INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

RESEARCH ARTICLE

Ishaq *et al.*, *Int. J. Syst. Evol. Microbiol.* 2024;74:006521
DOI 10.1099/ijsem.0.006521

MICROBIOLOGY SOCIETY

Microbulbifer spongiae sp. nov., isolated from marine sponge *Diacarnus erythraeanus*

Nabila Ishaq[1], Mimi Zhang[1], Luyao Gao[1], Micha Ilan[2] and Zhiyong Li[1,3,*]

Abstract

A novel bacterium, designated as MI-G^T, was isolated from marine sponge *Diacarnus erythraeanus*. Cells of strain MI-G^T are Gram-stain-negative, aerobic, and rod or coccoid-ovoid in shape. MI-G^T is able to grow at 10–40 °C (optimum, 28 °C), with 1.0–8.0% (w/v) NaCl (optimum, 4.0%), and at pH 5.5–9.0 (optimum, pH 8.0). The 16S rRNA gene sequence of strain MI-G^T shows 98.35, 97.32 and 97.25% similarity to those of *Microbulbifer variabilis* Ni-2088^T, *Microbulbifer maritimus* TF-17^T and *Microbulbifer echini* AM134^T, respectively. Phylogenetic analysis also exhibits that strain MI-G^T falls within a clade comprising members of the genus *Microbulbifer* (class *Gammaproteobacteria*). The genome size of strain MI-G^T is 4478124 bp with a G+C content of 54.51 mol%. The average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values between strain MI-G^T and other type strains are 71.61–76.44% (ANIb), 83.27–84.36% (ANIm) and 13.4–18.7% (dDDH), respectively. These values are significantly lower than the recommended threshold values for bacterial species delineation. Percentage of conserved proteins and average amino acid identity values among the genomes of strain MI-G^T and other closely related species are 52.04–59.13% and 67.47–77.21%, respectively. The major cellular fatty acids of MI-G^T are composed of summed feature 8 ($C_{18:1}$ $\omega7c$ or $C_{18:1}$ $\omega6c$), iso-$C_{11:0}$ 3-OH, iso-$C_{15:0}$, $C_{16:0}$, and summed feature 9 ($C_{17:1}$ iso $\omega9c$ or $C_{16:0}$ 10-methyl). The polar lipids of MI-G^T mainly consist of phosphatidylethanolamine, phosphatidylglycerol, aminolipid, and two glycolipids. The major respiratory quinone is Q-8. Based on differential phenotypic and phylogenetic data, strain MI-G^T is considered to represent a novel species of genus *Microbulbifer*, for which the name *Microbulbifer spongiae* sp. nov. is proposed. The type strain is MI-G^T (=MCCC 1K07826^T=KCTC 8081^T).

Microbulbifer is the only genus of family *Microbulbiferaceae* [1], and currently contains 35 species with validly published names as listed in the List of Prokaryotic names with Standing in Nomenclature. The genus *Microbulbifer* was first described by González *et al.* [2] to accommodate Gram-stain-negative, strictly aerobic, marine *Gammaproteobacteria* that can decompose variety of hydrocarbons [2]. Bacterial strains that belong to this genus were mainly isolated from salt-rich marine environments (sediment, estuarine, mangrove, salt marshes and coastal sand), marine organisms (algae, sea urchin and sponge), and rhizosphere of a halophyte [3–10]. *Pseudomonas elongata* was reclassified to genus *Microbulbifer* as *Microbulbifer elongatus* in 2003, because phylogenetic analysis based on 16S rRNA genes revealed that *P. elongata* is more closely related to the genus *Microbulbifer* than to *Pseudomonas* species [11]. Cells of most species of genus *Microbulbifer* possess a rod–coccus cell cycle during their growth phase [8], contain ubiquinone 8 (Q-8) as the major respiratory quinone, and have DNA G+C contents from 48.5 to 60.2% [7].

Marine sponges are considered a valuable source of novel microbes [12, 13]. It was reported that bacteria associated with marine sponges constitute an interesting source of novel-bioactive compounds with biotechnological potentials *i.e.*, enzymes, surfactants and antimicrobial substances [14, 15]. To date, various novel natural products, antibacterial substances, enzymes and plant hormones have been isolated from sponge-derived bacteria [16–18]. However, studies on marine sponge-derived novel microbes

Author affiliations: [1]Marine Biotechnology Laboratory, State Key Laboratory of Microbial Metabolism and School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai, PR China; [2]School of Zoology, Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv, Israel; [3]Yazhou Bay Institute of Deepsea Science and Technology, Shanghai Jiao Tong University, Shanghai, PR China.
***Correspondence:** Zhiyong Li, zyli@sjtu.edu.cn
Keywords: *Microbulbifer spongiae* sp. nov.; marine sponge; polyphasic taxonomy.
Abbreviations: AAI, average amino acid identity; ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; MA, marine agar; MB, marine broth; POCP, percentage of conserved proteins.
The GenBank accession numbers for the 16S rRNA gene sequence and the whole-genome sequence of the strain MI-G^T are ON849098 and CP098023. The raw data of the Whole Genome Shotgun project and the draft genome sequences of strain DSM18651^T and JCM30400^T have been deposited at GenBank/EMBL/DDBJ under accession numbers JBGMEK000000000 and JBGMEL000000000, respectively. Six supplementary figures are available with the online version of this article.
Six supplementary figures are available with the online version of this article.

are still in infancy [19–22]. During the screening of micro-organisms from the sponges [19], a novel bacterial strain MI-G^T was isolated and purified by repeated re-streaking on marine agar (MA) media and maintained at −80 °C in marine broth (MB; Difco) with 30% (v/v) glycerol. The aim of the current study was to determine the exact taxonomic position of strain MI-G^T.

Cells of strain MI-G^T were grown in MB at 28 °C and cell morphology was observed after 24 h, 48 h, 7 days and 14 days of incubation by using a light microscope (Eclipse 50i, Nikon) and a scanning electron microscope (Sirion 200, FEI). Colony appearance was observed on MA medium after 4 days of incubation at 28 °C. Gram-staining was performed using a Gram-stain kit (Hangzhou Tianhe Microorganism Reagent Co), according to the manufacturer's instruction. Motility was observed on motility agar under high-moisture conditions in a hanging-drop method under ×100 objective lens with oil immersion [20, 21]. Growth under anaerobic conditions was also verified after 7 days of incubation on MA medium at 28 °C using AnaeroPack-Anaero (MGC), according to the manufacturer's instructions [12]. The optimal growth conditions for strain MI-G^T were determined at different temperatures, NaCl and pH. The temperature range for growth was assessed by incubating isolate on MA at various temperatures 4, 10, 15, 20, 25, 28, 30, 35, 37 and 40 °C. The concentration range of sodium chloride suitable for growth was investigated by using MB medium (NaCl omitted) supplemented with appropriate concentrations of NaCl ranging from 1 to 16% (w/v; at increments of 1.0%). The pH range for growth was observed at pH 5.5–10.0 (intervals of pH 0.5 unit) in MB; and pH of the medium was adjusted using biological buffers MES (pH 5.5–6.0), PIPES (6.5–7.0), HEPES (7.5–8.0), Tricine (8.5) and CAPSO (9.0–10.0). These tests of growth conditions were performed in quadruplicate; OD_{600} measurements were taken after 24 h, 48 h, 72 h, 96 h and 7 days of incubation using a multifunctional enzyme marker (Spark, Tecan) [22].

Genomic DNA of strain MI-G^T was extracted using a TIANamp Bacteria DNA kit (Tiangen) following the manufacturer's instructions. PCR amplification of 16S rRNA gene was performed using universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) [23]. The PCR conditions were as follows: denaturation at 95 °C for 10 min, followed by 35 cycles of denaturation again at 95 °C for 30 s, annealing at 55 °C for 30 s, and final extension at 72 °C for 120 s. At the end of these cycles, the reaction mixture was kept at 72 °C for 10 min and then cooled to 4 °C. The amplified PCR products were recovered by agarose gel electrophoresis and cloned into vector pEASY -T5 Zero vector (TransGen Biotech). The recombinant plasmid was transformed into *Escherichia coli* DH5*a* for blue-white screening, positive clones on the plate were selected for culture, plasmids were extracted and commercially sequenced at Sangon Biotech (Shanghai, PR China) [24]. The resulting 16S rRNA gene sequence (1439 nt) of MI-G^T was compared by EzBioCloud (www.ezbiocloud.net) and BLAST (www.ncbi.nlm.nih.gov/blast/) [25, 26]. To perform phylogenetic analysis, the neighbour-joining, minimum-evolution and maximum-likelihood trees were reconstructed by using MEGA 11 after multiple alignment of sequences by the Clustal W program [27]. Evolutionary distance matrices of phylogenetic trees were calculated according to the algorithm of the Kimura two-parameter model, and bootstrap analysis was performed with 1000 replications [28, 29].

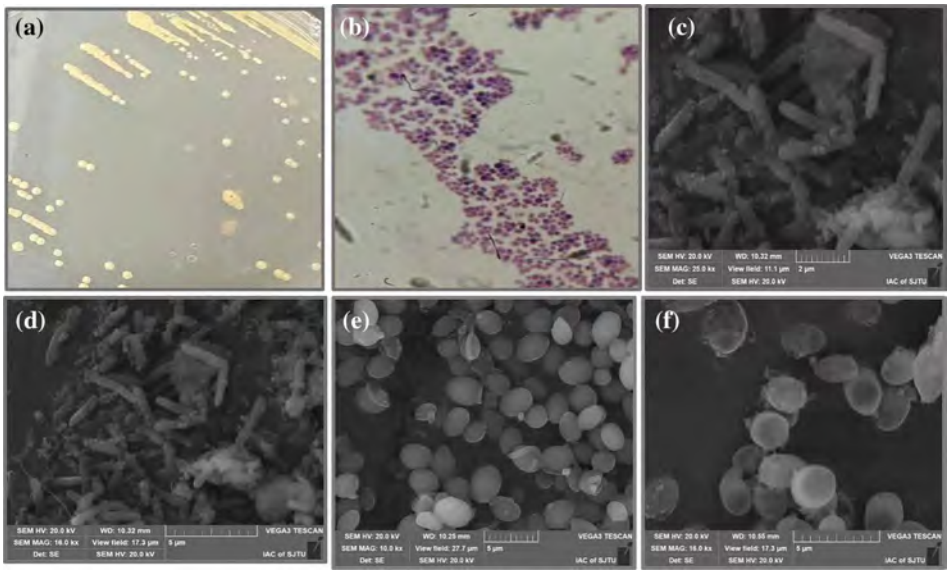

Fig. 1. Morphological observation of strain MI-G^T. (a) Colonies of strain MI-G^T cells on 2216E agar plate. (b) Light microscopic observation of strain MI-G^T. (c) Scanning electron microscope image of cells of strain MI-G^T after 12 h of growth (early growth phase) in liquid media 2216E at 28 °C. (d) After 24 h of growth (logarithmic growth phase); cells maintain rod shape. (e) After 48 h of growth (early stationary phase) cells morphology changes from rod shape to coccoid-ovoid shape. (f) After 14 days of incubation (late-stationary phase) cells maintain resting coccoid-ovoid shape.

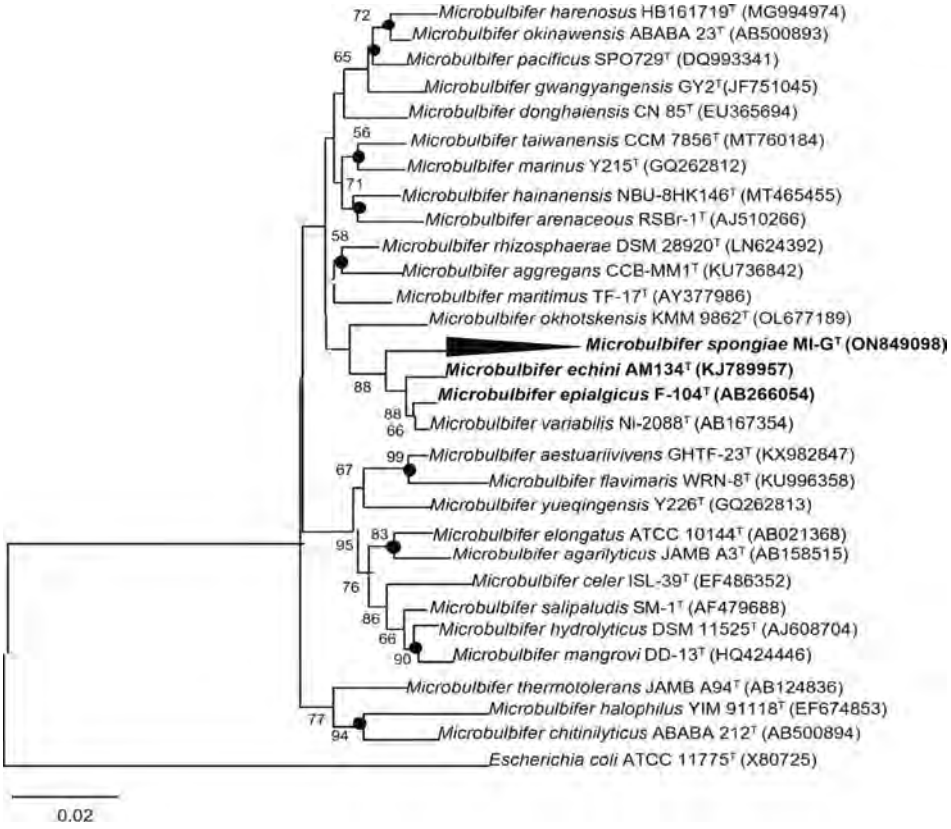

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain MI-G^T and the type strains of species of the genus *Microbulbifer*. Only bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at branching points. GenBank accession numbers are given in parentheses. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and minimum-evolution algorithms. *Escherichia coli* ATCC 11775^T (GenBank accession number X80725) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

The whole genome sequencing of strain MI-G^T was performed on the DNBSEQ (BGI) and Nanopore (ONT) platforms. Reads of each data set were filtered, and only high-quality filtered paired-end reads were assembled using Canu version 1.5 and GATK version 1.6–13. The gene prediction was performed on the genome assembly by Glimmer version 3.02 [30, 31]. Meanwhile, rRNA and tRNA genes were predicted by using RNAmmer version 1.2 and tRNAscan-SE version 1.3.1, respectively [32, 33]. Further, whole genomes of two closely related type strains, namely *Microbulbifer epialgicus* DSM18651^T and *Microbulbifer echini* JCM30400^T, were also sequenced using the DNBSEQ platform at the Beijing Genomics Institute (Shenzhen, PR China). The sequenced reads were assembled using SOAPdenovo version 1.05 software [34]. The phylogenomic analysis was performed by uploading genome sequence data of strain MI-G^T and other closely related type strains of genus *Microbulbifer* that were retrieved from the NCBI genome database to the Type Strain Genome Server (https://tygs.dsmz.de/) [35]. In addition, genome-based phylogeny of these strains was also calculated through multiple sequence alignments of 120-sinlge copy marker proteins generated by GTDB-tk software version 1.3.0 [36], as described by Steiner *et al.* [37]. The neighbour-joining phylogenomic tree was visualized using the MEGA version 11.0.13 [27]. The genome sequence similarity between MI-G^T and other *Microbulbifer* strains was evaluated by calculating average nucleotide identity (ANI) values by using JSpecies [38]. The digital DNA–DNA hybridization (dDDH) analysis was performed using the Genome-to-Genome Distance Calculator online service at https://ggdc.dsmz.de/distcalc2.php [39, 40]. Average amino acid identity (AAI) values was determined using the EzAAI pipeline, which is available at http://leb.snu.ac.kr/ezaai [41]. Percentage of conserved proteins (POCP) was also calculated as a mean similarity of orthologous genes [42–44].

Three phylogenetically closely related *Microbulbifer* strains were used as reference strains for the comparative studies of phenotypic characterization and fatty acid analysis. *M. epialgicus* DSM18651^T and *M. hydrolyticus* DSM 11525^T were purchased from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cultures, and *M. echini* JCM 30400^T was purchased from the Microbe Division/Japan Collection of Microorganisms RIKEN Bio Resource Research Center. All the subsequent biochemical, physiological and chemotaxonomical tests were carried out on strains *M. spongiae* MI-G^T, *M. echini* JCM30400^T, *M. epialgicus* DSM18651^T, and *M. hydrolyticus* DSM 11525^T after these bacteria were cultured in MB medium at 28 °C for 48 h. Catalase activity was evaluated by assessing the production of bubbles after adding a drop of 3% (v/v) H_2O_2 on the bacterial culture on

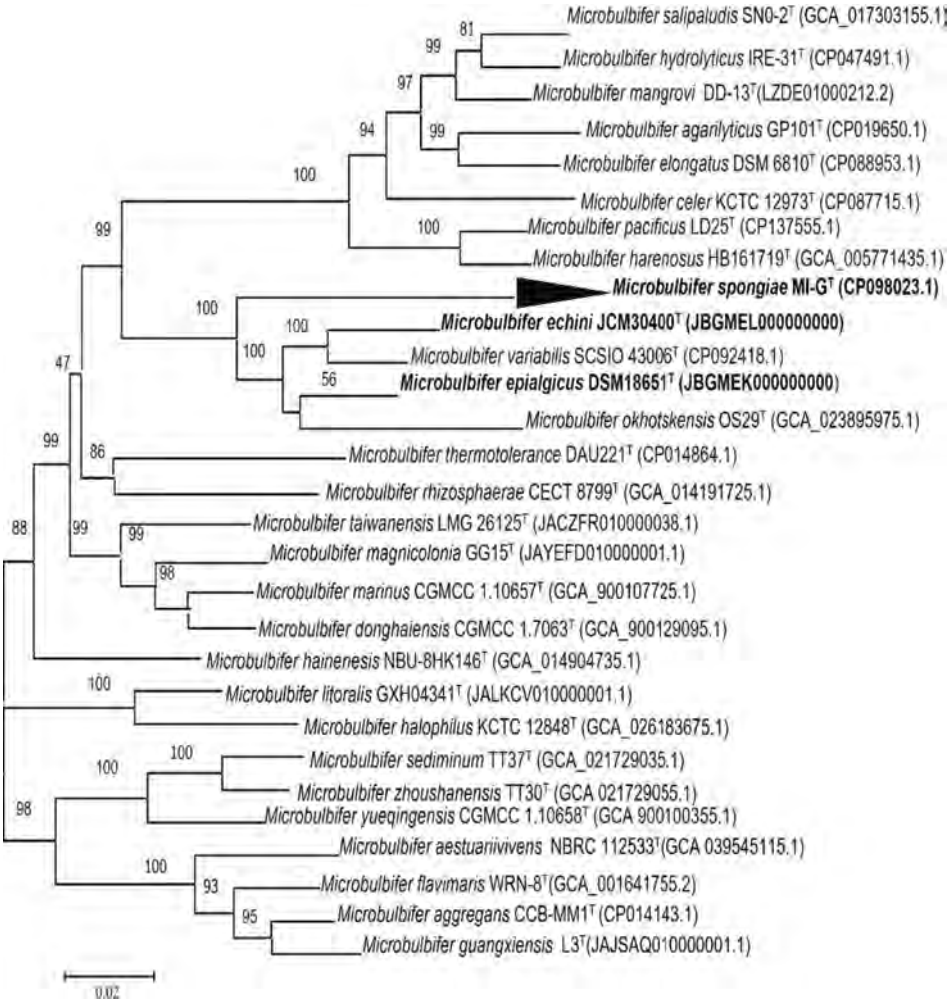

Fig. 3. Genome phylogeny of strain MI-G^T elucidated using the GTDB-tk pipeline version 1.3.0. The pipeline is based on 94759 reference genomes. For bacterial genomes, the taxonomic identification is based on 120 single-copy marker proteins. The pipeline employs MEGA version 11.0.13 to calculate the phylogenetic trees using the neighbour-joining method. Bootstrap values (≥50%) based on 1000 replications are shown next to the branches. Bar, 0.02 substitutions per nucleotide positions.

MA plates at 28 °C for 4 days. Oxidase activity was determined based on the oxidation of (w/v) tetramethyl-p-phenylenediamine [45], when the bacteria were cultured on MA plates at 28 °C for 4 days. API ZYM system (bioMérieux), API 20E, Biolog GEN III MicroPlate (Biolog), and API 20NE (bioMérieux) kits were used according to the manufacturers's instructions to further determine the biochemical characteristics of MI-G^T including enzyme activities, carbon source utilization, and other biochemical characteristics, respectively. All these experiments were carried out in triplicates and repeated twice to ensure reproducibility.

After culturing in MB medium for 4 days at 28 °C, MI-G^T cells were harvested and washed with sterile distilled water before freeze-drying. Respiratory quinones were extracted from freeze-dried cells (100 mg) with chloroform–methanol (2 : 1, v/v) and separated into their different functional classes by thin layer chromatography on silica gel, then further analysed by UPLC-MS system equipped with a diode array detector (SPD-M20A, Shimadzu) by using the previously described methods [46–48]. Polar lipids were extracted according to the method described by Kates [49]and separated on silica-gel 60 aluminium-backed thin-layer plates according to the method of Minnikin *et al.* [50]. These plates were spotted with sample and subjected to two-dimensional development, with chloroform–methanol-water (65 : 25 : 4, by volume) as the first solvent, followed by chloroform–methanol–acetic acid–water (85 : 12 : 15 : 4, by volume) as the second solvent. The TLC plates were sprayed with α-naphthol, ninhydrin, and phosphomolybdic acid to detect polar lipids according to Tindall [51]. To identify cellular fatty acid composition, cells were grown on MA medium at 28 °C for 4 days. Fully grown cells were harvested and fatty acid methyl esters were prepared as described previously [52]. The fatty acids were analysed by gas chromatography (Agilent Technologies 6850) and identified using the TSBA6.0 database of the Microbial Identification System (MIDI) [53, 54].

Table 1. The ANI and dDDH values between strain MI-G^T and related type strains of genus *Microbulbifer*

MI-G^T vs	Accession	Genome size (Mbp)	G+C content (mol%)	POCP (%)	AAI (%)	ANIb (%)	ANIm (%)	GGDC	
								dDDH	Model C.I. (%)
Microbulbifer agarilyticus GP101^T	CP019650	4.3	55	53.68	67.64	71.61	84.36	13.5	10.7–16.8
Microbulbifer aggregans CCB-MM1^T	CP014143	3.9	58.5	54.06	71.04	73.04	83.68	14.4	11.6–17.8
Microbulbifer donghiensis CGMCC 1.7063^T	FQVA01000004.1	4.3	59.7	56.89	73.71	74.54	83.64	15.3	12.4–18.8
Microbulbifer elongatues DSM 6810^T	CP088953	4.1	57.5	52.36	67.72	72.37	83.7	13.6	10.9–17
Microbulbifer flavimaris WRN-8 ZB100000^T	LRFG02000001	3.6	59.5	54.73	70.93	73.25	83.5	14.6	12.2–17.5
Microbulbifer hydrolyticus IRE-31^T	CP047491	4.2	57.6	52.74	68.25	72.37	83.94	13.4	10.9–17
Microbulbifer mangrovi DD-13 1000^T	LZDE01000212.1	4.5	57.2	53	68.15	72.52	83.75	13.5	10.8–16.9
Microbulbifer marinus CGMCC 1.10657^T	FNQO01000001	4	59.5	56.78	73.91	74.84	83.7	16	13–19.4
Microbulbifer pacificus LD25^T	PREV01000001	4	57.	52.04	67.96	72.53	83.91	13.7	10.9–17.1
Microbulbifer taiwanensis LMG 26125^T	JACZFR01000003	4.8	60.2	53.8	72.17	74.75	83.76	15.6	12.6–19
Microbulibfer hainenesis NBU-8HK146^T	GCA_014904735.1	4.7	58.9	52.29	70.55	73.68	83.27	14.6	11.8–18
Microbulibfer halophilus KCTC 12848^T	JAPIVK01000000	4.6	61.7	52.71	70.45	74.21	83.42	14.3	11.4–17.6
Microbulbifer celer KCTC 12973^T	CP087715	4.3	57.2	53.14	67.85	72.39	83.87	13.6	10.8–16.9
Microbulbifer thermotolerans DAU221^T	CP014864	3.9	56.6	55.96	72.76	73.5	83.27	14.3	11.5–17.7
Microbulbifer variabilis SCSIO 43006^T	CP092418	4.8	48.5	59.13	77.21	75.53	83.96	18.7	15.5–22.2
Microbulbifer harenosus HB161719^T	VANI01000010	4.6	58.2	52.26	67.47	72.46	83.59	13.7	10.9–17
Microbulbifer aestuariivivens NBRC112533^T	GCA_039545115.1	3.4	59.5	52.05	70.01	73.55	83.54	14.4	11.6–17.8
Microbulbifer echini JCM30400^T	JBGMEL000000000	4.4	52.23	53.69	71.65	75.91	83.61	18.9	15.8–22.5
Microbulbifer epialgicus DSM18651^T	JBGMEK000000000	5.6	50.07	55.12	73.12	76.44	83.58	18.8	15.7–22.4
Microbulbifer hainenesis NBU-8HK146^T	GCA_014904735.1	4.7	58.9	53.16	68.72	73.22	83.66	14.6	11.8–18
Microbulbifer guangxiensis L3^T	JAJSAQ010000001.1	3.7	60	52.89	69.86	73.31	83.56	14.4	11.5–17.7
Microbulbifer litoralis GXH04341^T	JALKCV010000001.1	5	62	53.76	72.67	73.91	83.53	14.5	11.7–17.9
Microbulbifer magnicolonia GG15^T	JAYEFD010000001.1	4.3	61.5	56.32	68.02	75.07	83.66	15.4	12.5–18.9
Microbulbifer okhotskensis OS29^T	GCA_023895975.1	5	50	54.29	72.01	74.4	83.63	17.1	14.1–20.6
Microbulbifer rhizosphaerae CECT8799^T	GCA_014191725.1	5.3	60	55.01	74.21	73.07	83.59	14.3	11.5–17.7
Microbulbifer salipaludis SN0-2^T	GCA_017303155.1	4	58.5	52.87	72.43	72.38	83.02	13.8	11.0–17.1

Continued

Table 1. Continued

MI-G^T vs	Accession	Genome size (Mbp)	G+C content (mol%)	POCP (%)	AAI (%)	ANIb (%)	ANIm (%)	GGDC	
								dDDH	Model C.I. (%)
Microbulbifer sediminum TT37^T	JAJSAP010000010.1	3.9	61	54.68	68.98	73.07	83.51	14.4	11.6–17.8
Microbulbifer yueqingensis CGMCC1.10658^T	GCA_900100355.1	3.7	62	53.38	71.98	73.2	83.88	14.7	11.9–18.1
Microbulbifer zhoushanensis TT30^T	JAJSAO010000010.1	4.1	61.5	54.9	72.54	73.39	83.42	14.4	11.6–17.8

*Formula 1 based on a generalized linear model (identities/high-scoring segment pair) was used for dDDH. GGDC, Genome-to-Genome Distance Calculator; ANIb, average nucleotide identity based on the blast; ANIm, average nucleotide identity based on MUMmer; dDDH, digital DNA–DNA hybridization; C.I., confidence interval; POCP, percentage of conserved protein; AAI, average amino acid identity.

Table 2. Differential phenotypic characteristics of strain MI-G^T and the type strains of four phylogenetically related species of the genus *Microbulbifer*

Strains: 1, MI-G^T; 2, *M. echini* JCM30400^T; 3, *M. epialgicus DSM* 18651^T; 4, *M. hydrolyticus* DSM11525^T. All the data are from this study, except where indicated. +, Positive; –, negative; w, weakly positive; NA, no data available.

Characteristic	1	2	3	4
Isolation source	Sponge (*Diacarnus erythraeanus*)	Sea urchin	marine algae	Pulp mill effluent
Pigmentation	Milky white (young culture) Yellowish brown (old culture)	Brown	Ivory (young culture) brown (old culture)	Cream
Cell morphology	Rod–coccus	Rod– coccus	Rod–coccus	Rod-shaped
Growth conditions:				
Temperature range (°C)	10–40	10–36	10–36	10–41
Optimum temperature (°C)	28	30	30	37
pH range	5.5–9.0	6.2–9	6.2–9.0	6.5–8.5
Optimum pH	8.0	7.0	7.0	7.5
NaCl range (%)	1–8.0	1–8.0	1–8.0	1–10
Optimum NaCl (%)	8.0	7.0	7.0	7.5
Nitrate reduction	+	+	+	–
Hydrolysis of:				
D-Glucose	–	w	–	–
Gelatinase	w	+	+	+
Assimilation of:				
D-Glucose	–	–	–	+
L-Arabinose	–	–	–	+
D-Mannitol	–	–	–	w
D-Mannose	–	–	–	w
Maltose	–	–	–	+
Adipic acid	–	–	–	w
Malic acid	–	–	–	w
N-Acetyl-glucosamine	–	–	–	+
Enzyme activity:				
Trypsin	+	–	+	w
α-Chymotrypsin	+	–	+	w
α-Mannosidase	–	–	+	–
Carbon source utilization:				
Trehalose	–	+	+	+
D-Terpinediase	–	+	+	+
Lactose	–	+	w	+
β-Formyl-D-glucoside	w	+	w	+
α-D-Glucose	w	+	+	+
D-Fructose	w	+	w	+
D-Glucose-6-phosphate	w	+	+	+
D-Aspartic acid	–	–	–	w
L-Alanine	w	+	+	+

Continued

Table 2. Continued

Characteristic	1	2	3	4
L-Aspartic acid	+	+	w	+
D-Gluconic acid	w	+	+	+
p-Hydroxy-phenylacetic acid	–	–	–	+
L-Lactic acid	–	–	–	+
β-Hydroxy-D,L butyric acid	+	+	–	+
DNA G+C content (mol%)	53.43	56*	56.1†	57.7‡

*Data from Lee *et al.* [7]. †Data from Nishijima *et al.* [8]. ‡Data from Gonzalez *et al.* [2].

Colonies of MI-G^T on MA were smooth, circular, white colour after 48 h of incubation at 28 °C and turned to yellowish-brown after 7–8 days of incubation under the same conditions. Cells of this strain were Gram-stain-negative, aerobic, and non-motile. Scanning electron microscopy showed that cells of MI-G^T exhibit a rod–coccus cycle during the growth phase; cells appeared rod-shaped after 24 h of incubation, and after 48 h of incubation almost all the cells changed to a coccoid-ovoid form, cells maintain the same ovoid shape even after 7 days of incubation (Fig. 1). A similar change in morphology was also reported in other species of genus *Microbulbifer* [8, 55–57]. Strain MI-G^T grew at 10–40 °C with an optimum at 28 °C. This strain showed growth in the presence of 1.0–8.0% (w/v) NaCl (optimum, 4.0% NaCl; Fig. S1, available in the online version of this article) and at pH 5.5–9.0 (optimum, pH 8.0.

EzBioCloud-based 16S rRNA gene sequence comparison results showed that strain MI-G^T probably represented a novel species of genus *Microbulbifer*, exhibiting the highest sequence similarity of 98.35% to *M. variabilis* Ni-2088^T, 97.32% to *M. maritimus* TF-17^T, 97.25% to *M. echini* AM134^T and 96.77% to *M. thermotolerance* JAMB A94^T, respectively. Phylogenetic analysis based on 16S rRNA gene sequences using the neighbour-joining (Fig. 2), minimum-evolution (Fig. S2) and maximum-likelihood algorithms (Fig. S3) revealed that strain MI-G^T formed a coherent cluster with the type strains *M. echini* AM134^T (KJ789957), *M. epialgicus* F-104^T (AB266054) and *M. variabilis* Ni-2088^T (AB167354) of the genus *Microbulbifer*. Furthermore, the phylogenomic tree (Fig. S4) and the neighbour-joining phylogenetic tree based on protein sequences (Fig. 3) also confirmed that strain MI-G^T belongs to the same genus.

The complete genome of strain MI-G^T was composed of 4478124 bp with 54.51 mol% G+C content. The complete genome sequence of this strain has been uploaded to NCBI GenBank under the accession number CP098023. The genome of strain MI-G^T contains 4433 protein-coding genes, 50 tRNAs, 12 sRNA, 74 ncRNA and 4 rRNA genes. The draft genome of *M. epialgicus* DSM18651^T is 5.6 Mb and is composed of a total of 300 contigs, whereas draft genome of *M. echini* JCM30400^T is 4.4 Mb consisting of 65 contigs. The draft genome sequences of strains DSM18651^T and JCM30400^T were deposited to GenBank with the accession numbers JBGMEK000000000 and JBGMEL000000000, respectively. The DNA G+C content of strain MI-G^T and related strains varies from 48.5 to 61.7 mol%. The overall genome relatedness indices among the genomes of strain MI-G^T and other related strains demonstrated that the ANIb and ANIm values are 71.61–76.44% and 83.27–84.36%, respectively (Table 1). These values are significantly lower than the recommended threshold (94–96%) for the delineation of bacterial species [38, 58]. The dDDH relatedness values between strain MI-G^T and other strains ranged from 13.4 to 18.7%, also far below the proposed cut-off borderline of 70% for species delineation, indicating that this is potentially represents a novel species in the genus *Microbulbifer* [59, 60]. The POCP values between the genomes of strain MI-G^T and other members of genus *Microbulbifer* are 52.04–59.13%, and the AAI values are 67.47–77.21%. However, there is no generally recognized genus boundary of AAI, only the proposed genus boundary (70%) for the family *Erythrobacteraceae*. The AAI values between strain MI-G^T and *M. variabilis* SCSIO 43006^T, *M. Thermotolerance* DAU221^T, *M. donghiensis* CGMCC 1.7063^T and *M. aggregans* CCB-MM1^T are greater than 70%, clearly representing that strain MI-G^T is a member of the genus *Microbulbifer* [61].

The oxidase and catalase activity of strain MI-G^T is positive, which is consistent with that of *M. echini* JCM30400^T, *M. epialgicus* DSM18651^T and *M. hydrolyticus* DSM11525^T. Strain MI-G^T is positive for nitrate reduction and enzyme activity of trypsin and *α*-chymotrypsin, and weakly positive for hydrolysis of gelatinase, but negative for assimilation of L-arabinose, D-mannitol, D-mannose, maltose, adipic acid, malic acid and *N* acetyl-glucosamine, and sole carbon source utilization of trehalose, D-terpinediase, lactose, D-aspartic acid, and *p*-hydroxy-phenylacetic acid. A detailed summary of some differential physiological and biochemical characteristics of strain MI-G^T is presented in the Table 2.

The main cellular fatty acids of strain MI-G^T (>5%) were $C_{16:0}$ (14.0%), iso-$C_{15:0}$ (28.9%), iso-$C_{11:0}$ 3-OH (8.6%), summed feature 8 ($C_{18:1}$ *ω7c* or $C1_{8:1}$ *ω6c*, 7.4%), and summed feature 9 ($C_{17:1}$ iso-*ω9c* or $C_{16:0}$ 10-methyl, 11.7%). The overall fatty acid profile of strain MI-G^T is similar to those of other reference strains of the genus *Microbulbifer* (Table 3). However, in contrast to strain

Table 3. Cellular fatty acid compositions and polar lipids (percentages) of MI-G^T and the type strains of three phylogenetically related species of the genus *Microbulbifer*

Strains: 1, MI-G^T; 2, *M. echini* JCM30400^T; 3, *M. epialgicus DSM* 18651^T; 4, *M. hydrolyticus* DSM11525^T. All the data is obtained from this study, except where indicated. TR, Trace amount (<0.5 %); –, not detected; NA, no data available; DPG, diphosphatidylglycerol; GL, unidentified glycolipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, phospholipid.

Components	1	2*	3†	4‡
Fatty acids				
Saturated:				
$C_{9:0}$	0.5	0.6	0.8	0.5
$C_{10:0}$	1.4	3.0	2.7	1.8
$C_{11:0}$	TR	0.5	0.3	–
$C_{12:0}$	0.9	2.2	2.0	0.7
$C_{14:0}$	1.7	8.7	3.3	3.0
$C_{16:0}$	14.0	19.8	18.3	12.4
$C_{17:0}$	0.8	0.8	1.0	1.9
$C_{18:0}$	0.6	0.6	0.8	1.2
$C_{17:0}$-cyclo	TR	1.1	1.9	4.0
$C_{19:0}$ 10-methyl	TR	0.9	0.5	–
Branched:				
iso-$C_{11:0}$	TR	0.7	1.6	4.2
iso-$C_{13:0}$	1.4	–	TR	TR
iso-$C_{15:0}$	28.8	1.2	3.5	18.2
iso-$C_{17:0}$	8.8	TR	0.6	5.2
iso-$C_{15:1}$ F	TR	TR	TR	1.7
Unsaturated:				
$C_{17:1}$ ω8*c*	TR	0.9	1.3	1.3
$C_{18:1}$ ω9*c*	TR	TR	TR	0.6
$C_{19:0}$ cyclo ω8*c*	TR	1.7	4.0	TR
Hydroxylated:				
$C_{10:0}$ 3-OH	1.2	2.2	1.9	2.2
$C_{11:0}$ 3-OH	TR	0.7	0.6	TR
$C_{12:0}$ 3-OH	TR	5.1	3.0	TR
$C_{10:0}$ 2-OH	–	–	0.2	0.7
$C_{16:0}$ 3-OH	0.8	0.8	0.6	–
iso-$C_{11:0}$ 3-OH	8.6	0.6	1.3	5.2
iso-$C_{13:0}$ 3-OH	TR	TR	–	0.5
iso-$C_{17:0}$ 3-OH	0.6	–	–	–
Summed features:§				
3	1.8	11.0	7.3	6.3
8	7.5	33.2	35.4	12.8
9	11.7	TR	4.5	11.0
Major polar lipids	GL1, GL2, PG, PE, AL	NA	PG, DPG, PL, AL[a]	PE, PG[b]

*Data from Lee *et al.* [7]. †Data from Nishijima *et al.* [8]. ‡Data from Gonzalez *et al.* [2]. §Summed features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total. Summed feature 3 comprises $C_{16:1}$ *ω7c* and /or $C_{16:1}$ *ω6c*. Summed feature 8 consists of $C_{18:1}$ *ω7c*. Summed feature 9 comprises $C_{17:1}$ iso *ω9c*.

MI-G^T, *M. echini* JCM30400^T, *M. epialgicus* DSM18651^T and *M. hydrolyticus* DSM11525^T do not produce iso-$C_{17:0}$ 3-OH. The hydroxy fatty acid $C_{10:0}$ 2-OH was detected in *M. epialgicus* DSM18651^T and *M. hydrolyticus* DSM11525^T but was not found in strain MI-G^T. The predominant respiratory quinones in strain MI-G^T is Q-8 (91.8%) and Q-9 (8.1%) in trace amounts (Fig. S6); Q-8 is also the major quinone in almost all members of the genus *Microbulbifer* [11, 62, 63]. The polar lipids of strain MI-G^T (Fig. S5) consisted of phosphatidylglycerol, phosphatidylethanolamine, aminolipid, and two glycolipids. The polar lipid profile of strain MI-G^T is quite distinct from those of the reference strains *M. epialgicus* DSM18651^T, *M. echini* JCM30400^T and *M. hydrolyticus* DSM11525^T, with the main difference being diphosphatidylglycerol, which is present only in *M. epialgicus* DSM18651^T.

According to the results of phylogenetic, phenotypic, chemotaxonomic, and biochemical studies obtained above, strain MI-G^T is allocated to genus *Microbulbifer*, class *Gammaproteobacteria*. However, the strain can be distinguished from other related strains by some phenotypic and genetic characteristics; so, the isolate should not be assigned to any already known species. Therefore, strain MI-G^T represents a novel species of the genus *Microbulbifer*, for which the name *Microbulbifer spongiae* sp. nov. is proposed.

DESCRIPTION OF *MICROBULBIFER SPONGIAE* SP. NOV.

Microbulbifer spongiae (spon'gi.ae. L. gen. n. *spongiae,* of a sponge, the source of the type strain)

Cells are Gram-stain-negative, aerobic, non-motile and exhibit a rod-coccoid–ovoid cell cycle during the growth phase. Colonies on marine agar 2216E (Difco) medium are smooth, circular, and white colour (young cultures) turned to yellowish brown (old cultures). The type strain can grow at 10–40 °C (optimum, 28 °C), with 1.0–8.0% (w/v) NaCl (optimum, 4.0%), and at pH 5.5–9.0 (optimum, pH 8.0). Positive for catalase and oxidase activity. In API 20NE tests, positive for reduction of nitrate, and weakly positive for hydrolysis of gelatinase. Negative for the assimilation of D-glucose, L-arabinose, D-mannitol, D-mannose, maltose, adipic acid, malic acid, and *N*-acetyl-glucosamine. In the API ZYM tests, positive for trypsin and α-chymotrypsin enzyme activity, negative for α-mannosidase. In the Biolog GEN III MicroPlate, strain MI-G^T is positive for L-aspartic acid, β-hydroxy-D,L-butyric acid, weakly positive for β-formyl-D-glucoside, α-D-glucose, D-fructose, D-glucose-6-phosphate, L-alanine, and D-gluconic acid, and negative for trehalose, D-terpinediase, lactose, D-aspartic acid, D-gluconic acid and *p*-hydroxy-phenylacetic acid. The predominant respiratory quinone is Q-8, which is distinctive of *Microbulbifer*. The principle fatty acid components of strain MI-G^T are (>5%) $C_{16:0}$, iso-$C_{15:0}$, iso-$C_{11:0}$ 3-OH, summed feature 8 ($C_{18:1}$ $\omega7c$ or $C_{18:1}$ $\omega6c$) and summed feature 9 ($C_{17:1}$ iso $\omega9c$ or $C_{16:0}$ 10-methyl). The major polar lipids primarily consist of phosphatidylethanolamine, phosphatidylglycerol, an aminolipid, and two glycolipids.

The type strain, MI-G^T (=MCCC 1K07826^T=KCTC 8081^T), was isolated from a sample of marine sponge *Diacarnus erythraeanus*, collected from mesophotic and shallow reefs in front of the interuniversity institute for marine sciences in Eilat, Israel. The DNA G+C content of the type strain is 54.51 mol%. The GenBank accession numbers of the 16S rRNA gene and whole genome sequences of strain MI-G^T are ON849098 and CP098023, respectively. The draft genome sequences of strain DSM18651^T and JCM30400^T have been deposited at GenBank/EMBL/DDBJ under accession numbers JBGMEK000000000 and JBGMEL000000000.

Funding information
This work was supported by grants from the National Key Research and Development Program of China (2022YFC2804101), National Natural Science Foundation of China (31861143020) and the Oceanic Interdisciplinary Program of Shanghai Jiao Tong University (SL2021PT202).

Author contributions
Z.L. designed the research and project outline. G.L, M.Z. performed microbial isolation. N.I. performed polyphasic taxonomy and performed genome analysis. N.I., M.I. and Z.L. drafted and revised the manuscript. All authors read and approved the final manuscript.

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

References

1. Spring S, Scheuner C, Göker M, Klenk H-P. A taxonomic framework for emerging groups of ecologically important marine *Gammaproteobacteria* based on the reconstruction of evolutionary relationships using genome-scale data. *Front Microbiol* 2015;6:281.
2. González JM, Mayer F, Moran MA, Hodson RE, Whitman WB. *Microbulbifer hydrolyticus* gen. nov., sp. nov., and *Marinobacterium georgiense* gen. nov., sp. nov., two marine bacteria from a lignin-rich pulp mill waste enrichment community. *Int J Syst Bacteriol* 1997;47:369–376.
3. Xiong Q, Wang D, Dong X, Liu D, Liu Y, *et al*. *Microbulbifer flavimaris* sp. nov., a halophilic *Gammaproteobacteria* isolated from marine sediment of the Yellow Sea, China. *Int J Syst Evol Microbiol* 2019;69:1135–1141.
4. Moh TH, Furusawa G, Amirul AA-A. *Microbulbifer aggregans* sp. nov., isolated from estuarine sediment from a mangrove forest. *Int J Syst Evol Microbiol* 2017;67:4089–4094.
5. Huang H, Mo K, Hu Y, Liu M, Zhu J, *et al*. *Microbulbifer harenosus* sp. nov., an alginate-degrading bacterium isolated from coastal sand. *Int J Sys Evol Microbiol* 2020;70:1639–1643.
6. Cheng Y, Zhu S, Guo C, Xie F, Jung D, *et al*. *Microbulbifer hainanensis* sp. nov., a moderately halopilic bacterium isolated from mangrove sediment. *Antonie van Leeuwenhoek* 2021;114:1033–1042.
7. Lee J-Y, Kim PS, Hyun D-W, Kim HS, Shin N-R, *et al*. *Microbulbifer echini* sp. nov., isolated from the gastrointestinal tract of a purple sea urchin, *Heliocidaris crassispina. Int J Syst Evol Microbiol* 2017;67:998–1004.

8. Nishijima M, Takadera T, Imamura N, Kasai H, An K-D, *et al*. *Microbulbifer variabilis* sp. nov. and *Microbulbifer epialgicus* sp. nov., isolated from Pacific marine algae, possess a rod-coccus cell cycle in association with the growth phase. *Int J Syst Evol Microbiol* 2009;59:1696–1707.
9. Camacho M, del Carmen Montero-Calasanz M, Redondo-Gómez S, Rodríguez-Llorente I, Schumann P, *et al*. *Microbulbifer rhizosphaerae* sp. nov., isolated from the rhizosphere of the halophyte *Arthrocnemum macrostachyum*. *Int J Syst Evol Microbiol* 2016;66:1844–1850.
10. Jeong SH, Yang S-H, Jin HM, Kim JM, Kwon KK, *et al*. *Microbulbifer gwangyangensis* sp. nov. and *Microbulbifer pacificus* sp. nov., isolated from marine environments. *Int J Syst Evol Microbiol* 2013;63:1335–1341.
11. Yoon JH, Kim IG, Shin DY, Kang KH, Park YH. *Microbulbifer salipaludis* sp. nov., a moderate halophile isolated from a Korean salt marsh. *Int J Syst Evol Microbiol* 2003;53:53–57.
12. Chen Y, Sang J, Sun W, Song Q, Li Z. *Mycetocola spongiae* sp. nov., isolated from deep-sea sponge *Cacospongia mycofijiensis*. *Int J Syst Evol Microbiol* 2022;72:005291.
13. Varijakzhan D, Loh J-Y, Yap W-S, Yusoff K, Seboussi R, *et al*. Bioactive compounds from marine sponges: fundamentals and applications. *Mar Drugs* 2021;19:246.
14. Santos-Gandelman JF, Giambiagi-deMarval M, Oelemann WMR, Laport MS. Biotechnological potential of sponge-associated bacteria. *Curr Pharm Biotechnol* 2014;15:143–155.
15. Fuerst JA. Diversity and biotechnological potential of microorganisms associated with marine sponges. *Appl Microbiol Biotechnol* 2014;98:7331–7347.
16. Liu C, Xiao Y, Xiao Y, Li Z. Marine urease with higher thermostability, pH and salinity tolerance from marine sponge-derived *Penicillium steckii* S4-4. *Mar Life Sci Technol* 2020;3:77–84.
17. Simmons TL, Coates RC, Clark BR, Engene N, Gonzalez D, *et al*. Biosynthetic origin of natural products isolated from marine microorganism-invertebrate assemblages. *Proc Natl Acad Sci U S A* 2008;105:4587–4594.
18. Zhang M, Song Q, Sang J, Li Z. *Paenibacillus spongiae* sp. nov. isolated from deep-water marine sponge *Theonella swinhoei*. *Int J Syst Evol Microbiol* 2023;73:006122.
19. Gao L, Song Q, Sang J, Xiao Y, Li Z. *Cytobacillus spongiae* sp. nov. isolated from sponge *Diacarnus spinipoculum*. *Int J Syst Evol Microbiol* 2023;73:005903.
20. Chen Y-G, Cui X-L, Pukall R, Li H-M, Yang Y-L, *et al*. *Salinicoccus kunmingensis* sp. nov., a moderately halophilic bacterium isolated from a salt mine in Yunnan, south-west China. *Int J Syst Evol Microbiol* 2007;57:2327–2332.
21. Tittsler RP, Sandholzer LA. The use of semi-solid agar for the detection of bacterial motility. *J Bacteriol* 1936;31:575–580.
22. Zhang M, Song Q, Sang J, Li Z. *Qipengyuania spongiae* sp. nov., isolated from marine sponge *Cinachyrella kuekenthali*. *Int J Syst Evol Microbiol* 2023;73:005800.
23. Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 1991;173:697–703.
24. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 1977;74:5463–5467.
25. 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.
26. Yoon SH, Ha SM, Lim JM, Kwon SJ, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 2017;110:1281–1286.
27. Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Mol Biol Evol* 2021;38:3022–3027.
28. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
29. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985;39:783–791.
30. Salzberg S, Delcher AL, Kasif S, White O. Microbial gene identification using interpolated Markov models. *Nucleic Acids Res* 1998;26:544–548.
31. Delcher AL, Bratke KA, Powers EC, Salzberg SL. Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics* 2007;23:673–679.
32. Lagesen K, Hallin P, Rødland EA, Stærfeldt H-H, Rognes T, *et al*. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* 2007;35:3100–3108.
33. 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.
34. Luo R, Liu B, Xie Y, Li Z, Huang W, *et al*. SOAPdenovo2: an empirically improved memory-efficient short-read *de novo* assembler. *Gigascience* 2012;1:18.
35. Meier-Kolthoff JP, Göker M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nat Commun* 2019;10:2182–2192.
36. Parks DH, Chuvochina M, Chaumeil PA, Rinke C, Mussig AJ, *et al*. A complete domain-to-species taxonomy for bacteria and archaea. *Nat Biotechnol* 2020;38:1079–1086.
37. Steiner LX, Wiese J, Rahn T, Borchert E, Slaby BM, *et al*. *Maribacter halichondriae* sp. nov., isolated from the marine sponge *Halichondria panicea,* displays features of a sponge-associated life style. *Antonie van Leeuwenhoek* 2024;117:56.
38. Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 2016;32:929–931.
39. 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.
40. Auch AF, Henz SR, Holland BR, Göker M. Genome BLAST distance phylogenies inferred from whole plastid and whole mitochondrion genome sequences. *BMC Bioinformatics* 2006;7:350.
41. Kim D, Park S, Chun J. Introducing EzAAI: a pipeline for high throughput calculations of prokaryotic average amino acid identity. *J Microbiol* 2021;59:476–480.
42. Han MX, Dong L, Asem MD, Jiao J-Y, Wang D, *et al*. *Botryobacter ruber* gen. nov., sp. nov., a novel member of the family *Hymenobacteraceae*. *Int J Syst Evol Microbiol* 2019;69:821–827.
43. Qin Q-L, Xie B-B, Zhang X-Y, Chen X-L, Zhou B-C, *et al*. A proposed genus boundary for the prokaryotes based on genomic insights. *J Bacteriol* 2014;196:2210–2215.
44. Hua Z-S, Qu Y-N, Zhu Q, Zhou E-M, Qi Y-L, *et al*. Genomic inference of the metabolism and evolution of the archaeal phylum *Aigarchaeota*. *Nat Commun* 2018;9:2832–2843.
45. Kovacs N. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature* 1956;178:703–704.
46. Komagata K, Suzuki K. Lipid, and cell wall analysis in bacterial systematics. *Methods Microbiol* 1987;19:161–207.
47. Collins MD, Jones D. Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. *Microbiol Rev* 1981;45:316–354.
48. Lai QL, Liu XP, Yuan J, Xie SC, Shao ZZ. *Pararhodobacter marinus* sp. nov., isolated from deep-sea water of the Indian Ocean. *Int J Syst Evol Microbiol* 2019;69:932–936.
49. Kates M. Lipid extraction procedures. In: Kates M (ed). *Techniques of Lipidology Isolation, Analysis, and Identification of Lipids*. Amsterdam: Elsevier Science Publisher; 1986. pp. 100–111.
50. Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, *et al*. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbial Methods* 1984;2:233–241.
51. Tindall BJ. Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* 1990;66:199–202.

52. Vancanneyti M, Witt S, Abraham WR, Kersters K, Fredrickson HL. Fatty acid content in whole-cell hydrolysates and phospholipid and phospholipid fractions of pseudomonads: a taxonomic evaluation. *Syst Appl Microbiol* 1996;19:528–540.
53. Sasser M. Technical note 101: identification of bacteria by gas chromatography of cellular fatty acids. Newark: MIDI, Inc. North 1990; 20: 1-7.
54. Sakamoto M, Sakurai N, Tanno H, Iino T, Ohkuma M, *et al*. Genome-based, phenotypic, and chemotaxonomic classification of faecalibacterium strains: proposal of three novel species *Faecalibacterium duncaniae* sp. nov., *Faecalibacterium hattorii* sp nov and *Faecalibacterium gallinarum* sp nov. *Int J Syst Evol Microbiol* 2022;72:005379.
55. Zhang DS, Huo YY, Xu XW, Wu YH, Wang CS. *Microbulbifer marinus* sp. nov. and microbulbifer yueqingensis sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* 2012;62:505–510.
56. Moh TH, Lau N-S, Furusawa G, Amirul A-AA. Complete genome sequence of Microbulbifer sp. CCB-MM1, a halophile isolated from Matang Mangrove Forest, Malaysia. *Stand in Genomic Sci* 2017;12:36.
57. Vashist P, Nogi Y, Ghadi SC, Verma P, Shouche YS. *Microbulbifer mangrovi* sp. nov., a polysaccharide-degrading bacterium isolated from an Indian mangrove. *Int J Syst Evol Microbiol* 2013;63:2532–2537.
58. Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, *et al*. Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics. *Int J Syst Evol Microbiol* 1987;37:463–464.
59. Konstantinidis KT, Tiedje JM. Genomic insights that advance the species definition for prokaryotes. *Proc Natl Acad Sci USA* 2005;102:2567–2572.
60. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci USA* 2009;106:19126–19131.
61. Xu L, Sun C, Fang C, Oren A, Xu XW. Genomic-based taxonomic classification of the family *Erythrobacteraceae Int J Syst Evol Microbiol* 2020;70:4470–4495.
62. Tang S-K, Wang Y, Cai M, Lou K, Mao P-H, *et al*. *Microbulbifer halophilus* sp. nov., a moderately halophilic bacterium from north-west China. *Int J Syst Evol Microbiol* 2008;58:2036–2040.
63. Wang C-S, Wang Y, Xu X-W, Zhang D-S, Wu Y-H, *et al*. *Microbulbifer donghaiensis* sp. nov., isolated from marine sediment of the East China Sea. *Int J Syst Evol Microbiol* 2009;59:545–549.