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ORIGINAL PAPER

Marinobacter alexandrii sp. nov., a novel yellow-pigmented and algae growth-promoting bacterium isolated from marine phycosphere microbiota

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Abstract The marine phycosphere harbors unique cross-kingdom associations with ecological relevance. During investigating the diversity of phycosphere microbiota of marine harmful algal blooms dinoflagellates, a faint yellow-pigmented bacterium, designated as strain LZ-8, was isolated from paralytic shellfish poisoning toxin-producing dinoflagellate *Alexandrium catenella* LZT09. The new isolate appeared to have growth-promoting potential toward its algal host. Molecular analysis using 16S rRNA gene, housekeeping *rpo*D gene and whole-genome sequence comparison indicated that strain LZ-8^T was a novel gammaproteobacterium of the family *Alteromonadaceae*. The major fatty acids of strain LZ-8^T were $C_{16:0}$, $C_{18:1}$ ω9c, $C_{12:0}$ 3-OH, summed feature 3, $C_{16:1}$ ω9c, $C_{12:0}$ and summed feature 9. The major isoprenoid quinone was Q-9. Polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, unidentified phospholipid, two unidentified aminolipids and six unidentified polar lipids. The genomic DNA G+C content was 57.36 mol%. Based on genome sequencing, several biosynthetic gene clusters responsible for bacterial biosynthesis of carotenoids and siderophores that may involve in algae–bacterial interactions were identified in the genome of strain LZ-8^T. The polyphasic characterization indicated that strain LZ-8^T represents a novel *Marinobacter* species. The name *Marinobacter alexandrii* sp. nov., type strain LZ-8^T (= CCTCC AB 2018386^T = KCTC 72198^T) is proposed.

The GenBank/EMBL/DDBJ accession numbers for 16S rRNA gene sequence of strain LZ-8^{T}is MK092986, and for draft genome sequences of strains LZ-8^{T}and *M. sediminum* R65^T are SWKM00000000 and JAEMQH000000000, respectively.

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Keywords *Marinobacter alexandrii* sp. nov. · Phycosphere microbiota · Marine toxic dinoflagellates · Algae growth-promoting bacteria · Harmful algal blooms · Algae–bacterial interactions

Abbreviations

ABI	Algae–bacteria interactions
AGP	Algae growth-promoting
AL	Aminolipid
ANI	Average nucleotide identity
APL	Aminophospholipids
BGC	Biosynthetic gene clusters
dDDH	Digital DNA-DNA hybridization
DPG	Diphosphatidylglycerol
GL	Glycolipid
HAB	Harmful algal blooms
MA	Marine agar
ML	Maximum likelihood
MP	Maximum parsimony
NJ	Neighbour joining
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PL	Phospholipids
PM	Phycosphere microbiota
PMP	Phycosphere Microbiome Project
PSTs	Paralytic shellfish poisoning toxins
UBCG	Up-to-date bacterial core gene

Introduction

The genus *Marinobacter* was established by Gauthier et al. (1992) as a member of the family *Alteromonadaceae* of the gammaproteobacteria. Currently, it comprises 54 species with validly published names (https://lpsn.dsmz.de/genus/marinobacter). Members of this genus were isolated from a wide variety of environments (Ahmad et al. 2020; Zhang et al. 2015b, 2020), and are halophilic or halotolerant. *Marinobacter* species can degrade a wide variety of hydrocarbons (Gauthier et al. 1992; Chernikova et al. 2020). *Marinobacter* can have a close association with various marine phytoplanktons including diatoms and dinoflagellates (Ahmed and Holmstrom 2014; Amin et al. 2012; Green et al. 2006; Romanenko et al. 2005; Kaeppel et al. 2012; Zhang et al. 2015b, 2020). The phycosphere is a distinctive microscopical niche which hosts unique microbiota with dynamic interactions between the algae and its closely associated bacteria consortium (Amin et al. 2012; Seymour et al. 2017; Zhang et al. 2015a, b, 2020). Understanding the complex structures and roles of the phycosphere microbiota (PM) derived from toxic harmful algal blooms (HAB) dinoflagellates is important to unveil the nature of their inter-kingdom associations (Amin et al. 2012; Duan et al. 2020; Seymour et al. 2017; Zhou et al. 2021; Yang et al. 2018a, b, 2020a, b, c; Zhang et al. 2015b, 2020). These studies have resulted in the isolation of various new bacterial species, including marine species belonging to the genus *Marinobacter* (Ahmed and Holmstrom 2014; Green et al. 2006; Romanenko et al. 2005; Kaeppel et al. 2012; Zhang et al. 2015b, 2020).

In order to obtain more insights, we have initiated the Phycosphere Microbiome Project (PMP) where we combine the culture-dependent and multi-omics approaches (Duan et al. 2020; Yang et al. 2018a, b, 2020a, b; Zhou et al. 2021; Zhang et al. 2015a, b, 2020). During the subsequent studies, a novel mobile, faint yellow-pigmented bacterium, strain LZ-8^T, was isolated from toxic HAB dinoflagellate *Alexandrium catenella* LZT09 which produces high levels of paralytic shellfish poisoning toxins (PSTs). The new isolate showed obvious growth-promoting potential toward its algal host. Here, we describe the polyphasic characterization of strain LZ-8^T which represents a novel species of the genus *Marinobacter*.

Materials and methods

Bacterial strains isolation and maintenance

Strains LZ-8 was isolated from *A. catenella* LZT09 using our optimized isolation procedure by spreading the algal culture on a modified marine 2216 medium supplemented with algal culture extract of LZT09 at approximate 0.05 mg/L (Yang et al. 2018a, b, 2020a, b). The isolated strain was purified and maintained on marine agar (MA, Difco) and preserved as a glycerol suspension (20%, v/v) at -80 °C for long term preservation. For the comparative analysis, three reference type strains, *M. sediminum* R65^T (= DSM 15400^T = KMM 3657^T) obtained from German Collection of Microorganisms and Cell Cultures (DSMZ, Germany), and *M. salinus* Hb8^T

(= JCM 31416^T = KCTC 52255^T) and *M. similis* A3d10^T (KMM 7501^T = CIP 110589^T = JCM 19398^T) obtained from Japan Collection of Microorganisms (JCM, Japan) were used. All the strains were maintained routinely on marine agar at 25 °C, and cultured under the same conditions as strain LZ-8^T.

Phenotypic analysis

Morphological characteristics were recorded via light (CX21, Olympus, Tokyo, Japan) and transmission electron microscopy (JEM-1200, JEOL, Tokyo, Japan) using cultures grown on MA at 25 °C for 3 days. Gram-staining test was determined as described previously (Beveridge et al. 2007). Motility was tested microscopically under the phase-contrast mode by the hanging drop technique. Growth at different temperatures from 5 to 50 °C at 5 °C increments, pH range from 4.0 to 12.0 in increments of 0.5 pH unit) were determined as reported previously (Zhou et al. 2021; Yang et al. 2018b; Zhang et al. 2020). NaCl tolerance was determined in marine 2216 medium supplemented with 0–10% NaCl (w/v) at 25 °C for 14 days on a rotary shaker. Utilization of carbon sources and enzyme activity tests of the strains were performed using API 20NE, ZYM (bioMérieux, Marcy-l'Étoile, France) and GEN III Microplate systems (BIOLOG) following the manufacturer's instructions.

Chemotaxonomic characteristics

Bacterial biomass for chemotaxonomic analysis was prepared by growing strain LZ-8^T and the reference type strains in shake flasks with marine broth (MB) at 180 r.p.m., 25 °C for 3 days, and thereafter the harvested cells were freeze-dried. The polar lipids were determined by two-dimensional TLC according to the method described by Minnikin et al. (1984). The respiratory quinone was isolated, purified and identified according to Hiraishi et al. (1996). Cellular fatty acids were extracted, methylated and analyzed following the instructions of the Microbial Identification System (MIDI) (Sherlock version 6.1; MIDI database TSBA6).

Phylogenetic analysis

Genomic DNA extraction, PCR amplification and sequencing of the 16S rRNA gene were performed as described previously (Yang et al. 2018b). The almost-complete 16S rRNA gene sequence was compared with the sequences of the type strains available from GenBank using the BLAST program and online EzTaxon server (https://www.ezbiocloud.net). Multiple sequence alignments and phylogenetic analysis of the 16S rRNA gene, housekeeping *rpoD* gene sequences from strain LZ-8^T and the related reference type strains (taken from the GenBank database) were performed using MEGA version 7 (Kumar et al. 2016). Phylogenetic trees were reconstructed using neighbour-joining (NJ), maximum likelihood (ML) and maximum parsimony (MP) algorithms using the bootstrap method with 1000 replications (Zhou et al. 2021; Zhang et al. 2020; Yang et al. 2020a). A distance matrix was generated by using Kimura's two-parameter model (Kimura 1980).

Genomic sequencing and phylogenomic calculations

The genome sequencing, assembling and annotation of strain *M. sediminum* R65^T was performed as reported previously (Zhang et al. 2020) at Major Bioscience (Shanghai, China) using an Illumina HiSeq 4000 system. The genome was assembled using SPAdes v3.5.0 (Anton et al. 2012). Gene prediction and genomic annotation were performed using NCBI PGAP v1.2.1 (Tatusova et al. 2016). The average nucleotide identity (ANI) and digital DNA-DNA genome hybridization (dDDH) values between strain LZ-8^T and the close relative were calculated using the ANI/AAIMatrix genome-based distance matrix calculator (http://enve-omics.ce.gatech.edu/g-matrix) and online genome-to-genome distance calculator (version 2.1) (http://ggdc.dsmz.de). The genome-based phylogenetic tree using an up-to-date bacterial core gene set (UBCG) was constructed according to the method described by Na et al (2018).

Table 1 Differential characteristics of strain LZ-8^T and close type strains of the genus *Marinobacter* with validly published names

Characteristic	1	2	3	4
Isolate source	Marine phycosphere	Marine sediment	Seawater	Seawater
Colony color	Light yellow	Whitish	Creamy	Transparent
Cell size (μm)	0.4–0.5 × 2.0–4.5	0.3–0.4 × 1.8–2.5[a]	0.40–0.45 × 1.3–2.1[b]	0.6–0.7 × 1.5–2.8[c]
Temperature growth range (°C, optimal)	15–40 (25)	10–40 (25)	4–35 (30)	10–35 (35)
pH growth range (optimal)	5.5–10 (7.5)	6.0–9.0 (7.0)	6.0–9.0 (7.5)	5.0–9.0 (8.0)
NaCl growth range (%, w/v)	0.5–9.5 (3.5)	0.5–16.0 (5.0)	0.5–18.0 (4.0)	1.0–15 (5.0)
API ZYM tests				
Alkaline phosphatase	+	+	+	−
Esterase(C4)	+	−	+	+
Esterase lipase(C8)	+	w	+	−
Lipase(C14)	−	−	w	−
Leucine arylamidase	+	+	+	−
Valine arylamidase	+	−	w	−
Cystine arylamidase	+	−	w	−
α-Chymotrypsin	−	−	−	w
Acid phosphatase	+	−	w	w
Naphthol-AS-BI-phosphohydrolase	+	+	+	−
α-Galactosidase	−	−	−	w
β-Galactosidase	−	−	−	+
α-Glucosidase	+	−	−	w
N-acetyl-β-glucosaminidase	−	+	+	−
α-Mannosidase	−	−	−	w
α-Fucosidase	−	−	−	w
API 20NE tests				
Fermentation of glucose	−	+	−	−
Hydrolysis of gelatine	−	−	−	+
β-Galactosidase	−	−	−	+
Assimilation of glucose	−	+	−	+
Arabinose	−	−	−	+
Mannose	−	−	−	+
Mannitol	−	−	−	+
N-acetyl-glucosamine	−	−	−	+
Maltose	−	−	−	+
Potassium gluconate	−	−	−	+
Capric acid	−	−	−	+
Phenylacetic acid	−	−	−	+
Major polar lipids	DPG, PG, PE, PL, AL, Ls	DPG, PG, PE, AL, PL, Ls	DPG, PG, PE, PL, Ls	DPG, PG, PE, PL, Ls
G+C mol%	57.4	57.9[a]/56.5[b]	57.6[c]	54.5[d]
16S rRNA gene similarity to LZ-8^T	–	99.0	98.7	97.6

Strains: 1, LZ-8^T; 2, *M. sediminum* R65^T; 3, *M. similis* JCM 19398^T; 4, *M. salinus* KCTC 52255^T

All data were obtained from this study unless otherwise indicated. All strains were strictly aerobic, motile, gram-negative and rod-shaped, but positive for oxidase and catalase activities. In API 20NE tests, all strains were negative for indole production, arginine dihydrolase,urease, adipic acid, malic acid, trisodium citrate and hydrolysis of aesculin, but positive for nitrate reduction. In API ZYM tests, all strains were negative for trypsin, β-glucuronidase and β-glucosidase. + , positive; -, negative; w, weakly positive, ND, not detected

Data from the original study, [a]this study. [b]Romanenko et al. (2005). [c]Ng et al. (2014). [d]Rani et al. (2017)

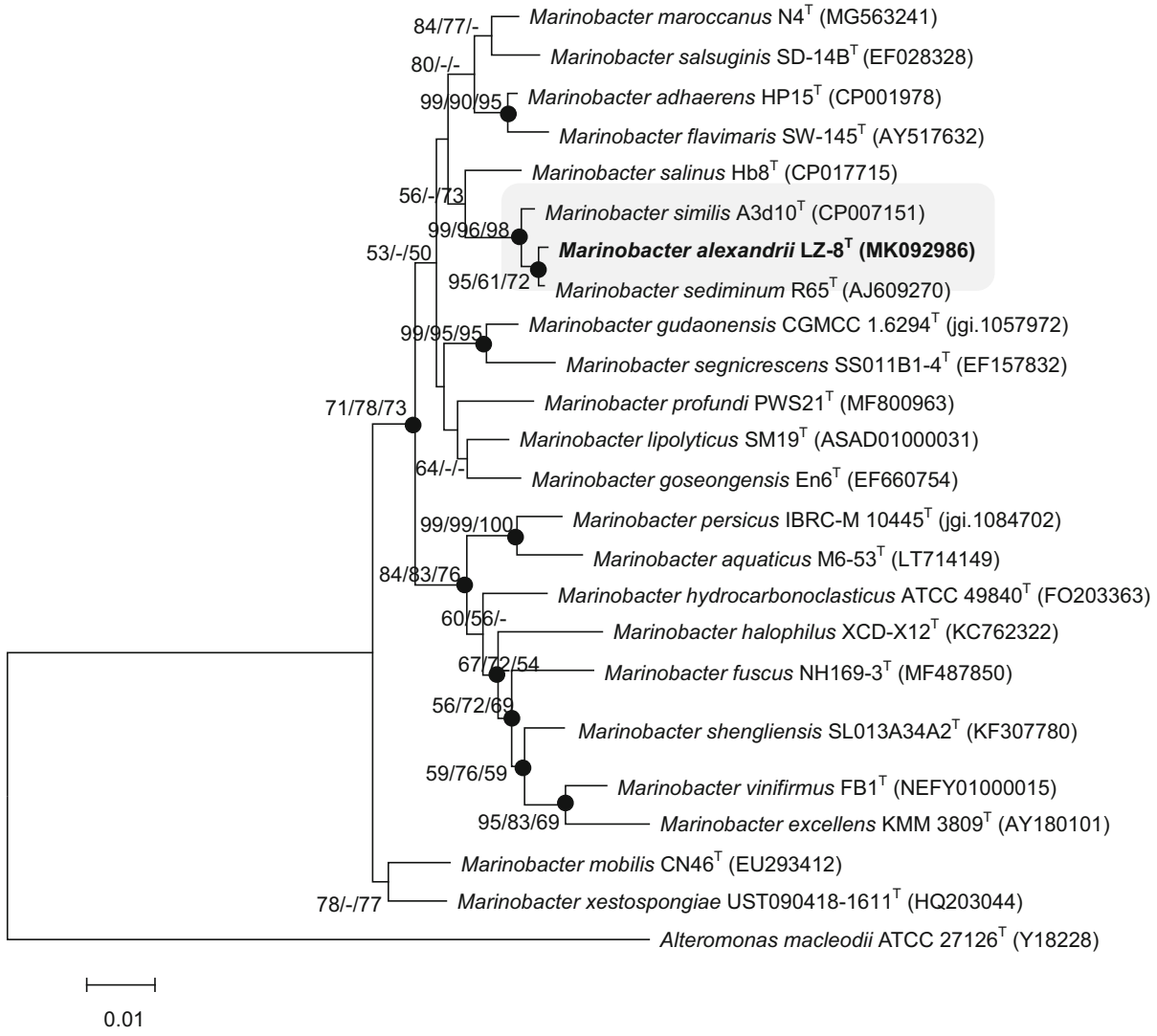

Fig. 1 Neighbor-joining (NJ) phylogenetic tree based on almost-complete 16S rRNA gene sequences showing the taxonomic position of strain LZ-8^T and related taxa of the genus *Marinobacter*. Filled circles indicate nodes that were also recovered in maximum-parsimony (MP) tree and maximum-likelihood (ML) tree based on the same sequences. Numbers at branch points indicate bootstrap percentages (based on 1000 replicates), only bootstrap values ($\geq$ 50%) are shown. 16S rRNA gene sequences of *Alteromonas macleodii* ATCC 27126^T is used as outgroup. Bar, 0.01 nucleotide substitutions per site

Results and discussion

Phenotypic characteristics

Strain LZ-8^T was isolated by spreading the algal culture on our modified isolation medium. The morphological characteristics of strain LZ-8^T were consistent with its classification as a member of the genus *Marinobacter*. Colonies of strain LZ-8^T grown on MA were faint yellow and creamy. Cells were 2.0–4.5 $\times$ 0.4–0.5 μm in size, rod-shaped, Gram-stain-negative, strictly aerobic, oxidase- and catalase-positive and motile by a flagellum (Fig. S1). Strain LZ-8^T grew at pH 5.5–10 (optimum, 7.5) and 15–40 °C (optimum, 25 °C) in the presence of 0.5–9.5% (w/v) NaCl (optimum, 3.5%). Compared to its closest relative *M. sediminum* R65^T, strain LZ-8^T was positive for the utilization of D-fructose, glycerol, D-malic acid, β-hydroxy-D, L-butyric acid, propionic acid, and acetic acid, but negative for the utilization of

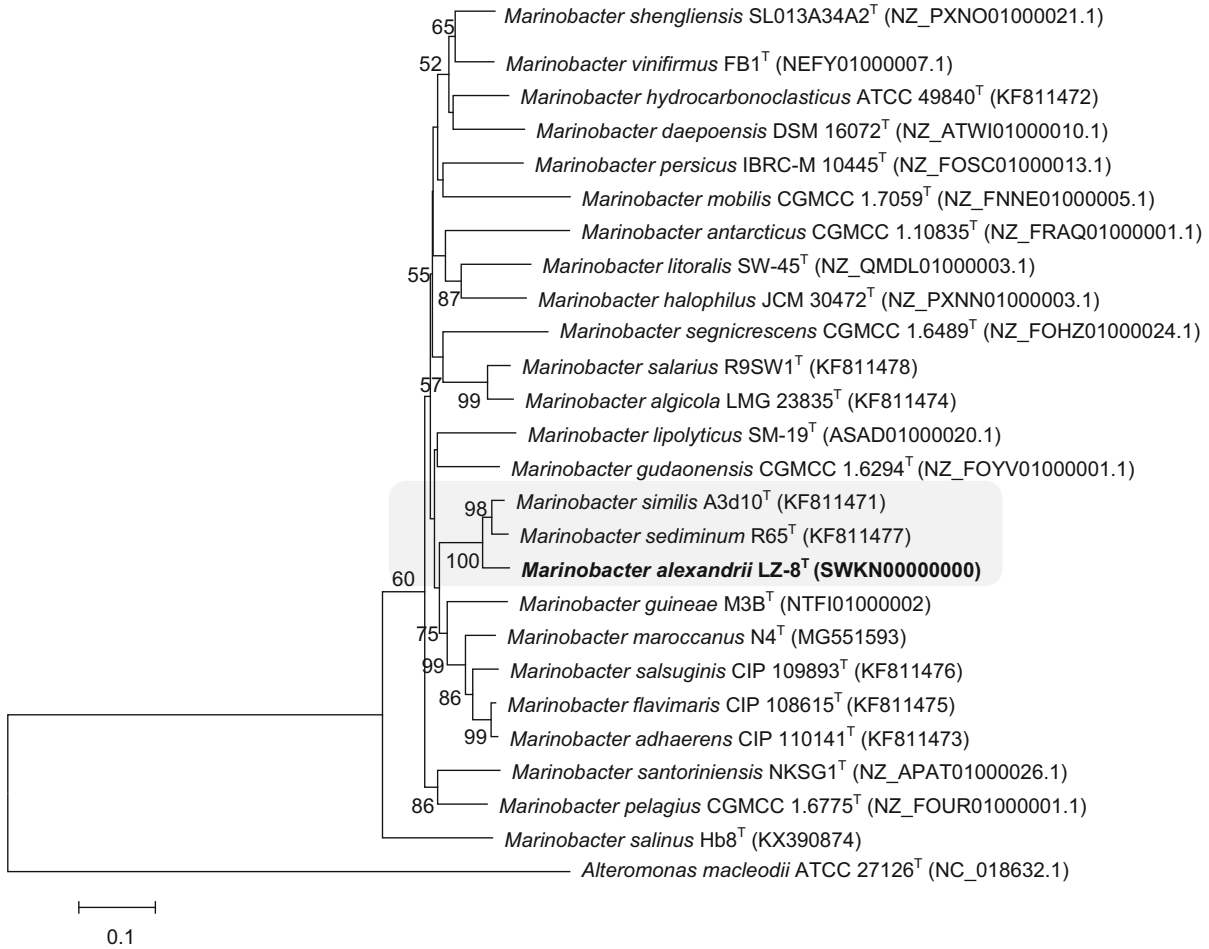

Fig. 2 Neighbor-joining phylogenetic tree based on the housekeeping *rpoD* gene sequences, showing the position of strain LZ-8^T with respect to other members of the genus *Marinobacter*. GenBank accession numbers are indicated in parentheses. Numbers at branch points indicate bootstrap percentages (based on 1000 replicates), only bootstrap values ($\geq$ 50%) are shown. *Alteromonas macleodii* ATCC 27126^T is included as outgroup. Bar, 0.1 nucleotide substitutions per site

D-cellobiose, α-D-glucose, D-mannose, or α-keto-butyric acid. In contrast to strain *M. sediminum* R65^T, strain LZ-8^T was positive for esterase (C4), valine arylamidase, cystine arylamidase, acid phosphatase and α-glucosidase, while negative for *N*-acetyl-*β*-glucosaminidase, and fermentation of glucose. These phenotypic characteristics can clearly distinguish strain LZ-8^T from *M. sediminum* R65^T (Table 1 and S1). The novel isolate did show growth-promoting potential toward its algal host LZT09 (Fig. S2).

Phylogenetic characteristics

Strain LZ-8^T showed high 16S rRNA gene sequence similarity values of 99.0% with *M. sediminum* R65^T (Romanenko et al. 2005), and 98.67% with *M. similis* A3d10^T (Ng et al. 2014), and low levels (< 98.0%) of similarities with other type strains of the genus *Marinobacter* (Ahmad et al. 2020). Based on 16S rRNA gene sequences analysis, strain LZ-8^T formed a phyletic line on the periphery of the 16S rRNA gene subclade of the type strains of *M. sediminum* and *M. similis* (Fig. 1), which was also supported by the MP and ML trees (Figs. S3 and S4). Additionally, comparison of the housekeeping *rpoD* (RNA polymerase sigma factor) gene sequences showed only 93.5% similarity between strains LZ-8^T and *M. sediminum* R65^T (Romanenko et al. 2005). Phylogenetic analysis using the housekeeping genes is beneficial because of its advantage of avoiding the

Table 2 Genomic features and signatures between strain LZ-8^T and its two close relatives, *M. sediminum* R65^T and *M. similis* A3d10^T

Attribute	*M. alexandrii* LZ-8^T (= CCTCC AB 2018386^T = KCTC 72198^T)[a]	*M. sediminum* R65^T (= DSM 15400^T = KMM 3657^T)[a]	*M. similis* A3d10^T (KMM 7501^T = CIP 110589^T = JCM 19398^T)[b]
GenBank accession no	SWKM00000000	JAEMQH000000000	CP007151
Assembly level	Draft	Draft	Complete
Genomic size (bp)	4,337,754	3,719,614	3,975,896
Number of contigs	20	11	1
N_{50} length (bp)	882,613	1,453,143	3,975,896
Coding genes	4,036	3,187	3,748
tRNA	49	36	46
rRNA	3	3	3
DNA G+C content (mol%)	57.4	57.9[a]/56.5[b]	57.6
ANI/dDDH values to LZ-8^T (%)	–/–	89.1/38.3	89.8/39.1

Data from the original study, [a]this study. [b]Rani et al. (2017)

possibility of nucleotide polymorphisms of 16S rRNA gene (Cilia et al. 1996). Phylogenetic tree constructed based on *rpoD* gene sequences showed strain LZ-8^T formed a separate branch outside the cluster formed by *M. sediminum* R65^T and *M. similis* A3d10^T (Figs. 2 and S5).

Due to the high 16S rRNA gene sequence similarity between strains LZ-8^T and *M. sediminum* R65^T, a genome-scale comparison between the two strains was carried out. Therefore, the genome of *M. sediminum* R65^T was sequenced and submitted to GenBank with the accession no. JAEMQH000000000. Its genome size was 3.719 Mbp consisting of 11 contigs with N_{50} value of 1,453 kbp. It had 3,187 protein-coding genes with the DNA G+C content of 57.9 mol% calculated from the genome. As shown in Table 2, the genome of strain LZ-8^T (GenBank accession no. SWKM00000000) (Zhang et al. 2020) had various different features compared to those of strains *M. sediminum* R65^T and *M. similis* A3d10^T. For strains LZ-8^T and *M. sediminum* R65^T, 3059 genes (77.1%) and 2681 genes (79.9%), respectively, were functionally annotated within COG database. However, only 2146 (54.1%) and 1558 genes (46.5%) were annotated by KEGG for metabolic pathways, indicating that a number of functional genes were still indistinct (Fig. S6). Additionally, several key biosynthetic gene clusters (BGCs) responsible for biosynthesis of bacterial secondary metabolites including photosynthesis and antioxidant carotenoids, and beneficial siderophores that may involve in algae–bacterial interactions (Amin et al. 2012; Green et al. 2006) were only identified in the genome of strain LZ-8^T.

Based on the phylogenomic calculations (Fig. S7), the ANI and dDDH values between strains LZ-8^T and *M. sediminum* R65^T, strains LZ-8^T and *M. similis* A3d10^T were 89.1 and 38.3%, 89.8% and 39.1%, respectively. All the phylogenomic values were below the threshold values for species delineation (Chun et al. 2018). Additionally, based on the constructed phylogenomic tree using the up-to-date bacterial core gene (UBCG) set (Fig. S8), strain LZ-8^T formed a separate branch while strain *M. sediminum* R65^T clustered with strain *M. similis* A3d10^T. This taxonomic feature was similar to the phylogenetic analysis based on the housekeeping *rpoD* gene (Figs. 2 and S5). Therefore, the phylogenomic analysis indicates that strain LZ-8^T represents a novel species within the genus *Marinobacter*.

Chemotaxonomic profiles

The major fatty acids of strain LZ-8^T were determined as $C_{16:0}$ (21.4%), $C_{18:1}$ ω9c (13.5%), $C_{12:0}$ 3-OH

Table 3 Cellular fatty acid composition of strain LZ-8^T and reference type strains of the genus *Marinobacter* with validly published names

Fatty acid	1	2	3	4
$C_{10:0}$	*tr*	–	–	–
$C_{11:0}$	*tr*	–	–	–
$C_{12:0}$	6.3	1.9	tr	6.9
$C_{11:0}$ 3-OH	*tr*	–	–	–
$C_{13:0}$	*tr*	–	–	–
$C_{12:0}$ 2-OH	*tr*	–	–	–
$C_{12:0}$ 3-OH	14.9	2.3	3.3	5.6
$C_{14:0}$	*tr*	*tr*	1.1	*tr*
$C_{16:0}$ N alcohol	2.6	–	–	–
$C_{16:1}$ ω9c	9.5	7.2	8.0	11.3
$C_{16:0}$	21.4	21.8	15.6	19.8
$C_{17:1}$ ω8c	1.4	5.3	5.1	3.2
$C_{17:0}$	*tr*	3.9	2.8	1.9
$C_{17:0}$ 10-methyl	2.0	–	–	–
$C_{18:3}$ ω6c (6,9,12)	1.7	–	–	–
$C_{18:1}$ ω9c	13.5	25.1	21.2	10.8
$C_{18:0}$	2.2	5.8	5.2	–
$C_{18:0}$ 10-methyl, TBSA	*tr*	–	–	–
$C_{18:0}$ 3-OH	*tr*	–	–	16.0
Summed features[a]				
1	*tr*	–	–	–
3	9.9	14.3	20.8	14.1
8	2.8	9.0	14.7	2.3
9	5.5	–	–	4.7

All of the data were from this study unless otherwise specified. 1, LZ-8^T; 2, *M. sediminum* R65^T; 3, *M. similis* JCM 19398^T; 4, *M. salinus* KCTC 52255^T. Values represent percentage of total fatty acids contents. –, not detected; *tr*, trace amounts (< 1%)

[a]Summed feature 1 contains $C_{13:0}$ 3-OH and/or iso-$C_{15:1}$ H; summed feature 3 contains $C_{16:1}$ ω7c and/or $C_{16:1}$ ω6c; summed feature 8 contains $C_{18:1}$ ω7c and/or $C_{18:1}$ ω6c; summed feature 9 contains iso-$C_{17:1}$ ω9c and/or $C_{16:0}$ 10-methyl

(14.9%), summed feature 3 (9.9%), $C_{16:1}$ ω9c (9.5%), $C_{12:0}$ (6.3%) and summed feature 9 (5.5%). The common fatty acids profiles were similar to other members of the genus *Marinobacter,* with some minor differences listed in Table 3. Both $C_{18:1}$ ω9c and $C_{16:0}$ were determined as the major fatty acids components (Table 3). The major isoprenoid quinone was ubiquinone-9 (Q-9). Polar lipids of strain LZ-8^T were determined as diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), unidentified phospholipid (PL), two unidentified aminolipids (ALs) and six unidentified polar lipids (Ls) (Fig. S9), which were also observed in strain *M. sediminum* R65^T (Romanenko et al. 2005).

Taxonomic conclusion

Based on the polyphasic characterization, phylogenetic and genome comparison, strain LZ-8^T clearly represents a novel species of the genus *Marinobacter*, for which the name *Marinobacter alexandrii* sp. nov. is proposed.

Description of *Marinobacter alexandrii* sp. nov.

Marinobacter alexandrii (a.le.xan´dri.i. N.L.gen. n. *alexandrii* of the dinoflagellate *Alexandrium catenella*, the source of isolation of the type strain).

Cells are Gram-stain-negative, strictly aerobic, oval or slightly curved rods, motile by flagellum with size 0.4–0.5 × 2.0–4.5 μm. They form faint yellow-pigmented, creamy, smooth-rounded colonies of about 1.0–2.0 mm in diameter on MA after 36 h incubation at 25 °C. Temperature, salt and pH ranges for growth are 15–40 °C, 0.5–9.5% (w/v) NaCl and pH 5.5–10, respectively. Optimal growth occurs at 4% (w/v) NaCl, 25 °C and pH 7.5. Positive for hydrolysis of Tween 40, but negative for hydrolysis of aesculin and gelatin. Oxidase and catalase activities are positive, and nitrate reduction to nitrite is variable. In the API ZYM tests, the following activities/tests are positive: alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase; lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, nacetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. In the API 20NE tests, negative for arginine dihydrolase, indole production, urease, oxidation and fermentation of D-glucose, assimilation of glucose, arabinose, mannose, mannitol, *N*-acetyl-glucosamine, maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate, phenylacetic acid, malate. Able to grow by using the following sole carbon sources in the Biolog GNIII MicroPlate: D-fructose, glycerol, L-arginine, glucuronamide, L-lactic acid, D-malic acid, L-malic acid, Tween 40, β-hydroxy- D, L-butyric acid,

acetoacetic acid, propionic acid, and acetic acid; and it can survive in environments which have potassium tellurite, aztreonam and sodium butyrate. The dominant fatty acids are $C_{18:1}$ ω9c, $C_{16:0}$, $C_{12:0}$ 3-OH and summed feature 3 (comprises $C_{16:1}$ ω7c and/ or $C_{16:1}$ ω6c). Minor amounts (2–6%) of $C_{17:0}$, summed feature 1 (comprises $C_{13:0}$ 3-OH and/or iso-$C_{15:1}$ H) and summed feature 3 (comprises $C_{16:1}$ ω7c and/ or $C_{16:1}$ ω6c) are also detected. The polar lipid profile comprises diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, unidentified phospholipid, two unidentified aminolipids and six unidentified polar lipids. The genomic DNA G+C content is 57.4 mol%.

The type strain, LZ-8^T (= CCTCC AB 2018386^T = KCTC 72198^T), was isolated from the cultivable phycosphere microbiota of highly-toxic dinoflagellate *Alexandrium catenella* LZT09 which was collected in Zhoushan Archipelago area in the East China Sea, China, during an algal bloom occurred in July of 2018, and routinely cultured in the ABI Laboratory. The GenBank/EMBL/DDBJ accession numbers for 16S rRNA gene sequence and draft genome sequence of strain LZ-8^T are MK092986 and SWKM00000000, respectively.

Author contributions XZ conceived the project and designed the experiments; QY, QF, BZ, JG and QX performed the experiments; QY, ZS, and XZ analyzed the data; QY and XZ drafted and revised the manuscript. All authors have read and approved the final version of the manuscript.

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Declarations

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

References

Ahmad W, Zheng Y, Li Y, Sun W, Hu Y, He X, Liu R, Xue CX, Zhang XH (2020) *Marinobacter salinexigens* sp. nov., a marine bacterium isolated from hadal seawater of the Mariana Trench. Int J Syst Evol Microbiol 70(6):3794–3800

Ahmed E, Holmstrom SJM (2014) Siderophores in environmental research: roles and applications. Microb Biotechnol 7:196–208

Amin SA, Parker MS, Armbrust EV (2012) Interactions between diatoms and bacteria. Microbiol Mol Biol Rev 76:667–684

Anton B, Sergey N, Dmitry A, Alexey AG, Mikhail D, Alexander SK, Valery ML, Sergey IN, Son P, Andrey DP, Alexey VP, Alexander VS, Nikolay V, Glenn T, Max AA, Pavel AP (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477

Beveridge TJ, Lawrence JR, Murray RGE (2007) Sampling and staining for light microscopy. In: Reddy CA, Beveridge TJ, Breznak TA et al (eds) Methods for general and molecular microbiology. American Society for Microbiol, Washington, pp 19–33

Chernikova TN, Bargiela R, Toshchakov SV, Shivaraman V, Lunev EA, Yakimov MM, Thomas DN, Golyshin PN (2020) Hydrocarbon-degrading bacteria *Alcanivorax* and *Marinobacter* associated with microalgae *Pavlova lutheri* and *Nannochloropsis oculata*. Front Microbiol 11:2650

Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, Rooney AP, Yi H, Xu XW, De Meyer S, Trujillo ME (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68:461–466

Cilia V, Lafay B, Christen R (1996) Sequence heterogeneities among 16S ribosomal RNA sequences, and their effect on phylogenetic analyses at the species level. Mol Biol Evol 13:451–461

Duan Y, Jiang Z, Wu Z, Sheng Z, Yang X, Sun J, Zhang X, Yang Q, Yu X, Yan J (2020) *Limnobacter alexandrii* sp. nov., a thiosulfate-oxidizing, heterotrophic and EPS-bearing *Burkholderiaceae* isolated from cultivable phycosphere microbiota of toxic *Alexandrium catenella* LZT09. Antonie Van Leeuwenhoek 113:1689–1698

Gauthier MJ, Lafay B, Christen R, Fernandez L, Acquaviva M, Bonin P, Bertrand JC (1992) *Marinobacter hydrocarbonoclasticus* gen. nov., sp. nov., a new, extremely halotolerant, hydrocarbon-degrading marine bacterium. Int J Syst Bacteriol 42:568–576

Green DH, Bowman JP, Smith EA, Gutierrez T, Bolch CJ (2006) *Marinobacter algicola* sp. nov., isolated from laboratory cultures of paralytic shellfish toxin-producing dinoflagellates. Int J Syst Evol Microbiol 56:523–527

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 42:457–469

Kaeppel EC, Gardes A, Seebah S, Grossart HP, Ullrich MS (2012) *Marinobacter adhaerens* sp. nov., isolated from marine aggregates formed with the diatom *Thalassiosira weissflogii*. Int J Syst Evol Microbiol 62:124–128

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M (1984) An integrated procedure for the extraction of bacterial iso-prenoid quinones and polar lipids. J Microbiol Methods 2:233–241

Na SI, Kim YO, Yoon SH, Ha SM, Baek I, Chun J (2018) UBCG: up-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction. J Microbiol 56:281–285

Ng HJ, López-Pérez M, Webb HK, Gomez D, Sawabe T, Ryan J, Vyssotski M, Bizet C, Malherbe F, Mikhailov VV, Crawford RJ, Ivanova EP (2014) *Marinobacter salarius* sp. nov. and *Marinobacter similis* sp. nov., isolated from sea water. PLoS ONE 9(9):e106514

Rani S, Koh HW, Kim H, Rhee SK, Park SJ (2017) *Marinobacter salinus* sp. nov., a moderately halophilic bacterium isolated from a tidal flat environment. Int J Syst Evol Microbiol 67(2):205–211

Romanenko LA, Schumann P, Rohde M, Zhukova NV, Mikhailov VV, Stackebrandt E (2005) *Marinobacter bryozoorum* sp. nov. and *Marinobacter sediminum* sp. nov., novel bacteria from the marine environment. Int J Syst Evol Microbiol 55:143–148

Seymour JR, Amin SA, Raina JB, Stocker R (2017) Zooming in on the phycosphere: the ecological interface for phytoplankton-bacteria relationships. Nat Microbiol 2:1–12

Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J (2016) NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624

Yang Q, Jiang Z, Huang C, Zhang R, Li L, Yang G, Feng L, Yang G, Zhang H, Zhang X, Mu J (2018a) *Hoeflea prorocentri* sp. nov., isolated from a culture of the marine dinoflagellate *Prorocentrum mexicanum* PM01. Antonie Van Leeuwenhoek 111:1845–1853

Yang Q, Zhang X, Li L, Zhang R, Feng L, Zhang R, Feng L, Mu J (2018b) *Ponticoccus alexandrii* sp. nov., a novel bacterium isolated from the marine toxigenic dinoflagellate *Alexandrium minutum*. Antonie Van Leeuwenhoek 111:995–1000

Yang Q, Jiang Z, Zhou X, Xie Z, Wang Y, Wang D, Feng L, Yang G, Ge Y, Zhang X (2020a) *Saccharospirillum alexandrii* sp. nov., isolated from the toxigenic marine dinoflagellate *Alexandrium catenella* LZT09. Int J Syst Evol Microbiol 70(2):820–826

Yang Q, Jiang Z, Zhou X, Zhang R, Xie Z, Zhang S, Wu Y, Ge Y, Zhang X (2020b) *Haliea alexandrii* sp. nov., isolated from phycosphere microbiota of the toxin-producing dinoflagellate *Alexandrium catenella*. Int J Syst Evol Microbiol 70(2):1133–1138

Yang X, Jiang Z, Zhang J, Zhou X, Zhang X, Wang L, Yu T, Wang Z, Bei J, Dong B, Dai Z, Yang Q, Chen Z (2020c) *Mesorhizobium alexandrii* sp. nov., isolated from phycosphere microbiota of PSTs-producing marine dinoflagellate *Alexandrium minutum* amtk4. Antonie Van Leeuwenhoek 113:907–917

Zhang X, Ma L, Tian X, Huang H, Yang Q (2015a) Biodiversity study of intracellular bacteria closely associated with paralytic shellfish poisoning dinoflagellates *Alexandrium tamarense* and *A. minutum*. Int J Environ Resour 4:23–27

Zhang X, Tian X, Ma L, Feng B, Liu Q, Yuan L, Fan C, Huang H, Yang Q (2015b) Biodiversity of the symbiotic bacteria associated with toxic marine dinoflagellate *Alexandrium tamarense*. J Biosci Med 3:23–28

Zhang X, Yang X, Wang S, Jiang J, Xie Z, Zhang L, Dai J, Fan C, Tian X, Yang Q (2020) Draft genome sequences of nine cultivable heterotrophic proteobacteria isolated from phycosphere microbiota of toxic *Alexandrium catenella* LZT09. Microbiol Resour Announc 9:e00281-e320

Zhou X, Zhang X, Jiang Z, Yang X, Zhang X, Yang Q (2021) Combined characterization of a new member of *Marivita cryptomonadis*, strain LZ-15-2 isolated from cultivable phycosphere microbiota of toxic HAB dinoflagellate *Alexandrium catenella* LZT09. Braz J Microbiol. https://doi.org/10.1007/s42770-021-00463-w