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SHORT COMMUNICATION

Draconibacterium aestuarii sp.nov., a Glycolipid-Producing Bacterium Isolated from Tidal Flat Sediment and Emended Description of the Genus *Draconibacterium*

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Abstract

A facultatively anaerobic, Gram-negative, curved rod-shaped bacterium (4.0–17.0 µm long, 0.6–0.9 µm wide), designated Z1-6^T, was obtained from tidal flat sediment collected from YueAo village in Zhoushan, Zhejiang, People's Republic of China. Strain Z1-6^T occurred at 15–45 °C (optimum 28–32 °C), pH 6.0–9.0 (optimum 7.0–7.5), and in the presence of 1–5% (w/v) NaCl (optimum 1–2%). The strain contained iso-$C_{15:0}$ and antesio-$C_{15:0}$ as the major fatty acids. An unsaturated menaquinone with seven isoprene units (MK-7) was the predominant respiratory quinone. The polar lipids included phosphatidylethanolamine (PE), one aminophospholipid (APL), two phospholipids (PL1 and PL2), three glycolipids (GL1, GL2, and GL3), and two unidentified lipids (L1 and L2). The genomic DNA G+C content of strain Z1-6^T was 39.2%, and the genome size was 6.4 Mb. The strain showed the highest average nucleotide identity (ANI) value of 73.5–74.6%, digital DNA–DNA hybridization (dDDH) value of 19.3–20%, average amino acid identity (AAI) value of 72.0–73.1% with the members of genus *Draconibacterium*. Phylogenetic analysis based on 16S rRNA gene sequences and genome revealed that strain Z1-6^T formed a distinct branch in the clade of the genus *Draconibacterium*. Based on the phenotypic, phylogenetic, chemotaxonomic analyses and genomic data, strain Z1-6^T represents a novel species of the genus *Draconibacterium*, for which the name *Draconibacterium aestuarii* sp. nov. (The type strain Z1-6^T = MCCC 1K07533^T = KCTC 92310^T) is proposed.

Introduction

The genus *Draconibacterium* (belongs to the family *Prolixibacteraceae*, order *Bacteroidales*, class *Bacteroidia*, Phylum *Bacteroidota*) was first proposed by Du et al. with *Draconibacterium orientale* as the type species [1]. Currently, the genus *Draconibacterium* comprised five species (four of the species names were validly published) at the time of writing (https://lpsn.dsmz.de/genus/draconibacterium, accessed Oct. 2023), all of which had been isolated from marine sediments [1–5]. Members of this genus are Gram-negative facultative anaerobic, straight or curved rods. The fatty acids are anteiso-$C_{15:0}$ and iso-$C_{15:0}$. The major respiratory quinone is seven isoprene units (MK-7) and the major polar lipid is phosphatidylethanolamine (PE) [1].

Due to the special characteristics of the marine environment (such as high pressure, high salt, poor nutrients), we initiated a study on the cultivable bacterial diversity of marine sediment in Zhoushan, Zhejiang, People's Republic of China. During our study, several bacterial strains were isolated. Among them, a facultatively anaerobic, designated strain Z1-6^T, was selected for further study.

Repositories The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Z1-6^T is OP882736. The GenBank/EMBL/DDBJ accession number for draft genome sequence of strain Z1-6^T is JAPOHD000000000.

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In this study, we report on the characterization of a novel bacterial strain Z1-6^T and propose that it is a novel member of the genus *Draconibacterium*.

Materials and Methods

Strain Isolation

In October 2022, sediment samples were collected from YueAo village in Zhoushan, Zhejiang, PR China (122.2° E, 29.6° N). To isolate bacterial strains, 10 g of sediment sample was suspended in 50-ml artificial seawater [3% (w/v) sea salt (Sigma)], the suspensions were serially diluted and spread on marine agar 2216 (MA; Difco).

After incubated at 25 ℃ for 2 weeks, a white colony was selected and purified by repeated streaking on MA plates, the purified strain was obtained and stored in 20% glycerol (v/v) at − 80 ℃.

Strain Z1-6^T has been deposited at the Marine Culture Collection of China (MCCC) and the Korean Collection for Type Cultures (KCTC) under the deposit numbers MCCC 1K07533^T and KCTC 92310^T, respectively. Two reference strains, *Draconibacterium orientale* MCCC 1A10579^T and *Draconibacterium sediminis* MCCC 1A00734^T, were obtained from MCCC.

16S rRNA Gene Sequencing and Phylogenetic Analysis

Genomic DNA of strain Z1-6^T was extracted using Quick Bacteria Genomic DNA Extraction Kit (Dongsheng Biotech; Guangzhou, P.R. China) according to the manufacturer's instructions. The 16S rRNA gene was amplified by PCR with universal primer set for bacteria (27F and 1492R) [6, 7]. PCR products were sequenced by Tsingke Biotechnology Co., Ltd. (Hangzhou, P.R. China).

The 16S rRNA gene sequences of closely related strains were obtained from the EzBioCloud database (https://www.ezbiocloud.net/) [8] and multiple sequence alignment was performed with the CLUSTALW program in the software MEGA version 11 [9] using the neighbor-joining (NJ) [10], maximum-likelihood (ML) [11], and maximum-evolution (ME) methods [10]. Genetic distances were calculated using the Kimura two-parameter model [12], and the bootstrap values were calculated based on 1000 replicates.

Whole Genome Sequencing and Genome Based Analyses

Genomic DNA of strain Z1-6^T was sequenced on Illumina HiSeq2500 platform by Novogene Biotech Co., Ltd (Beijing, PR China). The sequenced clean data was assembled using ABySS version 1.5.2 [13]. The quality check based on completeness and contamination rates was assessed by CheckM version 1.2.0 [14]. The draft genome sequence of strain Z1-6^T has been submitted in GenBank and annotated with NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [15]. The DNA G+C content of strain Z1-6^T was determined from the whole genomic sequence.

Genomic relatedness of strain Z1-6^T and other reference strains was determined by digital DNA-DNA hybridization (dDDH), average nucleotide identity (ANI) and average amino acid identity (AAI). The dDDH values were calculated by the DSMZ Genome-to-Genome Distance Calculator (GGDC 3.0; https://ggdc.dsmz.de/ggdc.php#) with the recommended local comparison tools (blast + and Eq. 2) [16]. The ANI values were determined in the JSpeciesWS Online Service version 3.9.7 (https://jspecies.ribohost.com/jspeciesws/#analyse) [17]. The AAI values were computed using the AAI calculator from the Kostas lab (http://enve-omics.ce.gatech.edu/aai/) [18]. Identified and concatenated alignment of 120 single-copy genes retrieved from the genome using GTDB-Tk v.1.5.1 [19], and then a phylogenomic tree was constructed using IQ-TREE 2.2.0 program and ModelFinder was used to determine the best-fit model [20].

The online tool BlastKOALA (https://www.kegg.jp/) [21] was used to reveal the metabolic pathways. Protein-encoding regions were identified with the Rapid Annotations using Subsystem Technology (RAST) server (https://rast.nmpdr.org/) [22]. Functional annotation was performed using eggNOG-Mapper version 2.1 (http://eggnog-mapper.embl.de/) [23].

Morphological, Biochemical, and Physiological Analyses

The morphological and physiological characteristics of strain Z1-6^T were examined after incubation on MA at 30 ℃. Cell morphology and flagella were observed using transmission electron microscopy (JEM-1230, JEOL). Gram staining was performed according to the method described by Buck et al. [24]. H_2S production was tested in MA supplemented with 0.5% (w/v) thiosulphate. Cell motility was observed in MA with 0.5% (w/v) agar. Catalase and oxidase activities were determined using 3% (v/v) H_2O_2 and oxidase reagent (bioMérieux), respectively [25]. Anaerobic growth was tested with a microaerobic system (AnaeroPack-MicroAero, 2.5 L; Mitsubishi Gas Chemical) using MA supplemented with $NaNO_3$ (20 mM), $NaNO_2$ (10 mM), Na_2SO_3 (5 mM), $Na_2S_2O_3$ (20 mM) or Na_2SO_4(20 mM) as potential electron acceptors.

The growth temperature range was tested in MB at 4, 10, 15, 20, 25, 28, 30, 35, 37, 40, 45, and 50 ℃. Salt tolerance was detected in NaCl-free MB (according to MB formula, but without NaCl) supplemented with 0–6% (w/v) NaCl

concentrations at 30 ℃. The pH range for growth was determined using the media from pH 5.0 to 9.5 with an interval of 0.5 pH unit and buffer system described by Du et al. [1]. The growth of strain Z1-6^T was examined after 14 days. Hydrolysis of CM-Cellulose, Tweens (20, 40, 60, and 80), starch, sodium alginate, and casein were evaluated as described by Chen et al. [26].

Acid production was tested using API 50CH systems with marine oxidation-fermentation (MOF) medium [27]. Enzymatic activities and other physiological and biochemical traits were tested using API ZYM and API 20NE stripes (bioMérieux) according to the manufacturer's instructions (except for salinity, which was adjusted to 2%). GEN III MicroPlates (Biolog) were used to determine the substrate utilization of strain Z1-6^T. All API and Biolog tests were done in triplicate, along with *D. sediminis* MCCC 1A00734^T and *D. orientale* MCCC 1A10579^T.

Chemotaxonomic Analyses

The polar lipids were extracted and separated via two-dimensional silica gel TLC on silica gel 60 F254 plates (10 × 10 cm, Merck) according to the method of Minnikin et al. [28, 29]. The identification of total lipids, aminolipids, phospholipids, and glycolipids were performed by spraying with molybdophosphoric acid, ninhydrin reagent, molybdenum blue and α-naphthol/sulfuric acid ethanol (1:1) solution, respectively. Isoprenoid quinones of strain Z1-6^T were extracted according to the method of Collins et al. [29] and analyzed by high-performance liquid chromatography-mass spectrometry system (Agilent 1200 and Thermo Finnigan LCQ DECA XP MAX mass spectrometer). Cellular fatty acids of strain Z1-6^T, *D. sediminis* MCCC 1A00734^T and *D. orientale* MCCC 1A10579^T were extracted and analyzed with reference to the protocol of MIDI (Sherlock version 6.0; MIDI database: ANAER6) with cells collected on MB for 3 days at 30 ℃.

Results and Discussion

Phylogenetic Relationship

Almost-complete 16S rRNA gene sequence (1508 bp) was uploaded to NCBI with the GenBank accession number of OP882736. The 16S rRNA similarities between strain Z1-6^T and members of the genus Draconibacterium ranged from 92.8 to 94.1%. Meanwhile, the phylogenetic tree using the NJ method (Fig. 1) showed strain Z1-6^T grouped with the members of the genus *Draconibacterium*, which suggested that Z1-6^T represented a novel species of the genus *Draconibacterium*. The results were consistent in the ML and ME trees (Supplementary Figs. S1, S2).

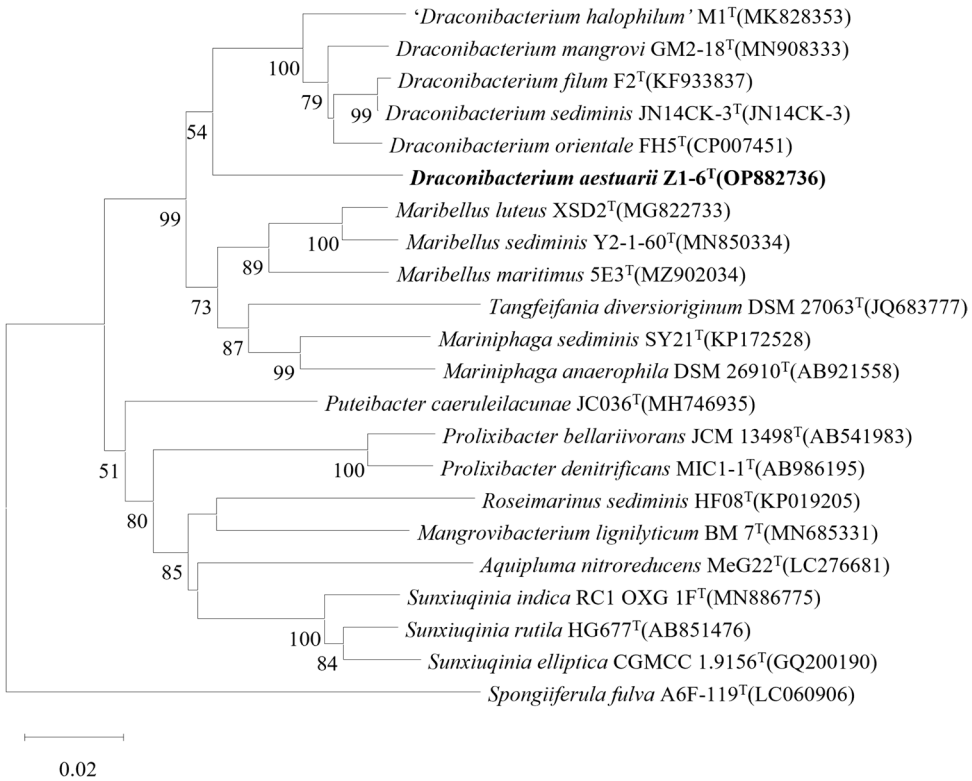

Fig. 1 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of strain Z1-6^T and the related species. Bootstrap values based on 1000 replicates are listed as percentages at branching points. Bar, 0.02 sequence divergence. *Spongiiferula fulva* A6F-119^T(LC060906) was used as an outgroup

Morphological, Biochemical, and Physiological Characteristics

Cells of strain Z1-6^T showed curved rod-shaped with 0.6–0.9 μm wide and 4.0–17.0 μm long (Supplementary Fig. S3A). Strain Z1-6^T colonies were white, circular, and rough with a diameter of 1.0–2.0 mm after 72 h of incubation on MA medium at 30 ℃ (Supplementary Fig. S3B). The isolate was Gram-negative, facultative anaerobic, and positive for alkaline phosphatase, esterase(C4), Esterase lipase(C8), α-glucosidase, *N*-acetyl-β-glucosaminidase, β-galactosidase and negative for arginine dihydrolase and urease, which were in accordance with the properties of the *Draconibacterium* strains.

The detailed phenotypic characteristics of strain Z1-6^T are summarized in the species description. As shown in Table 1 and Supplementary Table S1, several physiological and biochemical features could be used to distinguish strain Z1-6^T from other closely related type strains.

Chemotaxonomic Characteristics

The major fatty acids (> 10.0%) of strain Z1-6^T were iso-$C_{15:0}$ (25.5%) and anteiso-$C_{15:0}$ (17.7%), which were consistent with members of genus *Draconibacterium*, despite differences in content. In addition, $C_{16:0}$ was the major fatty acid in *D. sediminis* MCCC 1A00734^T, *D. filum* F2^T, and *D. orientale* MCCC 1A10579^T, iso $C_{17:0}$ 3-OH was the major fatty acid in *D. orientale* MCCC 1A10579^T and $C_{17:1}$ ω6c was used as the major fatty acid in *D. mangrovi* GM2-18^T, but not in strain Z1-6^T (Supplementary Table S2).The major polar lipids of strain Z1-6^T were phosphatidylethanolamine (PE), two unidentified phospholipid (PL1 and PL2), three unidentified glycolipids (GL1, GL2, and GL3), one unidentified aminoglycolipid (AGL), and two unidentified lipids (L), of which GL and APL were unique to strain Z1-6^T (Supplementary Fig. S4). The predominant quinone of strain Z1-6^T was MK-7, which is the typical menaquinone in the genus *Draconibacterium.*

Genomic Analyses

The whole genome sequence of strain Z1-6^T was 6,374,637 bp, size 6.4 Mb. The genome sequence was uploaded to NCBI with the registration number of JAPOHD000000000. The genome was obtained with 69 contigs, the value of N50 was 195,645 bp, the genome coverage was 217×, The genome completeness of strain Z1-6^T was 99.6% with 0.3% contamination. The DNA G+C content of strain Z1-6^T was 39.2%, which was similar to the members of *Draconibacterium*.

The ANI values, AAI values, and maximum values of the DNA–DNA hybridization between strain Z1-6^T with the members of *Draconibacterium* was 73.5–74.6%, 72.0–73.1%, 19.3–20.0%, respectively. All the values were lower than the threshold values for species delineation (> 95–96% for ANI [30], > 70% for dDDH [16], and > 65% for AAI [31]). The phylogenetic tree of the genome (Fig. 2) showed that strain Z1-6^T was placedin *Draconibacterium* but separated from other strains, hence confirming the distinct taxonomic status of Z1-6^T within the genus *Draconibacterium*.

The draft genome of strain Z1-6^T analyzed by the RAST server are shown in Supplementary Table S3, it revealed that the most abundant genes can be classified in the following categories: cofactors, vitamins, prosthetic groups, pigments (128 genes), nucleosides and nucleotides (64 genes), protein metabolism (148genes), DNA metabolism (52 genes), respiration (58 genes), amino acids and derivatives (249 genes), sulfur metabolism (65 genes), carbohydrates (254 genes).The analysis showed strain Z1-6^T had 55 unique common genes, as annotated by the COG database (Supplementary Table S4). KEGG results showed that strain Z1-6^T, *D. sediminis* MCCC 1A00734$^{T\,T}$, *D. orientale* MCCC 1A10579^T, *D. mangrovi* GM2-18^T, and '*D. halophilum'* M1^T had 52, 52, 51, 50, and 54 complete pathways (Supplementary Table S5), respectively, including carbohydrate metabolism, energy metabolism, lipid metabolism, nucleotide metabolism, amino acid metabolism, glycan metabolism, metabolism of cofactors and vitamins, and biosynthesis of terpenoids and polyketides.complete. One of them, "M00119 Pantothenate biosynthesis, valine/L-aspartate => pantothnate" is only present in strain Z1-6^T. According to the genome analysis, *panE, panD, panB, panC,* were found in the genome of strain Z1-6^T, which were assumed to be responsible for pantothenate biosynthesis. Gene *panE* has 2-dehydropantoate 2-reductase activity and was only present in strain Z1-6^T. In addition, gene mining of strain Z1-6^T revealed the presence of lipid A synthesis-related genes *fabZ*, *lpxD*, *lpxB*, and *waaM* and lipid A is a phosphorylated glycolipid that anchors the lipopolysaccharide to the outer membrane of the cell [32].

Taxonomic Conclusion

Based on phenotypic, chemotaxonomic, and genotypic characterization, it is concluded that strain Z1-6^T is distinct from related species and therefore represents a novel species of the genus *Draconibacterium*, for which the name *Draconibacterium aestuarii* sp. nov. is proposed.

Emended Description of the Genus *Draconibacterium*

In addition to the characteristics described for the genus *Draconibacterium* by Xu et al. [1], some species also exhibit

Table 1 Differential phenotypic characteristics between strain Z1-6^T and members of the genus *Draconibacterium*

Characteristic	1	2	3	4	5	6
Cell size (μm)	0.6–0.9×4.0–17.0	0.6–0.8×2.9–3.4	0.3–0.5×1.3–1.8	0.4×0.8–42.0	0.6×5.0–6.5	0.5×4.0
Temperature range (optimum) for growth (℃)	15–45 (28–32)	15–40 (30–35)	20–40 (28–32)	10–40 (30)	15–40 (35)	18–37 (30)
NaCl tolerance range (optimum) for growth (%, w/v)	1–5 (1–2)	3–5 (2)	1–7 (2–4)	0–8 (3)	0–5 (2)	1–7 (3)
PH range (optimum) for growth	6.0–9.0 (7.0–7.5)	5.0–8.0 (7.0)	5.5–9.0 (7.0–7.5)	6.0–8.5 (7.5)	5.0–9.0 (7.0)	5.5–9.0 (7.0)
Oxidase activity	+	+	+	−	+	+
Hydrolysis of Tween 40	+	+	−	ND	−	ND
Reduction of nitrates to nitrites	+	+	−	+	+	−
Hydrolysis of esculin	−	−	+	+	+	+
Hydrolysis of gelatin	−	+	−	+	−	
Utilization of:						
D-glucose, D-maltose, malate	+	+	+	+	−	−
L-arabinose, D-mannitol	+	+	+	+	+	−
D-mannose, *N*-acetyl-glucosamine	+	+	−	+	−	−
potassium gluconate	−	−	+	+	−	−
Capric acid	−	+	−	−	−	−
Adipic acid	−	+	+	+	+	−
phenylacetic acid	−	+	+	+	−	−
Enzyme activity:						
Lipase(C14)	−	−	−	−	w	−
Leucine aryl amidase	+	+	+	+	+	−
valine arylamidase	−	w	−	−	+	−
Cystine arylamidase	w	w	−	−	+	−
Trypsin	−	+	+	+	+	−
α-chymotrypsin	−	w	−	+	+	−
Acid phosphatase	+	+	+	−	+	−
Naphthol-AS-BI-phosphohydrolase	w	w	+	−	+	+
α-galactosidase	−	+	+	+	+	+
β-galactosidase	w	+	+	−	+	−
β-glucuronidase	−	−	−	+	w	−
β-glucosidase	w	+	−	+	+	+
α-mannosidase	−	−	−	−	w	+
α-fucosidase	+	+	−	+	+	+

Table 1 (continued)

Characteristic	1	2	3	4	5	6
Major fatty acid	iso-$C_{15:0}$, anteiso-$C_{15:0}$	iso-$C_{15:0}$, anteiso-$C_{15:0}$	iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{17:0}$ 2-OH, iso-$C_{17:0}$ 3-OH	iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{16:0}$,	iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{17:1}$ ω6c	iso-$C_{15:0}$, anteiso-$C_{15:0}$,
Major Polar lipids	PE, PL (1–2), GL (1–3), APL, L (1–2)	PE, PL, L (1–5)	PE, PL, L (1–2), AL	PE, PI, L (1–4)	PE, PL, L (1–4)	PE, PL, L (1–9)
DNA G+C content (%)	39.2	40.9	41.3	44.7	40.8	40

Strains: 1, strain Z1-6^T; 2, *Draconibacterium sediminis* MCCC 1A00734^T (Data from this study); 3, *Draconibacterium orientale* MCCC 1A10579^T (Data from this study); 4. *Draconibacterium filum* F2^T (Data from Gwak et al. [4]); 5. *Draconibacterium mangrovi* GM2-18^T(Data from Hu et al. [3]); 6. '*Draconibacterium halophilum*' M1^T (Data from Kim et al. [5]). All strains are positive for alkaline phosphatase, Esterase(C4), Esterase lipase(C8), α-glucosidase, *N*-acetyl-β-glucosaminidase and negative for arginine dihydrolase, all strains can utilize β-galactosidase

+ Positive, − negative, *w* weakly positive, *ND* no data, *PE* phosphatidylethanolamine, *PL* uncharacterized phospholipid, *GL* uncharacterized glycolipid, *APL* unidentified aminophospholipid, *L* uncharacterized lipids

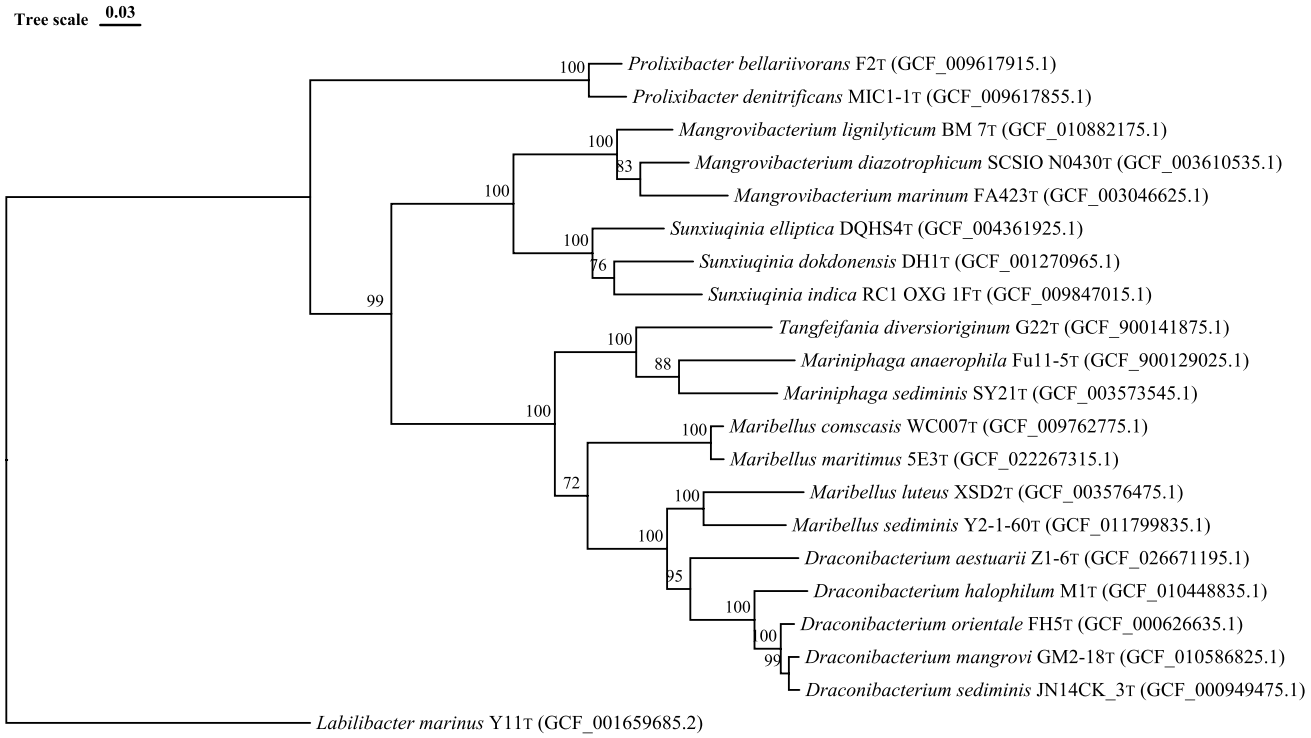

Fig. 2 Phylogenomic tree inferred from the concatenation of 120 single-copy bacterial marker genes showing the phylogenetic position of strain Z1-6^T. A number on nodes represent bootstrap values based on the 1000 replications. *Labilibacter marinus* Y11^T was used as the outgroup. Bootstrap values (≥ 50.0%) based on the 1000 replicates are shown at branch nodes

glycolipids as major polar lipids. DESCRIPTION OF DRACONIBACTERIUM AESTUARII SP. NOV.

Draconibacterium aestuarii (aes.tu.a' ri.i. L. gen. n. *aestuarii* of the tidal flat).

Cells are Gram-stain-negative, facultative anaerobic, oxidase- positive, catalase- positive, non- spore-forming, curved rod-shaped with 0.6–0.9 μm wide and 4.0–17.0 μm long. It can be grown at 15–45 ℃ (optimum 28–32 ℃), in 1–5% NaCl (optimum 1–2%), pH 6.0–9.0 (optimum 7.0–7.5). Nitrate can be reduced to nitrite. hydrolyzes Tween 40, but not Tween 20, 60, 80, CMC, starch, sodium alginate, and casein. In assays using the API ZYM system, positive results are obtained for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine

arylamidase, acid phosphatase, α-glucosidase, N-acetyl-β-glucosaminidase, and α-fucosidase; weekly positive for cystine arylamidase, Naphthol-AS-BI-phosphohydrolase, β-galactosidase, and β-glucosidase. In API 20 NE tests, positive for β-galactosidase, assimilating D-glucose, L-arabinose, D-mannose, D-maltose, D-mannitol, *N*-acetyl-glucosamine, and malic acid. Acid can be produced from the fermentation of D-arabinose, L-arabinose, D-ribose, D-xylose, D-galactose, D-galactose, D-glucose, D-fructose, D-mannose, methyl α-D-mannoside, methyl α-D-glucoside, *N*-acetyl-glucosamine, aesculin ferric citrate, D-salicin, cellobiose, D-maltose, lactose, melibiose, D-sucrose, D-trehalose anhydrous, inulin, melezitose, raffinose, gentiobiose. According to the Biolog GEN III MicroPlate test, positive for D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, stachyose, D-raffinose, D-lactose, D-melibiose, β-methyl-D-glucoside, D-salicin, *N*-acetyl-D-glucosamine, *N*-acetyl-β-D-mannosamine, *N*-acetyl-D-galactosamine, 1% NaCl, L-fucose, D-mannitol, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, Glucuronamide, Vancomycin, Tetrazolium violet, Tetrazolium blue, α-ketoglutaric acid, L-malic acid, Nalidixic acid, Potassium tellurite, Tween 40, Propionic acid, Acetic acid, Aztreonam, Sodium butyrate, Sodium bromate. Polar lipids include phosphatidylethanolamine (PE), an aminophospholipid (APL), two phospholipids (PL1 and PL2), three glycolipids (GL1, GL2, and GL3), and two unidentified lipids (L1, L2).

The type strain is Z1-6^T (= MCCC 1K07533^T = KCTC 92310^T) was obtained from tidal flat sediment collected from YueAo village in Zhoushan, Zhejiang, PR China. The DNA G+C content of the type strain is 39.2%.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and genomic DNA sequence are OP882736 and JAPOHD000000000, respectively.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00284-024-03682-0.

Author Contributions MW and GYF designed the experiments and guided the manuscript writing. JYW was responsible for the major experiments, data analysis and preparation of manuscripts. SSQ and WYP assisted in enzymatic experiments. TW assisted in the determination of fatty acids and polar lipids experiment. WWZ and YS are responsible for sample collection. All authors read and approved the manuscript.

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Declarations

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

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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