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# *Marinobacter caseinilyticus* sp. nov., Isolated from Saline Soil

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## Abstract

A Gram-stain-negative, motile, aerobic, rod-shaped bacterium with flagella, designated M3-13$^T$, was isolated from a saline soil in Zhoushan, China. According to phylogenetic analysis based on 16S rRNA gene sequences, strain M3-13$^T$ was assigned to the genus *Marinobacter* with highest 16S rRNA gene sequence similarity of 97.7% to *Marinobacter maroccanus* LMG 30466$^T$, followed by *Marinobacter sediminum* R65 $^T$ (97.5%) and *M. salsuginis* SD-14B$^T$ (97.2%). Digital DNA–DNA hybridization (DDH) and average nucleotide identity (ANI) were determined to evaluate the genomic relationship between strain M3-13$^T$ and *M. maroccanus* LMG 30466$^T$. Digital DDH estimation (19.8%) as well as ANI (72.98%) proved the dissimilarity of strain M3-13$^T$. Optimal growth of the strain M3-13$^T$ was at 28–30 °C and at pH 8.0–8.5, in the presence of 3–6% (w/v) NaCl. The major fatty acids detected in strain M3-13$^T$ were $C_{16:1}$ $\omega$7c/$C_{16:1}$ $\omega$6c, $C_{16:0}$, $C_{18:1}\omega$7c/$C_{18:1}$ $\omega$6c and $C_{12:0}$3-OH, and the predominant respiratory quinone was ubiquinone-9. The major polar lipids included diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, one unidentified aminophosphoglycolipid and one unidentified phosphoglycolipid. The DNA G+C content was 56.6%. A phylogenetic analysis based on 16S rRNA gene sequences showed that strain M3-13$^T$ belongs to the genus *Marinobacter*. Based on the polyphasic taxonomic characterization, strain M3-13$^T$ is considered to represent a novel species of the genus *Marinobacter*, for which the name *Marinobacter caseinlyticus* sp. nov. is proposed (type strain M3-13$^T$ = MCCC 1K04560$^T$ = KCTC 72043$^T$).

## Introduction

The genus *Marinobacter*, belonging to family *Alteromonadaceae*, was proposed by Gauthier et al. (1992) [1]. Currently, the genus *Marinobacter* is composed of 54 species with validly published names (https://lpsn.dsmz.de/genus/marinobacter), and the type species is *Marinobacter hydrocarbonoclasticus* which was isolated from sediment of the Gulf of Fos (French Mediterranean coast). Members of this genus were isolated from diverse environments such as saline soil [2], marine sediments [3], a junction between the ocean and a freshwater lake [4], marine sponge [5], Antarctic environment [6] and coastal hot springs [7]. Microorganisms requiring salt for growth are referred to as halophiles. According to the most widely used definition, that of Kushner (1978), one can distinguish between slight halophiles [many marine organisms; seawater contains about 3% (w/v) NaCl], moderate halophiles [optimal growth at 3–15% (w/v) salt] and extreme halophiles (optimal growth at 25% (w/v) NaCl [8]). And the cells of this genus were Gram-stain negative, rod shaped, flagellated, motile, aerobic, mesophilic and halophilic. They were halotolerant or halophilic microorganisms and may play roles in their potential biotechnological applications [9]. Moreover, most of *Marinobacter* strains harbour genes which encode wax ester synthases, and some *Marinobacter* strains were related to algal bloom or manganese oxidation [10]. In this study, we report the isolation and characterization of another novel and moderate halophilic strain M3-13$^T$ from saline soil.

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## Materials and Methods

### Isolation and Cultivation

Strain M3-13$^T$ was isolated from coastal saline soil (E122° 01′, N29° 57′) in Zhoushan, Zhejiang province, PR China. During a study of the bacterial diversity in the area, approximately 5 g soil sample was inoculated aseptically on marine agar 2216 (MA; Difco) at 20 °C by using 1:10 serial dilution plating methods. After 7 days of incubation, a white-coloured colony was collected and named M3-13$^T$. A pure colony of the strain was grown in fresh plates of the same medium. The morphological, physiological and biochemical characteristics of strain M3-13$^T$ were investigated using routine cultivation at 30 °C on MA supplemented with 4% (w/v) NaCl. The bacterial culture was then stored at -80 °C with 25% (v/v) glycerol. For long-term preservation, purified strains were preserved by lyophilization with 20% (w/v) skimmed milk. Strain M3-13$^T$ has been deposited at the Marine Culture Collection of China and the Korean Collection for Type Cultures.

### 16S rRNA Gene Sequence and Phylogenetic Analysis

Genomic DNA was extracted using a quick bacteria genomic DNA extraction kit (DongSheng Biotech). An almost complete 16S rRNA gene sequence of the isolate was obtained by PCR using the bacterial universal primers 27F (5′-GAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-AGAAAGGAGGTGATCCAGCC-3′), and the PCR products were cloned into pMD19-T vector (Takara) for sequencing. The almost complete 16S rRNA gene sequence (1506 bp) was obtained. In addition, complete 16S rRNA gene sequence was obtained from genome sequences of strain M3-13(1529 bp, MW342710) by RNAmmer 1.2 Sever [11]. There was no difference (100% similarity) between the two sequences. The latter was applied in 16S rRNA gene sequence similarity and phylogenetic analyses. Reference sequences were calculated by the identify tool included in the EzBioCloud portal [12] via the EzTaxon-e tool. Multiple sequences were aligned with CLUSTAL_W programme of the MEGA 7 package [13]. Phylogenetic trees were reconstructed using the neighbour-joining (NJ) [14], maximum-likelihood (ML) [15], and the maximum-parsimony (MP) [16] methods with the MEGA 7 programme package [13]. And their topological robustness was evaluated by bootstrap analysis based on 1000 replicates. Evolutionary distances were calculated according to the algorithm of Kimura's two-parameter model (Kimura 1980) for the neighbour-joining and maximum-likelihood trees [17].

### Genome Sequence and Analysis

The genomes of strain M3-13$^T$ and *M. salicampi* KCTC 12972$^T$ were sequenced using the Illumina HiSeq 4000 system at the Beijing Genomics Institute (Shenzhen, China). The sequencing generated approximate 1.1 Gb and 1.2 Gb clean data, respectively. The de novo assembly of the reads was performed by using ABySS 2.0.2 [18]. The assembly k-value was tested from 32 to 64 to find the optimal k-value using the abyss-pe script. The quality of microbial genomes was assessed using the bioinformatic tool CheckM [19]. Average nucleotide identity (ANI) was calculated with OrthoANI [20]. In silico DNA–DNA hybridization (DDH) value was calculated by using the Genome-to-Genome Distance Calculator [21]. The G+C content was obtained from the genomic sequences. The genomes of strain M3-13$^T$ and the reference strains were annotated by Rapid Annotation using Subsystem Technology (RAST https://rast.nmpdr.org/) [22].

### Morphology, Physiology, Biochemistry and Chemotaxonomy

Cell morphology and motility were determined by using optical microscopy (Olympus; BX40) and transmission electron microscopy (JEOL; JEM-1230) [23]. Cells grown on plates were suspended by distillation–distillation $H_2O$ and stained with uranyl acetate and then fixed on copper mesh before being observed by TEM. Furthermore, motility was determined by light microscopy and inoculation in semi-solid agar medium.

The salt tolerance was determined in NaCl-free MB added with various concentrations of NaCl (0, 0.5, 1.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, 20.0, 23.0 and 25.0%, w/v); The temperature for growth was tested at 4–55 °C (4, 15, 20, 25, 28, 30, 35, 37, 45, 50 and 55 in duplicates) in MA and for the pH range (from pH 5.5 to 10.0, at intervals of 0.5 pH units) was determined in MB with the addition of 30 mM buffering agents, including MES (pH 5.5–6.5), PIPES (pH 6.5–7.5), Tricine buffer (pH 7.5–8.5), and CAPSO (pH 9.0–10.0). After incubation for one day, $OD_{590}$ values were measured with a UV/visible spectrophotometer (Ultrospec 6300 pro; Amersham Biosciences) to determine the optical growth and the growth range were checked after two weeks. Anaerobic growth was performed in an anaerobic jar (Mitsubishi Gas Chemical) with an Anaero-Pack (Mitsubishi Gas Chemical) on modified MA supplemented with sodium thiosulphate (20 mM), sodium sulphite (5 mM), sodium sulphate (20 mM), sodium nitrite (5 mM), sodium nitrate (20 mM), L-arginine (5.0 g/L) as potential electron acceptors and examined after 20 days.

Antibiotic susceptibility tests were determined on MA plates for 3 days at 30 °C using antibiotic discs (Hangzhou Microbial Reagents) containing the following (mg per disc, unless indicated): lincomycin (2), carbenicillin (100), chloramphenicol (30), gentamicin (10), kanamycin (30), neomycin (30), novobiocin (30), penicillin G (10 IU), rifampicin (5), streptomycin (10), tetracycline (30), norfloxacin (10), ceftriaxone (30), ciprofloxacin (5), ofloxacin (5), vancomycin (30), ampicillin (10), and oxacillin (1). When the radius of the inhibition zone was over 2.0 mm, the strains were considered as sensitive.

Production of hydrogen sulphide was assessed with lead acetate paper [24]. Hydrolysis activities of starch, tyrosine, casein, xanthine, hypoxanthine, Tween 20, Tween 40, Tween 60 and Tween 80 were determined as described previously [25]. Oxidase and catalase activity, methyl-red and Voges–Proskauer tests were assayed according to a previous study [24]. Biomass for all analyses was obtained after cultivation on MA supplemented with 4% (w/v) NaCl at 30 °C for 2 days unless otherwise stated, and all experiments above were performed in triplicate. API 20NE and API ZYM strips (bioMérieux) were used to study the utilization of carbohydrates and enzyme activities. The inoculum was prepared by suspending colonies of strain M3-13$^T$ in a 6% (w/v) sea salts solution and transferred to the API strips following the manufacturer's instructions. For the acid production test, Leifson-modified oxidation–fermentation (MOF) medium [26] supplemented with 4% (w/v) NaCl was utilized to suspend the cells for the inoculation of API 50CH (bioMérieux) strips. To characterize the utilization of carbon sources, strains were inoculated in MB without peptone containing 0.2% diverse carbon sources including sugar, alcohol, organic acid and amino acid; yeast extract was added for providing trace elements at a concentration of 0.005 g/L; the detect method was described as previously [24].

Cells of strain M3-13$^T$ and reference strains grown on MB supplemented with 4% (w/v) NaCl for 2 days at 30 °C were used for polar lipid and isoprenoid quinone analysis. Polar lipids were analysed by two-dimensional thin-layer chromatography (TLC) with silica gel 60 F254 plates (Merck) as described previously [27]. Isoprenoid quinones were determined a high-performance liquid chromatography-mass spectrometry system (Agilent) [28]. For cellular fatty acid methyl esters (FAMEs), cells of strain M3-13$^T$, *M. maroccanus* LMG 30466$^T$, *M. salicampi* KCTC 12972$^T$ and *M. hydrocarbonoclasticus* DSM 8798$^T$ were harvested and freeze-dried at the exponential stage of growth after cultivated for 28 h at 30 °C on the MB supplemented with 4% (w/v) NaCl. And the exponential stage of growth was based on the growth curve of the strain M3-13 with a UV/visible spectrophotometer (Ultrospec 6300 pro; Amersham Biosciences) adjusted to a wavelength of 590 nm. Fatty acids were extracted according to the protocol of the Microbial Identification System (MIDI; Microbial ID), analysed by GLC with an Agilent model 6850 N device and identified based on the TSBA6 method [29].

## Results and Discussion

### 16S rRNA Gene Sequence and Phylogenetic Analysis

On the basis of 16S rRNA gene sequence alignment, strain M3-13$^T$ was most closely related to *Marinobacter maroccanus* LMG 30466$^T$ (97.7% 16S rRNA gene sequence similarity), *M. sediminum* R65$^T$ (97.5%), *M. salsuginis* SD-14B$^T$ (97.2%), *M. adhaerens* HP15$^T$ (97.1%), *M. similis* A3d10$^T$ (97.1%), and *M. salinus* Hb8$^T$ (97.1%), respectively.

The neighbour-joining phylogenetic tree based on 16S rRNA gene sequences is shown in Fig. 1. Strain M3-13$^T$ constituted a separated branch with *M. salicampi* KCTC 12972$^T$ in the clade of genus *Marinobacter* and closely related to *M. maritimus* CK47$^T$ and *M. aromaticivorans* D15-8P$^T$. The topologies of the maximum-likelihood (Fig. S1) and maximum-parsimony trees (Fig. S2) were almost the same as that of the neighbour-joining tree. Phylogenetic analyses based on the 16S rRNA gene sequence clearly suggested that strain M3-13$^T$ is a member of the genus *Marinobacter*.

### Genome Sequence and Analysis

A total genome length of 4,312,424 bp was obtained from 37 contigs in draft genomes of strain M3-13$^T$. The $N_{50}$ values of the genomes were 189,095 bp. The G+C content of strain M3-13$^T$ was 56.6%, which was similar to *M. salicampi* KCTC 12972$^T$ (57.4%), *M. maroccanus* LMG 30466$^T$ (57.3%) and *M. hydrocarbonoclasticus* ATCC 49840$^T$ (57.4%). The genome completeness of strain M3-13$^T$ was 99.57% with 0.3% contamination. Genome sequences estimated to be ≥ 95% complete, and with ≤ 5% contamination, were considered as excellent reference genomes for deeper analyses [12]. The ANI values between strain M3-13$^T$ and *M. salicampi* KCTC 12972$^T$, *M. maroccanus* LMG 30466$^T$ and *M. hydrocarbonoclasticus* ATCC 49840$^T$ were 73.55%, 72.98% and 72.51%, respectively, which were lower than the threshold values of the species boundary (94–96%) [30]. The in silico DDH values (the recommended results from formula 2) indicated that strain M3-13$^T$ shared 19.0%, 19.8%, and 19.8% DNA relatedness with *M. salicampi* KCTC 12972$^T$, *M. maroccanus* LMG 30466$^T$ and *M. hydrocarbonoclasticus* ATCC 49840$^T$, respectively, which were lower than the threshold values of GGDC (70%) [31]. Comparison of the genomic characteristics of strain M3-13$^T$ and the reference strains are shown in Table 1 via RAST. A total of 4085 coding sequences and 71 RNA genes were predicted in the genome of strain M3-13$^T$.

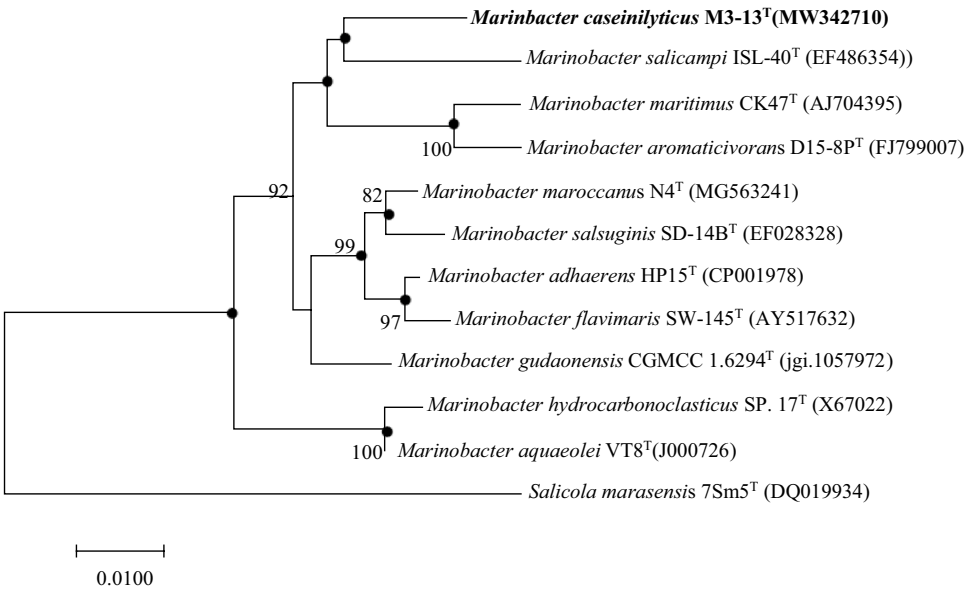


**Fig. 1** Neighbour-joining tree was constructed using the MEGA 7.0 programme package based on 16S rRNA gene sequences, showing the phylogenetic relationship of strain M3-13$^T$ with the related taxa. Filled circles indicate nodes also obtained in both maximum-likelihood and maximum-parsimony trees. Bootstrap values were based on 1000 replicates; only values ≥ 70% were shown. Bar, 0.0100 substitutions per nucleotide position. *Salicola marasensis* 7Sm5$^T$ was used as an outgroup

## Morphology, Physiology, Biochemistry and Chemotaxonomy

Cells of strain M3-13$^T$ were Gram-stain negative, rod shaped (0.2–0.7 μm wide and 1.3–2.5 μm long) and motile by a polar flagellum (Fig. S3). Strain M3-13$^T$ was positive for oxidase, catalase and hydrolysis of Tweens 20 and casein. Strain M3-13$^T$ was susceptible to chloramphenicol (30), norfloxacin (10), ampicillin (10), novobiocin (30), ofloxacin (5), carbenicillin (100), ciprofloxacin (5), rifampicin (5), penicillin G (10 IU) and ceftriaxone (30).

The main features of strain M3-13$^T$ are in line with the description of the genus *Marinobacter* according to Boujida et al. [2], such as Gram-stain negative, rod shaped, motile and halophilic. Strain M3-13$^T$ exhibited phenotypic similarities with the reference strains, such as they were all negative for the methyl-red and Voges–Proskauer tests, hydrolysis of gelatin, xanthine, hypoxanthine and tyrosine, and positive (or weakly positive) for catalase activity, oxidase activity and hydrolysis of Tween 20. However, strain M3-13$^T$ displayed certain distinct characteristics from other reference strains. Notably, strain M3-13$^T$ is positive for hydrolysis of casein and acid phosphatase while the results of other reference strains are opposite. Negative tests for reducing nitrate to nitrite, hydrolysis of Tween 80 and utilization of D-galactose, DL-Alanine could also be used to distinguish strain M3-13$^T$ from some of the reference strains. Details of morphological, physiological and biochemical analyses are provided in Table 2 and Table S1.

The fatty acid profiles of strain M3-13$^T$ and the reference strains are listed in Table 3. The major fatty acids (≥ 5% of the total fatty acids) detected in strain M3-13$^T$ are Sum in Feature 3 (comprising $C_{16:1}$ $\omega$7c/$C_{16:1}$ $\omega$6c) (41.1%), $C_{16:0}$ (21.6%), Sum in Feature 8 (comprising $C_{18:1}\omega$7c/$C_{18:1}$ $\omega$6c) (5.4%) and $C_{12:0}$ 3OH (5.0%). The four reference strains composed of similar major components (≥ 5.0%) such as Sum in feature 3 (comprising $C_{16:1}$ $\omega$7c/$C_{16:1}$ $\omega$6c) and $C_{16:0}$. However, there were some characteristics that distinguished strain M3-13$^T$ from the reference strains. $C_{18:1}$ $\omega$9c and $C_{12:0}$ were major fatty acids in other three strains but not in strain M3-13$^T$. In addition, contrast to *M. maroccanus* LMG 30466$^T$ and *M. hydrocarbonclasticus* DSM 8798$^T$, strain M3-13$^T$ contained $C_{15:1}$ $\omega$6c (0.4%), $C_{16:0}$ iso (0.3%) and $C_{17:0}$ iso (0.4%) as minor fatty acids. The ratios of $C_{16:0}$ N alcohol (4.7%) and $C_{16:1}$ $\omega$7c alcohol (3.62%) in strain M3-13$^T$ were higher than other reference strains, and $C_{16:1}$ $\omega$9c was not detected in strain M3-13$^T$ while other three references were done.

The predominant ubiquinone detected in strain M3-13$^T$ was Q-9, which is a common feature of most *Marinobacter* strains. The polar lipid profile of strain M3-13$^T$ was diphosphatidyglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), aminophosphoglycolipid (APGL), unidentified phosphoglycolipid (PGL), unidentified aminophospholipid (APL), unidentified phospholipid (PL1), two unidentified glycolipid (GL1, GL2) and two unidentified lipids (L1, L2) (Supplementary Fig. S4). DPG, PE, PG, APL, PL and AL were also detected in the three reference strains. This result is consistent with those reported previously for other *Marinobacter* species [32] which could be a significant feature to support that strain M3-13$^T$ belongs to genus *Marinobacter*. Strain M3-13$^T$ could be differentiated from *M. salicampi* KCTC 12972$^T$ by the presence of unidentified aminophospholipid (APL) and from *M. maroccanus* LMG 30466$^T$ by the presence of unidentified glycolipid GL1.

**Table 1** Comparison of the genomic characteristics of strain M3-13$^T$ and the reference strains by RAST

| Genomic characteristics | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| GenBank accession numbers | WUQJ00000000 | JAAMPF000000000 | PSSX01000000 | NC_017067 |
| Size (bp) | 4312424 | 4345659 | 4340695 | 3989480 |
| GC content (%) | 56.6 | 57.4 | 57.3 | 57.4 |
| Number of Contigs (with protein-encoding genes) | 37 | 36 | 61 | 1 |
| N50 | 189095 | 197262 | 222417 | 398948 |
| Number of coding sequences | 4085 | 4085 | 4040 | 3709 |
| Number of RNAs | 71 | 33 | 51 | 59 |
| Subsystem feature counts | | | | |
| Cofactors, vitamins, prosthetic groups, pigments | 168 | 158 | 138 | 149 |
| Cell wall and capsule | 11 | 28 | 24 | 26 |
| Virulence, disease and defence | 36 | 37 | 41 | 46 |
| Potassium metabolism | 9 | 7 | 13 | 9 |
| Miscellaneous | 7 | 10 | 6 | 11 |
| Phages, prophages, transposable elements, plasmids | 5 | 4 | 9 | 7 |
| Membrane transport | 112 | 108 | 105 | 115 |
| Iron acquisition and metabolism | 11 | 11 | 10 | 21 |
| RNA metabolism | 51 | 58 | 54 | 52 |
| Nucleosides and nucleotides | 63 | 58 | 60 | 60 |
| Protein metabolism | 197 | 176 | 189 | 196 |
| Motility and chemotaxis | 29 | 70 | 29 | 32 |
| Regulation and cell signalling | 18 | 27 | 30 | 24 |
| Secondary metabolism | 4 | 4 | 4 | 4 |
| DNA metabolism | 66 | 78 | 84 | 70 |
| Fatty acids, lipids and isoprenoids | 138 | 118 | 110 | 77 |
| Nitrogen metabolism | 13 | 55 | 49 | 58 |
| Dormancy and sporulation | 3 | 4 | 3 | 3 |
| Respiration | 103 | 107 | 63 | 74 |
| Stress response | 90 | 102 | 75 | 74 |
| Metabolism of aromatic compounds | 66 | 51 | 42 | 40 |
| Amino acids and derivatives | 302 | 313 | 298 | 269 |
| Sulphur metabolism | 8 | 6 | 42 | 7 |
| Phosphorus metabolism | 22 | 21 | 28 | 28 |
| Carbohydrates | 227 | 207 | 205 | 168 |

Strains: 1, M3-13$^T$; 2, *M. salicampi* KCTC 12972$^T$; 3, *M. maroccanus* LMG 30466$^T$; 4, *M. hydrocarbonoclasticus* ATCC 49840$^T$

## Taxonomic Conclusion

Based on the polyphasic taxonomic characterization presented in this study, strain M3-13$^T$ is proposed to represent a novel species of genus *Marinobacter*, with the name *Marinobacter caseinilyticus* sp. nov.

### Description of *Marinobacter caseinilyticus* sp.nov.

*Marinobacter caseinilyticus* (ca.se.i.ni.ly'ti.cus. N.L. neut. n.*caseinum* casein; N.L. masc. adj. *lyticus* (from Gr. masc. adj. *lytikos*) able to loosen, able to dissolve; N.L. masc. adj. *caseinilyticus* casein dissolving).

Cells are rod shaped, Gram-stain negative and facultative anaerobic. Colonies on MA supplemented with 4% (w/v) NaCl are circular, raised, smooth, white in colour and 0.8–2 mm in diameter after 7 days incubation at 30 °C. The pH and temperature ranges for growth are pH 5.5–9.5 and 15–42 °C (optimal at pH 8.0–8.5 and 28–30 °C). The NaCl concentration range for growth is 0.5–16% (w/v), and optimal growth occurs at 3–6% (w/v). Tween 20 and casein are hydrolysed. Starch, tyrosine, gelatin, xanthine, hypoxanthine, Tween 40, Tween 60 and Tween 80 are not degraded. In addition, catalase activity and oxidase activity are positive, while results from hydrogen sulphide production, methyl-red test and Voges–Prokauer test are negative. Growth is observed in anaerobic conditions with sulphate

**Table 2** Characteristics of strain M3-13$^T$ and closely related type strains

| Characteristic | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Cell size (μm) | 0.2–0.7 × 1.3–2.5 | 0.4–0.8 × 1.0–7.0[a] | 0.5 × 1.6–2.0[b] | 0.3–0.6 × 2.0–3.0[c] |
| NaCl range (optimum) (%, w/v) | 0.5–16 (3–6) | ND (8[a]) | 2–15 (5–7)[b] | 0.5–20[c] (ND) |
| Temperature range(optimum) (°C) | 4–42 (28–30) | 4–39[a] (ND) | 4–40 (30–37)[b] | 10–45[c] (ND) |
| pH range(optimum) | 5.5–9.5 (8.0–8.5) | ND (7–8[a]) | 6–9 (7–8) [b] | 5.5–9.5[c] (ND) |
| Nitrate reduction to nitrite | − | + | + | − |
| Nitrite reduction to $N_2$ | − | − | − | + |
| Hydrolysis of | | | | |
| Tween 40, Tween 60 | − | + | − | + |
| Tween 80 | − | + | + | + |
| Casein and arginine dihydrolase | + | − | − | − |
| API ZYM test | | | | |
| Lipase (C14) | − | + | − | + |
| Acid phosphatase | + | − | − | − |
| Acid production from | | | | |
| D-Xylose | + | − | − | − |
| D-Maltose and erythritol | − | − | + | − |
| D-Glucose, D-fructose and D-mannose | + | − | + | − |
| Utilization of | | | | |
| D-Galactose | − | W | W | W |
| D-Fructose | W | − | W | − |
| D-Mannose | W | − | + | − |
| DL-Alanine | − | W | + | W |
| D-Sorbitol | W | W | W | − |
| Formate | + | − | W | W |
| L-Lysine | − | + | W | − |
| L-Rhamnose and trehalose | − | − | W | + |
| Maltose | − | W | + | − |
| Sucrose | − | − | + | + |
| Trisodium citrate | + | − | + | W |

Strains: 1, M3-13$^T$; 2, *M. salicampi* KCTC 12972$^T$; 3, *M. maroccanus* LMG 30466$^T$; 4, *M. hydrocarbonoclasticus* DSM 8798$^T$. All data are obtained from this study unless indicated. In this study, all strains were negative for hydrolysis of starch, tyrosine, gelatin, xanthine, hypoxanthine and methyl-red test and the Voges–Prokauer test; and positive for catalase activity and Tween 20

+ positive, − negative, *W* weakly positive, *ND* no data available

[a]Data from Yoon, J., et al. [33]

[b]Data from Boujida, N., et al. [2]

[c]Data from Lee, O.O., et al. [5]

and nitrite as electron acceptors. The following carbon sources stimulated growth: D-fructose, D-glucose, D-mannose, D-sorbitol, malate, trisodium citrate, succinate and formate. In API 20NE test, cells are positive for fermentation of glucose. The API ZYM system revealed that cells are positive for acid phosphatase, alkaline phosphatase, esterase (C4), esterase (C8), cystine arylamidase, leucine arylamidase, valine arylamidase, naphthol-AS-BI-phosphohydrolase, *β*-glucosidase and *N*-acetyl-*β*-glucosaminidas. Using the API 50CH system, cells are able to oxidize D-glucose, D-fructose and D-xylose. The respiratory quinone is ubiquinone-9 (Q-9). Major fatty acids are $C_{16:1}$ *ω*7c/$C_{16:1}$ *ω*6c, $C_{16:0}$, $C_{18:1}$ *ω*7c/$C_{18:1}$ *ω*6c) and $C_{12:0}$ 3OH. The major polar lipids include diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, aminophosphoglycolipid and phosphoglycolipid. The DNA G+C content is 56.6% (by genome).

The type strain, M3-13$^T$ (= MCCC 1K04560$^T$ = KCTC 72043$^T$), was isolated from a saline soil in Zhejiang, China. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain M3-13$^T$ is MW342710. The GenBank accession numbers for the whole genome sequence of strain M3-13$^T$ are WUQJ00000000.

**Table 3** Cellular fatty acid contents of strain M3-13$^T$ and the reference strains

| Fatty acid | Percentages of total fatty acids | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| $C_{10:0}$ | 1.5 | 0.6 | 0.6 | 2.3 |
| $C_{11:0}$ | 0.3 | 0.4 | – | – |
| $C_{12:0}$ | 4.7 | 5.9 | 5.7 | 7.3 |
| $C_{13:0}$ | – | 0.2 | – | – |
| $C_{14:0}$ | 0.8 | 0.4 | 1.5 | 1.5 |
| $C_{16:0}$ | 21.6 | 14.0 | 21.0 | 25.6 |
| $C_{17:0}$ | 1.0 | 4.3 | 1.1 | 1.4 |
| $C_{18:0}$ | 0.4 | 2.5 | 2.6 | 3.4 |
| iso-$C_{13:0}$ | – | 0.7 | – | – |
| iso-$C_{14:0}$ | – | – | – | 1.2 |
| iso-$C_{16:0}$ | 0.3 | 0.6 | – | – |
| iso-$C_{17:0}$ | 0.4 | 1.2 | – | – |
| $C_{16:0}$ N-alcohol | 4.7 | 0.6 | 2.7 | – |
| $C_{12:0}$ 3OH | 5.0 | 4.1 | 4.7 | 6.5 |
| $C_{11:0}$ 3OH | – | 0.6 | – | – |
| $C_{13:0}$ 2OH | – | – | – | 0.9 |
| 10-Methyl $C_{17:0}$ | 3.0 | 0.9 | 1.2 | – |
| $C_{14:1}$ $\omega$5c | – | – | 0.4 | 2.2 |
| $C_{15:1}$ $\omega$6c | 0.4 | 0.5 | – | – |
| $C_{15:1}$ $\omega$8c | 0.3 | 0.6 | 0.4 | 1.6 |
| $C_{16:1}$ $\omega$5c | – | 0.4 | – | – |
| $C_{16:1}$ $\omega$7c alcohol | 3.6 | – | 0.9 | – |
| $C_{16:1}$ $\omega$9c | – | 4.9 | 10.0 | 9.3 |
| $C_{17:1}$ $\omega$6c | 0.6 | 0.7 | – | – |
| $C_{17:1}$ $\omega$8c | 3.0 | 6.4 | 2.4 | 1.8 |
| $C_{18:1}$ $\omega$5c | – | 0.5 | – | – |
| $C_{18:3}$ $\omega$6c (6, 9, 12) | 0.3 | – | 1.3 | – |
| $C_{18:1}$ $\omega$9c | 0.8 | 6.4 | 18.1 | 26.4 |
| Sum in feature 1* | 0.3 | 0.5 | 0.3 | 1.5 |
| Sum in feature 2* | – | 0.4 | – | – |
| Sum in feature 3* | 41.1 | 32.1 | 20.0 | 7.3 |
| Sum in feature 9* | 0.6 | 3.3 | 0.4 | – |
| Sum in feature 8* | 5.4 | 6.0 | 4.6 | – |

Strains: 1, M3-13$^T$; 2, *M. salicampi* KCTC 12972$^T$; 3, *M. maroccanus* LMG 30466$^T$; 4, *M. hydrocarbonoclasticus* DSM 8798$^T$; All data was obtained from this study

*Summed features represent one or more fatty acids that cannot be separated by MIDI system. Sum in Feature 1 composed of $C_{15:1}$ iso H and/or $C_{13:0}$ 3OH, Sum in Feature 2 composed of $C_{14:0}$ 3OH and/or $C_{16:1}$ iso I, Sum in Feature 3 composed of $C_{16:1}$ $\omega$7c and/or $C_{16:1}$ $\omega$6c, Sum in Feature 9 composed of $C_{17:1}$ iso $\omega$9c and/or $C_{16:0}$ 10-methyl, Sum in Feature 8 composed of $C_{18:1}$ $\omega$7c and/or $C_{18:1}$ $\omega$6c

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