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Marinobacterium zhoushanense sp. nov., isolated from surface seawater of the East China Sea

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Abstract:	A Gram-stain-negative, facultative anaerobic bacterium, designated WM3T, was isolated from surface seawater collected from the East China Sea. Cells were catalase and oxidase positive, short rods and motile by means of a single polar flagellum. Growth occurred at 15-43 ºC (optimum 37-40 ºC), pH 5.5-9.5 (optimum 6.5-7.5) and with 0.25-9.0% NaCl (optimum 1.0-1.5%, w/v). Chemotaxonomic analysis showed that the respiratory quinone was ubiquinone-8, the major fatty acids included C16:0 (23.6%), C18:1 ω7c (26.2%) and summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH, 22.1%). The phylogenetic analysis based on 16S rRNA gene sequences revealed that strainWM3T was most closely related to the genus Marinobacterium by sharing the highest sequence similarity of 95.5% with both M. litorale KCTC 12756T and M. mangrovicola DSM 27697T. The genomic DNA G+C content of the strain WM3T was 55.8 mol%. On the basis of its phenotypic, chemotaxonomic and genotypic characteristics presented in this study, strain WM3T is suggested to represent a novel species in genus Marinobacterium, for which the name Marinobacterium zhoushanense sp. nov. is proposed. The type strain is WM3T (=KCTC 42782T =CGMCC 1.15341T).

Marinobacterium zhoushanense sp. nov., isolated from surface seawater of the East China Sea

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Abbreviations: PHB, poly-*β*-hydroxybutyric.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain WM3^T is KT248536.

Summary

A Gram-stain-negative, facultative anaerobic bacterium, designated WM3^T, was isolated from surface seawater collected from the East China Sea. Cells were catalase and oxidase positive, short rods and motile by means of a single polar flagellum. Growth occurred at 15-43 ºC (optimum 37-40 ºC), pH 5.5-9.5 (optimum 6.5-7.5) and with 0.25-9.0% NaCl (optimum 1.0-1.5%, w/v). Chemotaxonomic analysis showed that the respiratory quinone was ubiquinone-8, the major fatty acids included $C_{16:0}$ (23.6%), $C_{18:1}$ *ω7c* (26.2%) and summed feature 3 ($C_{16:1}$*ω7c* and/or iso-$C_{15:0}$ 2-OH, 22.1%). The phylogenetic analysis based on 16S rRNA gene sequences revealed that strainWM3^T was most closely related to the genus *Marinobacterium* by sharing the highest sequence similarity of 95.5% with both *M. litorale* KCTC 12756^T and *M. mangrovicola* DSM 27697^T. The genomic DNA G+C content of the strain WM3^T was 55.8 mol%. On the basis of its phenotypic, chemotaxonomic and genotypic characteristics presented in this study, strain WM3^T is suggested to represent a novel species in genus *Marinobacterium*, for which the name *Marinobacterium zhoushanense* sp. nov. is proposed. The type strain is WM3^T (=KCTC 42782^T =CGMCC 1.15341^T).

The genus *Marinobacterium*, belonging to the family *Alteromonadaceae*, was originally proposed by Gonzalez *et al*. (1997). At the time of writing, the genus *Marinobacterium* contained 15 species and they were isolated from various environments: *M. georgiense* from pulp mill effluent (Gonzaez *et al.*, 1997), *M. halophilum* (Chang *et al.*, 2007), *M. rhizophilum* (Kim *et al.*, 2008), *M. lutimaris* (Kim *et al.*, 2010) and *M. aestuariivivens* (Park *et al.*, 2016) from tidal flats, *M. litorale* (Kim *et al.*, 2007) and *M. marisflavi* (Kim *et al.*, 2009a) from seawater, *M. nitratireducens*, *M. sediminicola* (Huo *et al.*, 2009), *M. maritimum* (Kim *et al*., 2009b) and *M. profundum* (Hwang *et al.*, 2016) from marine sediment, *M. coralli* from mucus of coral (Chimetto *et al.*, 2011), *M. mangrovicola* from mangrove roots (Alfaro-Espinoza & Ullrich, 2014), *M. jannaschii* and *M. stanieri* (Satomi *et al*., 2002) which were transferred from *Oceanospirillum jannaschii* and *Pseudomonas stanieri* were isolated from coastal seawater. Cells of the *Marinobacterium* members were Gram-negative, oxidase positive, rods and most of them motile by means of single polar flagellum. Colonies of most species in this genus were circular, smooth, convex, opaque and creamy white. $C_{18:1}$ *ω7c*, $C_{16:1}$*ω7c and* $C_{16:0}$ were the major fatty acids. The respiratory quinone was Q-8. The DNA G+C content of the genus *Marinobacterium* vary from 54.9 mol % to 62.5 mol%.

In this paper, we described a novel facultative anaerobic strain, designated WM3^T, isolated from the surface seawater collected in April 2014 around Zhoushan Islands (30°07′59.56″ N, 122°47′41.55″E) of the East China Sea. The pH of the seawater is 7.9 and the salinity is 1.5% (w/v). Based on the phenotypic and phylogenetic data presented in this study, the new isolate represented a novel species of the genus *Marinobacterium*. *M. litorale* KCTC 12756^T, *M. mangrovicola* DSM 27697^T, *M. lutimaris* DSM 22012^T and *M. georgiense* KCTC 12422^T were used as reference strains.

We obtained the novel isolate by the following procedure. The seawater was diluted and spread onto marine ager 2216 (MA) plates using a tenfold dilution series method.

Obvious colonies formed after 3 days incubation at 30 ºC. Distinctive colonies were picked out and purified by repeated restreaking. Purity was confirmed by the uniformity of cell morphology. The isolate was routinely cultured on marine broth 2216 (MB) medium and maintained at -80 ºC with 20% (v/v) glycerol.

Cell morphology and motility were examined by optical microscopy (BX40; Olympus) and transmission electron microscopy (JEM-1230; JEOL) during the late-exponential or stationary growth phases. Growth at various NaCl concentrations (0.25, 0.5, 1.0, 1.5 and 2.0-13.0%, at increments of 1%, w/v) was investigated in modified MB medium without Na^+ ions. Temperature range for growth was tested by incubating cells in MB medium at various temperatures (4, 15, 20, 25, 28, 32, 37, 40, 43, 45 and 50 ºC). The pH range (from pH 5.5 to 10.0, at intervals of 0.5 pH units) was determined in MB medium with the addition of 30 mM buffering agents, including MES (pH 5.5-6.5), PIPES (pH 6.5-7.5), Tricine buffer (pH7.5-8.5) and CAPSO (pH 9.0-10.0). Anaerobic growth was determined at 30 ºC for 15 days in modified MB medium supplemented with sodium thiosulfate (20 mM), sodium sulfite (5 mM), sodium sulfate (20 mM), sodium nitrite (5 mM) and sodium nitrate (20 mM) as electron acceptors under a gas phase of 100% N_2.

The utilization of single carbon source was performed using GN2 MicroPlate™ (Biolog) according to the manufacturer's instructions and the description of Park *et al*. (2009), with modified BM medium (Farmer & Hickman-Brenner, 2006). The medium contained (per litre distilled water): 0.5 g NH_4Cl, 0.0375 g $K_2HPO_4 \cdot 7H_2O$, 0.014 g $FeSO_4 \cdot 7H_2O$, 5.85 g NaCl, 6.15 g $MgSO_4 \cdot 7H_2O$, 0.375 g KCl, 0.725 g $CaCl_2$, 10 ml Tris-HCl (10 mM, pH 8.0), and 25 mM PIPES, pH 7.0. Catalase and oxidase activities, PHB production, H_2S production from thiosulfate and L-cysteine, hydrolysis of starch, casein, L-tyrosine and cellulose were tested according to Zhu *et al*. (2011). Acids production from carbonhydrates was performed using MOF medium (l^{-1} distilled water: NaCl optimal concentration, $MgCl_2 \cdot 6H_2O$ 3.87g, $MgSO_4$ 0.48g, KCl 0.5g, $CaCl_2$ 0.25g, $(NH_4)_2SO_4$ 0.5g, $FeSO_4$ trace, casitone (BD) 1.0g, yeast extract (BD)

0.1g, Tris 0.5g, and phenol red 0.01g; and adjusted to pH 7.5) (Leifson, 1963) at 30 °C. API ZYM and 20NE kits (Bio Mérieux) were also used according to the manufacturers' instructions. Antibiotic susceptibility tests were determined on MA plates at 30 ºC using antibiotic discs containing the following (μg per disc, unless indicated): amikacin (30), ampicillin (10), bacitracin (0.04 IU), carbenicillin (100), cefazolin (30), cefoxitin (30), cefradine (30), ceftriaxone (30), cephalothin (30), chloramphenicol (30), ciprofloxacin (5), erythromycin (15), furazolidone (100), gentamicin (10), kanamycin (30), lincomycin (2), macrodantin (300), nalidixan (30), neomycin (30), polymyxin B (300 IU), rifampicin (5), streptomycin (10), tetracycline (30) and vancomycin (30). The test on the plates was performed with 1.5 x 10^8 cells / ml (McFarland standard 0.5). The strains were considered susceptible, intermediate and resistant respectively when the diameter of the inhibition zone was >5 mm, 2~5 mm and <2 mm according to Nokhal & Schlegel (1983).

Quick bacteria genomic DNA extraction kit (DongSheng Biotech) was used to obtain high quality DNA template. An almost complete 16S rRNA gene sequence of strain WM3^T was obtained by PCR using the primer pair 27F (5'-AGAGTTTGATCCTGGCTCAG- 3') / 1492R (5'-GGTTACCTTGTTACGACTT-3') and the PCR products were cloned into pMD19-T vector (Takara) for sequencing (Xu *et al*, 2007). The primer pair 27F/1492R was also used for sequencing. The complete 16S rRNA sequence of strain WM3^T was identified on EzTaxon-e service (Kim *et al*., 2012) by using EzTaxon-e tool. Multiple sequences were aligned with clustal W1.8 (Thompson *et al.*, 1994). Phylogenetic trees were constructed using the neighbor-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and the maximum-parsimony (Fitch, 1971) methods with the MEGA 5 program package. Evolutionary distances were calculated according to the algorithm of Kimura's two-parameter model (Kimura, 1980) for the neighbor-joining method. In addition, the16S rRNA sequence of strain WM3^T was also compared with other relative sequences in the SILVA rRNA Databases Project libraries (http://www.arb-silva.de/) and subjected to phylogenetic analyses using ARB

(Ludwig *et al.*, 2004). The DNA G+C content was determined by reversed-phase HPLC as described by Mesbah & Whitman (1989).

Isoprenoid quinones were extracted from freeze-dried cells which were grown on MB medium for 24 h at 30 ºC with chloroform/methanol (2:1) and analysed by reversed-phase HPLC. For the preparation of cellular fatty acid methyl esters (FAMEs), those strains were harvested and freeze-dried at the exponential stage of growth according to Kuykendall *et al*. (1988). Identification and quantification of the FAMEs were performed by the Sherlock Microbial Identification System (MIDI) with the standard MIS Library Generation software (Microbial ID).

After 24 h incubation, strain WM3^T formed white, smooth, circular, elevated and cream colonies with diameters of approximately 0.5~1 mm. Cells of the strain WM3^T were Gram-negative, rod-shaped and motile by means of single flagellum (Fig. 1). Strain WM3^T grew at 15-43ºC (optimum 37-40ºC), pH 5.5-9.5 (optimum 6.5-7.5) and with 0.5-9.0% NaCl (optimum 0.5-1.0%, w/v). Detailed results of physiological and biochemical tests are given in Table 1 and species description.

Almost-complete 16S rRNA gene sequence (1486 nt) of strain WM3^T was obtained. The analysis of 16S rRNA gene sequence similarity between WM3^T and other representative species revealed that the novel isolate was closely related to the genus *Marinobacterium* and shared the highest sequence similarity of 95.5% both with *M. litorale* KCTC 12756^T and *M. mangrovicola* DSM 27697^T.The strain WM3^T was also closely related to *M. lutimaris* DSM 22012^T (95.4% similarity) but shared low sequence similarity (<94%) with all other species. Conformably, phylogenetic analysis based on the multiple sequences alignment indicated that strain WM3^T belonged to the genus *Marinobacterium* by clustering with *M. litorale* KCTC 12756^T, *M. mangrovicola* DSM 27697^T and *M. lutimaris* DSM 22012^T in neighbor-joining, maximum-likelihood and the maximum-parsimony trees (Fig.2). The result was consistent with the ARB tree (Fig S1). The DNA G+C content of strain WM3^T was

55.8 mol% (HPLC).

The fatty acid compositions of strain WM3^T and other related *Marinobacterium* strains are shown in Table S1. The major cellular fatty acids of strain WM3^T were $C_{16:0}$ (23.7%), $C_{18:1}$ $\omega 7c$ (26.2%) and summed feature 3 ($C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH, 22.1%), which was similar to those reference strains of the genus *Marinobacterium*, but there were also little differences in the proportions of some fatty acids. Ubiquinone-8 was detected as the sole respiratory quinone of the strain WM3^T which was in accordance with the members of the genus *Marinobacterium*.

Based on the phylogenetic, genomic and chemotaxonomic characteristics described above, strain WM3^T should belong to genus *Marinobacterium*. However, except for the low 16S rRNA gene sequence similarity value, the different characteristics between the strain WM3^T and its reference strains were evident. First and foremost, many carbon substrates such as $_L$-arabinose, $_D$-arabitol, $_D$-galactose, $_D$-mannitol, turanose, $_D$-gluconic acid and $_D$-glucuronic acid could be used by strain WM3^T, but were not assimilated by the other four strains. Secondly, the maximal and optimal temperatures for growth of strain WM3^T were higher than those of all other *Marinobacterium* type strains. Thirdly, acid production was detected from $_D$-glucose and $_D$-mannose, while the result was not observed from its all reference strains. Fourthly, strain WM3^T was resistant to amikacin, cefazolin and nalidixan, but the reference strains were all sensitive to those antibiotics. In addition, though the major fatty acids of strain WM3^T and other strains was the same, there was still some little difference between those strains. The content of $C_{12:0}$ in the strain WM3^T (9.6%) was higher than that in other strains and $C_{17:0}$ was only detected in the strain WM3^T (6.6%).

According to the physiological, biochemical and phylogenetic characteristics, strain WM3^T is proposed to represent a novel species of genus *Marinobacterium*, with the name *Marinobacterium zhoushanense* sp. nov..

Description of *Marinobacterium zhoushanense* sp. nov

Marinobacterium zhoushanense (zhou.shan.en'se. N.L. neut. adj. *zhoushanense* referring to the Zhoushan Islands in China, from which the type strain was isolated).

Cells are Gram-stain-negative, PHB accumulating, rod-shaped, approximately 0.4-0.6 μm wide and 1.0-2.0 μm long, motile by means of single polar flagellum. After incubation for 24 h on MA plates, colonies are 0.5-1.0 mm in diameter, cream-coloured, slightly convex, smooth and circular. Growth occurs at pH 5.5-9.5, optimum is 6.5-7.5. Growth temperature range is 15-43 ºC and no growth is detected at 4 or 45 ºC. The NaCl concentrations range for growth is 0.25-9.0% (w/v), and optimal growth occurs at 1.0-1.5% (w/v). Weakly growth was observed in anaerobic conditions. Strain WM3^T was positive in catalase, oxidase, PHB accumulation, methyl red reaction and hydrolysis of tyrosine. Negative in Voges-Proskauer reaction, indole production, H_2S production (from thiosulfate or $_L$- cysteine), hydrolysis of aesculin, casein, gelatin, starch or CM-cellulose. Acid is produced from arabinose, fructose, $_D$-glucose and $_D$-mannose. The following carbon substrates are utilized: $_L$-arabinose, $_D$-arabitol, $_D$-fructose, $_D$-galactose, α-$_D$-glucose, maltose, $_D$-mannitol, sucrose, $_D$-trehalose, turanose, formic acid, $_D$-galacturonic acid, $_D$-gluconic acid, $_D$-glucuronic acid, β-hydroxybutyric acid, *p*-hydroxyphenlyacetic acid, α-ketoglutaric acid, $_D$, $_L$-lactic acid, malonic acid, propionic acid, quinic acid, succinic acid, $_L$-alaninamide, $_D$-alanine, $_L$-alanine, $_L$-glutamic acid, $_L$-ornithine, $_L$-phenylalanine, $_L$-proline, $_L$-pyroglutamic acid, γ-aminobutyric acid, phenylethylamine, putrescine, glycerol. Those following carbon sources are utilized weakly: glycogen, Tween 40, Tween 80, pyruvic acid methyl ester, acetic acid, *cis*-aconitic acid, citric acid, γ-hydroxybutyric acid, $_D$-saccharic acid, bromosuccinic acid, $_L$-alanyl glycine, $_L$-asparagine and $_L$-aspartic acid. The strain WM3^T can reduce nitrate to nitrite but not to nitrogen. The following enzymic activities are present: alkaline and acid phosphatase, esterase (C4), leucine arylamidase and α-glucosidase. Weakly positive enzyme activity for esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase. Susceptible to ampicillin,

carbenicillin, cefradine, ceftriaxone, chloramphenicol, erythromycin, gentamicin , kanamycin, macrodantin, neomycin, rifampicin, streptomycin and tetracycline, but resistant to amikacin, bacitracin, cefazolin, lincomycin, nalidixan, polymyxin B and vancomycin. The respiratory quinone was Q-8. Major fatty acids are $C_{16:0}$ and $C_{18:1}$ $\omega 7c$ and summed feature 3 (comprising $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH). The DNA G+C content of the type strain is 55.8 mol% (determined by HPLC).

The type strain, WM3^T (=CGMCC 1.15341^T=KCTC 42782^T), was isolated from surface seawater around Zhoushan Islands (30° 07′ 59.56″ N, 122° 47′ 41.55″ E) of the East China Sea.

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References

Alfaro-Espinoza, G. & Ullrich, M. S. (2014). *Marinobacterium mangrovicola* sp. nov., a marine nitrogen-fixing bacterium isolated from mangrove roots of Rhizophora mangle. *Int J Syst Evol Microbiol* **64**, 3988-3993.

Chang, H. W., Nam, Y. D., Kwon, H. Y., Park, J. R., Lee, J. S., Yoon, J. H., An, K. G. & Bae, J. W. (2007). *Marinobacterium halophilum* sp. nov., a marine bacterium isolated from the Yellow Sea. *Int J Syst Evol Microbiol* **57**, 77-80.

Chimetto, L. A., Cleenwerck, I., Brocchi, M., Willems, A., De Vos, P. & Thompson, F. L. (2011). *Marinobacterium coralli* sp. nov., isolated from mucus of coral (Mussismilia hispida). *Int J Syst Evol Microbiol* **61**, 60-64.

Farmer, J. J., III & Hickman-Brenner, F. W. (2006). The genera *Vibrio* and *Photobacterium.* In *The Prokaryotes: a Handbook on the Biology of Bacteria*, 3rd edn, vol. 6, pp. 508-563. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer & E. Stackebrandt. New York: Springer.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368-376.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406-416.

Gonzaez, J. M., Mayer, F., Moran, M. A., Hodson, R., E. & Whitman, W. B. (1997). *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* **47**, 369-376.

Huo, Y. Y., Xu, X. W., Cao, Y., Wang, C. S., Zhu, X. F., Oren, A. & Wu, M. (2009). *Marinobacterium nitratireducens* sp. nov. and *Marinobacterium sediminicola* sp. nov., isolated from marine sediment. *Int J Syst Evol Microbiol* **59**, 1173-1178.

Hwang, C. Y., Yoon, S. J., Lee, I., Baek, K., Lee, Y. M., Yoo, K.-C., Yoon, H. I. & Lee, H. K. (2016). *Marinobacterium profundum* sp. nov., a marine bacterium from deep-sea sediment. *Int J Syst Evol Microbiol*. doi:10.1099/ijsem.0.000918

Kim, H., Choo, Y. J., Song, J., Lee, J. S., Lee, K. C. & Cho, J. C. (2007). *Marinobacterium litorale* sp. nov. in the order *Oceanospirillales*. *International Journal of Systematic and Evolutionary Microbiology* **57**, 1659-1662.

Kim, H., Oh, H. M., Yang, S. J., Lee, J. S., Hong, J. S. & Cho, J. C. (2009a). *Marinobacterium marisflavi* sp. nov., isolated from a costal seawater. *Current microbiology* **58**, 511-515.

Kim, J. M., Lee, S. H., Jung, J. Y. & Jeon, C. O. (2010). *Marinobacterium lutimaris* sp. nov., isolated from a tidal flat. *Int J Syst Evol Microbiol* **60**, 1828-1831.

Kim, O.S., Cho, Y.J., Lee, K., Yoon, S.H., Kim, M., Na, H., Park, S.C., Jeon, Y.S., Lee, J.H., Yi, H., Won, S., Chun, J. (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA Gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716-721.

Kim, S. J., Park, S. J., Yoon, D. N., Park, B. J., Choi, B. R., Lee, D. H., Roh, Y. & Rhee, S. K. (2009b). *Marinobacterium maritimum* sp. nov., a marine bacterium isolated from Arctic sediment. *Int J Syst Evol Microbiol* **59**, 3030-3034.

Kim, Y. G., Jin, Y. A., Hwang, C. Y. & Cho, B. C. (2008). *Marinobacterium rhizophilum* sp. nov., isolated from the rhizosphere of the coastal tidal-flat plant Suaeda japonica. *Int J Syst Evol Microbiol* **58**, 164-167.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111-120.

Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum. Int J Syst Bacteriol* **38**, 358-361.

Leifson, E. (1963). Determination of Carbohydrate Metabolism of Marine Bacteria. *J Bacteriol* **85,** 1183-1184.

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004). ARB: a software

environment for sequence data. *Nucleic Acids Res* **32**, 1363-1371.

Mesbah, M. & Whitman, W. B. (1989). Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. *J Chromatogr* **479**, 297-306.

Nokhal, T. H. & Schlegel, H. G. (1983). Taxonomic study of *Paracoccus denitrificans*. *Int J Syst Bacteriol* **33**, 26-37.

Park, S., Jung, Y.-T., Kim, S. & Yoon, J.-H. (2016). *Marinobacterium aestuariivivens* sp. nov., isolated from a tidal flat. *Int J Syst Evol Microbiol*. doi:10.1099/ijsem.0.000927.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406-425.

Satomi, M., Kimura, B., Hamada,T., Harayama, S & Fujii, T. (2002) Phylogenetic study of the genus *Oceanospirillum* based on 16S rRNA and gyrB genes: emended description of the genus *Oceanospirillum*, description of *Pseudospirillum* gen. nov., *Oceanobacter* gen. nov. and *Marinobacterium* gen. nov. and transfer of *Oceanospirillum jannaschii* and *Pseudomonas stanieri* to *Marinobacterium* as *Marinobacterium jannaschii* comb. nov. and M*arinobacterium stanieri* comb. nov. *Int J Syst Evol Microbiol* **52**, 739-747.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acid Res* **22**, 4673-4680.

Xu, X. W., Wu, Y. H., Zhou, P., Wang, C. S., Zhou, Y. G., Zhang, H. B., Wang, Y. & Wu, M. (2007). *Halomonas saccharevitans* sp. nov., *Halomonas arcis* sp. nov. and *Halomonas subterranea* sp. nov., halophilic bacteria isolated from hypersaline environments of China. *Int J Syst Evol Microbiol* **57,** 1619-1624.

Zhu, X.-F. , Jia, X.-M., Zhang, X.-Q., Wu, Y.-H., & Chen, Z.-Y. (2011). Modern experimental technique of microbiology. Hangzhou: Zhejiang University Press (English translation).

Table 1. Phenotypic and genotypic characteristics of strain WM3^T compared with other type strains of related *Marinobacterium* species. Taxa: 1, WM3 ^{T}; 2, *M. litorale* KCTC 12756^T; 3, *M. mangrovicola* DSM 27697^T; 4, *M. lutimaris* DSM 22012^T; 5, *M. georgiense* KCTC 12422^T. All strains were positive for catalase and oxidase activities. All strains were negative in indole production, glucose fermentation, arginine dihydrolase, Voges-Proskauer reaction, H_2S production from thiosulfate, hydrolysis of *β*-galactosidase, aesculin, gelatincasein, starch and CM-cellulose. The following substrates: *β*-hydroxybutyric acid , *α*-ketoglutaric acid, D, L-lactic acid, propionic acid, quinic acid, succinic acid, L-alaninamide, D-alanine, L-alanine, L-glutamic acid, L-proline, putrescine, pyruvic acid methyl ester, acetic acid, *cis*-aconitic acid and citric acid could be used by all strains as the sole carbon sources. All strains were sensitive to chloramphenicol, kanamycin, erythromycin, streptomycin, tetracycline, neomycin, cefradine, ceftriaxone, macrodantin and rifampicin, but resistant bacitracin and vancomycin.+, positive; -, negative; w, weakly positive.

Characteristic	**1**	**2**	**3**	**4**	**5**
Temperature for growth (°C)					
range	15-43	8-42	4-42	15-40	4-41
optimum	37-40	30	28-37	25-30	37
Relationship with O_2	facultatively anaerobic	facultatively anaerobic	strictly aerobic	strictly aerobic	strictly aerobic
PHB accumulation	+	-	+	+	+
Nitrate reduction	+	-	w	-	-
Methyl red test	+	-	-	-	-
Hydrolysis of:					
Tyrosine	+	-	-	-	-
Urea	-	+	+	+	-
Utilization of †:					

$_{L}$-Arabinose	+	-	-	-	-
$_{D}$-Arabitol	+	-	-	-	-
$_{D}$-Galactose	+	-	-	-	-
$_{D}$-Mannitol	+	-	-	-	-
Turanose	+	-	-	-	-
D-Trehalose	+	-	-	-	-
$_{D}$-Gluconic Acid	+	-	-	-	-
$_{D}$-Glucuronic Acid	+	-	-	-	-
Succinic Acid Mono-methyl Ester	-	+	+	+	+
Acid production from:					
Arabinose	+	-	-	+	+
Fructose	+	-	-	-	+
$_{D}$-Glucose	+	-	-	-	-
$_{D}$-Mannose	+	-	-	-	-
Antibiotics Susceptibility:					
amikacin	-	+	+	+	+
ampicillin	+	+	-	+	+
cefazolin	-	+	+	+	+
gentamicin	+	+	+	-	+
nalidixan	-	+	+	+	+
polymyxin B	-	+	+	-	-
API ZYM					
alkaline phosphatase	+	w	+	+	+
esterase lipase (C8)	w	+	+	+	+
valine arylamidase	-	w	-	-	-

acid phosphatase	+	+	+	+	w
naphthol-AS-BI-phosphohydrolase	w	+	+	+	+
α-glucosidase	+	-	-	-	-
β- glucosidase	-	-	-	w	-
DNA G+C content (mol%)	55.8%	60.7%	57.0%	58.0%	54.6%

341 † Utilization of $_{L}$-arabinose, $_{D}$-galactose, $_{D}$-mannitol, $_{D}$-gluconic acid and $_{D}$-trehalose was also tested in tubes with modified BM medium.

Legends to figures:

Fig.1. Transmission electron micrograph of a cell of strain WM3^{T}.

Fig.2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences，showing the relationships of strain WM3^{T} and related species. Bootstrap values based on 1000 replicates are listed as percentages at branching points. Only bootstrap values above 50% are shown. Filled circles indicate that the corresponding nodes were also recovered in both maximum-likelihood and maximum-parsimony trees. *Escherichia coli* ATCC 11775^{T} (GenBank accession no.X80725) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

Supplementary Materials

Supplementary table 1. Fatty acid composition of strain WM3^T and the reference strains.

Strains: 1, WM3^T; 2, *M. litorale* KCTC 12756^T; 3, *M. mangrovicola* DSM 27697^T; 4, *M. lutimaris* DSM 22012^T ; 5, *M. georgiense* KCTC 12422^T. Values are percentages of the total fatty acids. ECL, equivalent chain length; tr, trace (<0.5%); -, not detected. Major components (≥10%) are highlighted in bold.

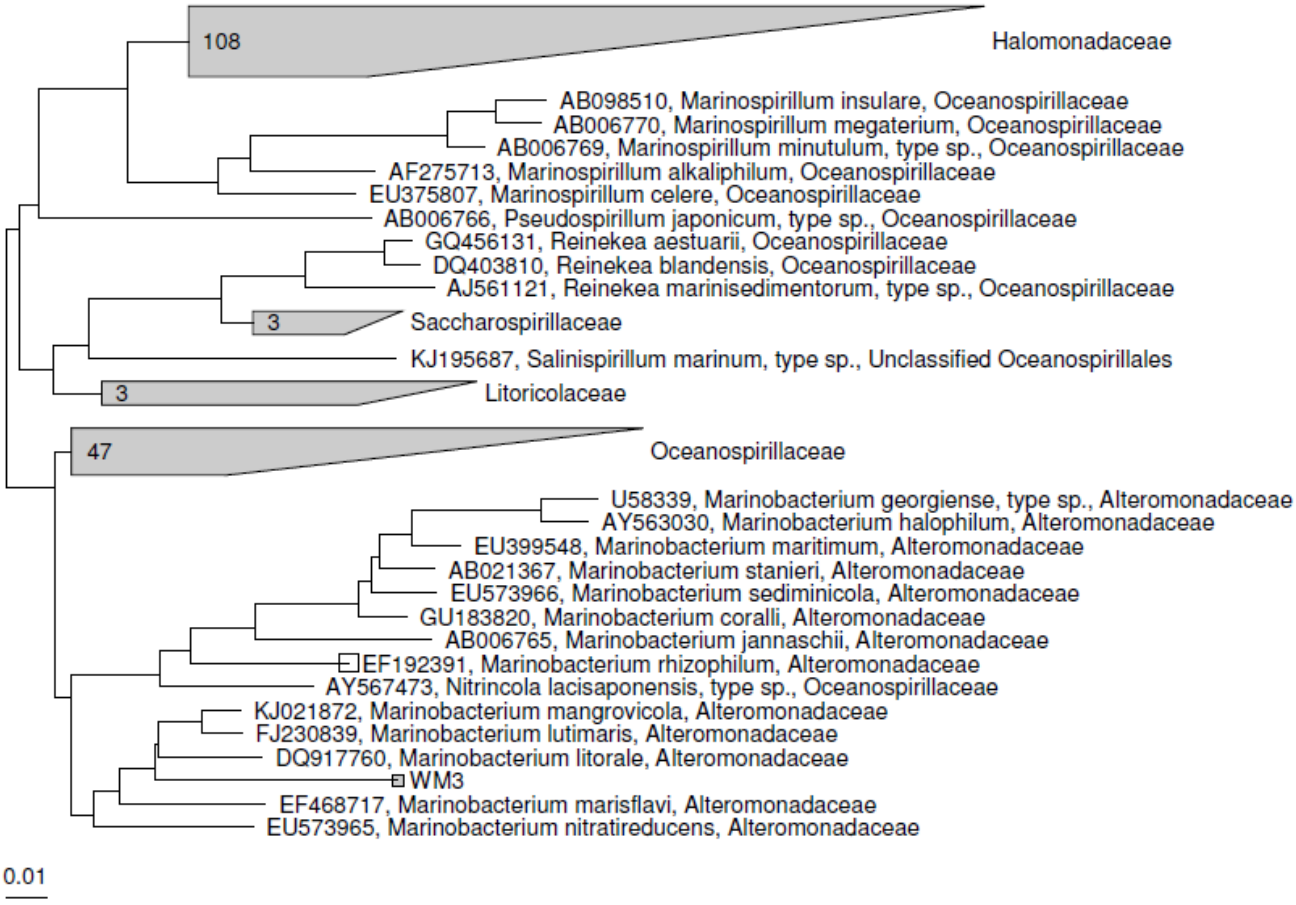
Fatty acid	1	2	3	4	5
Saturated					
$C_{10:0}$	1.2	0.8	tr	0.8	7.7
$C_{12:0}$	9.6	6.1	5.6	5.5	4.3
$C_{15:0}$	tr	-	-	1.5	-
$C_{16:0}$	**23.6**	**22.8**	**28.2**	**21.5**	**21.2**
C17:0	6.6	-	tr	-	-
Unsaturated					
$C_{18:1}$ *ω7c*	**26.2**	**31.6**	**31.2**	**33.0**	**27.0**
Hydroxy					
C10:0 3-OH	7.9	7.1	4.6	7.0	8.3
C12:0 2-OH	tr	tr	tr	3.0	-
Summed Feature 3*	**22.1**	**28.7**	**27.1**	**22.6**	**28.1**
Unknown (ELC 11.799)	1.4	1.8	tr	0.7	2.6

* Summed Feature 3 contained $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH.

Legends to the Supplementary Figures

Supplementary Fig. 1. ARB phylogenetic tree based on 16S rRNA gene sequences，showing the relationships of strain WM3 ^{T} and related species. The phylogenetic status of the genus *Marinobacterium* could also be clearly illustrated. Bar, 0.01 substitutions per nucleotide position.

Supplementary Fig. S1. ARB phylogenetic tree based on 16S rRNA gene sequences, showing the relationships of strain WM3 ^{T} and related species. The phylogenetic status of the genus *Marinobacterium* could also be clearly illustrated. Bar, 0.01 substitutions per nucleotide position.



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