuring 0.4–0.5 × 1.0–2.1 μm, and have a polar flagellum (Supplementary Figure S4). The morphological, physiological, and biochemical characteristics

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TABLE 1 Unique proteins in the genomes of bacteria isolated from marine or freshwater environments.

Pfam	Genes	Number of genes			
		Marine			Freshwater
		1	2[a]	3[b]	4[c]
Unique to SAORIC-580^T					
PF06594	Haemolysin-type calcium binding protein related domain	5	0	0	0
PF09828	ChrB, C-terminal domain	2	0	0	0
Unique to marine strains					
PF00043	Glutathione S-transferase, C-terminal domain	3	3	3	0
PF07411	Domain of unknown function (DUF1508)	2	3	2	0
PF00209	Sodium:neurotransmitter symporter family	2	2	2	0
PF02332	Methane/Phenol/Alkene Hydroxylase	2	2	2	0
PF03795	YCII-related domain	2	2	2	0
PF04214	Protein of unknown function (DUF411)	2	2	2	0
PF11804	Protein of unknown function (DUF3325)	2	2	2	0
Unique to freshwater strain					
PF13475	Domain of unknown function (DUF4116)	0	0	0	8
PF18914	Domain of unknown function (DUF5666)	0	0	0	6
PF00805	Pentapeptide repeats (8 copies)	0	0	0	4
PF02146	Sir2 family	0	0	0	2
PF06165	Glycosyltransferase family 36	0	0	0	2
PF09722	Antitoxin Xre/MbcA/ParS C-terminal toxin-binding domain	0	0	0	2
PF13641	Glycosyltransferase like family 2	0	0	0	2
PF14518	Iron-containing redox enzyme	0	0	0	2
PF16197	Ketoacyl-synthetase C-terminal extension	0	0	0	2
PF19570	Family of unknown function (DUF6088)	0	0	0	2

[a]Data from Duan et al., 2020.
[b]Data from Pinhassi and Berman, 2003.
[c]Data from Spring et al., 2001.
Strains: 1, SAORIC-580^T; 2, *Limnobacter alexandrii* LZ-4^T; 3, *Limnobacter* sp. MED105; 4, *Limnobacter thiooxidans* DSM 13612^T. Strains were isolated from deep-sea (this study), algae, surface seawater, and freshwater lake sediment, respectively. Only unique genes with more than two copies are presented.

of strain SAORIC-580^T are listed in Table 2, Supplementary Table S11, and in the species description. Strain SAORIC-580^T exhibited phenotypic differences from other *Limnobacter* species, such as growth range for temperature and NaCl concentration, catalase activity, several enzyme activities, and utilization of various carbon compounds. Phenol degradation by strain SAORIC-580^T was tested using phenol added in concentrations ranging from 0 to 4.5 mM in 0.5 mM intervals (Figure 6). Strain SAORIC-580^T could utilize phenol as a sole carbon source at concentrations up to 3.0–3.5 mM, which aligns with the phenol degradation pathway found in its genome sequence. Phenol degradation by marine *Limnobacter* strains and other marine bacteria has also been reported in other studies (Vedler et al., 2013; Li et al., 2019). Marine pollution from aromatic compounds poses serious threats to aquatic life and water quality, making it crucial to understand biodegradation processes by microorganisms (Anku et al., 2017). The ability of strain SAORIC-580^T to degrade phenol suggests that the strain could play an important role in phenol degradation in marine environments.

Fatty acid profiles of SAORIC-580^T and closely related species are listed in Table 3. The major fatty acids (>10%) of strain SAORIC-580^T were $C_{16:0}$ (30.6%), summed feature 3 ($C_{16:1}$ $\omega6c$ and/or $C_{16:1}$ $\omega7c$; 28.5%), and summed feature 8 ($C_{18:1}$ $\omega7c$ and/or $C_{18:1}$ $\omega6c$; 17.3%). $C_{16:0}$ and summed feature 3 were also major fatty acids of other *Limnobacter* species. However, strain SAORIC-580^T differed from other *Limnobacter* species in the proportion of several fatty acids such as $C_{14:0}$, $C_{17:0}$ cyclo, and summed feature 8. The respiratory quinones detected in strain SAORIC-580^T was Q-8. The major polar lipids were phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and diphosphatidylglycerol (DPG), similar to the type species of the genus *Limnobacter*, *L. thiooxidans* KACC 13837^T (Supplementary Figure S4).

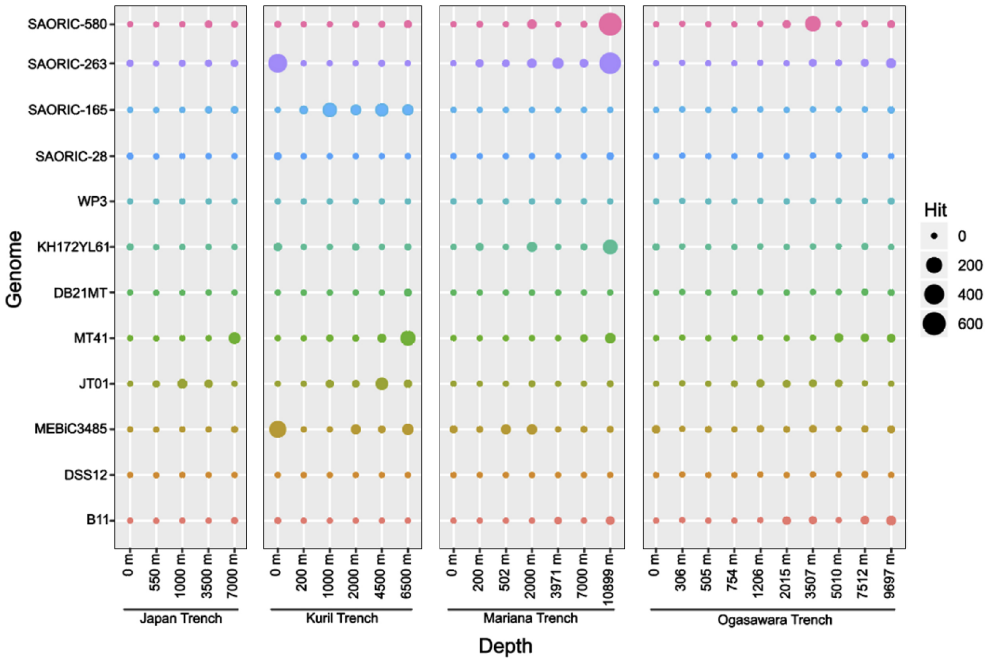

FIGURE 5
Relative abundance of deep-sea bacterial genomes analyzed by recruitment of metagenomic reads from four deep ocean trenches in the Pacific Ocean. Bubble plots show the hits of metagenomic reads from four trenches that matched genomic sequences of the deep-sea bacterial genomes. Full name of the bacterial strains: *Limnobacter profundi* sp. nov. SAORIC-580^T, *Sulfitobacter* sp. SAORIC-263, *Rubritalea* sp. SAORIC-165, *Rubrivirga* sp. SAORIC-28, *Shewanella* sp. WP3, *Psychrobacter* sp. KH172YL61, *Shewanella* sp. DB21MT, *Colwellia* sp. MT41, *Moritella* sp. JT01, *Pseudoalteromonas* sp. MEBiC 03485, *Shewanella* sp. DSS12, and *Altererythrobacter* sp. B11. Bubble sizes indicate the number of metagenomic reads recruited to the genomes (y-axis).

Chemotaxonomic characteristics and phylogenetic inference based on 16S rRNA gene and whole-genome sequences indicated that strain SAORIC-580^T belongs to the genus *Limnobacter*. The low ANI and dDDH values between strain SAORIC-580^T and the type strains of *Limnobacter* species suggest that strain SAORIC-580^T should be classified as a novel species within the genus. Phenotypic analyses also showed that strain SAORIC-580^T differs from other *Limnobacter* species in various characteristics. Therefore, the name *Limnobacter profundi* is proposed for this novel species, with SAORIC-580^T as the type strain.

TABLE 2 Phenotypic characteristics of strain SAORIC-580^T and four *Limnobacter* species.

Isolation source	1	2	3	4	5
	Deep seawater	Lake sediment[a]	Marine Algae[b]	Volcanic deposit[c]	Humus soil[d]
Cell size (μm)	0.4–0.5 × 1.0–2.1	0.6–1.5 × 1.5–3.7[a]	0.4–0.6 × 1.1–1.8[b]	0.4–0.6 × 1.0–3.0[c]	0.4–0.5 × 1.0–1.8[d]
Cell shape	Slightly curved and straight rod	Slightly curved rod[a]	Short rod[b]	Slightly curved rod[c]	Straight rod[d]
Growth range					
Temperature (°C)	4–40	4–38[a]	10–42[b]	10–44[c]	15–50[d]
NaCl (%)	0–4.0	0–6.0[a]	0–7.0[b]	0–8.0[c]	0–2.5[d]
pH	6.0–9.0	5.5–10[a]	5.5–9.0[b]	6.5–9.0[c]	5.5–9.0[d]
Catalase	–	–	+	+	+
API 20NE					
Arginine dihydrolase	–	–	+	– (+)	–
Urease	–	–	+	+	–

(Continued)

TABLE 2 Continued

Isolation source	1	2	3	4	5
	Deep seawater	Lake sediment[a]	Marine Algae[b]	Volcanic deposit[c]	Humus soil[d]
API ZYM					
Alkaline phosphatase	+	+	–	–	+
Naphthol-AS-BI-phosphohydrolase	+	+	–	+	+
Degradation					
Tween 20	+	–	–	+	+

[a]Data from Spring et al., 2001.
[b]Data from Duan et al., 2020.
[c]Data from Lu et al., 2011.
[d]Data from Nguyen and Kim, 2017.
Strains: 1, SAORIC-580^T; 2, *Limnobacter thiooxidans* KACC 13837^T; 3. *Limnobacter alexandrii* KCTC 72281^T; 4. *Limnobacter litoralis* NBRC105857^T; 5. *Limnobacter humi* KACC 18574^T. Data are from this study unless otherwise indicated. All strains are positive for: oxidase; esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase (API ZYM). All strains are negative for: nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, gelatinase and β-galactosidase (PNPG) (API 20NE); lipase (C14), valine arylamidase, cysteine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl β-glucosaminidase, α-mannosidase and α-fucosidase (API ZYM); degradation of DNA, starch, casein, CM-cellulose, Tween 80, and chitin. +, positive; –, negative.

3.5 Description of *Limnobacter profundi* sp. nov.

Limnobacter profundi (pro.fun'di L. gen. n. *profundi* of/from the depths of the sea, referring to the environment where the type strain was isolated.)

Cells are Gram-stain-negative, motile, strictly aerobic, rod-shaped (0.4–0.5 μm in diameter and 1.0–2.1 μm in length). Colonies on R2A agar are circular, smooth with entire margins, and beige in color after 3 days of incubation at 30°C. Growth occurs in the presence of 0–4% (w/v) NaCl (optimum 0% NaCl), at pH 6.0–9.0 (optimum pH 7.0–8.0), and at 4–40°C (optimum 30°C). Oxidase-positive and catalase-negative. In the API 20NE strip, negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, aesculin hydrolysis, gelatinase, and β-galactosidase (PNPG). In the API ZYM strip, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase but negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. All macromolecules (casein, chitin, DNA, starch, CM-cellulose, and Tween 80) are not hydrolyzed except for Tween 20. In a carbon source oxidation test (GEN III microplate; Biolog), positive for L-alanine, L-aspartic acid, L-glutamic acid, glucuronamide, methyl pyruvate, L-lactic acid, α-ketoglutaric acid, D-malic acid, L-malic acid, bromosuccinic acid, Tween 40, β-hydroxy- D, L-butyric acid, propionic acid, and acetic acid but negative for dextrin, D-maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, stachyose, D-raffinose, α- D-lactose, D-melibiose, β-methyl- D-glucoside, D-salicin, *N*-acetyl- D-

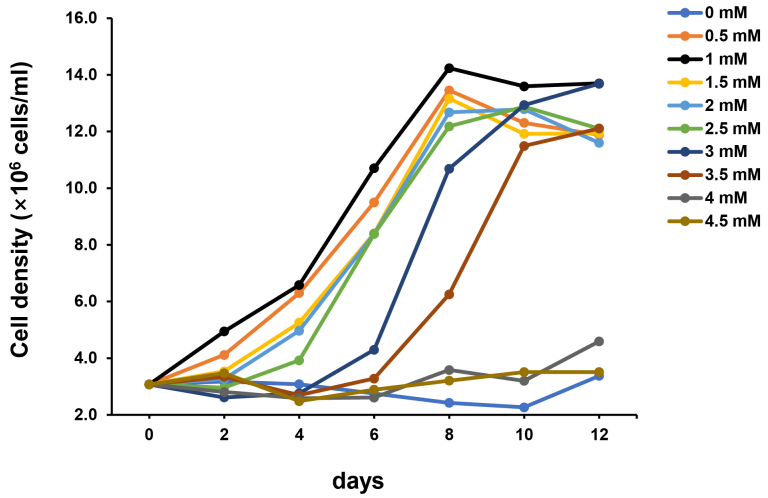

FIGURE 6
Growth of strain SAORIC-580^T using phenol as a sole carbon source at different concentrations. Phenol as a sole carbon source was added in concentrations ranging from 0 to 4.5 mM in 0.5 mM increments to basal medium supplemented with 10 μM NH_4Cl. The growth was measured at 30°C.

TABLE 3 Cellular fatty acids composition of strain SAORIC-580^T and four *Limnobacter* species.

Fatty acid (%)	1	2	3	4	5
Saturated					
$C_{12:0}$	Tr	–	–	–	–
$C_{14:0}$	1.1	3.7	1.4	Tr	5.9
$C_{16:0}$	**30.6**	**24.1**	**16.2**	**24.1**	**14.0**
$C_{18:0}$	3.1	5.2	1.4	5.6	4.3
Branched					
$C_{10:0}$ iso	Tr	–	–	–	–
$C_{10:0}$ 3-OH	5.7	5.3	8.4	5.1	6.5
$C_{15:0}$ anteiso	Tr	–	–	–	–
$C_{16:0}$ iso	1.8	–	1.8	3.1	2.8
$C_{17:0}$ cyclo	5.6	–	**15.9**	5.8	–
$C_{18:0}$ iso	–	–	Tr	–	Tr
$C_{19:0}$ iso	Tr	–	Tr	–	–
Mono-unsaturated					
$C_{14:1}$ *ω5c*	–	–	Tr	–	–
$C_{18:1}$ *ω9c*	4.3	1.0	3.1	1.4	–
Summed feature[a]					
3	**28.5**	**31.7**	**26.5**	**31.9**	**40.2**
7	–	3.2	–	–	–
8	**17.2**	**25.9**	**23.7**	5.6	**25.4**

[a]Summed features represent groups of two fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 consisted of $C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*, Summed feature 7 consisted of $C_{18:1}$ *ω7c*, $C_{18:1}$ *ω9t* or $C_{18:1}$ *ω12t* or any combination of these isomers. Summed feature 8 consisted of $C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*.
Strains: 1, SAORIC-580^T; 2, *Limnobacter thiooxidans* KACC 13837^T; 3. *Limnobacter alexandrii* KCTC 72281^T; 4. *Limnobacter litoralis* NBRC105857^T; 5. *Limnobacter humi* KACC 18574^T. All data were obtained in the present study. –, Not detected; Tr, <1%. Major fatty acids (>10%) are highlighted in bold.

glucosamine, *N*-acetyl-*β*- D-mannosamine, *N*-acetyl- D-galactosamine, *N*-acetylneuraminic acid, *α*- D-glucose, D-mannose, D-fructose, D-galactose, 3-methyl glucose, D-fucose, L-fucose, L-rhamnose, inosine, D-sorbitol, D-arabitol, *myo*-inositol, glycerol, D-glucose-6-phosphate, D-fructose-6-phosphate, D-aspartic acid, D-serine, gelatin, glycyl- L-proline, L-arginine, L-histidine, L-pyroglutamic acid, L-serine, pectin, D-galacturonic acid, L-galactonic acid lactone, D-gluconic acid, D-glucuronic acid, mucic acid, quinic acid, D-saccharic acid, *p*-hydroxyphenlyacetic acid, D-lactic acid methyl ester, citric acid, *γ*-amino-butyric acid, *α*-hydroxybutyric acid, *α*-ketobutyric acid, acetoacetic acid, and formic acid. The major fatty acids are $C_{16:0}$, summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*), and summed feature 8 ($C_{18:1}$ *ω7c* and/or $C_{18:1}$ *ω6c*). The polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, three unidentified phospholipids, two unidentified aminophospholipids, and four unidentified lipids. The major respiratory quinone is Q-8.

The type strain, SAORIC-580^T (= KACC 21400^T = NBRC 114111^T), was isolated from deep-seawater (4000 m) in the northwestern Pacific Ocean. The length of the complete genome sequence of the type strain is 3.3 Mbp with a DNA G+C content of 52.5%. GenBank accession numbers of the 16S rRNA gene sequence and the complete whole-genome sequence of the type strain are OQ777717 and CP053084, respectively.

4 Conclusion

In this study, a significant proportion of cultured bacteria from a depth of 4000 m in the Northwest Pacific Ocean were classified into the genus *Limnobacter* of the order *Burkholderiales*, with the representative strain SAORIC-580^T further characterized. This strain, identified as a novel species member of the genus *Limnobacter*, demonstrates a range of genomic adaptations to the extreme conditions of the deep sea, including unique or differentially abundant genes associated with cold adaptation, heavy metal resistance, and signal transduction. These genomic features suggest specialized survival strategies that enhance ecological fitness in the deep-sea environment. The SAORIC-580^T genome is the first deep-sea genome reported for the order *Burkholderiales*, providing an insight into the adaptation mechanisms of these bacteria in the deep-sea biosphere.

Additionally, taxonomic analyses confirmed the distinctiveness of strain SAORIC-580^T from other *Limnobacter* species, revealing differences in temperature and NaCl growth ranges, enzyme activities, and carbon compound utilization. The ability of SAORIC-580^T to degrade phenol, along with chemotaxonomic properties, further supports its classification as a novel species within the genus *Limnobacter*. Based on these findings, we propose the name *Limnobacter profundi* for this new species, with SAORIC-580^T as the type strain. This study not only enriches our understanding of the genetic and physiological diversity of deep-sea *Burkholderiales* but also underscores the specific adaptability of the *Limnobacter* isolates to extreme marine environments.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/genbank/, OQ777717 https://www.ncbi.nlm.nih.gov/genbank/, CP053084.

Author contributions

MK: Data curation, Formal analysis, Methodology, Writing – original draft. JS: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. SS: Investigation, Methodology, Writing – review & editing. KK: Conceptualization, Formal analysis, Investigation, Project administration, Writing – review & editing. IK: Conceptualization, Investigation, Resources, Software, Supervision, Writing – review & editing. J-CC:

Conceptualization, Funding acquisition, Investigation, Project administration, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2024.1449548/full#supplementary-material

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