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Flavobacterium algoriphilum sp. nov., *Flavobacterium arabinosi* sp. nov., *Flavobacterium cryoconiti* sp. nov., *Flavobacterium galactosi* sp. nov., *Flavobacterium melibiosi* sp. nov., and *Flavobacterium algoris* sp. nov., six novel cold-adapted bacteria isolated from glaciers

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Abstract

Background Six novel cold-adapted bacteria, LB3P122^T, LT1R49^T, ZT3R17^T, ZT3R25^T, XS2P12^T, and GB2R13^T, were isolated from glaciers on the Tibetan Plateau. This study aimed to characterize their taxonomic status and elucidate their molecular adaptations to cold environments using a polyphasic approach.

Results All strains were Gram-stain-negative, rod-shaped, and psychrophilic, growing at 0 °C with an optimum at 14–20 °C and at pH values of 6.0–8.0 (optimum pH 7.0). Analysis of the 16S rRNA gene sequences placed their taxonomic positions within the genus *Flavobacterium*, with similarities ranging from 97.2 to 98.4% to species with validly published names. Phylogenetic analysis of the 16S rRNA gene sequences revealed that the six strains formed distinct clades with *Flavobacterium gawalongense* GSP16^T. Phylogenomic analysis showed that these strains clustered with *Flavobacterium gawalongense* GSP16^T and exhibited a close relationship with *Flavobacterium urumqiense* CGMCC 1.9230^T and *Flavobacterium xinjiangense* CGMCC 1.2749^T. Average nucleotide identity (ANI) values ranging from 82.5 to 93.6% and digital DNA-DNA hybridization (dDDH) values ranging from 26.1 to 51.5% between these strains and their closest relatives were well below the bacterial species delineation thresholds (95–96% ANI, 70% dDDH). The predominant fatty acids were iso-$C_{15:0}$ and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). Genomic analysis identified genes associated with cryoprotection, oxidative stress response, cold-shock response, and osmoprotection in these strains, underscoring their adaptations to glacial environments.

Conclusions Based on polyphasic taxonomic evidence, the strains represent six novel species within the genus *Flavobacterium*, with the proposed names *Flavobacterium algoriphilum* sp. nov. (LB3P122^T = CGMCC 1.11443^T = NBRC

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114820^T), *Flavobacterium arabinosi* sp. nov. (LT1R49^T = CGMCC 1.11617^T = NBRC 114822^T), *Flavobacterium cryoconiti* sp. nov. (ZT3R17^T = CGMCC 1.11707^T = NBRC 114824^T), *Flavobacterium galactosi* sp. nov. (ZT3R25^T = CGMCC 1.11711^T = NBRC 114825^T), *Flavobacterium melibiosi* sp. nov. (XS2P12^T = CGMCC 1.23198^T = NBRC 114826^T), and *Flavobacterium algoris* sp. nov. (GB2R13^T = CGMCC 1.24741^T = NBRC 114830^T). These findings enhance our understanding of *Flavobacterium* diversity and cold adaptation in cryospheric ecosystems.

Keywords Flavobacterium, glacier, psychrophilic, cold-adapted

Background

The genus *Flavobacterium*, within the family *Flavobacteriaceae*, was first delineated by Bergey et al. [1] and subsequently refined by Bernardet et al. [2], Dong et al. [3], and Kang et al. [4]. Strains of *Flavobacterium* are broadly distributed across diverse habitats, such as aquatic systems [5], rhizospheres [6], compost [7], glaciers [8], and soils [3], highlighting their exceptional ecological adaptability. In cold environments, *Flavobacterium* emerges as one of the predominant bacterial genera, thriving in the polar regions and high-altitude mountain glaciers [9, 10]. This dominance underscores their vital ecological contributions in extreme ecosystems, where they contribute to nutrient cycling and microbial community stability under challenging conditions. Currently, the genus *Flavobacterium* encompasses 324 species with validly published names [11]. These species are typically characterized as Gram-stain-negative, aerobic, rod-shaped, yellow-pigmented, non-spore-forming cells, often exhibiting gliding motility. Their primary respiratory menaquinone is MK-6, with common polar lipids including phosphatidylethanolamine and aminophospholipids, and DNA G + C contents ranging from 30 to 52 mol% [12]. Driven by the ecological importance of glacier-dwelling bacteria and the need to deepen our understanding of microbial diversity in cold environments, we examined the taxonomic status of six strains, LB3P122^T, LT1R49^T, ZT3R17^T, ZT3R25^T, XS2P12^T, and GB2R13^T, isolated from glacier surfaces on the Tibetan Plateau. Using a polyphasic taxonomic approach, we propose these strains as six novel species within *Flavobacterium*, contributing to the growing catalog of psychrophilic bacteria and their roles in glacier ecosystems.

Methodology

Sample processing

During our research on glacier biodiversity, six strains were successfully isolated from four glaciers on the Tibetan Plateau (Table S1). Strains LB3P122^T and LT1R49^T were isolated from ice and cryoconite samples of Laigu glacier (96°49′7.30″ E, 29°18′31.62″ N), respectively. Strains ZT3R17^T and ZT3R25^T were isolated from a cryoconite sample of Zepu glacier (95°15′3.02″ E, 30°16′35.60 N). Strain XS2P12^T was isolated from a meltwater sample of Zhuxi glacier (95°34′58.33″ E, 30°2′42.75″ N). Strain GB2R13^T was isolated from an ice sample of Gawalong glacier (95°42′37.26″ E, 29°45′57.34″ N). Standard plating techniques on peptone, yeast extract and glucose (PYG) agar, and Reasoner's 2 A (R2 A) agar (BD Difco) were used for isolation. The isolates were continuously cultured at 14 °C on PYG agar and stored in aqueous glycerol suspensions (10%, v/v) in a liquid nitrogen storage tank. For comparative taxonomic study, strain *Flavobacterium gawalongense* CGMCC 1.24642^T was obtained from China General Microbiological Culture Collection Center (CGMCC) and used as a reference strain.

16S rRNA gene sequencing

For 16S rRNA gene sequencing, genomic DNA was extracted using the TaKaRa MiniBEST Bacteria Genomic DNA Extraction Kit Ver. 3.0 (TaKaRa, Japan) following the manufacturer's instructions. The 16S rRNA gene was amplified and sequenced using the universal primer pairs 27 F and 1492R [13]. The almost complete 16S rRNA gene sequences of the six strains were identified using EzBioCloud to determine their taxonomic positions [14]. Multiple sequences were aligned with the CLUSTAL_W program implemented in the MEGA software package version 12 [15]. Neighbor-joining (NJ) [16] and maximum-likelihood (ML) [17] phylogenetic trees were reconstructed and evaluated using the MEGA software. Kimura's two parameter model [18] was used to calculate the genetic distances for the NJ analysis. Tree topologies were evaluated by the bootstrap values based on 1000 resamplings.

Genome sequencing and Functional analysis

For genomic analysis, genome sequencing of the six strains was conducted on the Illumina Hiseq 4000 platform (Illumina, San Diego, CA, USA) with 150 bp paired-end reads, following the manufacturer's protocols. Short reads were assembled de novo using the SPAdes program to generate draft genomes [19]. Genome quality was assessed based on completeness and contamination rates using CheckM2 version 1.0.2 [20] and QUAST version 5.2 [21]. Annotation was conducted with Bakta v1.9.4 [22], and rRNA prediction was performed using Barrnap within Prokka v1.14 [23]. The 92 core genes

were extracted by UBCG program [24] from genomic sequences, and alignments were generated with MAFFT software v7.520 [25]. A phylogenetic tree was constructed using the ML algorithm in IQ-TREE software V2.3.4 [26], based on concatenated core gene sequences with 1000 bootstrap replicates and the best nucleotide substitution model $GTR+F+I+$ R10. Average nucleotide identity (ANI) values were calculated using the FastANI program [27], and digital DNA–DNA hybridization (dDDH) values were determined using the Type (Strain) Genome Server (TYGS) [28].

Physical, biochemical and chemotaxonomic analysis

Colony morphology of the six strains was observed on PYG agar plates after incubation at 14 °C. Cellular morphology was visualized by a JEM-1400 transmission electron microscopy (TEM, JEOL Ltd., Tokyo, Japan). Bacteria cultured on PYG agar were suspended in deionized water, dropped onto copper grids, and stained with phosphotungstic acid for 5–10 s for TEM observation. Gram staining was performed as described by Gerhardt et al. [29]. Gliding motility was tested by direct microscopic examination according to Bernardet et al. [30]. The presence of flexirubin-type pigments was assessed with 20% (w/v) KOH. Growth was assessed at temperatures of 0, 4, 10, 14, 20, 25, and 28 °C in PYG broth over 7 days. NaCl tolerance was tested in PYG broth supplemented with 0–4.0% (w/v) NaCl at 0.5% intervals. pH tolerance was examined in filter-sterilized PYG broth adjusted to pH 5.0–10.0 at 1-unit intervals, using Na_2HPO_4/NaH_2PO_4 (pH 5.0–8.0) and Na_2CO_3/$NaHCO_3$ (pH 9.0–10.0) buffers, with incubation at 14 °C for 7 days. Growth ranges for temperature, pH, and NaCl were confirmed by monitoring optical density under controlled conditions. Hydrolysis of casein, starch, and Tween 80 was tested according to Smibert & Krieg [31]. Oxidase activity was evaluated using 3% (v/v) H_2O_2, and cytochrome oxidase activity was determined using 1% (w/v) tetramethyl-*p*-phenylenediamine (bioMérieux). Utilization of various carbon sources was tested in a basal medium (0.2% $(NH_4)_2SO_4$, 0.05% $NaH_2PO_4{\cdot}H_2O$, 0.05% K_2HPO_4, 0.02% $MgSO_4{\cdot}7H_2O$, and 0.01% $CaCl_2{\cdot}2H_2O$) with 1% (w/v) of each carbon compound. Enzyme activities and additional biochemical characteristics were performed using the API 20E, 20 NE, and ZYM strips (bioMérieux, Marcy-l'Étoile, France). Fatty acid profiles were analyzed using cells grown on PYG agar at 14 °C for 4 days. Fatty acids were extracted according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.0), and identified using a 6890 N Gas Chromatograph (Agilent) with the TSBA6 database [32]. Cell masses of the six strains were harvested from PYG medium after 4 days of incubation at 14 °C for menaquinone and polar lipid analyses. Menaquinones were extracted and purified following Collins et al. [33] and detected via an HPLC system. Polar lipids were extracted and analyzed by two-dimensional thin-layer chromatography on silica gel 60 plates (Merck 1.05553) [34].

Results and discussion

The nearly complete 16S rRNA gene sequences of six strains were analyzed in the EzBioCloud database [14], revealing their affiliation with the genus *Flavobacterium*. These strains shared 16S rRNA gene sequence similarities ranging from 98.2 to 99.6%. Strains LT1R49^T, GB2R13^T, ZT3R25^T, and XS2P12^T demonstrated the highest sequence similarities with *F. sinopsychrotolerans* 0533^T (97.7–98.4%). The closest relative of ZT3R17^T was *F. gawalongense* GSP16^T (98.0% similarity), while LB3P122^T was most closely related to *F. urumqiense* Sr25^T (97.2% similarity). Phylogenetic analysis based on 16S rRNA gene sequences indicated that the six strains clustered with *F. gawalongense* GSP16^T, forming a distinct branch in the NJ tree (Fig. 1). Within this branch, GB2R13^T and ZT3R17^T each formed an independent clade, whereas LB3P122^T, XS2P12^T, LT1R49^T, and ZT3R25^T clustered together. The topologies of the ML and NJ trees were similar with those of the NJ tree (Fig. 1).

Genome features of the six strains, including draft genome sizes, gene counts, and G+ C contents, were listed in Table S2. De novo assembly of the genome sequencing data yielded 41–82 contigs with N50 values of 85,572–568,252 bp. CheckM2 analysis confirmed genome completeness of 99.98–99.99% and contamination rates of 0.14–0.35%. Genome sizes ranged from 3.47 to 4.26 Mb, with DNA G+ C contents of 33.75–34.28%. Bakta annotation identified 2,981–3,726 protein-coding sequences, 6 rRNA genes, and 43–46 tRNA genes per genome. Additionally, 10–12 ncRNA regions and 80–118 ncRNAs were detected.

In the phylogenomic tree (Fig. 2), the six strains, alongside *F. gawalongense* GSP16^T, *F. urumqiense* CGMCC 1.9230^T, and *F. xinjiangense* CGMCC 1.2749^T, formed an independent lineage with 100% bootstrap support. Each strain constituted a distinct clade within this lineage. The ANI values between the six new strains and their closely relatives, *F. gawalongense* GSP16^T, *F. urumqiense* CGMCC 1.9230^T, and *F. xinjiangense* CGMCC 1.2749^T, ranged from 82.5 to 93.6%, below the 95–96% species delineation threshold [35]. The dDDH values between them were 26.1–51.5%, below the 70% threshold [36]. The detailed pairwise ANI and dDDH valus between the six new strains and their closest relative, *F. gawalongense* GSP16^T, were shown in Table S3. A TYGS-generated genome BLAST distance phylogeny tree supported these results (Fig. 3). Therefore, these data support the

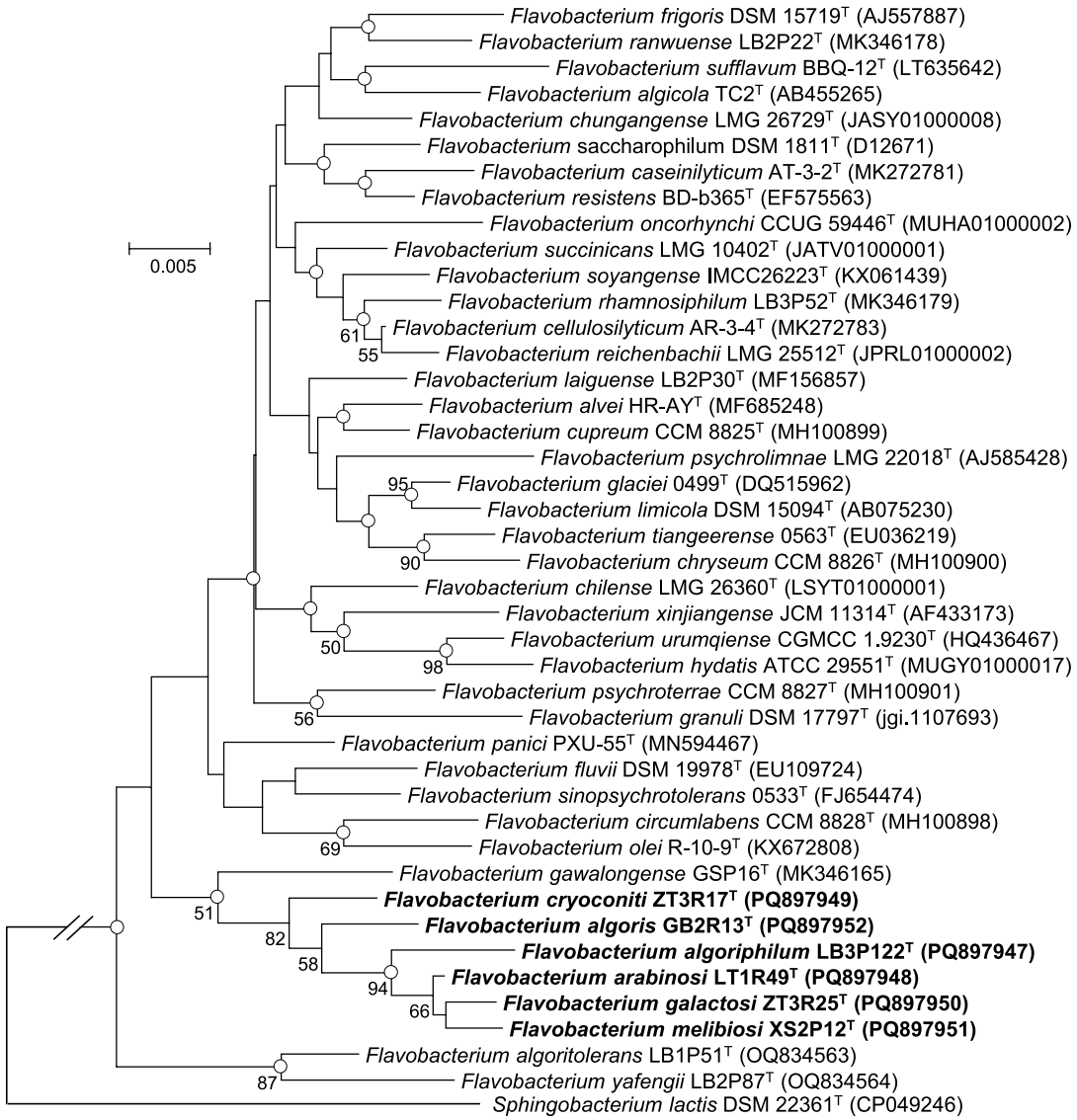

Fig. 1 Phylogenetic tree of the six strains and related taxa based on the 16S rRNA gene sequences comparisons using the NJ method. GenBank accession numbers of the 16S rRNA gene sequences are given in parentheses. Open circles indicate that the corresponding branches were also recovered in the ML tree. Bootstrap values (> 50%) based on 1,000 replicates are shown at the branch nodes. Bar, 0.02 substitutions per nucleotide position

classification of strains LB3P122^T, LT1R49^T, ZT3R17^T, ZT3R25^T, XS2P12^T, and GB2R13^T as six novel genospecies of the genus *Flavobacterium*.

Cells of the six strains were Gram-stain-negative, rod-shaped, non-sporulating, and lacked gliding motility (Fig. S1). Colonies were yellow, convex, round, and smooth with entire margins after incubation on PYG plates at 14 °C. All strains grew at 0 °C, with a maximum growth temperature of 21–27 °C, indicating that they are psychrophiles [37]. Growth occurred at pH 6.0–8.0 and NaCl concentrations of 0–0.5% (w/v). None of them reduced nitrate, produced indole or H_2S, or exhibited citrate utilization, urease, arginine dihydrolase, tryptophan deaminase, lysine decarboxylase, or ornithine decarboxylase. Flexirubin-type pigments were absent. However, a zeaxanthin synthesis pathway, comprising *crtB* (phytoene synthase), *crtI* (phytoene dehydrogenase), *crtY* (lycopene β-cyclase), and *crtZ* (β-carotene hydroxylase), was identified in their genomes. This suggests that these strains, similar to most of the glacier-derived *Flavobacterium* strains reported by Liu et al. [38], are likely capable of producing zeaxanthin. All strains were oxidase-positive,

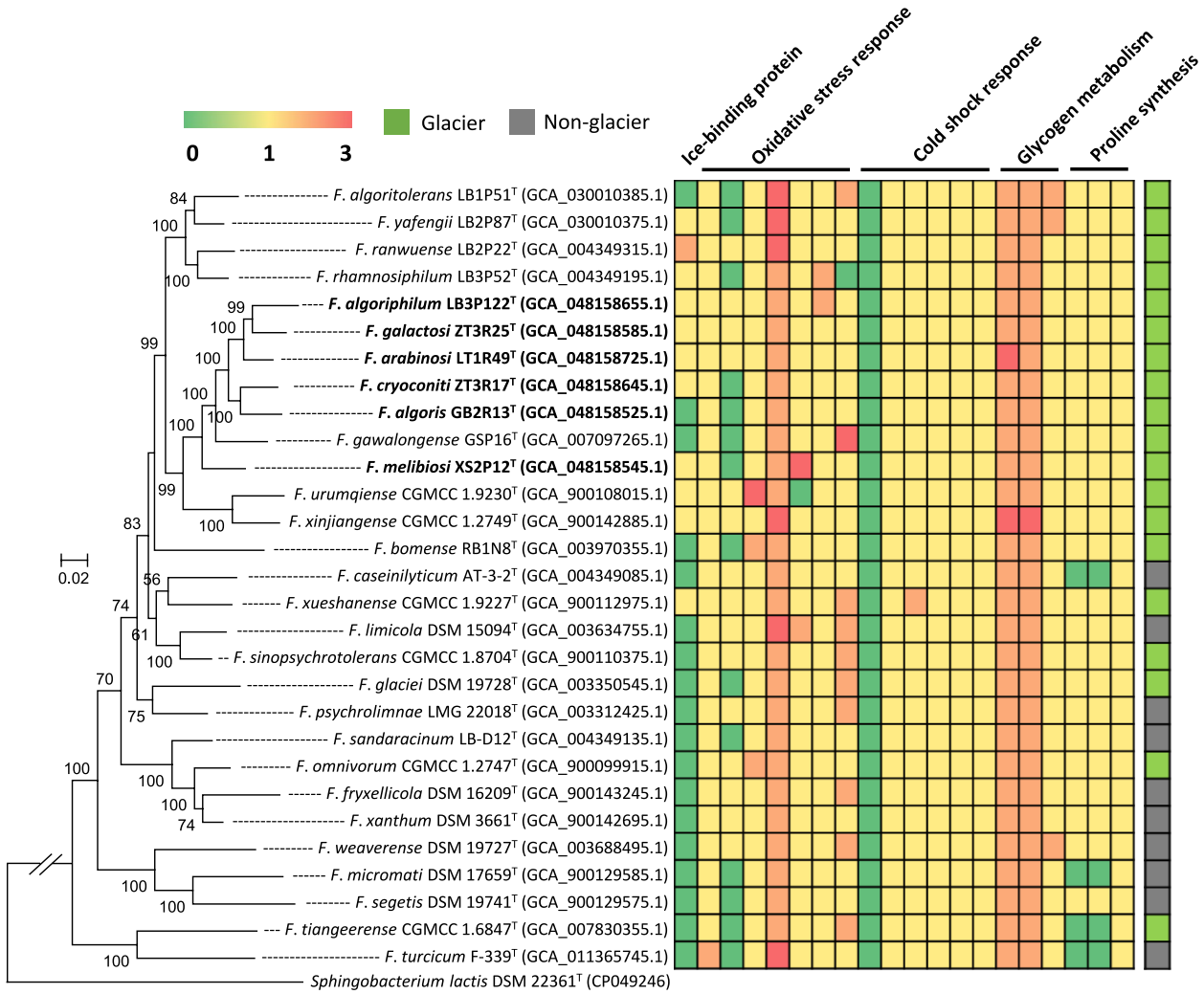

Fig. 2 ML phylogeny of six strains and related taxa, inferred using IQ-TREE based on a concatenated alignment of 92 core genes. Bootstrap values (> 50%) from 1,000 replicates are shown at branch nodes. Bar, 0.02 substitutions per nucleotide position. The heatmap displays genes associated with ice-binding protein, oxidative stress response (*sodA*, *katE*, *katG*, *bcp*, *osmC*, *trxB*, *trxA*), cold-shock response (*cspA*, *nusA*, *pnp*, *rbfA*, *infA*, *infB*), glycogen metabolism (*glgA*, *glgB*, *glgC*), and proline synthesis (*proA*, *proB*, *proC*)

hydrolyzed esculin, and yielded positive Voges-Proskauer test results. Differential physiological and biochemical characteristics compared to *F. gawalongense* GSP16^T are detailed in Table 1. The carbon source utilization profiles, as well as other physiological and biochemical characteristics detected by API strips, are presented in Tables S4 and S5.

Fatty acid compositions of the six strains were compared with *F. gawalongense* GSP16^T, *F. xinjiangense* CGMCC 1.2749^T, and *F. urumqiense* CGMCC 1.9230^T (Table S6). All these strains contained summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) and/or iso-$C_{15:0}$ as the predominant fatty acids. Notably, strain LB3P122^T showed a high proportion of iso-$C_{15:0}$ (17.7%) and iso-$C_{17:0}$−3OH (11.2%); strain ZT3R17^T contained a significant amount of iso-$C_{14:0}$ (6.2%), similar to *F. urumqiense* CGMCC 1.9230^T. Strain ZT3R25^T was notable for its high level of anteiso-$C_{15:0}$ (15.3%). *F. gawalongense* GSP16^T and *F. xinjiangense* CGMCC 1.2749^T exhibited a high proportion of summed feature 3 (22.4 and 24.3%, respectively). *F. urumqiense* CGMCC 1.9230^T maintained a distinct fatty acid profile with a notable presence of $C_{15:1}$ *ω6c* (11.4%), and several hydroxylated fatty acids, including iso-$C_{16:0}$−3OH (11.0%), iso-$C_{16:1}$-H (8.2%), and $C_{16:0}$−3OH (11.0%). Menaquinone-6 (MK-6) was the only isoprenoid quinone identified in all six strains, consistent with the characteristics of the genus *Flavobacterium* [39]. Phosphatidylethanolamine (PE), unidentified aminolipid

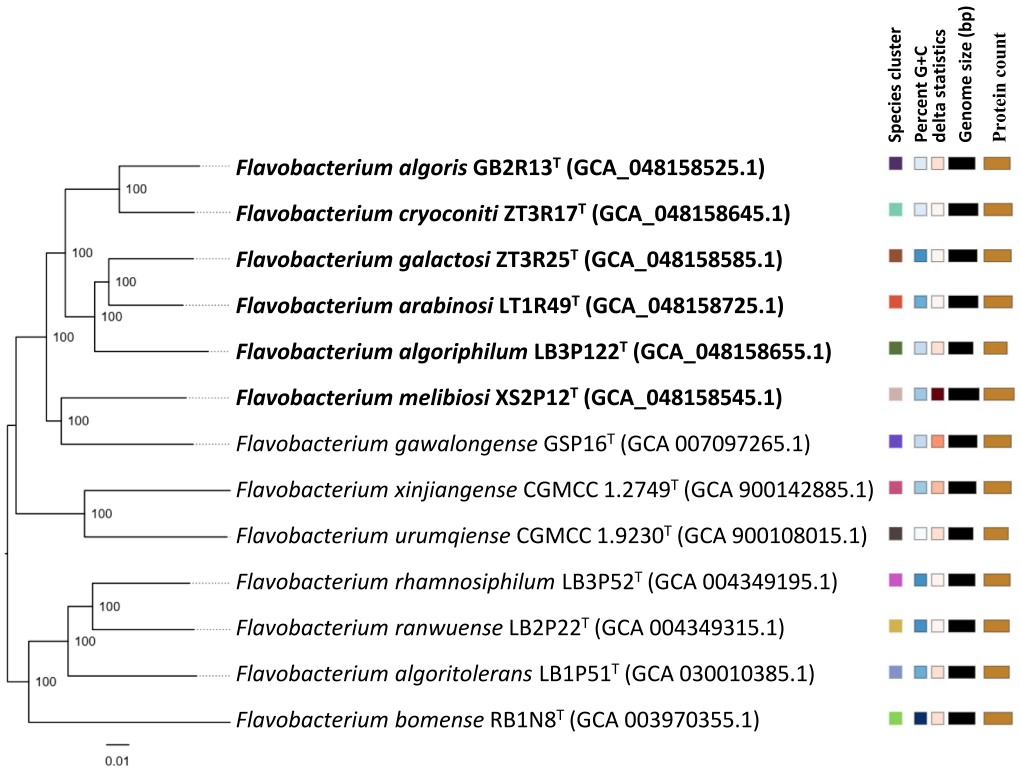

Fig. 3 Genome BLAST distance phylogeny (GBDP) of selected genomes inferred by TYGS webserver. The node values indicated are based on 100 bootstrap pseudo-replicates

Table 1 Phenotypic characteristics that differentiate the six strains and *F. gawalongense* GSP16^T

Characteristic	1	2	3	4	5	6	7
Maximum Growth temperature (°C)	21	25	27	25	23	25	20
Highest salt tolerance (%, w/v)	0.5	0.5	1.0	0.5	0.5	0.5	0.5
Hydrolysis of:							
starch	–	–	–	–	–	–	+
casein	+	+	–	–	–	–	–
gelatin	+	+	–	+	+	–	+
Enzyme activity:							
trypsin	–	–	–	–	+	–	–
α-chymotrypsin	–	+	–	–	–	–	–
α-galactosidase	–	–	+	–	–	+	–
β-galactosidase	+	+	+	+	–	+	+
Utilization of:							
melibiose	+	+	+	+	+	–	+
L-rhamnose	+	+	+	+	+	–	–
G + C content (%)	33.8	34.1	33.6	34.3	34.0	33.8	33.8
Polar lipids	PE, AL, 3 ULs	PE, 2 ALs, 6 ULs	PE, 2 ALs, 4 ULs	PE, 2 ALs, 3 ULs	PE, 2 ALs, 4 ULs	PE, 2 ALs, 4 ULs	PE, 2 ALs, 4 ULs[a]

Strains: 1, LB3P122^T; 2, LT1R49^T; 3, ZT3R17^T; 4, ZT3R25^T; 5, XS2P12^T; 6, GB2R13^T; 7, *F. gawalongense* GSP16^T. +, Positive; –, negative

[a] data from Liu et al., [48]

(AL), and unidentified lipid (UL) were common polar lipid components across these strains, though variations in AL and UL counts indicated differences in their polar lipid profiles (Fig. S2). Strain LB3P122^T contained PE, an AL, and three ULs; LT1R49^T had PE, two ALs, and six ULs; while ZT3R17^T, ZT3R25^T, XS2P12^T, and GB2R13^T each possessed PE and two ALs, with UL counts of four (ZT3R17^T, XS2P12^T, GB2R13^T) or three (ZT3R25^T).

To elucidate the molecular basis of cold adaptation in bacterial strains, genomic annotation and comparative analysis were conducted on six novel *Flavobacterium* strains and 23 related taxa (Fig. 2). Of these, only *F. xanthum* DSM 3661^T, *F.* weaverense DSM 19727^T, and *F. turcicum* F-339^T were isolated from terrestrial environments, and *F. limicola* DSM 15094^T from freshwater, while the remaining 25 strains originated from cryospheric habitats, including glaciers (19) and polar regions (6) [40]. The analysis identified numerous genes associated with cold adaptation, encompassing cryoprotection, oxidative stress response, cold-shock response, and osmoprotection. These genes provide critical insights into the molecular mechanisms underpinning survival in extreme cold [41].

Ice-binding protein (IBP) gene, which inhibit ice crystal growth to mitigate freeze–thaw damage, was detected in five of the six novel strains (single copy each), but was absent in GB2R13^T and most relatives, except *F. ranwuense* LB2P22^T (two copies), *F. yafengii* LB2P87^T, *F. rhamnosiphilum* LB3P52^T, *F. urumqiense* CGMCC 1.9230^T, *F.* xinjiangense CGMCC 1.2749^T, and *F. xueshanense* CGMCC 1.9227^T (one copy each). The prevalence of IBP gene in glacier-isolated strains underscores its role in maintaining cellular integrity in subzero conditions, while its absence in non-glacial strains suggests specificity to cryospheric stressors [42].

Genes mediating oxidative stress response, including *sodA* (superoxide dismutase), *katE* and *katG* (catalase), *bcp* (peroxiredoxin), *osmC* (organic hydroperoxide reductase), *trxB* (thioredoxin reductase), and *trxA* (thioredoxin) [43], were abundant across all strains, indicating their fundamental role in diverse environments. Similarly, cold-shock response genes (*nusA*, *pnp*, *rbfA*, *infA*, *infB*), present across all taxa, support protein synthesis under cold stress [44]. Notably, the cold-shock protein gene *cspA* was absent in all strains, suggesting reliance on alternative mechanisms or that *cspA* homologs were not detected due to potential sequence divergence, warranting further investigation.

Osmoprotection and glycogen metabolism genes were also prevalent. Glycogen synthesis genes (*glgA*, *glgB*, *glgC*) [45], typically in two or three copies for *glgA* and *glgB* and one for *glgC*, were universal, facilitating carbon storage in nutrient-scarce glacial habitats. Proline synthesis genes (*proA*, *proB*, *proC*), detected in single copies across most strains, promote osmoprotection by accumulating proline as a cryoprotectant [46]. Exceptions included *F. caseinilyticum* AT-3-2^T, *F. micromati* DSM 17659^T, and *F. tiangeerense* CGMCC 1.6847^T, primarily from non-glacial environments, highlighting the role of these genes in cryospheric adaptation.

The genomic profiles of the novel *Flavobacterium* strains reveal a suite of adaptations tailored to glacial environments. The unique presence of IBP genes in certain glacier isolates distinguishes these psychrophilic taxa from their relatives. While genes for oxidative stress response, cold-shock response, and glycogen metabolism are broadly conserved across glacial and non-glacial strains, their roles may be modulated at the transcriptional or translational level [47]. Collectively, these findings elucidate the molecular strategies enabling *Flavobacterium* survival in extreme cold, providing a foundation for further studies of psychrophilic adaptation.

Conclusion

This study employed a polyphasic taxonomic approach to characterize six *Flavobacterium* strains (LB3P122^T, LT1R49^T, ZT3R17^T, ZT3R25^T, XS2P12^T, GB2R13^T) isolated from glaciers in China, confirming their classification as novel species within the genus *Flavobacterium*. Phylogenetic analyses of 16S rRNA gene sequences revealed similarities of 98.2–99.6% with related species and distinct clustering with *Flavobacterium gawalongense* GSP16^T. Phylogenomic trees, supported by ANI (82.5–93.6%) and dDDH (26.1–51.5%) values below species thresholds, substantiated their taxonomic novelty. These psychrophilic strains, capable of growth at 0 °C, exhibited genomic adaptations to glacial environments, as revealed by cold-adaptive gene annotations.

Based on integrated taxonomic evidence, these strains were classified as six novel *Flavobacterium* species, with the following proposed names: *Flavobacterium algoriphilum* sp. nov. (type strain =LB3P122^T= CGMCC 1.11443^T= NBRC 114820^T), *Flavobacterium arabinosi* sp. nov. (type strain =LT1R49^T= CGMCC 1.11617^T= NBRC 114822^T), *Flavobacterium cryoconiti* sp. nov. (type strain =ZT3R17^T= CGMCC 1.11707^T= NBRC 114824^T), *Flavobacterium galactosi* sp. nov. (type strain =ZT3R25^T= CGMCC 1.11711^T= NBRC 114825^T), *Flavobacterium melibiosi* sp. nov. (type strain =XS2P12^T= CGMCC 1.23198^T= NBRC 114826^T), and *Flavobacterium algoris* sp. nov. (type strain =GB2R13^T= CGMCC 1.24741^T= NBRC 114830^T). These findings enhance our understanding of *Flavobacterium* diversity and shed light on microbial adaptation within cryospheric habitats.

Description of Flavobacterium algoriphilum sp. nov.

Flavobacterium algoriphilum (al.go.ri'phi.lum. L. masc. n. algor, the cold; N.L. masc. adj. philus (from Gr. neut. adj. philos), loving; N.L. neut. adj. algoriphilum, cold-loving.)

Cells are Gram-stain-negative, rod-shaped, non-gliding, and devoid of flagella, measuring 0.7–0.8 μm ×1.2–3.5 μm. Colonies are circular, yellow, convex, and round on PYG plates at 14 °C. Growth occurs at temperatures between 0–21 °C (optimum 14 °C), at pH 6.0–8.0 (optimum pH 7.0), and in the presence of 0–0.5% (w/v) NaCl. Flexirubin-type pigments are absent. Positive for catalase and negative for oxidase. Cells hydrolyze casein, esculin, and gelatin, but do not hydrolyze starch or Tween 80. Indole and H_2S are not formed. Positive for the Voges-Proskauer test, alkaline phosphatase, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *α*-glucosidase, *β*-glucosidase, N-acetyl-*β*-glucosaminidase, esterase (C4), esterase lipase (C8), lipase (C14), and *β*-galactosidase. Negative for glucose fermentation, citrate utilization, nitrate reduction, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, urease, trypsin, *α*-chymotrypsin, *α*-galactosidase, *β*-glucuronidase, *α*-mannosidase, and *α*-fucosidase. Utilize the following carbohydrates as the sole carbon source: D-glucose, melibiose, maltose, lactose, D-mannose, D-turanose, glycogen, L-rhamnose, L-proline, D-raffinose, sucrose, D-trehalose, cellobiose, and L-arabinose. Cannot utilize the following carbohydrates: D-galactose, D-mannitol, D-xylose, D-fructose, propionate, tartrate, L-sorbose, citrate, myo-inositol, succinate, and D-ribose. The major fatty acids are iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). The menaquinone is MK-6. The polar lipids are phosphatidylethanolamine, aminolipid, and three lipids. The genomic DNA G+ C content of the type strain is 33.8%.

The type strain LB3P122^T (= CGMCC 1.11443^T = NBRC 114820^T) was isolated from an ice sample collected from the Laigu glacier on the Tibetan Plateau, P.R. China. The NCBI accession numbers for the 16S rRNA gene and genome sequences are PQ897947 and JBLNKY000000000, respectively.

Description of *Flavobacterium arabinosi* sp. nov.

Flavobacterium arabinosi (a.ra.bi.no'si. N.L. gen. n. arabinosi, pertaining to arabinose.)

Cells are Gram-stain-negative, rod-shaped, non-gliding, and devoid of flagella, measuring 0.8–0.9 μm ×1.1–2.2 μm. Colonies are circular, yellow, convex, round on PYG plates at 14 °C. Growth occurs at temperatures between 0–25 °C (optimum 14–20 °C), at pH 6.0–8.0 (optimum pH 7.0), and in the presence of 0–0.5% (w/v) NaCl. Flexirubin-type pigments are absent. Positive for catalase and oxidase. Cells hydrolyze casein, esculin, and gelatin, but do not hydrolyze starch or Tween 80. Indole and H_2S are not formed. Positive for the Voges-Proskauer test, *β*-galactosidase, *α*-chymotrypsin, alkaline phosphatase, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *α*-glucosidase, *β*-glucosidase, esterase (C4), esterase lipase (C8), lipase (C14), and N-acetyl-*β*-glucosaminidase. Negative for glucose fermentation, citrate utilization, nitrate reduction, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, urease, trypsin, *α*-galactosidase, *β*-glucuronidase, *α*-mannosidase, and *α*-fucosidase. Utilize the following carbohydrates as the sole carbon source: D-glucose, melibiose, maltose, lactose, D-mannose, D-turanose, glycogen, L-rhamnose, L-proline, D-raffinose, sucrose, D-trehalose, cellobiose, L-arabinose, D-galactose, D-xylose, and D-fructose. Cannot utilize the following carbohydrates: D-mannitol, propionate, tartrate, L-sorbose, citrate, myo-inositol, succinate, and D-ribose. The major fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). The menaquinone is MK-6. The polar lipids are phosphatidylethanolamine, two aminolipids, and six lipids. The genomic DNA G+ C content of the type strain is 34.1%.

The type strain LT1R49^T (= CGMCC 1.11617^T = NBRC 114822^T) was isolated from a cryoconite sample collected from the Laigu glacier on the Tibetan Plateau, P.R. China. The NCBI accession numbers for the 16S rRNA gene and genome sequences are PQ897948 and JBLNKX000000000, respectively.

Description of *Flavobacterium cryoconiti* sp. nov.

Flavobacterium cryoconiti (cry.o.co.ni'ti. N.L. gen. n. cryoconiti, of cryoconite.)

Cells are Gram-stain-negative, rod-shaped, non-gliding, and devoid of flagella, measuring 0.7–0.8 μm ×1.5–3.4 μm. Colonies are circular, yellow, convex, round on PYG plates at 14 °C. Growth occurs at temperatures between 0–27 °C (optimum 14–20 °C), at pH 6.0–8.0 (optimum pH 7.0), and in the presence of 0–1.0% (w/v) NaCl. Flexirubin-type pigments are absent. Positive for catalase and oxidase. Cells hydrolyze esculin, but do not hydrolyze starch, casein, gelatin, or Tween 80. Indole and H_2S are not formed. Positive for the Voges-Proskauer test, alkaline phosphatase, esterase(C4), esterase lipase(C8), lipase(C14), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *α*-galactosidase, *β*-galactosidase, *α*-glucosidase, *β*-glucosidase, and N-acetyl-*β*-glucosaminidase. Negative for glucose fermentation,

citrate utilization, nitrate reduction, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, urease, trypsin, *α*-chymotrypsin, *β*-glucuronidase, *α*-mannosidase, and *α*-fucosidase. Acids are produced from D-glucose, L-rhamnose, and L-arabinose. Utilize the following carbohydrates as the sole carbon source: D-glucose, D-galactose, melibiose, maltose, lactose, D-mannose, D-xylose, D-turanose, D-fructose, glycogen, L-rhamnose, L-proline, D-raffinose, sucrose, D-trehalose, cellobiose, and L-arabinose. Cannot utilize the following carbohydrates: D-mannitol, propionate, tartrate, L-sorbose, citrate, myo-inositol, succinate, and D-ribose. The major fatty acids are iso-$C_{15:0}$ and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). The polar lipids are phosphatidylethanolamine, two aminolipids, and four lipids. The genomic DNA G+ C content of the type strain is 33.6%.

The type strain ZT3R17^T (= CGMCC 1.11707^T = NBRC 114824^T) was isolated from a cryoconite sample collected from the Zepu glacier on the Tibetan Plateau, P.R. China. The NCBI accession numbers for the 16S rRNA gene and genome sequences are PQ897949 and JBLNKW000000000, respectively.

Description of *Flavobacterium galactosi* sp. nov.

Flavobacterium galactosi (ga.lac.to'si. N.L. gen. n. galactosi, pertaining to galactose.)

Cells are Gram-stain-negative, rod-shaped, non-gliding, and devoid of flagella, measuring 0.7–0.8 μm ×2.1–5.4 μm. Colonies are circular, yellow, convex, round on PYG plates at 14 °C. Growth occurs at temperatures between 0–25 °C (optimum 14–20 °C), at pH 6.0–8.0 (optimum pH 7.0), and in the presence of 0–0.5% (w/v) NaCl. Flexirubin-type pigments are absent. Positive for catalase and negative for oxidase. Cells hydrolyze esculin and gelatin, but do not hydrolyze starch, casein, or Tween 80. Indole and H_2S are not formed. Positive for the Voges-Proskauer test, alkaline phosphatase, esterase(C4), esterase lipase(C8), lipase(C14), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *β*-galactosidase, *α*-glucosidase, *β*-glucosidase, and N-acetyl-*β*-glucosaminidase. Negative for glucose fermentation, citrate utilization, nitrate reduction, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, urease, trypsin, *α*-chymotrypsin, *α*-galactosidase, *β*-glucuronidase, *α*-mannosidase, and *α*-fucosidase. Utilize the following carbohydrates as the sole carbon source: D-glucose, D-galactose, melibiose, maltose, lactose, D-mannose, D-xylose, D-turanose, D-fructose, glycogen, L-rhamnose, L-proline, D-raffinose, sucrose, D-trehalose, cellobiose, and L-arabinose. Cannot utilize the following carbohydrates: D-mannitol, propionate, tartrate, L-sorbose, citrate, myo-inositol, succinate, and D-ribose. The major fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). The polar lipids are phosphatidylethanolamine, two unidentified aminolipids, and three unidentified lipids. The genomic DNA G+ C content of the type strain is 34.2%.

The type strain ZT3R25^T (= CGMCC 1.11711 ^{T} = NBRC 114825 ^{T}) was isolated from a cryoconite sample collected from the Zepu glacier on the Tibetan Plateau, P.R. China. The NCBI accession numbers for the 16S rRNA gene and genome sequences are PQ897950 and JBLNKV000000000, respectively.

Description of *Flavobacterium melibiosi* sp. nov.

Flavobacterium melibiosi (me.li.bi.o'si. N.L. gen. n. melibiosi, pertaining to melibiose.)

Cells are Gram-stain-negative, rod-shaped, non-gliding, and devoid of flagella, measuring 0.6–0.7 μm ×1.8–3.9 μm. Colonies are circular, yellow, convex, round on PYG plates at 14 °C. Growth occurs at temperatures between 0–23 °C (optimum 14–20 °C), at pH 6.0–8.0 (optimum pH 7.0), and in the presence of 0–0.5% (w/v) NaCl. Flexirubin-type pigments are absent. Positive for catalase and negative for oxidase. Cells hydrolyze esculin and gelatin, but do not hydrolyze starch, casein, or Tween 80. Indole and H_2S are not formed. Positive for the Voges-Proskauer test, alkaline phosphatase, esterase(C4), esterase lipase(C8), lipase(C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *α*-glucosidase, *β*-glucosidase, and N-acetyl-*β*-glucosaminidase. Negative for glucose fermentation, citrate utilization, nitrate reduction, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, urease, *α*-chymotrypsin, *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *α*-mannosidase, and *α*-fucosidase. Utilize the following carbohydrates as the sole carbon source: D-glucose, D-galactose, melibiose, maltose, lactose, D-mannose, D-turanose, D-fructose, glycogen, L-rhamnose, L-proline, D-raffinose, sucrose, D-trehalose, cellobiose, and L-arabinose. Cannot utilize the following carbohydrates: D-mannitol, D-xylose, propionate, tartrate, L-sorbose, citrate, myo-inositol, succinate, and D-ribose. The major fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). The polar lipids are phosphatidylethanolamine, two aminolipids, and four lipids. The genomic DNA G+ C content of the type strain is 34.0%.

The type strain XS2P12^T (= CGMCC 1.23198^T = NBRC 114826^T) was isolated from a melt water sample collected from the Zhuxi glacier on the Tibetan Plateau,

P.R. China. The NCBI accession numbers for the 16S rRNA gene and genome sequences are PQ897951 and JBLNKU000000000, respectively.

Description of *Flavobacterium algoris* sp. nov.

Flavobacterium algoris (al'go.ris. L. gen. n. algoris, of the cold.)

Cells are Gram-stain-negative, rod-shaped, non-gliding, and devoid of flagella, measuring 0.8–0.9 μm ×2.2–3.4 μm. Colonies are circular, yellow, convex, round on PYG plates at 14 °C. Growth occurs at temperatures between 0–25 °C (optimum 14–20 °C), at pH 6.0–8.0 (optimum pH 7.0), and in the presence of 0–0.5% (w/v) NaCl. Flexirubin-type pigments are absent. Positive for catalase and negative for oxidase. Cells hydrolyze esculin, but do not hydrolyze starch, casein, gelatin, or Tween 80. Indole and H_2S are not formed. Positive for the Voges-Proskauer test, alkaline phosphatase, esterase(C4), esterase lipase(C8), lipase(C14), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *α*-galactosidase, *β*-galactosidase, *α*-glucosidase, *β*-glucosidase, and N-acetyl-*β*-glucosaminidase. Negative for glucose fermentation, citrate utilization, nitrate reduction, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, urease, trypsin, *α*-chymotrypsin, *β*-glucuronidase, *α*-mannosidase, and *α*-fucosidase. Acids are produced from D-glucose, D-melibiose, and L-arabinose. Utilize the following carbohydrates as the sole carbon source: D-glucose, D-galactose, maltose, lactose, D-mannose, D-turanose, D-fructose, glycogen, L-proline, D-raffinose, sucrose, D-trehalose, cellobiose, and L-arabinose. Cannot utilize the following carbohydrates: melibiose, D-mannitol, D-xylose, L-rhamnose, propionate, tartrate, L-sorbose, citrate, myo-inositol, succinate, and D-ribose. The major fatty acids are iso-$C_{15:0}$ and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). The polar lipids are phosphatidylethanolamine, two aminolipids, and four lipids. The genomic DNA G + C content of the type strain is 33.8%.

The type strain GB2R13^T (= CGMCC 1.24741^T = NBRC 114830^T) was isolated from an ice sample collected from the Gawalong glacier on the Tibetan Plateau, P.R. China. The NCBI accession numbers for the 16S rRNA gene and genome sequences are PQ897952 and JBLNKT000000000, respectively.

Abbreviations

ML	Maximum-likelihood
NJ	Neighbor-joining
PYG	Peptone, yeast extract and glucose
ANI	Average nucleotide identity
dDDH	Digital DNA–DNA hybridization
R2 A	Reasoner's 2A
TEM	Transmission electron microscopy
PE	Phosphatidylethanolamine
AL	Unidentified aminolipid
UL	Unidentified lipid

Supplementary Information

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Supplementary Material 1.

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Authors' contributions

Y.H. Xin and Q. Liu designed the project and analyzed the data. Y.H. Xin and Q. Liu purified the strains. L.L. Yang and Q. Liu performed bioinformatic analysis of the genome sequences, and the phenotypic analysis. L.L. Yang, Y.H. Xin, and Q. Liu wrote the manuscript.

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Data availability

The GenBank accession numbers for the 16S rRNA gene sequence of strains LB3P122 T, LT1R49 T, ZT3R17 T, ZT3R25 T, XS2P12 T, and GB2R13 T are PQ897947-PQ897952. The genome sequences have been deposited at DDBJ/ENA/GenBank under the accession numbers JBLNKY000000000, JBLNKX000000000, JBLNKW000000000, JBLNKV000000000, JBLNKU000000000, and JBLNKT000000000, respectively.

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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