Membranicola marinus gen. nov., sp. nov., a new member of the family Saprospiraceae isolated from a biofilter in a recirculating aquaculture system

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A Gram-staining-negative bacterial strain (termed CZ-AZ5T) was isolated from a biological filter in a marine recirculating aquaculture system in Tianjin, China. Its taxonomic status was determined using a polyphasic approach. CZ-AZ5T cells were non-spore-forming, non-motile rods, 0.6–0.7 μm wide and 3.0–3.7 μm long. CZ-AZ5T was strictly heterotrophic, aerobic, oxidase-negative and catalase-positive. Growth occurred in the temperature range 20–40 °C (optimal: 30 °C), pH range 6.0–8.5 (optimal: pH 7.5) and salinity range 0–5 % (w/v) NaCl (optimal: 1 %). In phylogenetic analyses based on 16S rRNA gene sequences, CZ-AZ5T was assigned to the family Saprospiraceae (phylum Bacteroidetes) and was clustered with the genera Saprospira and Aureispira within this family. It showed highest sequence similarity to ‘Candidatus Haliscomenobacter caldificugens’ (86.2 %), followed by Saprospira grandis ATCC 23119T (85.7 %) and Lewinella persica T-3T (85.6 %). DNA G + C content was 40.1 mol%, the major menaquinone was MK-7, and the major cellular fatty acids (>10 %) were C16:0, 10/7c and iso-C15:0. Our phenotypic, chemotaxonomic and phylogenetic observations, taken together, led us to conclude that strain CZ-AZ5T represents a novel species and genus of the family Saprospiraceae, for which the name Membranicola marinus gen. nov., sp. nov. is proposed. The type strain is CZ-AZ5T (=CGMCC 1.13179T=JCM 18886T).

The bacterial family Saprospiraceae (Garrity & Holt, 2001), within the phylum Bacteroidetes, comprises seven genera: Saprospira (type genus), Aureispila, Haliscomenobacter, Lewinella, Phaeodactylibacter, ‘Rubidimonas’ and Portibacter. Four of these genera contain a single species, i.e. Saprospira grandis (Reichenbach, 1989, 2006), Haliscomenobacter hydrossis (van Veen et al., 1973), ‘Rubidimonas crustatorum’ (Yoon et al., 2012a) and Portibacter latus (Yoon et al., 2012b). The genus Aureispira (Hosoya et al., 2006) contains two species: Aureispira marina (Hosoya et al., 2006) and Aureispira maritima (Hosoya et al., 2007). The genus Phaeodactylibacter comprises Phaeodactylibacter xiamensis (Chen et al., 2014) and Phaeodactylibacter luteus (Lei et al., 2015). The genus Lewinella (originally called Herpetosiphon) contains eight species: Lewinella cohaerens (Khan et al., 2007), L. agarilytica (Lee, 2007), L. antarctica (Oh et al., 2009), L. lutea (Khan et al., 2007), L. marina (Khan et al., 2007), L. nigricans (Khan et al., 2007), L. persica (Khan et al., 2007) and L. xylanilytica (Sung et al., 2015). We isolated an unusual strain of the family Saprospiraceae from a biological filter in a recirculating aquaculture system in Tianjin, China. Results of a polyphasic analysis indicated that the strain belongs to a novel species and genus within the family Saprospiraceae.

For isolation of bacterial strains, biofilm samples were dispersed in sterilized detachment buffer [0.1 % sodium pyrophosphate in phosphate buffer (130 mM NaCl, 10 mM Na2HPO4, 10 mM NaH2PO4; pH 7.4)] and serially 10-fold diluted with sterile saline solution. Dilutions of 10−4, 10−5 and 10−6 (each 100 μl, in triplicate) were spread on diluted marine agar 2216 (DMA; per litre seawater: 0.25 g tryptone, 0.05 g yeast extract, 0.1 g iron...
citrate, 15 g agar; pH 7.4) and incubated at 30 °C for 2 weeks. Strain CZ-AZ5<sup>T</sup> was isolated and purified by repeated restreaking on the same plates. Purity of the strain was confirmed, based on uniformity of colony morphology and microscopic examination.

To evaluate phylogenetic relationships of CZ-AZ5<sup>T</sup>, its 16S rRNA gene was PCR-amplified with universal primers 27F (5’-AGAGTTTGATCCTGGCTCAG-3’) and 1492R and sequenced by Nuosai (Beijing, China). A nearly complete 16S rRNA gene sequence (1396 nt) was obtained and compared with available 16S rRNA gene sequences in GenBank databases using (i) the BLAST program (Altschul et al., 1990) on the National Center for Biotechnology Information (NCBI) database (www.ncbi.nlm.nih.gov), and (ii) identity analysis (Chun et al., 2007; Kim et al., 2012) on the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net). Sequence data were aligned using the DNAMAN program, v. 5.0 (Lynnon Biosoft). Phylogenetic trees were reconstructed using neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods in the MEGA 5 program package (Tamura et al., 2011). Robustness of tree topologies was assessed by bootstrap analyses based on 1000 resamplings.

Analysis of 16S rRNA gene sequences revealed highest sequence similarity of CZ-AZ5<sup>T</sup> with ‘Candidatus Haliscomenobacter calcifugius’ (86.2 %), followed by S. grandis ATCC 23119<sup>T</sup> (85.7 %), L. persica T-3<sup>T</sup> (85.6 %), Aureispira maritima 59SA<sup>T</sup> (85.3 %) and Haliscomenobacter hydrossis DSM 1100<sup>T</sup> (85.2 %). Sequence similarity with all other members of the family Saprospiraceae was <84 %. Phylogenetic analyses indicated that CZ-AZ5<sup>T</sup> belongs to the family Saprospiraceae of the phylum Bacteroidetes and is clustered with the genera Saprospira and Aureispira within this family (Fig. 1).

Strains were cultured routinely at 30 °C on marine agar 2216 (MA; Difco), or under other conditions as indicated. Reference strains, S. grandis JCM 21750<sup>T</sup>, L. persica NBRC 102663<sup>T</sup>, Aureispira marina JCM 23197<sup>T</sup> and Haliscomenobacter hydrossis DSM 1100<sup>T</sup>, were cultured and analysed in parallel.

Gram staining was performed as described by Dong & Cai (2001). Cell morphology was examined by light microscopy (model BH-2; Olympus) and flagellation was determined by transmission electron microscopy (model JEM-1400; JEOL) of cells grown for 2 days on MA. Oxidase activity was evaluated using oxidase reagent (bioMérieux). Catalase activity, amylase and hydrolysis of casein, tyrosine and Tweens 20, 40, 60 and 80 were evaluated as described by Dong & Cai (2001). Oxygen consumption was measured in cells grown on MA at 25 °C for 1 week by incubation in an Oxoid AnaeroGen system. H₂S production was measured using lead acetate paper. For pigment characterization, strain CZ-AZ5<sup>T</sup> was grown on MA for 48 h at 30 °C. Absorption spectra between 200 and 800 nm of pigments extracted by acetone/methanol (7 : 2, v/v) were determined using a UV spectrophotometer (model UV-2802H; UNICO). The presence of flexirubin-like pigments was assessed as described by Bernardet et al., (2002).

![Fig. 1](https://www.microbiologyresearch.org/figure.png)

**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing relationships among strain CZ-AZ5<sup>T</sup> and representative members of the family Saprospiraceae. Only bootstrap values >50 % (based on 1000 resamplings) are shown at branching points. *Escherichia coli* ATCC 1177<sup>T</sup> was used as an outgroup. Bar: 0.01 nucleotide substitutions per site.
Growth temperature range was evaluated by incubating cells in marine broth medium 2216 (MB; laboratory prepared; per litre distilled water: 5.0 g tryptone, 1.0 g yeast extract, 19.45 g NaCl, 5.9 g MgCl₂, 3.24 g Na₂SO₄, 1.8 g CaCl₂, 0.55 g KCl, 0.16 g Na₂CO₃, 0.1 g iron citrate, 0.08 g KBr, 0.034 g SrCl₂, 0.022 g boric acid, 0.004 g Na₂SiO₃, 0.0024 g NaF, 0.0016 g NH₄NO₃, 0.008 g Na₂HPO₄; pH 7.6) for 1 week, in 5 °C increments from 10 to 45 °C. Salinity range for growth was evaluated in modified MB supplemented with 0–6 % (w/v) NaCl (in 1 % increments). pH range for growth was evaluated in MB (Difco; inoculum size 0.01 %) with pH 5.0–10.0 (increments of 0.5 unit) using three different buffers (final concentration, 50 mM): sodium acetate buffer (for pH 5.0–5.5), sodium phosphate buffer (for pH 6.0–8.0) and Tris/HCl buffer (for pH 8.0–10.5).

Strain CZ-AZ5T was further characterized using API ZYM and API 20NE systems (bioMérieux) according to the manufacturer’s instructions. Carbon source utilization was assessed using API 50CH test strips (bioMérieux) and artificial seawater (per litre distilled water: 24 g NaCl, 7 g MgSO₄·7H₂O, 5.3 g MgCl₂·6H₂O, 0.7 g KCl, 0.1 g CaCl₂·2H₂O; pH 7.5) supplemented with trace element solution (per litre distilled water: 0.08 g KBr, 0.034 g SrCl₂, 0.022 g boric acid, 0.004 g Na₂SiO₃, 0.0024 g NaF, 0.0016 g NH₄NO₃, 0.008 g Na₂HPO₄), 0.05 g yeast extract and 0.4 % (w/v) carbon source. Antibiotic susceptibility was evaluated by the agar diffusion method using filter paper discs (Beijing Pharmaceutical) containing various antibiotics as specified in the species description.

Strain CZ-AZ5T showed some important properties that are in agreement with those reported for the family Saprospiraceae (Mcilroy & Nielsen, 2014), such as exhibiting an isoprenoid quinone (23197T; from L. n. incola) a dweller, inhabitant; N.L. masc. n. Membranicola a dweller of filters).

Strictly heterotrophic and aerobic. Cells are Gram-staining-negative, non-spore-forming, non-motile rods (Fig. S1 available in the online Supplementary Material). Oxidase-negative and catalase-positive. Flexirubin-type pigments are absent. The major respiratory quinone is menaquinone 7 (MK-7). The major fatty acids are C₁₆:₁ω7c and iso-C₁₃:₀. The type species is Membranicola marinus.

For fatty acid analysis, CZ-AZ5T and three closely related strains (‘phylogenetic neighbors’; S. grandis JCM 21750T, L. persica NBRC 102663T, Aureispira marina JCM 23197T) were incubated separately on MA plates for 3 days at 25 °C to late exponential phase, and harvested. Cellular fatty acids were analysed using the standard MIDI Sherlock Microbial Identification System (v. 6.0). Isoprenoid quinones were extracted and purified as described by Collins (1985), analysed by HPLC (Wu et al., 1989), and identified by comparison with known quinones from reference strain Algoriphagus aquatilis A8-7T. Genomic DNA of CZ-AZ5T was extracted and purified using a bacterial genomic DNA Purification kit (Sigma). DNA G+C content was analysed by a thermal denaturation method (Marmur & Doty, 1962). The major fatty acid components of CZ-AZ5T were C₁₆:₁ω7c (38.2 %) and iso-C₁₃:₀ (20.4 %), in striking contrast to the three phylogenetic neighbours (Table 2). The predominant respiratory quinone in CZ-AZ5T was MK-7, as also observed in other strains representing the family Saprospiraceae, for which the name Membranicola marinus gen. nov., sp. nov. is hereby proposed.

**Description of Membranicola gen. nov.**

*Membranicola* [Mem.bra.ni´co.la. N.L. n. membrana membrane; L. suff. -cola (from L. n. incola) a dweller, inhabitant; N.L. masc. n. Membranicola a dweller of filters].

Strictly heterotrophic and aerobic. Cells are Gram-staining-negative, non-spore-forming, non-motile rods (Fig. S1 available in the online Supplementary Material). Oxidase-negative and catalase-positive. Flexirubin-type pigments are absent. The major respiratory quinone is menaquinone 7 (MK-7). The major fatty acids are C₁₆:₁ω7c and iso-C₁₃:₀. The type species is Membranicola marinus.

**Description of Membranicola marinus sp. nov.**

*Membranicola marinus* (ma.ri´nus. L. masc. n. marinus of the sea, where the type strain was first isolated).

Colonies grown on MA, at 1 week, are 0.5–1.0 mm in diameter, circular, smooth, convex, opaque and orange in colour. Growth occurs in the temperature range 20–40 °C (optimal, 30 °C), pH range 6.0–8.5 (optimal, pH 7.5) and salinity range 0–5 % (w/v) NaCl (optimal, 1 %). H₂S is produced. Aesculin and Tween 20 and 40 and 60 are hydrolysed. Casein, trypsin, starch, Tween 80, urea, arginine and gelatin are not hydrolysed. In the API ZYM system, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, N-acetyl-β-glucosaminidase, β-glucosidase, N-acetyl-β-glucosaminidase, β-glucosidase, α-mannosidase and α-fucosidase activities are present. Esterase (C4), lipase (C14), trypsin, α-chymotrypsin and β-glucuronidase activities are absent. Negative for nitrate reduction, indole production and

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Table 1. Differential characteristics of strain CZ-AZ5\textsuperscript{T} and other members of the family Saprospiraceae

Strains: 1, CZ-AZ5\textsuperscript{T}; 2, S. grandis ICM 21750\textsuperscript{T}; 3, L. persica NBRC 102663\textsuperscript{T}; 4, 'L. xylanilytica' 13-9-B8; 5, L. cohaerens II-2\textsuperscript{T} (data from Sung et al., 2015); 6, L. nigricans SS-2\textsuperscript{T} (Khan et al., 2007); 7, L. agarlytica SST-19\textsuperscript{T} (Lee, 2007); 8, L. antarctica IMCC3223\textsuperscript{T} (Oh et al., 2009); 9, L. lutea FYK2402M69\textsuperscript{T} (Chen et al., 2014); 10, L. marina MKG-38\textsuperscript{T} (Khan et al., 2007); 11, Aureispira marina JCM 23197\textsuperscript{T}; 12, Aureispira maritima 59SAT (Hosoya et al., 2007); 13, Haliscomenobacter hydrossis DSM 1100\textsuperscript{T}; 14, Phaeodactylibacter xiamenensis KD52\textsuperscript{T} (Chen et al., 2014); 15, Phaeodactylibacter luteus GYP20\textsuperscript{T} (Lei et al., 2015); 16, Portibacter luteus YM8-076\textsuperscript{T} (Yoon et al., 2012b).

+ Positive; −, negative; W, weakly positive; V, variable; ND, no data available.

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<td>Tween 20</td>
<td>+</td>
<td>W</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
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<td>+</td>
<td>W</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Tween 80</td>
<td>−</td>
<td>W</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>40.1</td>
<td>49.8</td>
<td>53</td>
<td>59.1</td>
<td>44.9</td>
<td>53.1</td>
<td>51.3</td>
<td>50.3</td>
<td>56</td>
<td>61</td>
<td>38-39</td>
<td>38.7</td>
<td>47.8</td>
<td>51</td>
<td>53</td>
<td>53.7</td>
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</table>

aData from this study except for DNA G+C content result.
Table 2. Cellular fatty acids of CZ-AZ5\textsuperscript{T} and three closely related strains

<table>
<thead>
<tr>
<th>Fatty acid</th>
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<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td>C\textsubscript{16}:0</td>
<td>3.6</td>
<td>1.3</td>
<td>5.5</td>
<td>15.7</td>
</tr>
<tr>
<td>iso-C\textsubscript{13}:0</td>
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<td>TR</td>
<td>1.8</td>
<td>–</td>
</tr>
<tr>
<td>iso-C\textsubscript{15}:0</td>
<td>20.4</td>
<td>28.8</td>
<td>14.2</td>
<td>9.1</td>
</tr>
<tr>
<td>iso-C\textsubscript{16}:0</td>
<td>TR</td>
<td>2.2</td>
<td>TR</td>
<td>7.6</td>
</tr>
<tr>
<td>iso-C\textsubscript{17}:0</td>
<td>TR</td>
<td>–</td>
<td>TR</td>
<td>25.9</td>
</tr>
<tr>
<td>iso-C\textsubscript{15}:1 G</td>
<td>–</td>
<td>11.7</td>
<td>–</td>
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<tr>
<td>iso-C\textsubscript{15}:1 F</td>
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<td>–</td>
<td>15.3</td>
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<tr>
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<td>–</td>
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<tr>
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<td>3.7</td>
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<tr>
<td>anteiso-C\textsubscript{15}:0</td>
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<td>1.5</td>
<td>2.1</td>
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</tr>
<tr>
<td>C\textsubscript{15}:0 10\text{c}c</td>
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<td>–</td>
<td>7.1</td>
<td>–</td>
</tr>
<tr>
<td>C\textsubscript{15}:1 10\text{c}c</td>
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<td>1.7</td>
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<tr>
<td>C\textsubscript{16}:0 N alcohol</td>
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<td>–</td>
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<tr>
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<td>1.1</td>
<td>–</td>
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<tr>
<td>C\textsubscript{16}:1 7\text{c}c</td>
<td>38.2</td>
<td>5.5</td>
<td>31.3</td>
<td>1.9</td>
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<tr>
<td>C\textsubscript{18}:0 7\text{c}c\textsubscript{18}:1 \text{c}c</td>
<td>1.6</td>
<td>–</td>
<td>–</td>
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<tr>
<td>iso-C\textsubscript{17}:1 10\text{c}c\textsubscript{16}:0 10-methyl</td>
<td>7.8</td>
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<td>1.3</td>
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<td>C\textsubscript{20}:5\text{c}c</td>
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<td>TR</td>
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</tr>
<tr>
<td>C\textsubscript{17}:1 9\text{c}c</td>
<td>8.6</td>
<td>–</td>
<td>30.1</td>
<td>–</td>
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<tr>
<td>C\textsubscript{17}:0 2\text{OH}</td>
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<td>–</td>
<td>1.3</td>
<td>–</td>
</tr>
<tr>
<td>C\textsubscript{18}:0 3\text{OH}</td>
<td>TR</td>
<td>2.3</td>
<td>TR</td>
<td>TR</td>
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<tr>
<td>C\textsubscript{17}:0 3\text{OH}</td>
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<td>TR</td>
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<td>–</td>
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<td>6.3</td>
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</table>

References


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Membranicola marinus gen. nov., sp. nov.

glucose fermentation. Growth occurs on pectin, trehalose, D-sorbitol and L-glutamate. No growth occurs on glucose, pyruvate, mannitol, D-mannose, D-galactose, lactose, maltose, D-xylene, cellobiose, glycine, citrate, succinate, malate or fumarate. The major fatty acid components are C\textsubscript{16}:0 10\text{c}c and iso-C\textsubscript{15}:0. The predominant respiratory quinone is MK-7.

The type strain is CZ-AZ5\textsuperscript{T} (≡CGMCC 1.13179\textsuperscript{T} = JCM 18886\textsuperscript{T}), isolated from biofilm of a biological filter in a recirculating aquaculture system in Tianjin, China. The DNA G+C content of the type strain is 40.1 mol%.

Acknowledgements

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