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TAXONOMIC DESCRIPTION

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Lysobacter antarcticus sp. nov., an SUF-system-containing bacterium from Antarctic coastal sediment

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Abstract

A Gram-stain-negative, heterotrophic, aerobic, non-motile, rod-shaped bacterial strain (GW1-59^T) belonging to the genus *Lysobacter* was isolated from coastal sediment collected from the Chinese Great Wall Station, Antarctica. The strain was identified using a polyphasic taxonomic approach. The strain grew well on Reasoner's 2A media and could grow in the presence of 0–4% (w/v) NaCl (optimum, 1%), at pH 9.0–11.0 and at 15–37 °C (optimum, 30 °C). Strain GW1-59^T possessed ubiquinone-8 as the sole respiratory quinone. The major phospholipids were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. The major fatty acids were summed feature 9 (10-methyl $C_{16:0}$ and/or iso-$C_{17:1}$ ω9*c*), iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$, $C_{16:0}$ and iso-$C_{11:0}$ 3-OH. DNA–DNA relatedness with *Lysobacter concretionis* Ko07^T, the nearest phylogenetic relative (98.5% 16S rRNA gene sequence similarity) was 23.4% (21.1–25.9%). The average nucleotide identity value between strain GW1-59^T and *L. concretionis* Ko07^T was 80.1%. The physiological and biochemical results and low level of DNA–DNA relatedness suggested the phenotypic and genotypic differentiation of strain GW1-59^T from other *Lysobacter* species. On the basis of phenotypic, phylogenetic and genotypic data, a novel species, *Lysobacter antarcticus* sp. nov., is proposed. The type strain is GW1-59^T (=CCTCC AB 2019390^T=KCTC 72831^T).

The genus *Lysobacter* was firstly proposed by Christensen and Cook [1] and then emended by Park *et al.* in 2008 [2]. *Lysobacter* owes its name to the lytic activity against bacteria, fungi and algae [3]. At the time of writing, the genus *Lysobacter* comprised 66 recognized species with validly names (https://lpsn.dsmz.de/genus/Lysobacter). *Lysobacter* strains are ubiquitously distributed in various environments, such as seawater [3], deep-sea sponge [4], soil [5–8], freshwater [9, 10] and freshwater sediments [11]. Species of the genus *Lysobacter* are Gram-stain-negative, aerobic, non-fruiting, gliding organisms with a high genomic DNA G+C content (61.7–70.7mol%) and contain ubiquinone 8 (Q-8) as the major respiratory quinone. All validly named species of the genus *Lysobacter* show negative activities for urease and indole production [6]. A few *Lysobacter* species show positive reactions in tests for flexirubin-type pigments [12–15]. Flagella were detected in some members of this genus, enclosed in the periplasm and capable of motility (e.g. *Lysobacter xanthus* and *Lysobacter helvus*) [16]. Some members of the genus are proteolytic and have potential as new sources of bioactive compounds and new antibiotics against plant and human pathogens [3, 17, 18]. New antibiotics were isolated from species such as *Lysobacter antibioticus* 76^T, *Lysobacter capsici* 55^T, *Lysobacter enzymogenes* C3^T and *Lysobacter antibioticus* ATCC 29479^T, that contain more secondary metabolite gene clusters than others in the genus. Secondary metabolites such as cyclodepsipeptides, cyclic lipodepsipeptides, cephem-type beta-lactams and polycyclic tetramate macrolactams have been reported from members of this genus [18, 19].

During an investigation of the microbial community in a nearshore Antarctic marine ecosystem, a Gram-stain-negative, aerobic bacterium, designated strain GW1-59^T, was isolated from coastal sediment collected at a depth of 95 m near the Chinese Great Wall Station and identified as a member of the genus *Lysobacter*. Here, we report the taxonomic position and characterization of the bacterial strain by genotypic, chemotaxonomic and phenotypic approaches. This is the first novel *Lysobacter* species obtained from polar marine environment.

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Abbreviations: AANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; DPG, Diphosphatidylglycerol; NA, nutrient agar; PE, phosphatidylinositol; PG, phosphatidylglycerol; Q-8, ubiquinone 8; R2A, Reasoner's 2A; SUF, sulphur utilization factor.
The genome sequences of *Lysobacter antarcticus* GW1-59^T has been deposited in the GenBank database under the accession number JAIEUJ000000000. The GenBank accession number of the 16S rRNA gene sequence of strain GW1-59^T is MN650237.
Two supplementary tables and six supplementary figures are available with the online version of this article.

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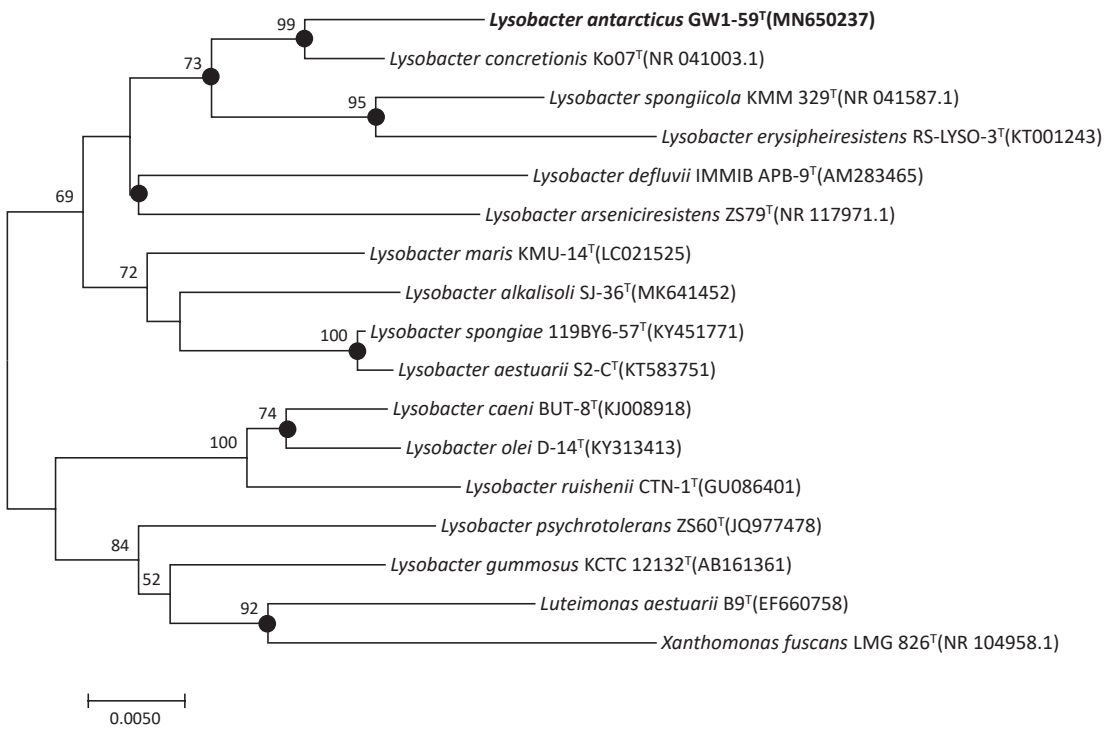

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of GW1-59^T. Bootstrap values (expressed as percentages of 1000 replications) of above 50% are shown at the branch points. Solid circles indicate that nodes are also recovered using the maximum-likelihood and maximum-parsimony methods. *Xanthomonas fuscans* NCPPB 381^T(NR_104958.1) was used as the outgroup. Bar, T% sequence divergence.

ISOLATION AND ECOLOGY

The coastal sediment sample was collected in Antarctica (62 ° 13′ 34″ S, 58 ° 55′ 31″ W) in December 2016. One gram of sample was diluted in sterile distilled water and plated over nutrient agar (NA) medium (Difco) with 1.5% (w/v) agar and incubated at 15 °C for up to 7–14 days. Reasoner's 2A (R2A) medium (Difco) with 1.5% (w/v) agar was used to purify and cultivate the strain at 15 °C for 7 days for physiology analysis. The long-term preservation of the strain was kept in 20% (v/v) glycerol at −20 °C and −80 °C.

16S rRNA PHYLOGENY

For phylogenetic analysis, a standard procedure was used to extract genomic DNA from the test strain [20]. The 16S rRNA gene sequence was obtained from strain GW1-59^T by PCR amplification [21] and then submitted to the EzBioCloud database(www.ezbiocloud.net) for comparison and retrieval of the most similar sequences of recognized bacteria [22]. Maximum-likelihood [23], neighbour-joining [24] and maximum-parsimony [23] trees were reconstructed using MEGA 7.0 [25] after multiple alignment of data by ClustalW [26]. Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by performing 1000 resamplings [27].

The 16S rRNA gene sequence comparisons showed that strain GW1-59^T showed highest similarity to *Lysobacter concretionis* Ko07 ^{T} (98.5%) and less than 97% identities to other type strains. Maximum-likelihood and neighbour-joining trees showed that strain GW1-59^T was located within the genus *Lysobacter* and formed a closely branch with the type strain *L. concretionis* Ko07 ^{T} with 99 and 66% bootstrap support (Figs 1 and S1a, available in the online version of this article), which was strongly supported by the genomic evolutionary tree (Fig. 2). Strain GW1-59^T and *L. concretionis* Ko07 ^{T} are on the same branch on the genomic evolutionary tree with 100% bootstrap support. Strain GW1-59^T formed a separate cluster in the maximum-parsimony tree, but branched close to *L. concretionis* Ko07 ^{T} (Fig. S1b). Phylogenetic analysis confirmed that strain GW1-59^T represents a novel strain of genus *Lysobacter* and type strains *L. concretionis* Ko07^T, *L. spongiicola* DSM 21749^T and *L. enzymogenes* DSM 2043^T were chosen as the reference strains for further tests.

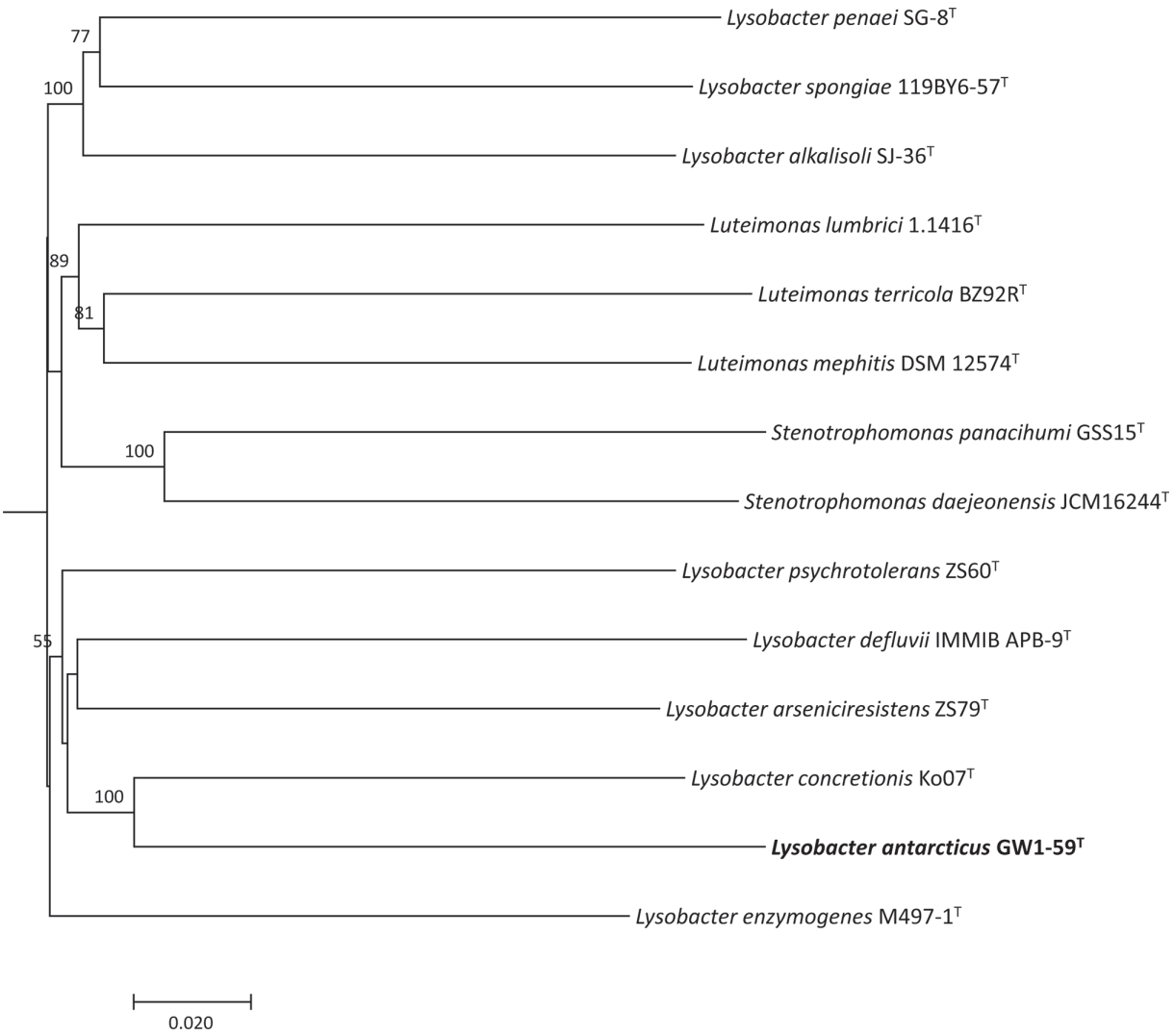

Fig. 2. Phylogenetic tree based on genome sequences in the TYGS for GW1-59^T. Distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d5. The numbers above branches are GBDP pseudo-bootstrap support values > 50% from 100 replications. The tree was rooted at the midpoint.

GENOME FEATURES

A standard procedure was used to extract genomic DNA from the test strain [20]. The genome of strain GW1-59^T was sequenced by Beijing Novogene Bioinformatics Technology using the Illumina PE150 platform. All good- quality paired reads were assembled using the SOAPdenovo software (version 2.04) into a number of scaffolds [28]. The authenticity of the genome sequence of the novel strain and the absence of contamination were confirmed according to the proposed Jongsik Chun minimal standards [28–30]. The tRNA genes were identified by tRNAscan-SE [31], rRNA genes were predicted by Barrnap [32], secondary metabolism were predicted by antiSMASH [33]. GeneMarkS software was used to predict the protein-coding genes of the bacterial genome [34]. Comparing the protein sequence of the predicted gene with the NR (Non-Redundant Protein Database) [35], COG (Cluster of Orthologous Groups of proteins), KEGG (Kyoto Encyclopedia of Genes and Genomes) [36], eggNOG (evolutionary genealogy of genes: Non-supervised Orthologous Groups) [37], Swiss-Prot [38] and GO (Gene Ontology) [39] databases was performed by Diamond BLASTP. For this analysis, the comparison result with the highest score was selected for annotation and the following cutoff values were applied (e-value <1e-6 and amino acid sequence identity of at least 40%). Orthologous average nucleotide identity (ANI) [40] and digital DNA–DNA hybridization (dDDH) [41] values were calculated using the ANI calculator tool from the EzBioCloud (www.ezbiocloud.net/tools/ani) [42] and the Genome-to-Genome Distance Calculator (http://ggdc.dsmz.de/ggdc) [43], respectively. The genomic DNA G+C content was determined from whole genome sequence data of strain GW1-59^T [44]. The genome of strain GW1-59^T was compared to those of the nearest *Lysobacter* species to better understand

the genetic background of the novel strain. Gene orthology was predicted by OrthoFinder [45]. eggNOG-mapper was used to annotate the strain GW1-59^T-specific genes [46].

The genome data of strain GW1-59^T was acquired and deposited at NCBI under JAIEUJ000000000. The pairwise identity between the one 16S rRNA gene copy in the genome of strains GW1-59^T was 100%, suggesting that the genome was not contaminated [29]. The size of the draft genome is 2.8 Mb with six contigs, 46 tRNA genes, one 5S rRNA gene, one 16S rRNA gene and one 23S rRNA gene (Table 1). *In silico* secondary metabolite detection using the AntiSMASH tool found at least two gene clusters within the genome sequences of the strains (Table S2). The arylpolyene type cluster was more prominent in all the four strains while resorcinol-type metabolites were detected only in *L. antarcticus* GW1-59^T (Table S2). The arylpolyene cluster of strain GW1-59^T had 50% similarity to the defined cluster 'xanthomonadin I', which contains membrane transport (Orf4) and acyl transferases (Orf6). These genes are related to the xanthomonadin I biosynthetic gene cluster reported by Goel *et al.* [47]. These results supported the xanthomonadin I-producing potential of strain GW1-59^T and the reference strains.

The genome contains 2487 coding sequences, which accounts for 90.1% of the total genome. A total of 2429 annotations were found in the NR database, 2210 in COG, 1390 in KEGG, 1856 in SwissProt and 1881 in GO. Of the total GO terms assigned, 5409, 2547 and 3910 were assigned to biological processes, cellular components and molecular functions, respectively. Biological process, cell and molecular function were the most abundant among each category (Fig. S3a). Analysis of COG annotation data found that most of the proteins were categorized as being involved in cell wall/membrane/envelope biogenesis, followed by amino acid transport and metabolism, and translation, ribosomal structure and biogenesis (Fig.S3b). The ANI value between strain GW1-59^T and its closest relative *L. concretionis* Ko07^T was 80.1%, which was below the threshold used to distinguish between the closely related strains (Fig. S4) [48, 49]. The dDDH value (average and confidence intervals in parentheses) between strain GW1-59^T and *L. concretionis* Ko07^T was 23.4% (21.1–25.9%), a value significantly below the 70% threshold used to assign strains to the same genomic species [50]. The G+C content of strain GW1-59^T was calculated to be 67.2 mol% from genome data (Table 1).

The core genomes of the novel strain GW1-59^T and the nearest *Lysobacter* species contained 1650 genes, and 220 genes were detected to be specific to the novel strain (Fig. S5). Annotation of the specific genes of GW1-59^T indicated a N_2O reduction pathway in the GW1-59^T genome, which included periplasmic copper-binding protein NosD, ABC transporter-related protein NosF, Cu chaperones NosL, nitrous oxide expression regulator NosR and nitrous oxide reductase NosZ (1.7.2.4). GW1-59^T was isolated from coastal sediment at a depth of 95 m in Great Wall Bay, Antarctics, where high concentrations of organic matter and low oxygen were reported [51, 52]. In environments such as sediment with low oxygen concentration, although microbial metabolism is limited by the diffusive supply of electron acceptors [53], obligate aerobic micro-organisms with *nos*Z genes can utilize N_2O as a temporary surrogate for O_2 to survive periodic anoxia [54]. In addition, nitric oxide reductase large subunit (1.7.2.5) and protein involved in response to NO were also detected in the genome of GW1-59^T. The nitrogen metabolism genes detected in GW1-59^T reveal its strong ability to adapt to low oxygen concentrations and organotrophy [55, 56].

Furthermore, the small heat shock protein (HSP20) responding to heat shock or other environmental stresses [57] was detected in the specific genes of GW1-59^T. The cyanobacterial small heat-shock protein HspA was found to play roles in enhancing bacterial tolerance to oxidative stress. Overexpression of Hsp20 in *Deinococcus radiodurans* in *Escherichia coli* could enhance tolerance to hydrogen peroxide (H_2O_2) stress [58]. Three sHsps paralogs of Archaea *M. psychrophilus* R15 all increase transcriptions at lower temperatures [59, 60], suggesting that these sHsps may play a role in the cold adaptation of Archaea.

Genes of thriving under stress conditions such as oxidation and iron starvation were detected in GW1-59^T, which contains Fe-S cluster scaffold complex subunit SufB, Fe-S cluster assembly ATPase SufC, Fe-S cluster assembly protein SufD, cysteine desulfuration protein SufE (3.1.3.5) and cysteine desulfurase SufS for the mobilization of sulphur from cysteine (4.4.1.16). The sulphur utilization factor (SUF) system is operative only under conditions of iron limitation or oxidative stress [61]. Recently a genomic analysis of a Verrucomicrobial endosymbiont from a marine-derived Antarctic lake illustrated that the SUF system is more resistant to reactive oxygen species than the iron–sulfur cluster (ISC) system [62]. The SUF system was also detected in the genome of GW1-59^T, based on literature reports, the SUF system appears to maintain Fe-S cluster assembly under oxidative stress conditions such as NO [63].

The genetic ability of encoding, *nos* operon, Hsp20 and SUF proteins for GW1-59^T may be especially relevant to the prevailing Antarctic environmental conditions and coastal sediments (low oxygen concentrations, freezing temperatures, oxidation and enhanced UV irradiation).

Genomic screening for carbohydrate-active enzymes (CAZymes) revealed a shared profile for strain GW1-59^{T}and *L. concretionis* Ko07^T, which were clustered together in the phylogenetic trees (Table S1). Compared with *L. enzymogenes* DSM 2043^T, the two strains lost amylase, chitinase, cellulase and other high-molecular-weight sugar polymer-degrading enzymes, while the abundant lysozyme type G (3.2.1.17) or peptidoglycan lyase (4.2.2 .n1) were kept in the genomes. GH109 family (α-*N*-acetylgalactosaminidase), which is quite rare in marine bacteria [64], was only detected in the genome of novel strain GW1-59^T. The presence of glycoside hydrolase family GH103, 23, 94 and GH109 suggests the possible ability of GW1-59^T to degrade cell-derived carbohydrates such as peptidoglycan, glycolipids, glycopeptides and glycoproteins. The loss of genes encoding

Table 1. Phenotypic comparison of strain GW1-59^T with its phylogenetically closest *Lysobacter* relatives

Strain:1, GW1-59^T; 2, *L. concretionis* Ko07^T; 3, *L. spongiicola* DSM 21749^T; 4, *L. enzymogenes* DSM 2043^T. All data are from this study, except for the G+C content, polar lipid and quinone profiles of the reference strains [4, 5, 75]. Experiments were performed under the same conditions using the same methodology in this study. +, Positive; –, negative.

Characteristic	1	2	3	4
Hydrolysis of				
Aesculin	–	–	–	+
Gelatin	+	+	+	+
Casein	–	+	+	+
Tween 80	+	–	–	+
Starch	–	–	–	–
Assimilation of (from API 50CHB):				
D-Glucose	–	–	–	+
L-Arabinose	–	–	–	+
D-Mannose	+	–	–	+
D-Mannitol	–	–	–	+
N-Acetylglucosamine	–	–	–	+
Maltose	–	–	–	+
Malic acid	–	–	–	+
Trisodium citrate	–	–	–	+
Galactose	–	–	–	+
Methyl α-D-glucoside	–	–	–	+
Melibiose	–	–	–	+
Sucrose	–	–	–	+
Glycogen	–	+	–	+
5-Ketogluconate	–	–	+	–
Enzyme activities:				
Catalase	+	+	+	+
Oxidase	+	+	+	+
Urease	+	–	–	–
β-Galactosidase	–	–	–	+
Nitrate reduction	+	–	–	–
Polar lipids*	PG, DPG, PE, UPL, UL	DPG, PE, PG, L, GL	PE, PG, DPG	DPG, PME, PE, PG, UAL, UAPL, UPL
Cell size (μm)	0.6–0.8×1.4–1.7	0·7×1·0–13·5	4.6×12.9	0.5×38.0
Temperature range for growth (°C)	15–37	25–30	5–41	5–40
pH range for growth	9–11	6.8–7.5	5.5–9.5	4.0–10.0
Salinity range for growth (%)	0–4	ND	0–6	0–1
rRNA	3	6	4	10
5S rRNA	1	1	ND	3
16S rRNA	1	1	ND	1

Continued

Table 1. Continued

Characteristic	1	2	3	4
23S rRNA	1	1	ND	1
tRNA	45	91	46	108
G+C content (mol%)	63.9	67.0	67.0	69.0
Genome size (nt)	2784373	3032117	3099673	6263953

*PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PME, phosphatidylmonomethylethanolamine; UAL, unidentified aminolipid; UAPL, unidentified aminophospholipid; UL, unidentified lipid; UPL, unidentified phospholipid.

high-molecular-weight sugar polymer-degrading enzymes and the presence of Xaa-Pro aminopeptidase PepP (3.4.11.9) and Xaa-Pro dipeptidase PepQ (3.4.13.9) encoding genes in the genome of GW1-59^T, combined with the physiologic result of high gelatin hydrolysis, indicated that it could also acquire carbon and energy from proteinaceous resources, as previously reported for *Idiomarina* bacteria, which were also isolated from the polar marine environment [65]. The genome sequence analysis supports that strain GW1-59^T is within the genus *Lysobacter*, but different from current valid species of *Lysobacter*.

PHYSIOLOGY AND CHEMOTAXONOMY

Morphological characteristics of strain GW1-59^T were viewed with a JEOL transmission electron microscope (JEM-1200EX) at 80 kV under standard operating conditions. An aliquot of culture was stained with crystal violet and Gram's iodine. Cell motility determination was conducted by observing the development of turbidity in a tube using R2A semisolid medium containing 0.4% agar [66]. Anaerobic growth conditions were tested as described by Hoffmann *et al.* [67]. Catalase and oxidase activity were determined by bubble production in 3% (v/v) hydrogen peroxide solution and 1% (w/v) *N-N-N′-N′*-tetramethyl-*p*-phenylenediamine, respectively. Casein, starch, gelatin (Sigma) and Tween 60/80 degradation was tested on R2A plates containing milk powder (5%, w/v), starch (0.2%, w/v), gelatin (12%, w/v) and Tween 60/80 (1%, v/v), respectively. Nitrate reduction was tested in nitrate broth containing 0.2% KNO_3. The ability of GW1-59^T to utilize citrate was tested in Simmons citrate agar. Urease activity was evaluated in Christensen's medium. The sulphur reduction test was performed using sulfide indole motility medium. The assimilation of substrates and other physiological properties were examined using API 20NE strips (bioMérieux) following the instructions of the manufacturer. Acid production from different carbohydrates was determined with the API CHB50 system. Glycine, L-arginine and aspartic acid were added to a basic medium consisting of components (KH_2PO_4 1.36 g l^{-1}, Na_2HPO_4 1.97 g l^{-1}, $MgSO_4$ 0.12 g l^{-1}, $FeSO_4$ 0.1 g l^{-1}; $CaCl_2$ 0.2 g l^{-1}, D-mannose 10 g l^{-1}, respectively) to investigate the utilizations of substrates as nitrogen sources. Tolerance to NaCl was tested on R2A agar at concentrations of 0–15%. The pH tolerance (pH range 4–10) was tested in R2A broth using the buffer system described by Xu *et al.* [68], and temperature tolerance (4, 10, 15, 20, 28, 30, and 37 °C) was tested on R2A agar.

Strain GW1-59^T exhibited a colour shift from yellow to brown when flooded with 20% KOH and an acidic solution, indicating that it produces a flexirubin-type pigment [69]. For analysis of cellular fatty acids, strain GW1-59^T and the reference strains were grown in R2A broth at 30 °C and cells were harvested in the late exponential phase. The fatty acids were prepared and identification was carried out according to the standard protocol of the Sherlock Microbial Identification System [70]. Respiratory quinones were extracted and separated as described by Collins *et al.* [71] and subsequently analysed as described by Wink *et al.* [72]. Polar lipids were extracted using the method described by Minnikin *et al.* [73] and identified by two-dimensional TLC as described by Collins and Jones [74].

Strain GW1-59 ^{T} was Gram-stain-negative, heterotrophic, aerobic, non-motile, rod-shaped and pale-yellow-coloured when routinely cultured on R2A agar at 30 °C. GW1-59^T was capable of growing on R2A and tryptic soy agar, tolerated a pH range of pH 9.0–11.0 and NaCl concentration of 0–4% (w/v) with optimal growth at 1 % NaCl. The cells grew on R2A agar over a temperature range of 15–37 °C, optimum at 30 °C. The cells were 0.6–0.8×0.7–1.7 µm (Fig. S1). The ability of GW1-59^T to hydrolyse gelatin and Tween 80/60 was demonstrated. The oxidase activity, catalase activity, urease activity and nitrate reduction activity of GW1-59^T were positive. In the API 20NE system, D-mannose, D-gluconic acid, adipic acid and phenylacetic acid were assimilated. The novel strain showed negative activities for urease and indole production. In the API 50CH system, acid was produced from ribose and D-mannose. Strain GW1-59^T had the capability of assimilating glycine, L-arginine and aspartic acid. The phenotypic, physiological and biochemical characteristics differentiating strain GW1-59^T from its closely related type strains are summarized in Table 1. The respiratory quinone of strain GW1-59^T was Q-8, which was consistent with other type strains in the genus *Lysobacter*.

Table 2. Cellular fatty acid compositions (%) of strain GW1-59^T and the three most closely related *Lysobacter* type strains

Strain:1, GW1-59^T; 2, *L. concretionis* Ko07^T; 3, *L. spongiicola* DSM 21749^T; 4, *L. enzymogenes* KCTC 12131^T. GW1-59^T data are from this study. Data are expressed as percentages of total fatty acids. Data are expressed as percentages of total fatty acids. Major components (>5.0%) are highlighted in bold. Fatty acids amounting to less than 0.5% in all strains are not shown. TR, Trace amount (<1%); –, not detected.

Fatty acid	1	2	3	4
$C_{14:0}$	–	TR	–	1.2
$C_{16:0}$	**6.9**	2.4	–	**5.1**
$C_{18:0}$	1.3	–	–	–
iso-$C_{11:0}$	4.3	**6.4**	**9.5**	4.3
iso-$C_{11:0}$ 3OH	**5.1**	**5.6**	**15.5**	**6.0**
iso-$C_{14:0}$	–	2.6	3.3	–
iso-$C_{15:0}$	**18.4**	**36.1**	**23.0**	**43.0**
anteiso-$C_{15:0}$	1.3	1.2	–	1.9
iso-$C_{16:0}$	**12.7**	**19.9**	**32.5**	3.0
iso-$C_{17:0}$	**7.2**	2.9	2.8	4.4
iso-$C_{17:1}$	–	–	**13.2**	–
iso-$C_{17:1}$ $\omega 9c$	–	**13.9**	–	**8.8**
$C_{17:0}$ cyclo	1.9	2.5	–	**10.6**
Summed feature 3*	3.5	–	–	–
Summed feature 4†	–	TR	–	**8.3**
Summed feature 7‡	–	–	–	1.6
Summed feature 9§	**35.9**	–	–	–

*Summed feature 3 consists of $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$.
†Summed feature 4 consists of $C_{15:0}$ iso 2OH and/or $C_{16:1}$ $\omega 7c$.
‡Summed feature 7 consists of $C_{18:1}$ $\omega 7c/\omega 9t/\omega 12t$ and/or $C_{18:1}$ $\omega 7c/\omega 9c/\omega 12t$.
§Summed feature 9 consists of 10-methyl $C_{16:0}$ and/or iso-$C_{17:1}$ $\omega 9c$.

The major phospholipids found in strain GW1-59^T were diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine, which are commonly found in most *Lysobacter* species (Table 1, Fig. S6) [3]. Strain GW1-59^T also contained three unidentified phospholipids and two unidentified lipids. The polar lipid profile of strain GW1-59^T was clearly different from those of the reference strains. An unknown glycolipid was only detected in *L. concretionis* Ko07^T. Phosphatidylmonomethylethanolamine, two unidentified aminophospholipids and an unidentified aminolipid were present in *L. enzymogenes* KCTC 12131^T.

The fatty acid profile of strain GW1-59^T was characterized by summed feature 9 (10-methyl $C_{16:0}$ and/or iso-$C_{17:1}$ $\omega 9c$), iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$, $C_{16:0}$ and iso-$C_{11:0}$ 3-OH (Table 2). However, some qualitative and quantitative differences (shown in Table 2) in the fatty acid profiles distinguished strain GW1-59^T from the reference strains. The major fatty acids (>10% of the total fatty acids) found in strain GW1-59^T were iso-$C_{15:0}$, iso-$C_{16:0}$ and summed feature 9. The significant differences between GW1-59^T and the other strains were observed in summed feature 9 as well as a lower iso-$C_{11:0}$ 3OH and iso-$C_{15:0}$ content. Compared to others strains, the contents of $C_{16:0}$ and iso-$C_{17:0}$ were slightly higher in GW1-59^T, while the amounts of iso-$C_{16:0}$ and iso-$C_{11:0}$ were lower than detected in *L. concretionis* Ko07^T and *L. spongiicola* DSM 21749^T. Strain GW1-59^T was also distinguishable from *L. enzymogenes* KCTC 12131^T in the amounts of $C_{17:0}$ cyclo and summed feature 4. Compared with *L. concretionis* Ko07^T and *L. enzymogenes* KCTC 12131^T, iso-$C_{17:1}$ $\omega 9c$ was not detected in GW1-59^T.

Strain GW1-59^T showed the highest similarity to *L. concretionis* Ko07^T in the 16S rRNA gene sequence analysis, and formed a close cluster with *L. concretionis* Ko07^T in the phylogenetic analysis. The major chemotaxonomic data were consistent with the assignment of strain GW1-59^T to the genus *Lysobacter*. Strain GW1-59^T could be distinguished from its closely related strains by several phenotypic and chemotaxonomic characteristics (Tables 1 and 2). Strain GW1-59^T showed nitrate reduction and was urease-positive, but *L. concretionis* Ko07^T, *L. spongiicola* DSM 21749^T and *L. enzymogenes* DSM 2043^T were negative for these characteristics. Strain GW1-59^T showed different tolerances to temperature, pH and salt from the reference strains. Based on its distinct phylogenetic position within the genus *Lysobacter*, together with physiological and

chemotaxonomic properties, it is concluded that strain GW1-59^T represents a novel species in the genus *Lysobacter*, for which the name *Lysobacter antarcticus* sp. nov. is proposed.

DESCRIPTION OF *LYSOBACTER ANTARCTICUS* SP. NOV.

Lysobacter antarcticus (ant.arc'ti.cus. L. masc. adj. *antarcticus*, of the Antarctic, pertaining to the Antarctica, the geographical origin from which the type strain was first isolated).

Cells are aerobic, Gram-stain-negative, rod-shaped, non-flagellated, non-motile, approximately 0.6–0.8 µm wide and 1.4–1.7 µm long. Strain GW1-59^T grows well on NA and R2A agar. Colonies are circular, convex, opaque and pale yellow, with a smooth surface when grown on R2A agar. The pH range for growth is pH 9.0–11.0. The maximum concentration of NaCl for growth is 4% (w/v) supplemented on R2A. Growth occurs at 15–37 °C on R2A agar. Positive for oxidase, catalase, nitrate reduction and urease, as well as gelatin and Tween 60/80 hydrolysis. Negative for hydrogen sulphide production, citrate utilization, starch and casein hydrolysis. The following substrates in the API 50 CHB/E medium are utilized: ribose and D-mannose. In the API 20NE system, D-mannose, D-gluconic acid, adipic acid and phenylacetic acid are assimilated. Glycine, L- arginine and aspartic acid are utilized in the basic medium. The major phospholipids are phosphatidylglycerol, diphosphatidylglycerol and phosphatidylinositol. The predominant quinone is Q-8. The major fatty acids are summed feature 9 (10-methyl $C_{16:0}$ and/or iso-$C_{17:1}$ $\omega 9c$), iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$, $C_{16:0}$ and iso-$C_{11:0}$ 3-OH.

The type strain is GW1-59^T (=CCTCC AB 2019390^T=KCTC 72831^T), which was isolated from a coastal sediment sample collected near the Chinese Great Wall Station, Antarctica. The genome of the type strain is characterized by a size of 2.8 Mbp and a G+C content of 67.2mol%.

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Author contributions
Z.L., P.J., G.N. and W.W. equally contributed to this work.

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

References

1. Christensen P, Cook FD. *Lysobacter*, a new genus of nonfruiting, gliding bacteria with a high base ratio. *Int J Syst Bacteriol* 1978;28:367–393.
2. Park JH, Kim R, Aslam Z, Jeon CO, Chung YR, *et al*. *Lysobacter capsici* sp. nov., with antimicrobial activity, isolated from the rhizosphere of pepper, and emended description of the genus *Lysobacter*. *Int J Syst Evol Microbiol* 2008;58:387–392.
3. de Bruijn I, Cheng X, de Jager V, Expósito RG, Watrous J, *et al*. Comparative genomics and metabolic profiling of the genus *Lysobacter*. *BMC Genomics* 2015;16:991.
4. Yoon J. Polyphasic characterization of *Lysobacter maris* sp. nov., a bacterium isolated from seawater. *Curr Microbiol* 2016;72:282–287.
5. Romanenko LA, Uchino M, Tanaka N, Frolova GM, Mikhailov VV, *et al*. *Lysobacter spongiicola* sp. nov., isolated from a deep-sea sponge. *Int J Syst Evol Microbiol* 2008;58:370–374.
6. Luo G, Shi Z, Wang G. *Lysobacter arseniciresistens* sp. nov., an arsenite-resistant bacterium isolated from iron-mined soil. *Int J Syst Evol Microbiol* 2012;62:1659–1665.
7. Zhang L, Bai J, Wang Y, Wu G-L, Dai J, *et al*. *Lysobacter korlensis* sp. nov. and *Lysobacter bugurensis* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* 2011;61:2259–2265.
8. Liu M, Liu Y, Wang Y, Luo X, Dai J, *et al*. *Lysobacter xinjiangensis* sp. nov., a moderately thermotolerant and alkalitolerant bacterium isolated from a gamma-irradiated sand soil sample. *Int J Syst Evol Microbiol* 2011;61:433–437.
9. Siddiqi MZ, Im W-T. *Lysobacter pocheonensis* sp. nov., isolated from soil of a ginseng field. *Arch Microbiol* 2016;198:551–557.
10. Fukuda W, Kimura T, Araki S, Miyoshi Y, Atomi H, *et al*. *Lysobacter oligotrophicus* sp. nov., isolated from an Antarctic freshwater lake in Antarctica. *Int J Syst Evol Microbiol* 2013;63:3313–3318.
11. Fang B-Z, Xie Y-G, Zhou X-K, Zhang X-T, Liu L, *et al*. *Lysobacter prati* sp. nov., isolated from a plateau meadow sample. *Antonie van Leeuwenhoek* 2020;113:763–772.
12. Lin S-Y, Hameed A, Wen C-Z, Liu Y-C, Hsu Y-H, *et al*. *Lysobacter lycopersici* sp. nov., isolated from tomato plant *Solanum lycopersicum*. *Antonie van Leeuwenhoek* 2015;107:1261–1270.
13. Singh H, Du J, Ngo HTT, Won K, Yang J-E, *et al*. *Lysobacter fragariae* sp. nov. and *Lysobacter rhizosphaerae* sp. nov. isolated from rhizosphere of strawberry plant. *Antonie van Leeuwenhoek* 2015;107:1437–1444.
14. Singh H, Won K, Du J, Yang J-E, Akter S, *et al*. *Lysobacter agri* sp. nov., a bacterium isolated from soil. *Antonie van Leeuwenhoek* 2015;108:553–561.
15. Ngo HTT, Won K, Du J, Son H-M, Park Y, *et al*. *Lysobacter terrae* sp. nov. isolated from *Aglaia odorata* rhizosphere soil. *Int J Syst Evol Microbiol* 2015;65:587–592.
16. Kim I, Choi J, Chhetri G, Seo T. *Lysobacter helvus* sp. nov. and *Lysobacter xanthus* sp. nov., isolated from soil in South Korea. *Antonie van Leeuwenhoek* 2019;112:1253–1262.
17. Ahmed K, Chohnan S, Ohashi H, Hirata T, Masaki T, *et al*. Purification, bacteriolytic activity, and specificity of beta-lytic protease from *Lysobacter* sp. IB-9374. *J Biosci Bioeng* 2003;95:27–34.
18. Panthee S, Hamamoto H, Paudel A, Sekimizu K. *Lysobacter* species: a potential source of novel antibiotics. *Arch Microbiol* 2016;198:839–845.
19. Xie Y, Wright S, Shen Y, Du L. Bioactive natural products from *Lysobacter*. *Nat Prod Rep* 2012;29:1277–1287.
20. Sun W, Dai S, Jiang S, Wang G, Liu G, *et al*. Culture-dependent and culture-independent diversity of Actinobacteria associated with the marine sponge *Hymeniacidon perleve* from the South China Sea. *Antonie van Leeuwenhoek* 2010;98:65–75.

21. Benndorf R, Guo H, Sommerwerk E, Weigel C, Garcia-Altares M, *et al*. Natural products from actinobacteria associated with fungus-growing termites. *Antibiotics* 2018;7:83.
22. Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y, *et al*. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
23. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.
24. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
25. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
26. Thompson J. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 1997;25:4876–4882.
27. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 2017;39:783–791.
28. Li R, Zhu H, Ruan J, Qian W, Fang X, *et al*. *De novo* assembly of human genomes with massively parallel short read sequencing. *Genome Res* 2010;20:265–272.
29. Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, *et al*. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol Microbiol* 2018;68:461–466.
30. Lee I, Chalita M, Ha S-M, Na S-I, Yoon S-H, *et al*. ContEst16S: an algorithm that identifies contaminated prokaryotic genomes using 16S RNA gene sequences. *Int J Syst Evol Microbiol* 2017;67:2053–2057.
31. Lowe TM, Eddy SR. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* 1997;25:955–964.
32. Kalvari I, Argasinska J, Quinones-Olvera N, Nawrocki EP, Rivas E, *et al*. Rfam 13.0: shifting to a genome-centric resource for non-coding RNA families. *Nucleic Acids Research* 2018;46:D335–D342.
33. Blin K, Shaw S, Kloosterman AM, Charlop-Powers Z, van Wezel GP, *et al*. antiSMASH 6.0: improving cluster detection and comparison capabilities. *Nucleic Acids Research* 2021;49:W29–W35.
34. Besemer J. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. *Nucleic Acids Research* 2001;29:2607–2618.
35. Bagheri H, Dyer R, Severin A, Rajan H. Comprehensive analysis of non redundant protein database. *In Review* 2020.
36. Moriya Y, Itoh M, Okuda S, Yoshizawa AC, Kanehisa M, *et al*. KAAS: an automatic genome annotation and pathway reconstruction server. *Nucleic Acids Res* 2007;35:W182-5.
37. Huerta-Cepas J, Szklarczyk D, Heller D, Hernández-Plaza A, Forslund SK, *et al*. eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. *Nucleic Acids Res* 2019;47:D309–D314.
38. UniProt Consortium. UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Res* 2019;47:D506–D515.
39. Conesa A, Götz S. Blast2GO: A comprehensive suite for functional analysis in plant genomics. *Int J Plant Genomics* 2008;2008:1–12.
40. Lee I, Ouk Kim Y, Park S-C, Chun J. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Microbiol* 2016;66:1100–1103.
41. Meier-Kolthoff JP, Göker M, Spröer C, Klenk H-P. When should a DDH experiment be mandatory in microbial taxonomy? *Arch Microbiol* 2013;195:413–418.
42. Yoon S-H, Ha S-M, Lim J, Kwon S, Chun J, *et al*. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 2017;110:1281–1286.
43. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
44. Meier-Kolthoff JP, Klenk H-P, Göker M. Taxonomic use of DNA G+C content and DNA-DNA hybridization in the genomic age. *Int J Syst Evol Microbiol* 2014;64:352–356.
45. Emms DM, Kelly S. OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. *Genome Biol* 2015;16:157.
46. Cantalapiedra CP, Hernández-Plaza A, Letunic I, Bork P, Huerta-Cepas J, *et al*. eggNOG-mapper v2: functional annotation, orthology assignments, and domain prediction at the metagenomic scale. *Mol Biol Evol* 2021;38:5825–5829.
47. Goel AK, Rajagopal L, Nagesh N, Sonti RV. Genetic locus encoding functions involved in biosynthesis and outer membrane localization of xanthomonadin in *Xanthomonas oryzae* pv. *oryzae*. *J Bacteriol* 2002;184:3539–3548.
48. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* 2009;106:19126–19131.
49. Chun J, Rainey FA. Integrating genomics into the taxonomy and systematics of the Bacteria and Archaea. *Int J Syst Evol Microbiol* 2014;64:316–324.
50. Moore WEC, Stackebrandt E, Kandler O, Colwell RR, Krichevsky MI, *et al*. Report of the *ad hoc* Committee on Reconciliation of Approaches to Bacterial Systematics. *Int J Syst Evol Microbiol* 1987;37:463–464.
51. Li J, Gu X, Gui Y. Prokaryotic diversity and composition of sediments from Prydz Bay, the Antarctic Peninsula Region, and the Ross Sea, Southern Ocean. *Front Microbiol* 2020;11:783.
52. Ward BB. Chapter 5 - Nitrification in marine systems. In: Capone DG, Bronk DA, Mulholland MR and Carpenter EJ (eds). *Nitrogen in the Marine Environment*, 2nd edn. San Diego: Academic Press; 2008. pp. 199–261.
53. Shaw DR, Ali M, Katuri KP, Gralnick JA, Reimann J, *et al*. Extracellular electron transfer-dependent anaerobic oxidation of ammonium by anammox bacteria. *Nat Commun* 2020;11:2058.
54. Park D, Kim H, Yoon S. Nitrous oxide reduction by an obligate aerobic bacterium, *Gemmatimonas aurantiaca* strain T-27. *Appl Environ Microbiol* 2017;83:e00502-17.
55. Garber AI, Zehnpfennig JR, Sheik CS, Henson MW, Ramírez GA, *et al*. Metagenomics of antarctic marine sediment reveals potential for diverse chemolithoautotrophy. *mSphere* 2021;6:e0077021.
56. Smith JA, Graham AGC, Post AL, Hillenbrand C-D, Bart PJ, *et al*. The marine geological imprint of Antarctic ice shelves. *Nat Commun* 2019;10:5635.
57. Burdon RH. The heat shock proteins. *Endeavour* 1988;12:133–138.
58. Sakthivel K, Watanabe T, Nakamoto H. A small heat-shock protein confers stress tolerance and stabilizes thylakoid membrane proteins in cyanobacteria under oxidative stress. *Arch Microbiol* 2009;191:319–328.
59. Chen Z, Yu H, Li L, Hu S, Dong X, *et al*. The genome and transcriptome of a newly described psychrophilic archaeon, *Methanolobus psychrophilus* R15, reveal its cold adaptive characteristics. *Environ Microbiol Rep* 2012;4:633–641.
60. Li J, Qi L, Guo Y, Yue L, Li Y, *et al*. Global mapping transcriptional start sites revealed both transcriptional and post-transcriptional regulation of cold adaptation in the methanogenic archaeon *Methanolobus psychrophilus*. *Sci Rep* 2015;5:9209.
61. Pérard J, Ollagnier de Choudens S. Iron-sulfur clusters biogenesis by the SUF machinery: close to the molecular mechanism understanding. *J Biol Inorg Chem* 2018;23:581–596.
62. Williams TJ, Allen MA, Ivanova N, Huntemann M, Haque S, *et al*. Genome analysis of a verrucomicrobial endosymbiont with a tiny genome discovAnalysis of a verrucomicrobial endosymbiont with a tiny genome discovered in an Antarctic lake. *Front Microbiol* 2021;12:674758.
63. Anand K, Tripathi A, Shukla K, Malhotra N, Jamithireddy AK, *et al*. *Mycobacterium tuberculosis* SufR responds to nitric oxide via its 4Fe-4S cluster and regulates Fe-S cluster biogenesis for persistence in mice. *Redox Biol* 2021;46:102062.

64. Alex A, Antunes A. Genus-wide comparison of *Pseudovibrio* bacterial genomes reveal diverse adaptations to different marine invertebrate hosts. *PLoS One* 2018;13:e0194368.
65. Qin Q-L, Li Y, Sun L-L, Wang Z-B, Wang S, *et al*. Trophic specialization results in genomic reduction in free-living marine *Idiomarina* bacteria. *mBio* 2019;10:e02545-18.
66. Leifson E. *Atlas of Bacterial Flagellation*. New York: Academic Press; 1960.
67. Hoffmann T, Frankenberg N, Marino M, Jahn D. Ammonification in *Bacillus subtilis* utilizing dissimilatory nitrite reductase is dependent on resDE. *J Bacteriol* 1998;180:186–189.
68. Xu P, Li W-J, Tang S-K, Zhang Y-Q, Chen G-Z, *et al*. *Naxibacter alkalitolerans* gen. nov., sp. nov., a novel member of the family "Oxalobacteraceae" isolated from China. *Int J Syst Evol Microbiol* 2005;55:1149–1153.
69. Fautz E, Reichenbach H. A simple test for flexirubin-type pigments. *FEMS Microbiol Lett* 1980;8:87–91.
70. Sasser M. Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids.Technical Note 101. *Microbial ID, inc*. Newark, Del, 2001.
71. Collins MD, Pirouz T, Goodfellow M, Minnikin DE. Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* 1977;100:221–230.
72. Wink J, Schumann P, Atasayar E, Klenk H-P, Zaburannyi N, *et al*. "*Streptomyces caelicus*", an antibiotic-producing species of the genus *Streptomyces*, and *Streptomyces canchipurensis* Li *et al.* 2015 are later heterotypic synonyms of *Streptomyces muensis* Ningthoujam *et al.* 2014. *Int J Syst Evol Microbiol* 2017;67:548–556.
73. Minnikin DE, Collins MD, Goodfellow M. Fatty acid and polar lipid composition in the classification of cellulomonas. *J Appl Bacteriol* 1979;47:87–95.
74. Collins MD, Jones D. Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2, 4-diaminobutyric acid. *J Appl Bacteriol* 1980;48:459–470.
75. Lin S-Y, Hameed A, Shahina M, Liu Y-C, Hsu Y-H, *et al*. Description of *Luteimonas pelagia* sp. nov., isolated from marine sediment, and emended descriptions of *Luteimonas aquatica*, *Luteimonas composti, Luteimonas mephitis, Lysobacter enzymogenes* and *Lysobacter panaciterrae*. *Int J Syst Evol Microbiol* 2016;66:645–651.

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