

***Maricaulis alexandrii* sp. nov., a novel dimorphic prosthecate and active biofloculants-bearing bacterium isolated from phycosphere microbiota of laboratory cultured highly-toxic *Alexandrium catenella* LZT09**

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Research Article

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Abstract

An aerobic, Gram-stain-negative, straight or curved rods, prosthecate bacterium designated as LZ-16-1^T was isolated from phycosphere microbiota of highly-toxic and laboratory cultured dinoflagellate *Alexandrium catenella* LZT09. This new isolate produces active bioflocculating exopolysaccharides (EPS). Cells were dimorphic with non-motile prostheca, or non-stalked and motile by a single polar flagellum. Growth occurred at 10-40 °C, pH 5–9 and 1–8% (w/v) NaCl, with optimum growth at 25 °C, pH 7–8 and 2-4% (w/v) NaCl, respectively. Phylogenetic analysis based on 16S rRNA indicated that strain LZ-16-1^T was affiliated to the genus *Maricaulis*, and closely related to *Maricaulis parjimensis* MCS 25^T (99.48%) and *M. virginensis* VC-5^T (99.04%),. However, based on genome sequencing and phylogenomic calculations, the average nucleotide identity (ANI) and digital DNA-DNA genome hybridization (dDDH) values between the two strains were only 85.0 and 20.9%, respectively. Strain LZ-16-1^T owned Q-10 as predominant isoprenoid quinone; summed feature 8, C_{16:0}, C_{17:0}, C_{18:0}, C_{18:1} ω_{9c} and summed feature 9 as dominant fatty acids; and sulfoquinovosyl diacylglycerol, glycolipids and unidentified phospholipid as major polar lipids. The genomic DNA G+C content is 63.6 mol%. Physiological and chemotaxonomic characterization further confirmed the distinctiveness of strain LZ-16-1^T from other *Maricaulis* members. Thus, strain LZ-16-1^T represents a novel species of the genus *Maricaulis*, for which the name *Maricaulis alexandrii* sp. nov. (type strain LZ-16-1^T=KCTC 72194^T=CCTCC AB 2019006^T) is proposed .

Introduction

The genus of *Maricaulis* was originally derived from the genus *Caulobacter* within the family *Hyphomonadaceae* of the order *Rhodobacterales*, and composed of dimorphic and prosthecate bacteria (Abraham et al. 1999; 2002). Recently, the genus *Maricaulis* has been transferred to the newly-proposed family *Maricaulaceae* in the order *Maricaulales* by Kevbrin et al (2021). At the time of writing, the genus of *Maricaulis* contains five validly named species all isolated from seawater (<https://lpsn.dsmz.de/genus/maricaulis>) (Abraham et al. 2002). The division mode representing unique regulation feature of prokaryotic reproduction cycle of the dimorphic prosthecate bacteria comes from their evolution to oligotrophic habitats by minimizing competition under limited resources circumstances. *Maricaulis* are ubiquitous in aquatic environments with vital ecologically importance responsible for considerable mineralization of dissolved organic material (DOM) in water especially when the nutrient concentrations are low (Jannasch and Jones, 1960).

Marine phycosphere as the boundary of phytoplankton holobionts harbors dynamic host-microbe interactions and play crucial roles in aquatic ecosystems (Amin et al. 2012; Seymour et al. 2017; Zhang et al. 2020). To unveil the nature of those cross-kingdom associations, we initiated the Phycosphere Microbiome Project (PMP) to convey the microbial structures of phycosphere microbiota (PM) of diverse harmful algal blooms (HAB) dinoflagellates (Duan et al. 2020; Yang et al. 2018a, 2018b, 2020a, 2020b; Yang et al. 2020; Zhang et al. 2015b, 2020; Zhou et al. 2021). During the subsequent culture-dependent investigation which is a crucial prerequisite for the interactions study, a novel cultivable dimorphic and

prothecate bacterium designated as LZ-16-1^T was isolated from one dominant PM of *Alexandrium catenella* LZT09 (Fig. S1), which is a routinely laboratory cultured HAB dinoflagellate that produces high levels of paralytic shellfish poisoning toxins (PSTs). This new isolate produces active bioflocculating exopolysaccharides (EPS) (Fig. S2) demonstrating potential environmental and biological applications (Mu et al. 2019). In this study, we described the polyphasic characterization of strain LZ-16-1^T to represent a novel species of the genus *Maricaulis*.

Materials And Methods

Bacterial strains and culture conditions

Strain LZ-16-1^T was isolated from *Alexandrium catenella* LZT09 by spreading the algal culture on marine agar (MA, Difco) plates according to our protocol described previously (Yang et al. 2018a, 2018b, 2020). The strain was purified and maintained on MA and preserved as a glycerol suspension (20%, v/v) at -80 °C for long term preservation. For the comparative analysis to ensure the taxonomic position of the new isolate, five type strains of the genus *Maricaulis* were used as the reference strains for phenotypic and chemotaxonomic analysis: *M. parjimensis* MCS 25^T (=CIP 107440^T =DSM 16078^T =LMG 19863^T), *M. virginensis* VC-5^T (=CIP 107438^T =DSM 16079^T =LMG 21018^T =VKM B-1513^T), *M. maris* CM 11^T (=ATCC 15268^T =CIP 106103^T =DSM 4729^T =DSM 4734^T =JCM 21108^T =NBRC 102484^T =VKM B-1510^T), *M. washingtonensis* MCS 6^T (=CIP 107441^T =DSM 16076^T =LMG 19865^T), and *M. salignorans* MCS 18^T (=CIP 107439^T =DSM 16077^T =LMG 19864^T) were all obtained from German Collection of Microorganisms and Cell Cultures (DSMZ, Germany).

Phenotypic characterizations

Phenotypic characterizations were performed as described previously by Macián et al. (2005). Cell morphology of an exponentially growing culture of strain LZ-16-1^T was observed by transmission electron microscopy (JEM-1200; JEOL, Tokyo, Japan) using cultures grown on MA at 25 °C for 3 days. Motility was examined microscopically under the phase-contrast mode by the hanging drop technique. Gram-staining test was determined as described previously (Beveridge et al. 2007). 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 MB medium supplemented with 0–12% NaCl (w/v) at 25 °C for two weeks on a rotary shaker. Utilization of carbon sources and enzyme activities were tested using the API 20E and API ZYM (bioMérieux, Marcy-l'Étoile, France) strips following the manufacturer's instructions.

16S rRNA gene phylogenetic analysis

The extraction of genomic DNA, PCR amplification of the 16S rRNA gene using the universal primer of 27F/1492R, and sequencing of the PCR product were performed as described previously (Yang et al. 2018a, 2018b). The 16S rRNA gene sequence similarities were compared with the sequences of the type

strains available from GenBank using the BLAST and Ez-Taxon server (<https://www.ezbiocloud.net>). Phylogenetic analysis of the 16S rRNA gene sequences of strain LZ-16-1^T (GenBank accession MK100326) and the reference type strains were performed using MEGA software version 7.0 (Kumar et al. 2016). Phylogenetic trees were reconstructed by neighbour-joining (NJ), maximum likelihood (ML) and maximum parsimony (MP) algorithms using bootstrap analysis based on 1000 replications (Yang et al. 2018a, 2018b). The Kimura's two-parameter model is used to generate the distance matrix (Kimura 1980).

Genomic sequencing and phylogenomic comparison

The genome sequencing, assembling and annotation of the genome of strain *M. parjimensis* MCS 25^T was performed as reported previously (Zhang et al. 2020; Zhou et al. 2021) at Major Bioscience (Shanghai) 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 using the default parameters (Tatusova et al. 2016). The average nucleotide identity (ANI) and digital DNA-DNA genome hybridization (dDDH) values between strain LZ-16-1^T and the close relatives were calculated using the ANI/AAIMatrix genome-based distance matrix calculator (<http://enve-omics.ce.gatech.edu/g-matrix>) and the online genome-to-genome distance calculator (version 2.1) (<http://ggdc.dsmz.de>).

Chemotaxonomic characterization

For the extraction of the quinones and polar lipids, the strains were harvested at stationary growth phase after growth in marine broth (MB) for 2 days at 25 °C. Polar lipids were extracted and separated by two-dimensional TLC on silica gel 60 F254 plates (Merck 5554) and then analyzed by the method of Minnikin et al. (1984). The respiratory quinone was isolated, purified and identified according to the method (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).

Results And Discussion

Morphological, physiological and biochemical analyses

The morphological observation of strain LZ-16-1^T were performed on MA plates and formed circular, smooth and white colonies with 1.5–2.5 mm in diameter after 48h incubation at 25 °C. Cells of strain LZ-16-1^T were Gram-stain-negative, aerobic, oxidase-negative but catalase-positive, straight or curved rod-shaped. Cells usually possess a prostheca ca. 0.15 µm in diameter and of varying length extending from one pole as a continuation of the long axis of the cell (Fig. 1). At the time of separation one cell possess a prostheca and the other a single polar flagellum. Based on the phenotypic characteristics (Table 1), it can distinguish strain LZ-16-1^T from other type strains of the genus *Maricaulis*. Strain LZ-16-1^T grew at a temperature range of 10–40°C and a pH range of 5.0–9.0 (Table 1). The optimal conditions turned out to

be pH 7.0 and 25-30°C. The observed optimum temperature of LZ-16-1^T is lower compared to *M. parjimensis* MCS 25^T (30-40°C) and *M. virginensis* VC-5^T (20-40°C).

Chemotaxonomic profiles

The major cellular fatty acids of strain LZ-16-1^T were summed feature 8 (C_{18:1} ω7c and/or C_{18:1} ω6c, 41.6%), C_{16:0} (10.4%), C_{18:0} (10.5%), C_{18:1} ω9c (6.1%) and summed feature 9 (iso-C_{17:1} ω9c and/or C_{16:0} 10-methyl, 5.9%). Detailed fatty acid profiles of strain LZ-16-1^T and five type strains of the genus *Maricaulis* were shown in Table 2. The major fatty acids compositions of strain LZ-16-1^T were similar to those of other *Maricaulis* members (Abraham *et al.*, 2002). Moreover, both C_{15:0} and C_{16:1} ω9c were absent in strains LZ-16-1^T, *M. parjimensis* MCS 25^T and *M. virginensis* VC-5^T. However, total 17 trace amounts fatty acids components, C_{10:0} 3OH, C_{11:0}, C_{14:0}, C_{16:1} ω5c, C_{18:1} ω5c, C_{20:1} ω7c, C_{20:1} ω9c, iso-C_{15:1} F, iso-C_{17:1} ω5c, so-C_{15:0}, anteiso-C_{17:0}, iso-C_{19:1}, iso-C_{19:0}, C_{10:0} 2-OH, C_{11:0} 3-OH, C_{12:1} 3-OH and C_{18:1} ω7c 11-methyl, were only found in strain LZ-16-1^T. That's a notably different profile for strain LZ-16-1^T compared with the other *Maricaulis* members. The predominant isoprenoid quinone was Q-10, which is identical to other *Maricaulis* members (Abraham *et al.*, 1999, 2002). Polar lipids of strain LZ-16-1^T were composed of one sulfoquinovosyl diacylglycerol (SQDG), six glycolipids (GLs), one unidentified phospholipid (PL) and one unidentified polar lipid (L) (Fig. S3), which were also observed in other *Maricaulis* species (Abraham *et al.*, 1999, 2002).

Phylogenetic analysis

Base on the 16S rRNA gene sequence similarity comparison, strain LZ-16-1^T was affiliated to the genus *Maricaulis* and shared high 16S gene similarities with *M. parjimensis* MCS 25^T (99.5%), *M. virginensis* VC-5^T (99.0%), *M. maris* CM 11^T (96.1%), *M. washingtonensis* MCS 6^T (93.3%) and *M. salignorans* MCS 18^T (93.2%), respectively. As shown in the neighbour-joining (NJ) tree based on 16S rRNA gene sequences, strain LZ-16-1^T felled within the clade comprising type species of the genus *Maricaulis*, and occupied a branch related to a cluster formed by *M. virginensis* VC-5^T and *M. parjimensis* MCS 25^T (Fig. 2).

Comparative genomic characteristics

Due to the high 16S gene sequence similarity between strains LZ-16-1^T and *M. parjimensis* MCS 25^T, the whole-genome of the type strain of *M. parjimensis* was sequenced and submitted to GenBank with the accession no. JAEMQH000000000. As shown in Table 3, the draft genome size of strain *M. parjimensis* MCS 25^T was 3,348,930 bp with 17 contigs with an N_{50} of 406 kb. It has 3,157 protein-coding genes with the DNA G+C content of 62.9 mol% calculated from the genome. The genome of LZ-16-1^T has a size of 3,348,699 bp which is very close to *M. parjimensis* (3,348,930 bp), whereas the GC content of strain LZ-16-1^T (63.6%) is slightly higher. Automated gene prediction and annotation identified 3,146 putative protein-encoding genes, of which 26.04% (834 genes) are annotated as hypothetical proteins. For strains

LZ-16-1^T and *M. parjimensis* MCS 25^T, total 2,286 genes (72.6%) and 2,263 genes (73.0%) were functionally annotated within COG database, respectively (Fig. S4, pane a). However, only 1,486 (47.2%) and 1,205 genes (38.9%) were annotated by KEGG for metabolic pathways, indicating that a number of functional genes were still indistinct (Fig. S4, pane b). In addition, strain LZ-16-1^T showed lesser number of genes encoding for carbohydrate-active enzymes (CAZymes) compared to strain *M. parjimensis* MCS 25^T (Fig. S4, pane c). Similar profile was also observed for the comparison of gene annotation by COG database which resulted in 27 functional categories (Fig. S3, pane b), although the two strains demonstrated only 231 bp difference between their genomic sizes.

In order to identify the multiple maximal matches and local collinear blocks (LCBs), a multiple whole-genome alignment of genome sequences of strains LZ-16-1^T and *M. parjimensis* MCS 25^T were performed using PATRIC software (www.patricbrc.org). The LCBs alignments of two strains resulted in 34 rearranged pieces larger than 1 kb, and it demonstrated obviously difference from each other (Fig. S5). It indicates a clear-cut genomic dissimilarity between the two strains. Moreover, the phylogenomic calculations of ANI and dDDH values between strains LZ-16-1^T and *M. parjimensis* MCS 25^T were only 85.0 and 20.9%, respectively (Table 1). Both values were clearly far below the threshold values for species delineation (Chun et al. 2018). Therefore, it strongly indicated that strain LZ-16-1^T represents a novel species of the genus *Maricaulis*.

Based on the genome annotation, the discrete biosynthetic components for holdfast polysaccharide synthesis, flagellar motility and type II pilus assembly were identified in the genome of strain LZ-16-1^T (Table S1). EPS has been revealed to serve as one vital chemical intermedia within microscopic phycosphere niches, and mediates the host-microbe interactions involving in those cross-kingdom exchanges of nutrients, infochemicals and gene transfer agents (Amin et al. 2012; Seymour et al. 2017; Zhang et al. 2020). Remarkably, the biofloculating activity of EPS produced by strain LZ-16-1^T was discovered by our bioactivity assay (Fig. S1) (Mu et al. 2019). Correspondingly, series of genes (*wza*, *exo* and *muc*) responsible for bacterial EPS biosynthesis were found in the genome of strain LZ-16-1^T (Table S1). Thus, we proposed that strain Z10-6^T could serve as a novel bacterial candidate with natural potential for the production of promising and versatile biofloculants (Duan et al. 2020; Yang et al. 2020; Zhang et al. 2020).

Taxonomic conclusion

Based on the polyphasic evidences by phenotypic characterization, phylogenetic and genome comparison, and chemotaxonomic analysis, strain LZ-16-1^T clearly represents a novel species of the genus *Maricaulis*, for which the name *Maricaulis alexandrii* sp. nov. is proposed.

Description of *Maricaulis alexandrii* sp. nov.

Maricaulis alexandrii (a.le.xan'dri.i. N.L. gen. n. *alexandrii* of the dinoflagellate *Alexandrium catenella*, the source of the 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. Cells usually possess a prostheca. ca. 0.15 μm in diameter and of varying length extending from one pole as a continuation of the long axis of the cell. At the time of separation one cell possess a prostheca and the other a single polar flagellum. They form white, smooth-rounded colonies of about 1.0-2.0 mm in diameter on MA after 48 h incubation at 25 °C. Temperature, salt and pH ranges for growth are 10-40 °C, 1.0-8.0% (w/v) NaCl and pH 5.0-9.0, respectively. Optimal growth occurs at 2-4% (w/v) NaCl, 25-30 °C and pH 7.0. Negative for oxidase and positive for catalase activities. Do not reduce nitrate, oxidize tryptophan to indole or hydrolyse arginine, urea, aesculin and gelatin. Cells are positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase; but negative for valine arylamidase, cystine arylamidase, acid phosphatase, α-glucosidase, lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. Cells are negative for H₂S production, urease, indole production, eelatinase, acetoin production, β-galactosidase, lysine decarboxylase, ornithine decarboxylase, fermentation of glucose, mannitol and rhamnose. Able to grow using D-glucose-6-PO₄ or D-melibiose as sole carbon source. Dominant fatty acids are summed feature 8, C_{16:0}, C_{18:0}, C_{18:1} ω_{9c} and summed feature 9. Polar lipids are sulfoquinovosyl diacylglycerol and glycolipids, also minor amount of unidentified phospholipid and unidentified polar lipid. The genomic DNA G+C content is 63.6 mol%.

The type strain, LZ-16-1^T (=CCTCC AB 2018386^T =KCTC 72198^T), was isolated from the cultivable phycosphere microbiota of highly-toxic harmful algal blooms dinoflagellate *Alexandrium catenella* LZT09, which was collected in Zhoushan Archipelago ares in the East China Sea during an algal bloom occurred in July of 2018, and then routinely cultured in ABI Laboratory. The GenBank/EMBL/DDBJ accession numbers for 16S rRNA gene sequence and draft genome sequence of strain LZ-16-1^T are MK100326 and SWKP00000000, respectively.

Electronic Supplementary Materials

The online version of this article contains supplementary material, which is available to authorized users.

Declarations

- Author contributions

QY designed the experiments; XZ, MQ and QL performed the experiments; XZ, ZC and QY analyzed the data; XZ and QY drafted and revised the manuscript. All authors have read and approved the final version of the manuscript.

- Funding

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- Conflicts of interest/Competing interests

The authors declare that there are no conflicts of interest.

- Ethics approval (include appropriate approvals or waivers)

Not applicable

- Consent to participate (include appropriate statements)

Not applicable

- Consent for publication (include appropriate statements)

All authors have read and approved the manuscript.

- Availability of data and material (data transparency)

Strain LZ-16-1^T has been deposited in two culture centers (CCTCC in China, and KCTC in South Korea) with the deposition no. CCTCC AB 2019006^T and KCTC 72194^T. The GenBank/EMBL/DDBJ accession numbers for 16S rRNA gene sequence of strain LZ-16-1^T is MK100326, and for draft genome sequences of strains LZ-16-1^T and *Maricaulis parjimensis* MCS 25^T are SWKP000000000 and JADOTT000000000, respectively.

- Code availability (software application or custom code)

Not applicable

The GenBank/EMBL/DDBJ accession numbers for 16S rRNA gene sequence of strain LZ-16-1^T is MK100326, and for draft genome sequences of strains LZ-16-1^T and *Maricaulis parjimensis* MCS 25^T are SWKP000000000 and JADOTT000000000, respectively.

Abbreviations

ABI, Algae-bacteria interaction

ANI, Average nucleotide identity

dDDH, digital DNA-DNA hybridization

EPS, Exopolysaccharides

GL, Glycolipid

HAB, Harmful algal blooms

LCBs, local collinear blocks

MA, Marine agar

MP, Maximum parsimony

NJ, Neighbour joining

PG, Phosphatidylglycerol

PL, Phospholipids

PM, Phycosphere microbiota

PMP, Phycosphere Microbiome Project

PSTs, Paralytic shellfish poisoning toxins

SQDG, sulfoquinovosyl diacylglycerol

References

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

Abraham WR, Strompl C, Meyer H, Lindholst S, Moore ERB, Christ R, Vancanneyt M, Tindall BJ, Bannasar A, Smit J, Tesar M (1999) Phylogeny and polyphasic taxonomy of *caulobacter* species. proposal of *maricaulis* gen. nov. with *maricaulis maris* (poindexter) comb. nov. as the type species, and emended description of the genera *brevundimonas* and *caulobacter*. *Int J Syst Bacteriol* 49(3):1053-1073

Abraham, W. R., Strmpl, C., Bannasar, A., Vancanneyt, M., & Moore, E. R. B. (2002). Phylogeny of *maricaulis abraham* et al. 1999 and proposal of *maricaulis virginensis* sp. nov. *M. parjimensis* sp. nov. *M. washingtonensis* sp. nov. and *M. salignorans* sp. nov. *Int J Syst Evol Microbiol* 52(6):2191-2201

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 p19-33

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

- 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
- 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
- Jannasch HW and Jones GE (1960) *Caulobacter* in sea water. *Limnol Oceanogr* 5:432-433
- Kevbrin V, Boltyanskaya Y, Koziyeva V, Uzun M, Grouzdev D (2021) *Alkalicaulis satelles* gen. nov., sp. nov., a novel haloalkaliphile isolated from a laboratory culture cyanobacterium *Geitlerinema* species and proposals of *Maricaulaceae* fam. nov., *Robiginitomaculaceae* fam. nov., *Maricaulales* ord. nov. and *Hyphomonadales* ord. nov. *Int J Syst Evol Microbiol* 71(1) <https://doi.org/10.1099/ijsem.0.004614>.
- Kimura M (1980) A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. *J Mol Evol* 16:111-120
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870-1874
- Macián MC, Arahál DR, Garay E, Ludwig W, Schleifer KH, Pujalte MJ (2005) *Thalassobacter stenotrophicus* gen. nov., sp. nov., a novel marine α -*proteobacterium* isolated from Mediterranean sea water. *Int J Syst Evol Microbiol* 55:105–110
- 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
- Mu J, Wang D, Yang G, Cui X, Yang Q (2019) Preparation and characterization of a substitute for *Ruditapes philippinarum* conglutination mud as a natural biofloculant. *Bioresour Technol* 281:480-484
- Seymour JR, Amin SA, Raina JB, Stocker R (2017) Zooming in on the phycosphere: the ecological interface for phytoplankton-bacteria relationships. *Nat Microbiol* 2:17065
- 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, Feng Q, Zhang B, Gao J, Sheng Z, Xue Q, Zhang X (2021) *Marinobacter alexandrii* sp. nov., a novel yellow-pigmented and algae growth-promoting bacterium isolated marine phycosphere microbiota. *Antonie van Leeuwenhoek*, 2021.

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 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 (2020) *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 Env 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:e00281-20

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* In Press

Tables

Table 1. Differential characteristics of strain LZ-16-1^T and other type strains of the genus *Maricaulis* with validly published names.

Strains: 1, LZ-16-1^T; 2, *M. parjimensis* MCS 25^T; 3, *M. virginensis* VC-5^T; 4, *M. maris* CM 11^T; 5, *M. washingtonensis* MCS 6^T; 6, *M. salignorans* MCS 18^T.

All data were obtained from this study unless otherwise indicated. All strains were aerobic and rod-shaped and positive for catalase. For API 20E tests, all strains were negative for H₂S production, urease, indole production, eelatinase, acetoin production, but positive for arginine dihydrolase, citrate utilization; For API ZYM test, all strains were negative for α -galactosidase, β -galactosidase, α -glucuronidase, α -mannosidase, α -fucosidase and N-acetyl- β -glucosaminidase, but positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase. +, Positive; -, negative; w, weak positive

Characteristic	1	2	3	4	5	6
Isolate source	Marine phycosphere microbiota	Seawater, Indian Ocean off Goa, India	Deep sea, Virgin Islands, USA	Filtered seawater	Seawater, Inner Marina, Edmonds, WA, USA	Seawater, Salsbury Point County Park, WA, USA
Temperature growth range (°C, optimal)	10-40 (25-30)	15-45 (30-40)	15-40 (30-40)	15-45 (30-40)	15-45 (30-40)	10-50 (20-40)
pH growth range (optimal)	5.0-9.0 (7.0)	6.0-8.0 (7.0)	6.0-8.0 (7.0)	6.0-8.0 (7.0)	6.0-8.0 (7.0)	6.0-8.0 (7.0)
NaCl growth range (% w/v)	1.0-8.0 (2-4)	0.5-10 (2-8)	0.5-10 (2-6)	0.5-10 (2-6)	0-8 (2-4)	0-8 (2-6)
Oxidase	-	+	+	+	+	+
API 20E tests						
β-Galactosidase	-	+	-	+	+	+
Lysine decarboxylase	-	+	-	+	-	-
Ornithine decarboxylase	-	-	-	+	+	+
Tryptophane deaminase	+	-	-	+	+	+
Fermentation of glucose	-	-	-	-	-	-
Mannitol	-	-	-	-	+	-
Inositol	+	+	-	W	-	-
Sorbitol	+	-	W	-	W	-
Rhamnose	-	+	-	+	-	-
Sucrose	+	-	+	W	-	W
Melibiose	+	W	-	-	-	-
Amygdalin	+	+	-	-	W	-
Arabinose	+	-	W	-	+	-
API 20 ZYM tests						
Lipase (C14)	-	W	W	W	W	W

Valine arylamidase	-	+	+	+	w	+
Cystine arylamidase	-	+	+	+	w	+
Trypsin	-	+	+	w	+	+
α -Chymotrypsin	-	+	+	+	+	+
Acid phosphatase	-	+	w	-	-	-
β -Glucuronidase	-	w	+	+	+	+
α -Glucosidase	-	w	-	-	-	-
β -Glucosidase	-	+	-	-	-	-
DNA G+C mol%	63.6	62.9 ^a / 63.0 ^b	65.2 ^b	63.3 ^c	63.0 ^b	63.3 ^b

Data from: ^a, this study, ^bAbraham *et al.* (2002); c, Abraham *et al.* (1999).

Table 2. Cellular fatty acid compositions of strain LZ-16-1^T and the phylogenetically related type strains of the genus *Maricaulis*

All of the data were from this study. Strains: 1, LZ-16-1^T; 2, *M. parjimensis* MCS 25^T; 3, *M. virginensis* VC-5^T; 4, *M. maris* CM 11^T; 5, *M. washingtonensis* MCS 6^T; 6, *M. salignorans* MCS 18^T. Values represent percentage of total fatty acids contents. -, not detected; *tr*, trace amounts (<1 %)

Fatty acid	1	2	3	4	5	6
Saturated						
C _{10:0} 3OH	<i>tr</i>	-	-	-	-	-
C _{11:0}	<i>tr</i>	-	-	-	-	-
C _{14:0}	<i>tr</i>	-	-	-	-	-
C _{15:0}	-	-	-	<i>tr</i>	<i>tr</i>	<i>tr</i>
C _{16:0}	10.4	3.2	10.3	16.7	10.5	8.5
C _{17:0}	7.7	7.4	15.0	5.1	9.5	8.1
C _{18:0}	10.5	7.2	3.8	1.5	<i>tr</i>	<i>tr</i>
Unsaturated						
C _{16:1} ω 5c	<i>tr</i>	-	-	-	-	-
C _{16:1} ω 9c	-	-	-	1.2	<i>tr</i>	<i>tr</i>
C _{17:1} ω 8c	3.9	4.5	9.4	4.1	10.2	10.3
C _{17:1} ω 6c	1.6	1.5	1.1	<i>tr</i>	1.2	1.4
C _{18:1} ω 5c	<i>tr</i>	-	-	-	-	-
C _{18:1} ω 9c	6.1	6.0	3.4	6.4	10.7	7.7
C _{20:1} ω 7c	<i>tr</i>	-	-	-	-	-
C _{20:1} ω 9c	<i>tr</i>	-	-	-	-	-
Branched-chain						
iso-C _{15:1} F	<i>tr</i>	-	-	-	-	-
iso-C _{17:1} ω 5c	<i>tr</i>	-	-	-	-	-
iso-C _{15:0}	<i>tr</i>	-	-	-	-	-
iso-C _{17:0}	2.7	1.5	7.2	7.5	9.1	10.2
anteiso-C _{17:0}	<i>tr</i>	-	-	-	-	-
iso-C _{19:1}	<i>tr</i>	-	-	-	-	-
iso-C _{19:0}	<i>tr</i>	-	-	-	-	-

Hydroxy							
C _{10:0} 2-OH	<i>tr</i>	-	-	-	-	-	-
C _{11:0} 3-OH	<i>tr</i>	-	-	-	-	-	-
iso-C _{11:0} 3-OH	1.8	<i>tr</i>	5.8	2.2	<i>tr</i>	<i>tr</i>	
C _{12:1} 3-OH	<i>tr</i>	-	-	-	-	-	-
Methyl							
C _{18:1} ω 7c 11-methyl	<i>tr</i>	-	-	-	-	-	-
Summed feature*							
3	2.2	2.5	2.2	6.8	3.8	2.5	
8	41.6	47.1	12.8	22.8	15.8	10.3	
9	5.9	4.5	13.1	17.1	21.2	27.4	

* summed feature 3 contains C_{16:1} ω 7c and/or C_{16:1} ω 6c; summed feature 8 comprises C_{18:1} ω 7c and/or C_{18:1} ω 6c; summed feature 9 comprises iso-C_{17:1} ω 9c and/or C_{16:0} 10-methyl.

Table 3. Genomic features and comparison between strains LZ-16-1^T and *M. parjimensis* MCS 25^T

Attribute	<i>M. alexandrii</i> LZ-16-1 ^{T a}	<i>M. parjimensis</i> MCS 25 ^T
GenBank accession no.	SWKP000000000	JADOTT000000000
Genomic size (bp)	3,348,699	3,348,930
Number of contigs	12	17
<i>N</i> ₅₀ length (bp)	558,629	406,752
Protein coding genes	3,146	3,157
Coding ratio (%)	90.78	91.79
Repeat Region Count/rate (%)	0/0	255/0.63
rRNA	3	3
tRNA	42	45
Other RNA	4	4
Pseudogene	24	23
G+C content (mol%)	63.63	62.94
ANI/AAI/dDDH to LZ-16-1 ^T (%)	-/-/-	85.0/20.9

Figures

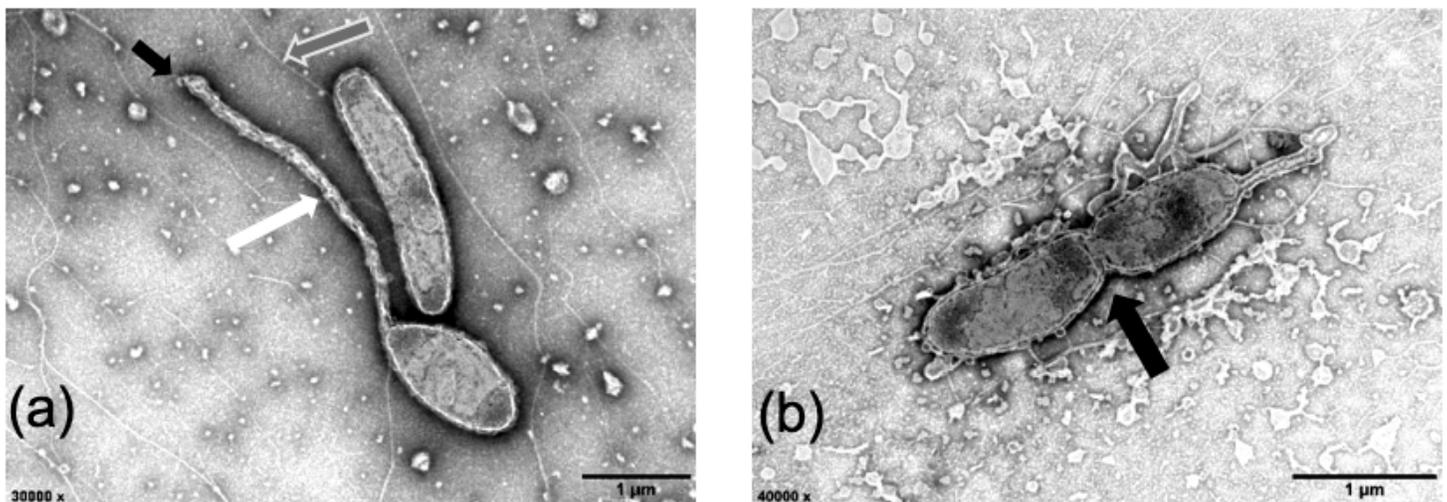


Figure 1

Transmission electron micrographs of the cells of strain LZ-16-1T (a) The two morphologies observed for strain Z-16-1T. First, a motile swarmer cell with monotrichous and monopolar flagellation (green arrowheads). Note the holdfast at the end of the stalk (black arrow). And, second, a non-motile, stalked

cell in a state of binary fission (white arrow). (b) The division of the cell. The black arrow indicates where binary fission is occurring. Bars, 1.0 μm .

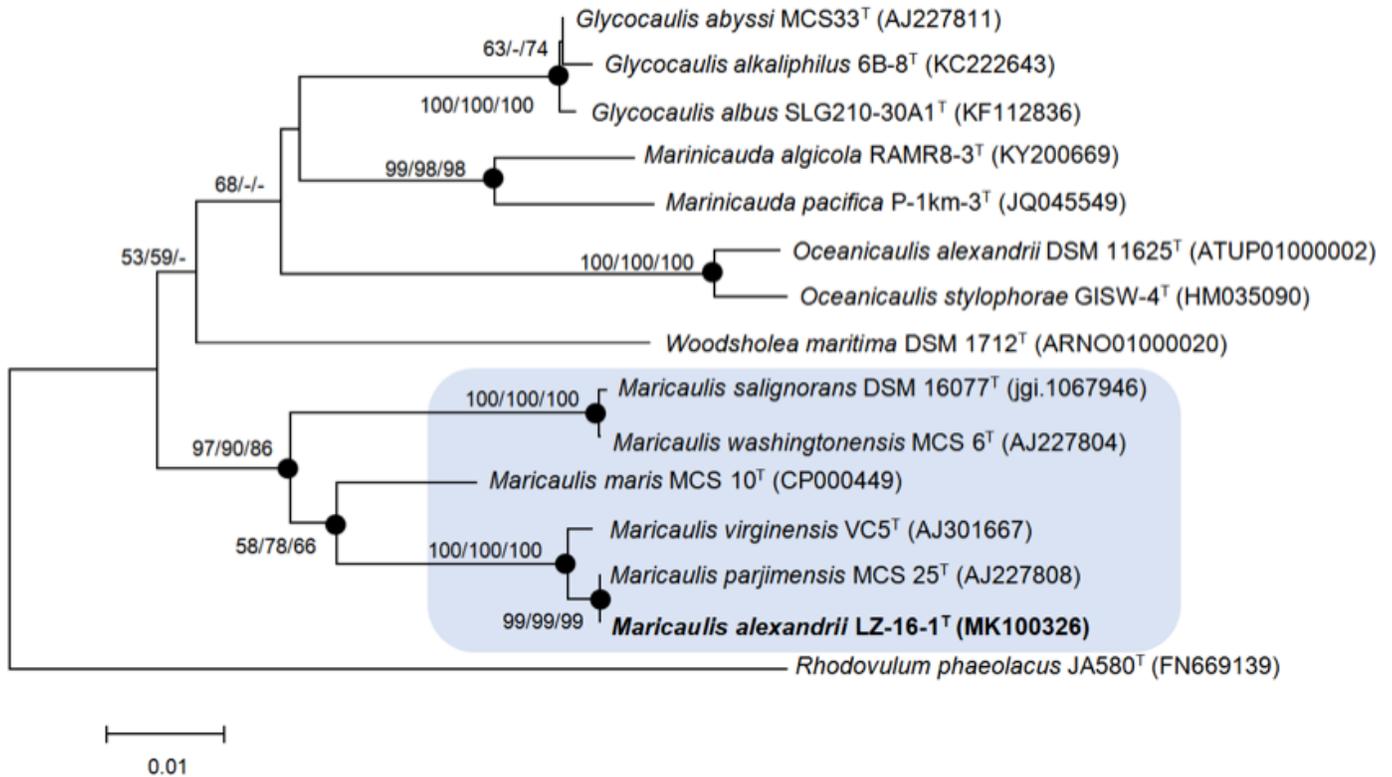


Figure 2

The constructed neighbour-joining (NJ) phylogenetic tree showing the phylogenetic position of strain LZ-16-1T and representatives of other related taxa based on 16S rRNA gene sequences. Filled circles indicate nodes that were also recovered in the maximum-parsimony (MP) tree and the maximum-likelihood (ML) tree based on the same gene sequences. Bootstrap values (expressed as percentages of 1000 replications) > 50% are shown at branching points for NJ/ML/MP trees. *Rhodovulum phaeolacus* JA580T was used as an outgroup. Bar, 0.01 nt substitution rate (Knuc) units.

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