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SHORT COMMUNICATION

Shimia fortis sp. nov., Isolated from Marine Sediment

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Abstract

A Gram-stain-negative, obligately aerobic, beige and ovoid bacterium, designated strain SDUM112013^T, was isolated from intertidal sediments of Xiaoshi Island, Weihai, China. Growth occurred at 20–37 °C (optimal 28–35 °C), at pH 6.0–9.0 (optimal pH 7.0), in the presence of 1–7% (w/v) NaCl (optimal 3–4%). Based on 16S rRNA gene sequence comparisons, strain SDUM112013^T showed highest similarity to *Shimia sediminis* KCTC 62578^T (98.12%), followed by *Shimia aestuarii* JCM 14332^T (97.91%). Phylogenetic analysis confirmed its affiliation within the genus *Shimia*. The digital DNA-DNA hybridization (dDDH) values between strain SDUM112013^T and *S. aestuarii* JCM 14332^T (20.5%), *S. marina* JCM 13038^T (19.70%) were significantly below the 70% species threshold. Consistently, average nucleotide identity (ANI) values between strain SDUM112013^T and *S. aestuarii* JCM 14332^T (75.82%), *S. marina* JCM 13038^T (73.10%) also fell below the novel species boundary (95%). The genome of strain SDUM112013^T (3,823,137 bp; G+C content 59.8%) contained a complete ectoine biosynthesis (M00033) pathway, and represents the first characterized member of the genus *Shimia* possessing this genetic trait, potentially reflecting unique adaptive evolution. Chemotaxonomic analysis identified ubiquinone-10 as the sole respiratory quinone. Major fatty acids (>5.0%) included $C_{16:0}$, $C_{17:0}$, $C_{18:1}$ ω7c 11-methyl and summed feature 8 ($C_{18:1}$ ω7c and/or $C_{18:1}$ ω6c). Polar lipids were consisted of phosphatidylglycerol, phosphatidyldimethylethanolamine diphosphatidylglycerol and phos-phatidylethanolamine. Based on the evidence presented in this study, strain SDUM112013^T represents a novel species of the genus *Shimia*, for which the name *Shimia fortis* sp. nov. is proposed. The type strain is SDUM112013^T (=KCTC 8307^T=MCCC 1H01481^T).

Abbreviations

KCTC	Korean Collection for Type Cultures
MCCC	Marine Culture Collection of China
MEGA	Molecular Evolutionary Genetics Analysis
HPLC	High-performance liquid chromatography
TLC	Thin-layer chromatography
dDDH	Digital DNA-DNA hybridization
GGDC	Genome-to-genome distance calculator
ANI	Average nucleotide identity
AAI	Average amino acid identity
MA	Marine agar 2216
MB	Marine broth 2216

Introduction

The genus *Shimia*, first described by Choi and Cho [1], belongs to the family *Roseobacteraceae* in the class *Alphaproteobacteria*. At the time of writing, the genus *Shimia* contained 13 validly published species (https://lpsn.dsmz.de/genus/shimia), five of which were reclassified: *Pseudopelagicola gijangensis* as *Shimia gijangensis* comb. nov. [2]; *Thalassobius abyssi*, *Thalassobius aestuarii* and *Thalassobius aquaeponti* as *Shimia abyssi* comb. nov., *Shimia aestuarii* comb. nov. and *Shimia aquaeponti* comb. nov. [3]; *Pelagicola litoralis* as *Shimia litoralis* comb. nov. [4]. *Shimia* species inhabit diverse marine environments, including coastal fish farms [1], seawater [2, 5, 6], sea sediments

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[7–9], even reef-building coral [10] and marine animals [11, 12]. Members of the genus *Shimia* are defined as Gram-stain-negative, rod-shaped, comprised of ubiquinone-10 (Q-10) as the predominant quinone, with high proportion of $C_{18:1}$ ω7c or summed feature 8 ($C_{18:1}$ ω7c and/or $C_{18:1}$ ω6c), and DNA G + C content ranging from 54.9 to 61.2% [13]. Notably, the recently described *Shimia ponticola* uniquely harbors bacteriochlorophyll-a (BChla)-based photosynthetic capacity [14], a trait previously unreported in this genus. In this paper, we characterize a novel strain, designated SDUM112013^T, isolated from intertidal sediments of Xiaoshi Island, Weihai, China. Based on polyphasic taxonomic analysis, we propose that strain SDUM112013^T represents a novel species of the genus *Shimia.*

Materials and Methods

Bacterial Isolation and Cultivation

Strain SDUM112013^T was isolated from intertidal sediments of Xiaoshi Island, Weihai, China (37° 33′ 57.60″ N, 122° 01′ 2.50″ E) in November 2019. For bacterial isolation, an enrichment culture technique was employed by incubating sediment samples (20 g) in separate 500-mL sealed glass bottles containing low-nutrient medium (consisting of 0.1% NH_4Cl, 0.2% CH_3COONa, 0.02% $MgSO_4 \cdot 7H_2O$, 0.02% yeast extract, 0.02% peptone, 0.1% EDTA-Na_2, and 0.11% sodium pyruvate in seawater) at 33 °C for 30 days [15]. Enrichment cultures were 10-fold serially diluted to 10^{-2} with sterile seawater and 100 µL aliquot of each dilution was spread onto the surface of marine agar 2216 (MA; Becton Dickinson). After 14-day incubation at 33 °C, a pure culture designed strain SDUM112013^T was obtained by purification process. The strain SDUM112013^T was stored at −80 °C in sterile 1% (w/v) saline supplemented with 15% (v/v) glycerol. The type strains *Shimia aestuarii* JCM 14332^T (obtained from Japan Collection of Microorganisms, Japan) and *Shimia marina* JCM 13038^T (obtained from Japan Collection of Microorganisms, Japan) were used as reference strains.

Phenotypic, Physiological, and Biochemical Characteristics

The morphological and physiological features of strain SDUM112013^T were examined after 5-day incubation on MA plates at 33 °C; unless otherwise indicated, a 5-day cultivation period at 33 °C was routinely used for experiments with the strain SDUM112013^T. Cell morphology and size were observed by light microscopy (E600, Nikon) and scanning electron microscopy (model Nova NanoSEM450, FEI). For scanning electron microscopy preparation, exponential-phase cells were washed with phosphate buffered saline (PBS, 0.1 M), resuspended to a standardized concentration, and fixed with 2.5% glutaraldehyde (1:1 v/v) at 4 °C for over 4 h. Fixed cells were deposited on glass slides, gently heat-dried over an alcohol lamp flame, and dehydrated through a graded ethanol series (50%, 70%, 80%, 90% and 100%; 8–10 min per step) before oven drying. The Gram reaction was determined using the method described by Tindall [16]. Gliding motility was examined on MA (0.3% agar) according to the method described by Bowman [17]. Growth range and optimum of temperature were investigated at various temperatures (0, 4, 10, 15, 20, 25, 28, 30, 33, 37, 40, and 45 °C). Salt tolerance was tested in the medium (0.1% yeast extract, 0.5% peptone and 1.8% agar), with artificial seawater (0.32% $MgSO_4$, 0.23% $MgCl_2$, 0.12% $CaCl_2$, 0.07% KCl and 0.02% $NaHCO_3$, all w/v) and containing different concentrations of NaCl (0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12%, w/v). The pH range (from pH 5.5 to 9.5, in 0.5 intervals) and optimal pH for growth were evaluated in marine broth 2216 (MB; Becton Dickinson) buffered with MES (pH 5.5 and 6.0), PIPES (pH 6.5 and 7.0), HEPES (pH 7.5 and 8.0), Tricine (pH 8.5), and CAPSO (pH 9.0 and 9.5). Growth under anaerobic (10% H_2, 10% CO_2, and 80% N_2) conditions was determined after incubation for 14 days on MA in an anaerobic jar with 0.1% (w/v) KNO_3. Catalase activity was detected by adding 3% (v/v) H_2O_2 to the plate with fresh colonies. Antibiotic susceptibility of strain SDUM112013^T was investigated by disk diffusion method as described previously on MA plates after 7-day incubation at 33 °C [18]. Hydrolysis Tweens (20, 40, 60, and 80), agar, DNA, alginate, starch, casein and Carboxymethyl-cellulose (CM-cellulose) followed Smibert and Krieg [19]. Additional physiological and biochemical characteristics and enzyme-producing activity were determined using API 20E and API ZYM kits (bioMérieux) according to the manufacturer's instructions. Acid production from carbohydrates was checked by using the API 50CH fermentation kits (bioMérieux). Oxidation of substrate was examined in Biolog GEN III MicroPlates. All API tests were performed according to the manufacturer's instructions (except for salinity, which was adjusted to 3%).

16S rRNA Gene Sequence Analysis

The 16S rRNA gene was amplified by PCR using the Taq DNA polymerase with the universal primers 27F and 1492R [20]. An initial 1,328-bp 16S rRNA gene sequence of strain

SDUM112013^T (Genbank accession number: PP555935) was obtained by RuiBiotech (Qingdao, China). Subsequently, a 1,464-bp 16S rRNA gene sequence (Genbank accession number: PP892595) extracted from the genome, demonstrated 100.0% identity to the PCR fragment over their comparable regions in NCBI BLAST analysis and was employed for phylogenetic analysis and comparative analyses. Both 16S rRNA gene sequences were deposited in GenBank and compared in the National Centre for Biotechnology Information (NCBI) GenBank database (https://www.ncbi.nlm.nih.gov) and the EzBioCloud databases (https://www.ezbiocloud.net/) for preliminary confirmation of taxonomic status. The 16S rRNA gene sequence similarities were calculated using the EzBioCloud Database [21]. Multiple sequences were aligned using the CLUSTALW program [22]. Phylogenetic analysis utilized MEGA 11 [23–25] with three approaches: neighbor-joining (NJ) under the Kimura 2-parameter model, maximum-likelihood (ML) with the general time reversible + gamma + invariant (GTR + G + I) model and maximum-parsimony (MP) using subtree-pruning-regrafting (SPR) heuristic search. Bootstrap support was evaluated with 1,000 replicates for all tree topologies.

Chemotaxonomic Characterization

In order to determine the chemotaxonomic properties, cells of strain SDUM112013^T were cultivated in MB medium at 28°C for 3 days, harvested by centrifugation, and freeze-dried. Fatty acids were extracted from 30 mg of freeze-dried biomass, methyl-esterified, and analyzed by the Agilent 6890N gas chromatograph with the Microbial Identification System (MIDI database: TSBA40) [26]. Respiratory quinones were obtained using a silica-gel TLC plate and analyzed by HPLC [27]. Polar lipids were extracted using a chloroform-methanol-water system (2.5:5:2, v/v/v) according to the procedures described by Liang and Xu with two-dimensional TLC silica-gel aluminium-backed thin-layer plates (8 × 8 cm) [28]. The TLC plates were sprayed with phosphomolybdic acid with 5% in ethanol to reveal total lipids, α-naphthol/H_2SO_4 to reveal glycolipids, phosphate to reveal phospholipids and ninhydrin to reveal aminolipids [29].

Genomic Analysis

Genomic DNA of strain SDUM112013^T was extracted by using a genomic DNA extraction kit (Takara) according to the manufacturer's recommendations. The draft genome sequence of strain SDUM112013^T was sequenced on the Illumina HiSeq PE150 platform at Beijing Novogene Bioinformatics Technology (Beijing, China). The genome sequence was deposited in the GenBank database, following quality assessment using BUSCO with the bacteria_odb10 database [30]. The phylogenomic tree was inferred using the Genome Taxonomy Database Toolkit (GTDB-Tk) pipeline [31]. Within this pipeline, the 120 bacterial core marker genes were aligned with HMMER and resulting concatenated alignment was used for phylogenetic inference with IQ-TREE under the GTR + F + I + G4 model, with node support assessed through 1,000 bootstrap replicates. The resultant tree was visualized by the ChiPlot [32]. Genome annotation was conducted through the NCBI Prokaryotic Genome Annotation Pipeline, supplemented by KEGG metabolic pathway analysis [33]. Protein-coding regions were identified via the Rapid Annotations using Subsystem Technology (RAST) server [34]. Functional categorization utilized eggNOG-mapper (http://www.eggnog-mapper.embl.de/) for Clusters of orthologous groups (COGs) [35], and dbCAN2 for carbohydrate-active enzyme annotation [36]. Reference genomes were obtained from NCBI genome database. The ANI was calculated with the online ANI calculator (https://www.ezbiocloud.net/tools/ani) [37], while AAI was determined using the online AAI calculator (http://enve-omics.ce.gatech.edu/aai/) [38]. Additionally, the dDDH value was obtained using the Genome-to-Genome Distance Calculator version 3.0 (GGDC) [39].

Results and Discussion

Morphological, Physiological, and Biochemical Characteristics

Scanning electron microscopy showed that the cells of strain SDUM112013^T were ovoid and lacked flagella (Fig. S1), consistent with the morphology of its close relative *S. aestuarii* JCM 14332^T. Strain SDUM112013^T exhibited susceptibility to chloramphenicol, penicillin, ampicillin, carbenicillin, cefotaxime and ceftriaxone, but resistance to vancomycin and lincomycin. Although the strain SDUM112013^T hydrolyzed starch, alginate and casein, it did not produce catalase and showed no hydrolytic activity against CM-cellulose or Tweens (20, 40, 60, and 80). According to the results of commercial kits, all the three strains produced alkaline phosphatase and acid phosphatase, but did not produce α-mannosidase, α-fucosidase. Given these results, the ability of strain SDUM112013^T to

Table 1 Physiological and biochemical characteristics of strain SDUM112013^T and closely related *Shimia* species. Strains: 1, SDUM112013^T; 2, *S. marina* JCM 13038^T 3, *S. aestuarii* JCM 14332^T. Reference data for other strains came from published studies, only the data from strain SDUM112013^T came from this research. -, negative; +, positive; W, weakly positive

Characteristic	1	2	3
Habitat (isolation)	Intertidal zone (sediment)	Coastal fish farm, (biofilm)[a]	Tidal flat, (sediment)[b]
Cell morphology	Ovoid	Rod[a]	Ovoid[b]
Cell size	0.6–1.0 × 1.2–1.8	0.3–0.6 × 0.8–3.6[a]	0.4–0.6 × 1.1–5.3[b]
Pigmentation	Cream	Colourless or beige[a]	Cream[b]
Motility	-	+[a]	-[b]
Temperature range (optimum) for growth (°C)	20–37 (28–35)	15–35 (30–35)[a]	15–35 (35)[b]
pH range (optimum) for growth	6.0–9.0 (7.0)	6.0–10.0[a]	6.0–11.0 (7.0)[b]
NaCl range (optimum) for growth (%, w/v)	1–7 (3–4)	3–7[a]	1–7 (2)[b]
API 20NE test results			
Indole production	-	-[a]	-[b]
Fermentation of D-glucose	-	-[a]	-[b]
Urease	-	-[a]	-[b]
API ZYM test results			
Alkaline phosphatase	+	+[a]	+[b]
Esterase (C4), esterase lipase (C8)	+	W[a]	W[b]
Leucine arylamidase	+	+[a]	+[b]
Valine arylamidase	W	-[a]	-[b]
Acid phosphatase	+	W[a]	+[b]
Naphthol-AS-Biphosphohydrolase	+	+[c]	+[b]
α-mannosidase	-	-[a]	-[b]
α-fucosidase	-	-[a]	-[b]
Utilization of:			
D-ribose	+	-[a]	W[b]
D-xylose	W	-[a]	+[b]
D-fructose	+	-[a]	-[b]

Data from: [a] [1]; [b] [7]; [c] [14]

utilize substrate was stronger than *S. aestuarii* JCM 14332^T and *S. marina* JCM 13038^T. Detailed phenotypical features were listed in species description as well as in Table 1. Unexpectedly, during routine cultivation experiments, strain SDUM112013^T demonstrated remarkable resilience by maintaining viability after 180 days of laboratory incubation at 33°C, and successfully reactivating with robust metabolic activity.

Table 2 Cellular fatty acid contents (%) of strains

Fatty acid	1	2	3
Saturated			
$C_{16:0}$	5.3	4.2	6.8
$C_{17:0}$	8.7	ND	1.4
$C_{18:0}$	2.1	4.1	3.0
Branched chain			
Anteiso-$C_{15:0}$	0.6	ND	ND
Iso-$C_{16:0}$	ND	ND	ND
Unsaturated acids			
$C_{17:1}\omega8c$	0.6	ND	ND
$C_{17:1}\omega6c$	0.7	ND	ND
$C_{18:1}\omega7c$	ND	64.1	68.0
$C_{18:1}\omega7c$ 11-methyl	11.3	10.6	12.0
$C_{20:1}\omega7c$	0.9	ND	ND
Hydroxy acids			
$C_{10:0}$ 3-OH	2.1	2.1	1.3
$C_{11:0}$ 3-OH	0.5	ND	ND
$C_{12:0}$ 3-OH	ND	1.0	ND
$C_{15:0}$ 2-OH	ND	ND	ND
$C_{16:0}$ 2-OH	2.4	3.9	3.8
$C_{17:0}$ 2-OH	1.2	ND	ND
Iso-$C_{17:0}$ 3-OH	ND	ND	ND
$C_{18:0}$ 2-OH	0.6	ND	ND
Summed features [a]			
1	0.5	ND	ND
8	56.8	ND	ND

Strains:1, SDUM112013^T; 2, *S. marina* JCM 13038^T; 3, *S. aestuarii* JCM 14332^T. ND, not detected or less than 0.5%. Reference data for other strains came from published studies, only the data from strain SDUM112013^T came from this research

The data about *S. marina* JCM 13,038^T 3, *S. aestuarii* JCM 14,332^T were taken from Choi and Cho [1], Hana Yi and Jongsik Chun [7], respectively

[a] Summed feature refer to groups of two or three fatty acids that could not be separated by the Microbial Identification System. Summed feature 1 comprises iso-$C_{15:1}$ H and/or $C_{13:0}$ 3-OH; summed feature 8 comprises $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$

Chemotaxonomic Characteristics

The major fatty acids (>5%) of strain SDUM112013^T included $C_{16:0}$ (5.3%), $C_{17:0}$ (8.7%), $C_{18:1}$ $\omega7c$ 11-methyl (11.3%), summed feature 8 ($C_{18:1}$ $\omega7c$ and/or $C_{18:1}$ $\omega6c$) (56.8%) which were similar in composition to those of other species in genus *Shimia* (Table 2). Notably, $C_{18:1}$ $\omega7c$ or summed feature 8 ($C_{18:1}$ $\omega7c$ and/or $C_{18:1}$ $\omega6c$) consistently dominates over 50% of the total fatty acids in each of the three species. The sole respiratory quinone was identified as ubiquinone-10, consistent with reference strains. The dominant polar lipids of strain SDUM112013^T were comprised of phosphatidylglycerol

(PG), phosphatidyldimethylethanolamine (PME), diphosphatidylglycerol (DPG) and phosphatidylethanolamine (PE) (Fig. S2).

Phylogenetic Analysis of 16S rRNA Gene Sequences

The 16S rRNA gene of strain SDUM112013^T was successfully amplified and sequenced, yielding a 1,328 bp sequence deposited in Genbank (accession number: PP555935). Through whole genome sequencing, an additional 16S rRNA (1,464 bp; Genbank accession number: PP892595) exhibiting 100.0% identity with the PCR amplification (1,328 bp) across comparable regions was extracted and employed for comparative analysis. BLAST analysis revealed that strain SDUM112013^T exhibited the highest sequence similarity with *Shimia sediminis* KCTC 62578^T (98.12%), followed by *Shimia aestuarii* JCM 14332^T (97.91%). In addition, strain SDUM112013^T showed the 16S rRNA gene sequence similarity of 96.82% with *S. marina* JCM 13038^T, which was the type species of the genus *Shimia* (Fig. 1). In phylogenetic trees constructed using neighbor-joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) methods, strain SDUM112013^T was placed within the clade of genus *Shimia* (Fig. S3). Both the sequence similarities and phylogenetic relationships establish the strain SDUM112013^T's affiliation with the genus *Shimia* and support its designation as a novel species.

Genomic Analysis

The draft genome of strain SDUM112013^T (Genbank accession number: JBBPEU000000000) comprises 3,823,137 bp

	16S rRNA (%)	ANI (%)	AAI (%)	dDDH (%)
***Shimia fortis* SDUM112013^T**	**100**	**100**	**100**	**100**
Shimia abyssi	97.76	73.14	73.53	19.3
Shimia aestuarii	97.91	75.82	75.81	20.5
Shimia biformata	96.75	73.78	73.65	19.6
Shimia gijangensis	96.25	76.98	76.98	18.5
Shimia haliotis	96.46	73.1	73.11	19.5
Shimia isoporae	96.73	73.25	72.98	19
Shimia litoralis	95.45	76.31	76.19	17.8
Shimia marina	96.82	73.1	73.06	19.7
Shimia ponticola	96.97	67.12	67.15	19.7
Shimia sagamensis	96.31	72.56	72.58	19.1
Shimia sediminis	98.12	77.1	77.03	19.9
Shimia thalassica	96.9	82.75	82.65	19.7
Thiosulfatihalobacter marinus	97.62	74.54	74.35	19.8
Cognatishimia activa	96.82	71.44	71.13	19
Cognatishimia maritima	96.68	71.91	71.35	19
Sulfitobacter brevis	94.55	68.12	68.03	18
Sulfitobacter delicatus	94.72	68.56	68.5	18.9
Sulfitobacter donghicola	94.79	67.83	67.75	19
Sulfitobacter dubius	95.06	68.5	68.17	18.5
Sulfitobacter faviae	95.44	68.69	68.7	18.9
Sulfitobacter geojensis	96.1	68.2	68.08	18.4
Sulfitobacter guttiformis	94.39	67.13	66.98	18.1
Sulfitobacter indolifex	94.71	68.25	68.24	19.1
Sulfitobacter marinus	94.45	68.33	68.27	18.8
Sulfitobacter mediterraneus	95.06	68.6	68.44	18.2
Sulfitobacter pacificus	95.76	68.06	67.86	18.5
Sulfitobacter undariae	95.31	67.22	66.99	18.8
Tateyamaria armeniaca	96.28	68.65	68.41	19.2
Tateyamaria omphalii	95.82	68.52	68.4	19
Tateyamaria pelophila	94.86	68.09	67.8	18.4
Paracoccus denitrificans	91.95	62.18	61.96	18.6

Fig. 1 Heatmap comparing genomic relatedness metrics between *Shimia fortis* SDUM112013^T (designated as 100% for all metrics) and 31 closely related type strains from the phylogenomic tree. Metrics shown are 16S rRNA gene sequence similarity, ANI, AAI and dDDH values. All compared strains are type strains. *Paracoccus denitrificans* at the bottom of the heatmap serves as the phylogenetic root and genomic reference point, exhibiting consistently low values across all metrics

Table 3 Genome statistics of strain SDUM112013^T and the related *Shimia* species

	1	2	3
Genome size (bp)	3,823,137	4,059,992	4,226,671
N_{50} value (bp)	276,378	323,790	312,137
Contigs (no.)	53	32	27
Genome coverage	150.0×	270.0×	252.0×
G+C content (%)	59.8	57.5	60.5
Annotation by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP)			
Genes (no.)	3,686	3,952	4,136
Protein-coding genes (no.)	3,617	3,866	4,035
tRNA (no.)	47	47	50
rRNA (no.)	3	5	9
ncRNA (no.)	3	3	3
DDBJ/ENA/GenBanK accession number	JBB-PEU000000000	FOMU00000000	FOTQ00000000

Strains:1, SDUM112013^T; 2, *S. marina* JCM 13038^T; 3, *S. aestuarii* JCM 14332^T. All data were obtained from this study

(53 contigs; N_{50} = 276,378 bp; longest contig 683,808 bp) with 150× coverage depth. Evaluation of genome quality indicated 97.6% completeness and a 0.8% contamination level, fulfilling the required standards of >95% completeness and <5% contamination [40]. The genome annotation of *S. aestuarii* JCM 14332^T, *S. marina* JCM 13038^T and their comparative analysis with strain SDUM112013^T are summarized in Table 3. Only one 16S rRNA gene sequence (1,464 bp; Genbank accession number: PP892595) was extracted from the genome. The DNA G + C content of strain SDUM112013^T was 59.8%, which was similar to *S. aestuarii* JCM 14332^T (60.5%) and *S. marina* JCM 13038^T (57.5%). Comparative genomic analysis (Fig. 1) revealed that strain SDUM112013^T exhibited 16S rRNA gene sequence similarities, average nucleotide identity (ANI), average amino acid identity (AAI), and digital DNA-DNA hybridization (dDDH) values below established species delineation thresholds when compared to all currently recognized type strains within the genus *Shimia* and other closely related taxa. Specifically, the highest 16S rRNA gene sequence similarity was observed with *Shimia sediminis*^{T} (98.12%), the highest ANI value was observed with *Shimia thalassica*^{T} (82.75%), and the highest dDDH value was observed with *Shimia aestuarii*^{T} (20.50%), all significantly below the respective thresholds of ≥ 98.65% for 16S rRNA [41], ≥ 95% for ANI [42], and ≥ 70% for dDDH [43]. Furthermore, the complete all-vs-all numerical matrices for these four metrics are provided in Supplementary Dataset S1 (an Excel file containing separate sheets for 16S rRNA gene sequence similarities, ANI, AAI and dDDH values). These results support that the new isolate represents a novel species of the genus *Shimia*.

Detailed information on gene number and metabolic function annotated by KEGG is available (Fig. S4). The IQ tree conducted using the genomes is shown in Fig. 2, which further corroborates the conclusion drawn from the analyses based on 16S rRNA gene sequences.

Metabolic Pathways

The metabolic pathways were analyzed for all *Shimia* species except *Shimia aquaeponti* whose genome is not available in NCBI (Fig. 3). All thirteen strains share identical ATP synthesis, sulfur metabolism and partial fatty acid metabolism. Furthermore, all *Shimia* members possess similar ubiquinone biosynthesis (M00117), potentially explaining their shared respiratory quinone, ubiquinone-10. Comparative analysis revealed an unusual feature within energy metabolism: only *S. ponticola* possesses the complete anoxygenic photosystem II (M00597), explaining its unique, genetically encoded photosynthetic capacity utilizing BChla. In particular, within the genus *Shimia*, strain SDUM112013^T is unique in possessing a complete ectoine biosynthesis (M00033) pathway, a metabolic capability absent in all other characterized *Shimia* species. Ectoine, a well-characterized compatible solute, acts as a potent osmoprotectant by stabilizing cellular macromolecules and maintaining membrane integrity under extreme conditions such as high salinity, desiccation, or temperature fluctuations [44]. In strain SDUM112013^T, ectoine biosynthesis proceeds via a five-step enzymatic cascade mediated by aspartate kinase (EC 2.7.2.4), aspartate-semialdehyde dehydrogenase (EC 1.2.1.11), diaminobutyrate-2-oxoglutarate transaminase (EC 2.6.1.76), L−2,4-diaminobutyric acid acetyltransferase (EC 2.3.1.178) and L-ectoine synthase (EC:4.2.1.108) (Fig. 4). This pathway not only enables osmotic balance under laboratory cultivation but also enhances survival in complex natural habitats.

Significantly, this metabolic trait may directly correlate with the observed experimental phenomenon wherein strain SDUM112013^T retained viability after 6 months of extended laboratory cultivation and could be successfully reactivated. Ectoine's role in preserving enzymatic activity and preventing protein aggregation during prolonged cultivation provides a plausible mechanistic basis for this revival capacity.

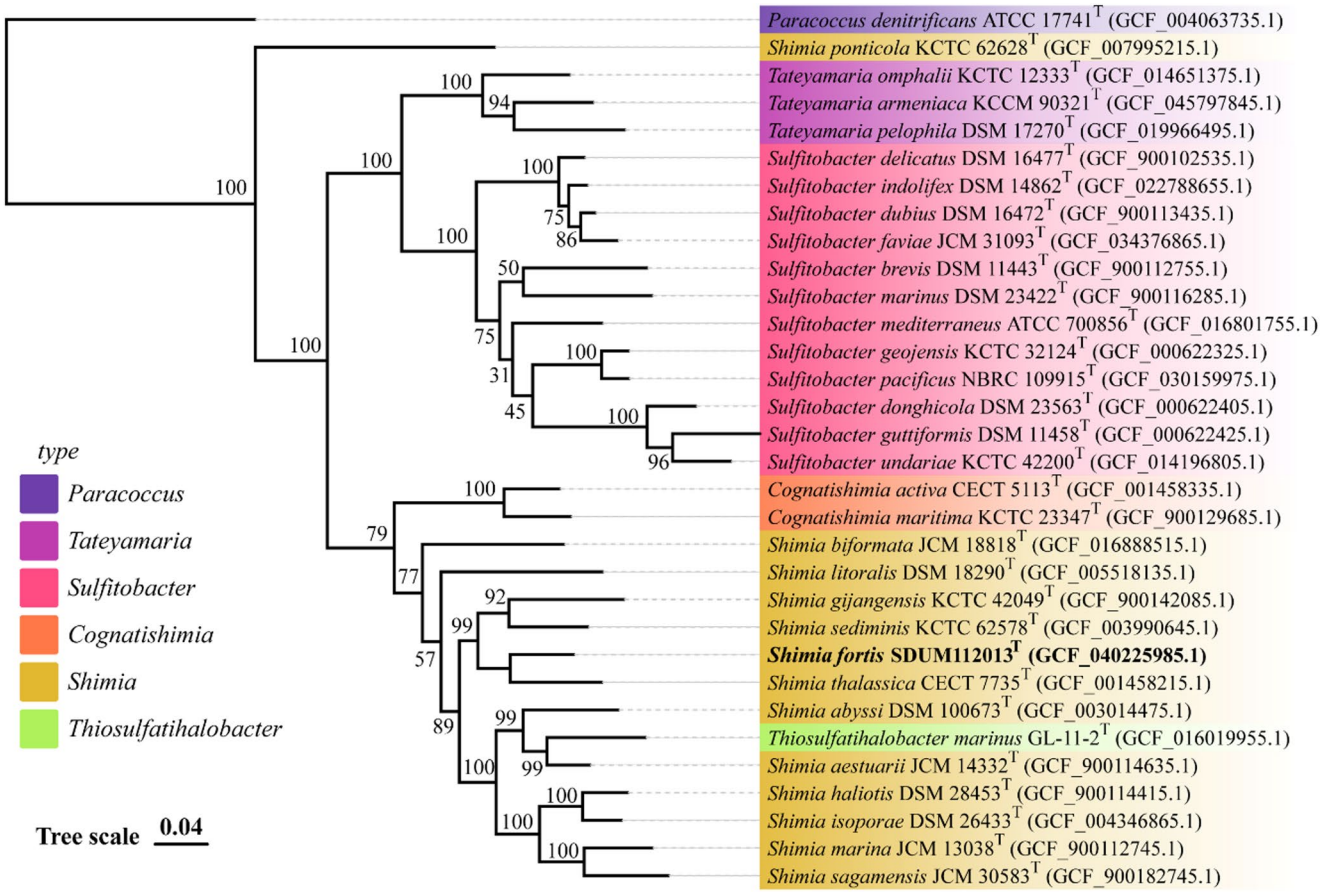

Fig. 2 Phylogenomic tree of strain SDUM112013^T and closely related taxa. Constructed with IQ-TREE, visualized with ChiPlot [32]. All bootstrap values are indicated on the nodes, based on 1000 replicates. *Paracoccus denitrificans* ATCC 17741^T (GCF_000203895.1) was employed as the outgroup. Bar indicates substitutions per nucleotide site

Furthermore, the absence of ectoine biosynthesis genes in closely related *Shimia* species suggests a unique evolutionary adaptation in strain SDUM112013^T, positioning it as a valuable model for studying stress tolerance mechanisms in marine bacteria. To deepen this insight, future investigations targeting ectoine-hydroxyectoine transcriptional regulation under extended cultivation conditions could further elucidate this connection. Building upon these metabolic advantages, the distinctive feature of *Shimia fortis* SDUM112013^T may enable biotechnological applications, particularly as a candidate for osmoprotectant production in biostabilization formulation.

Turning to functional evidence, the cluster of orthologous groups of proteins predictions based on the COG database annotation is shown in Fig. S5. The proteins related to amino acid transport and cell cycle control, cell division, chromosome partitioning, are much richer than other proteins which indicated the abundant metabolic activities. Simultaneously, analysis using the CAZy database showed significant enrichment of glycosyltransferases (GT dominance in CAZy, Fig. S6), suggesting an enhanced capacity for complex glycoconjugate synthesis. High gene counts in secondary metabolite biosynthesis (COG category Q: 99 genes, Fig. S5) and cofactor/vitamin metabolism (KEGG: 54 genes) indicate feasibility for novel bioactive compound discovery. Coupled with conserved sulfur metabolism across *Shimia* (Fig. 3), these traits merit targeted investigation into antimicrobial or enzymatic applications inherent to marine-derived bacteria.

Taxonomic Conclusion

Based on comprehensive physiological, chemotaxonomic, phylogenetic, genomic, and metabolic analysis, strain SDUM112013^T is considered to represent a novel species of the genus *Shimia*, for which the name *Shimia fortis* sp. nov. is proposed.

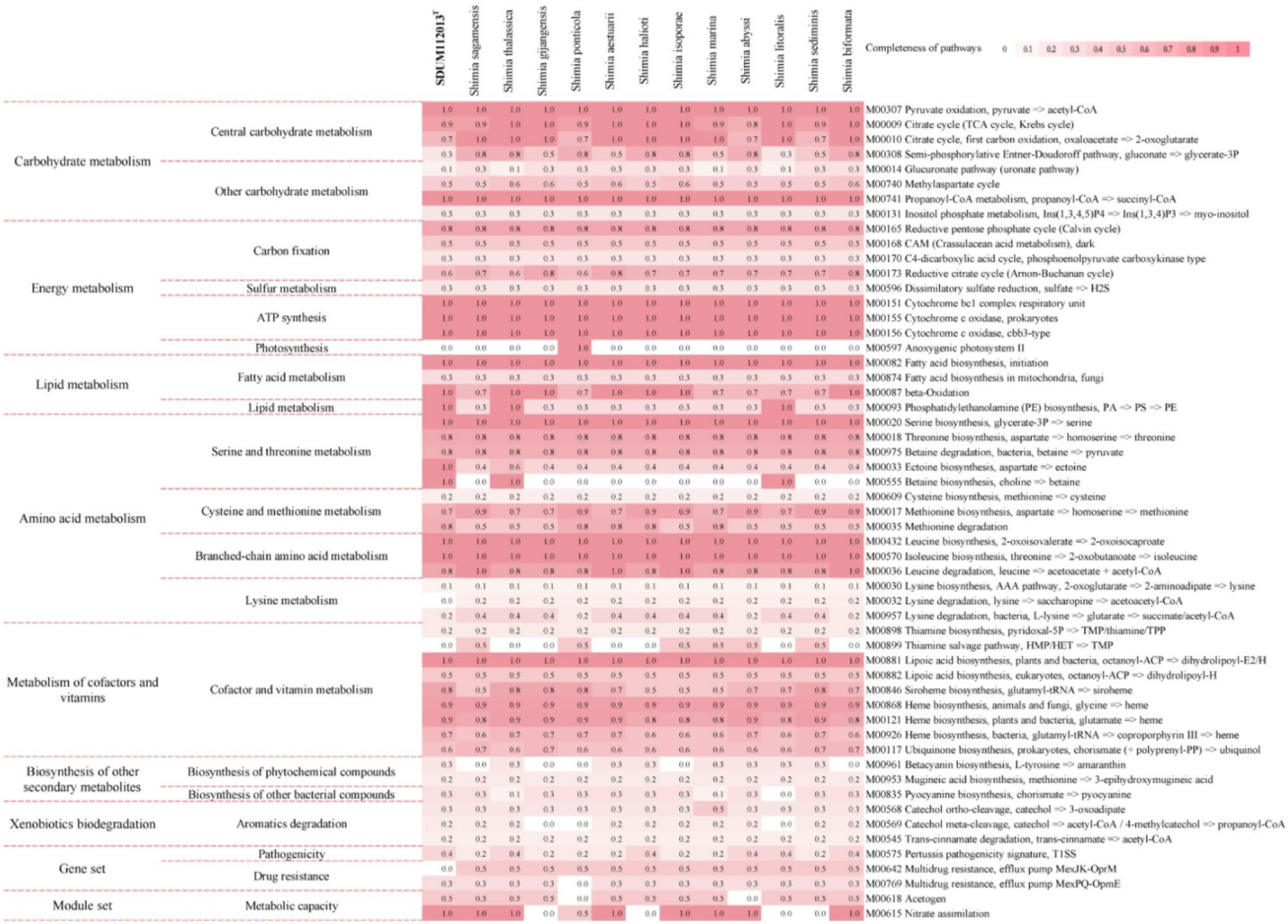

Fig. 3 A heatmap of metabolic pathway completeness in the genomes of strain SDUM112013^T and type strains of all other published *Shimia* species (except *Shimia aquaeponti*) based on KEGG annotations

Description of *Shimia fortis* sp. nov

Shimia fortis (for' tis. L. fem. adj. *fortis*, strong, referring to the fact that the strain is still viable after six months of laboratory cultivation).

Colonies are beige, circular, convex and smooth at 33 °C on MA. Cells are Gram-stain-negative, non-motile and aerobic with ovoid shape. Growth occurs at pH 6.0–9.0 (optimum 7.0), temperatures of 20–37 °C (optimum, 28–35 °C) and with 1–7% (w/v) NaCl (optimum, 3–4%). Strains produce alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase; show weak activity for valine arylamidase. Starch, alginate and casein are hydrolyzed, but CM-cellulose and Tweens (20, 40, 60, and 80) are not. Catalase is not produced. Acid is produced from D-arabinose (weakly), L-arabinose (weakly), D-ribose, D-xylose (weakly), L-xylose (weakly), fructose, L-sorbose, esculin, D-tagatose, D-turanose (weakly), D-lyxose (weakly), potassium 5-ketogluconate, potassium 2-ketogluconate (weakly). Oxidizes the following sole carbon sources: glucuronamide, D-glucuronic acid (weakly), L-malic acid, bromo-succinic acid, acetic acid, acetoacetic acid, α-hydroxy-butyric acid. Major cellular fatty acids (≥5%) include $C_{16:0}$, $C_{17:0}$, $C_{18:1}$ ω7c 11-methyl and summed feature 8 ($C_{18:1}$ ω7c and/or $C_{18:1}$ ω6c). The sole respiratory quinone is Q-10. The polar lipids consist of PG, PME, DPG and PE.

The type strain is SDUM112013^T (=KCTC 8307^T=MCCC 1H01481^T), which was isolated from intertidal sediments of Xiaoshi Island, Weihai, China. The GenBank accession number for the more complete 16S rRNA gene sequence extracted from the genome of strain SDUM112013^T is PP892595 and the draft genome data has been deposited in Genbank under the accession number JBBPEU000000000. The DNA G+C content of type strain is 59.8% with a genome size of 3.8 Mbp.

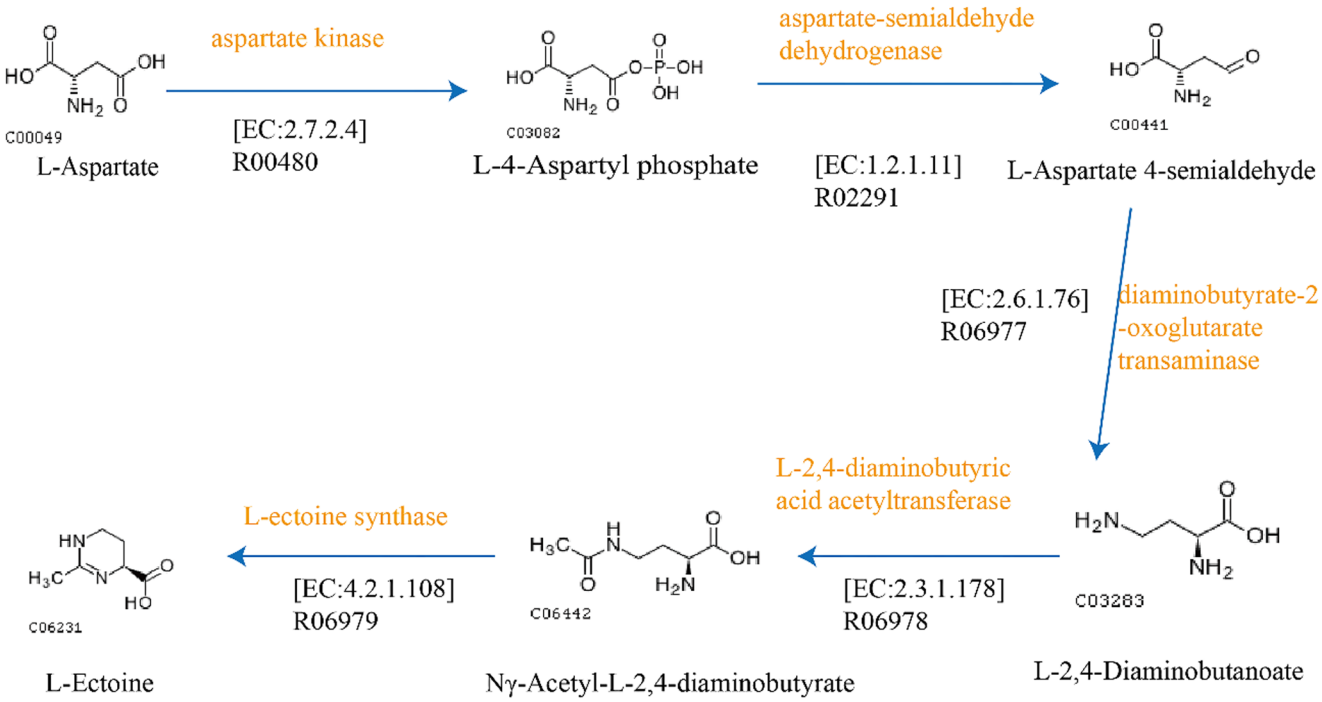

Fig. 4 Complete Ectoine biosynthesis (M00033) pathway in strain SDUM112013^T based on KEGG analysis. Other reactants and products have been omitted

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00284-025-04686-0.

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The nomenclature of the novel species was formulated in accordance with the International Code of Nomenclature of Prokaryotes (ICNP). The authors acknowledge the use of the guidelines provided in Chap. 3, Sect. 9, and Appendix 9 of the ICNP for the correct formation and etymology of the species name.

Author Contributions Xiao-Yu Dong and Jia-Qi Li isolated the strain SDUM112013^T, Xiao-Yu Dong, Pei-Ran Lin, Xin-Yun Tan and Jia-Qi Li performed material preparation, experimental operation, data collection and analysis. Pei-Ran Lin helped process cell samples and photograph cell morphology. Xiao-Yu Dong finished the experiment and manuscript. Zong-Jun Du and Meng-Qi Ye offered experiment guidance and critical revision of manuscripts. All authors contributed to the article and approved the submitted version.

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Data Availability All data generated or analysed during this study are included in this published article or through supplementary information files.

Declarations

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

Repositories The GenBank accession number for the 16S rRNA gene sequence of strain SDUM112013^T is PP892595. The draft genome sequence accession number is JBBPEU000000000.

References

1. Choi DH, Cho BC (2006) *Shimia marina* gen. nov., sp. nov., a novel bacterium of the *Roseobacter* clade isolated from biofilm in a coastal fish farm. Int J Syst Evol Microbiol 56:1869–1873. https://doi.org/10.1099/ijs.0.64235-0
2. Arahal DR, La Mura A, Lucena T, Rodrigo-Torres L, Aznar R, Pujalte MJ (2019) *Shimia thalassica*sp. nov., and reclassification of *Pseudopelagicola gijangensis*as *Shimia gijangensis*comb. nov., and *Thalassobius activus*as *Cognatishimia activa*comb. nov. Int J Syst Evol Microbiol 69:3405–3413. https://doi.org/10.1099/ijsem.0.003629
3. Wirth JS, Whitman WB (2018) Phylogenomic analyses of a clade within the Roseobacter group suggest taxonomic reassignments of species of the genera *Aestuariivita, Citreicella, Loktanella, Nautella, Pelagibaca, Ruegeria, Thalassobius, Thiobacimonas* and *Tropicibacter*, and the proposal. Int J Syst Evol Microbiol 68:2393–2411. https://doi.org/10.1099/ijsem.0.002833
4. Zhang DF, He W, Shao Z et al (2023) Phylotaxonomic assessment based on four core gene sets and proposal of a genus definition among the families *Paracoccaceae* and *Roseobacteraceae*. Int J Syst Evol Microbiol 73:1–13. https://doi.org/10.1099/ijsem.0.006156

5. Hameed A, Shahina M, Lin SY et al (2013) *Shimia biformata* sp. nov., isolated from surface seawater, and emended description of the genus *Shimia* Choi and Cho 2006. Int J Syst Evol Microbiol 63:4533–4539. https://doi.org/10.1099/ijs.0.053553-0
6. Park S, Jung YT, Won SM, Park JM, Yoon JH (2014) *Thalassobius aquaeponti* sp. nov., an alphaproteobacterium isolated from seawater. Antonie Van Leeuwenhoek 106:535–542. https://doi.org/10.1007/s10482-014-0225-1
7. Yi H, Chun J (2006) *Thalassobius aestuarii* sp. nov., isolated from tidal flat sediment. J Microbiol 44:171–176
8. Nogi Y, Mori K, Uchida H, Hatada Y (2015) *Shimia sagamensis* sp. nov., a marine bacterium isolated from cold-seep sediment. Int J Syst Evol Microbiol 65:2786–2790. https://doi.org/10.1099/ijs.0.000333
9. Nogi Y, Mori K, Makita H, Hatada Y (2016) *Thalassobius abyssi* sp. nov., a marine bacterium isolated from cold-seep sediment. Int J Syst Evol Microbiol 66:574–579. https://doi.org/10.1099/ijsem.0.000758
10. Chen MH, Sheu SY, Chen CA, Wang JT, Chen WM (2011) *Shimia isoporae* sp. nov., isolated from the reefbuilding coral Isopora palifera. Int J Syst Evol Microbiol 61:823–827. https://doi.org/10.1099/ijs.0.022848-0
11. Hyun DW, Kim MS, Shin NR et al (2013) *Shimia haliotis* sp. nov., a bacterium isolated from the gut of an abalone, Haliotis discus hannai. Int J Syst Evol Microbiol 63:4248–4253. https://doi.org/10.1099/ijs.0.053140-0
12. Kim YO, Park S, Nam BH, Kim DG, Yoon JH (2014) *Pseudopelagicola gijangensis* gen. nov., sp. nov., isolated from the sea squirt Halocynthia roretzi. Int J Syst Evol Microbiol 64:3447–3452. https://doi.org/10.1099/ijs.0.062067-0
13. Zhu S, Chen C, Cheng Y et al (2021) *Shimia sediminis* sp. nov., a bacterium isolated from marine sediment in the East China sea. Int J Syst Evol Microbiol 71(4). https://doi.org/10.1099/ijsem.0.004782
14. Chen Q, He B, Lin TH, Jiao N, Zheng Q (2023) *Shimia ponticola* sp. nov., an aerobic anoxygenic photoheterotrophic bacterium, isolated from surface seawater in the South China Sea. Int J Syst Evol Microbiol 73:1–7. https://doi.org/10.1099/ijsem.0.005859
15. Mu DS, Liang QY, Wang XM et al (2018) Metatranscriptomic and comparative genomic insights into resuscitation mechanisms during enrichment culturing. Microbiome 6:1–15. https://doi.org/10.1186/s40168-018-0613-2
16. Tindall BJ, Sikorski J, Smibert RA, Krieg NR (2007) Phenotypic characterization and the principles of comparative systematics. In: Reddy CA, Beveridge TJ et al (eds) Methods gen mol microbiol, 3rd edn. Wiley, New York. https://doi.org/10.1128/9781555817497.ch15
17. Bowman JP (2000) Description of *Cellulophaga algicola* sp. nov., isolated from the surfaces of Antarctic algae, and reclassification of *Cytophaga uliginosa* (ZoBell and Upham 1944) Reichen bach 1989 as *Cellulophaga uliginosa* comb. nov. Int J Syst Evol Microbiol 50:1861–1868. https://doi.org/10.1099/00207713-50-5-1861
18. James H, Jorgensen JDT (2015) Susceptibility test methods: Dilution and disk diffusion methods. In: James H, Jorgensen JDT et al (eds) Man clin microbiol, 11th edn. Wiley, New York. https://doi.org/10.1128/9781555817381.ch71
19. Smibert RM, Krieg NR (1994) Phenotypic characterization. In: Ger-hardt P, Murray R, Wood W, Krieg N (eds) Methods Gen Mol Bacteriol American Society for Microbiology, Washington, DC; 607–654
20. Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Good-fellow M (eds) Nucleic acid tech Bact syst. Wiley, Chichester, pp 115–175
21. Yoon SH, Ha SM, Kwon S et al (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67:1613–1617. https://doi.org/10.1099/ijsem.0.001755
22. Li KB (2003) ClustalW-MPI: ClustalW analysis using distributed and parallel computing. Bioinformatics 19:1585–1586. https://doi.org/10.1093/bioinformatics/btg192
23. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454
24. Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst Biol 20:406–416. https://doi.org/10.1093/sysbio/20.4.406
25. Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376. https://doi.org/10.1007/BF01734359
26. Sasser M (1990) MIDI technical note 101. Identification of bacteria by gas chromatography of cellular fatty acids. MIDI, Newark. DE, pp. 1–7
27. Hiraishi A, Ueda Y, Ishihara J, Mori T (1996) Comparative lipoquinone analysis of influent sewage and activated sludge by high-performance liquid chromatography and photodiode array detection. J Gen Appl Microbiol 42:457–459. https://doi.org/10.2323/jgam.42.457
28. Liang QY, Xu ZX, Zhang J, Chen GJ, Du ZJ (2018) *Salegentibacter sediminis* sp. nov., a marine bacterium of the family *Flavobacteriaceae* isolated from coastal sediment. Int J Syst Evol Microbiol 68:2375–2380. https://doi.org/10.1099/ijsem.0.002849
29. Zhang XQ, Sun C, Wang CS et al (2015) *Sinimarinibacterium flocculans* gen. nov., sp. nov., a gammaproteobacterium from offshore surface seawater. Int J Syst Evol Microbiol 65:3541–3546. https://doi.org/10.1099/ijsem.0.000452
30. Manni M, Berkeley MR, Seppey M et al (2021) BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. Mol Biol Evol 38:4647–4654. https://doi.org/10.1093/molbev/msab199
31. Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH (2019) Gtdb-tk: a toolkit to classify genomes with the genome taxonomy database. Bioinformatics 36:1925–1927. https://doi.org/10.1093/bioinformatics/btz848
32. Xie J, Chen Y, Cai G, Cai R, Hu Z, Wang H (2023) Tree visualization by one table (tvBOT): a web application for visualizing, modifying and annotating phylogenetic trees. Nucleic Acids Res 51(W1):W587–W592. https://doi.org/10.1093/nar/gkad359
33. Kanehisa M, Sato Y (2020) KEGG mapper for inferring cellular functions from protein sequences. Protein Sci 29:28–35. https://doi.org/10.1002/pro.3711
34. Aziz RK, Bartels D, Best A et al (2008) The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:1–15. https://doi.org/10.1186/1471-2164-9-75
35. Huerta-Cepas J, Forslund K, Coelho LP et al (2017) Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. Mol Biol Evol 34:2115–2122. https://doi.org/10.1093/molbev/msx148
36. Zhang H, Yohe T, Huang L et al (2018) dbCAN2: a meta server for automated carbohydrate-active enzyme annotation. Nucleic Acids Res 46:W95–W101. https://doi.org/10.1093/nar/gky418
37. Yoon SH, Ha S, min, Lim J, Kwon S, Chun J (2017) A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110:1281–1286. https://doi.org/10.1007/s10482-017-0844-4
38. Rodriguez-R LM, Konstantinidis KT (2014) Bypassing cultivation to identify bacterial species. Microbe Magazine 9:111–118
39. Meier-Kolthoff JP, Carbasse JS, Peinado-Olarte RL, Göker M (2022) TYGS and LPSN: a database tandem for fast and reliable

genome-based classification and nomenclature of prokaryotes. Nucleic Acids Res 50:D801–D807. https://doi.org/10.1093/nar/gkab902

40. Bowers RM, Kyrpides NC, Stepanauskas R et al (2017) Minimum information about a single amplified genome (MISAG) and a metagenome-assembled genome (MIMAG) of bacteria and archaea. Nat Biotechnol 35:725–731. https://doi.org/10.1038/nbt.3893
41. Kim M, Oh H-S, Park S-C, Chun J (2014) Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int J Syst Evol Microbiol 64:346–351. https://doi.org/10.1099/ijs.0.059774-0
42. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:60. https://doi.org/10.1186/1471-2105-14-60
43. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM (2007) DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91. https://doi.org/10.1099/ijs.0.64483-0
44. Pastor JM, Salvador M, Argandona M, Bernal V, Reina-Bueno M, Csonka LN, Iborra JL, Vargas C, Nieto JJ, Cánovas M (2010) Ectoines in cell stress protection: uses and biotechnological production. Biotechnol Adv 28:782–801. https://doi.org/10.1016/j.biotechadv.2010.06.005