Reclassification of *Salegentibacter catena* Ying *et al.* 2007 as *Salinimicrobium catena* gen. nov., comb. nov. and description of *Salinimicrobium xinjiangense* sp. nov., a halophilic bacterium isolated from Xinjiang province in China

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A Gram-negative, non-motile and moderately halophilic rod-shaped bacterium, designated strain BH206T, was isolated from a saline lake of Xinjiang province in China. The isolate showed catalase-positive and oxidase-negative reactions and did not reduce nitrate. Phylogenetic analysis based on 16S rRNA gene sequences showed that the isolate was most closely related to [Salegentibacter] catena HY1T with 95.8% 16S rRNA gene sequence similarity, and formed a tight phyletic group with [Salegentibacter] catena HY1T with a bootstrap value of 99% within the family Flavobacteriaceae. However, strain BH206T and [Salegentibacter] catena HY1T formed a phyletic lineage distinct from other Salegentibacter species. The 16S rRNA gene sequence similarities of strain BH206T with other related type species were lower than 94.6%. On the basis of physiological and molecular properties, it is clear that [Salegentibacter] catena should be reclassified in the new genus *Salinimicrobium* as *Salinimicrobium catena* gen. nov., comb. nov. (type strain HY1T=CGMCC 1.6101T=JCM 14015T) and that strain BH206T represents a novel species within the genus *Salinimicrobium*, for which the name *Salinimicrobium xinjiangense* sp. nov. is proposed. The type strain of *Salinimicrobium xinjiangense* is BH206T (=KCTC 12883T=DSM 19287T).

The genus *Salegentibacter*, a member of the family Flavobacteriaceae, was first proposed by McCammon & Bowman (2000) to accommodate moderately halophilic, yellow-pigmented, non-gliding bacteria that were isolated from a hypersaline meromictic lake in Antarctica. At the time of writing, the genus comprises five recognized species, *Salegentibacter holothuriorum*, isolated from the edible holothurian *Apostichopus japonicus* (Nedashkovskaya *et al.*, 2004), *Salegentibacter mishustinae*, from the sea urchin *Strongylocentrotus intermedius* (Nedashkovskaya *et al.*, 2005a), *Salegentibacter agarivorans*, associated with a sponge (Nedashkovskaya *et al.*, 2006), and *Salegentibacter flavus* (Ivanova *et al.*, 2006) and *Salegentibacter catena* (Ying *et al.*, 2007) from sea sediment.

In the course of screening halophilic bacteria, a non-motile, Gram-negative, moderately halophilic bacterium, designated strain BH206T, was isolated from soil sediment of a salt lake. Comparative analysis of 16S rRNA gene sequences indicated that the closest relative of strain BH206T was *Salegentibacter catena* HY1T, with 95.8% 16S rRNA gene sequence similarity, and strain HY1T should be reclassified as a new genus on the basis of phylogenetic and phenotypic characteristics.

Strain BH206T was isolated on marine agar 2216 (MA; Difco) with the addition of 8% (w/v) NaCl [final...
urea, casein, Tween 80, hypoxanthine, L-tyrosine and reduction and hydrolysis of starch, aesculin, CM-cellulose, family Flavobacteriaceae (Bernardet et al., 2002). Nitrate reduction and hydrolysis of starch, ascesulin, CM-cellulose, urea, casein, Tween 80, hypoxanthine, L-tyrosine and xanthine were determined on MA or in marine broth according to the methods described previously (Lanyi, 1987; Smibert & Krieg, 1994). Acid production from galactose, d-glucose, lactose, maltose, mannose, l-arabinose, d-fructose, glycerol, d-mannitol, melibiose, raffinose, salicin and sucrose was determined as described by Leifson (1963). Additional enzyme activities were determined using API ZYM strips (bioMérieux) at 32°C. Cell morphology, flagella and gliding motility were studied using phase-contrast microscopy and transmission electron microscopy (JEM-1010: JEOL) as described previously (Bernardet et al., 2002; Jeon et al., 2005). Strain BH206T was Gram-negative, catalase-positive, oxidase-negative and did not reduce nitrate to nitrite. Cells of the isolate were non-motile rods (0.6–1.0 μm wide and 1.2–2.4 μm long) (Supplementary Fig. S1; available in IJSEM Online). Strain BH206T hydrolysed ascesulin, casein, starch and L-tyrosine, but hydrolysis of CM-cellulose, hypoxanthine, Tween 80, xanthine and urea was not observed. Other phenotypic features of strain BH206T are presented in Table 1 and in the description of the novel species. Whole-cell fatty acids of strain BH206T were analysed according to the instructions of the Microbial Identification System (MIDI; Microbial ID) after cultivation on MA for 2 days at 32°C. Analyses of polar lipids and isoprenoid quinones were carried out using the methods described by Komagata & Suzuki (1987). The genomic DNA G+C content of strain BH206T was determined using an HPLC fitted with a reversed-phase column (GROM-SIL 100 ODS-2FE; GROM) according to the method of Tamaoka & Komagata (1984). The major respiratory lipoquinone of strain BH206T was menaquinone-6 (MK-6). The predominant cellular fatty acids of strain BH206T were iso-C15:0 (16.17%), anteiso-C15:0 (11.98%), iso-C16:0 (10.21%), iso-C17:0 3-OH (8.18%) and summed feature 3 (6.78%), comprising C16:0 7c and/or iso-C15:0 2-OH, which resemble those determined for other related type strains in the family Flavobacteriaceae (Supplementary Table S1). The major polar lipid of the test strain was phosphatidyl-ethanolamine (PE). The genomic DNA G+C content of strain BH206T was 42.1 mol%. A study of the phenotype of strain BH206T is summarized and compared with that of phylogenetically related type relatives in Table 1. Many of them are in accordance with those of [Salegentibacter catena HY1T and [Salegentibacter catena HY1T from Salegentibacter species. The sequencing and assembly of the 16S rRNA gene of strain BH206T was carried out as described previously (Lane, 1991). The resulting 16S rRNA gene sequence (1404 nt) of strain BH206T was compared with available 16S rRNA gene sequences from GenBank using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) to determine an approximate phylogenetic affiliation, and gene sequences were aligned with those of closely related species by using the CLUSTALW software program (Thompson et al., 1994). Phylogenetic trees were constructed using three different methods, neighbour-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP) algorithms, which are available in the PHYLIP software, version 3.6 (Felsenstein, 2002). Sequence similarity values were computed using Similarity Matrix version 1.1 (Ribosomal Database Project II; http://rdp.cme.msu.edu/; Cole et al., 2003) between the novel strain and other related members. Bootstrap analysis was performed according to the Kimura two-parameter model (Kimura, 1980) of the NJ method in the PHYLIP package. Phylogenetic analysis based on the 16S rRNA gene sequences indicated that the isolate was most closely related to [Salegentibacter catena HY1T, with 95.8% 16S rRNA gene sequence similarity, and formed a tight phylectic group with [Salegentibacter catena HY1T with 99% bootstrap value within the family Flavobacteriaceae (Fig. 1). However, strain BH206T and [Salegentibacter catena HY1T formed a phylectic lineage distinct from other Salegentibacter species. The 16S rRNA gene sequence similarities of strain BH206T with other related type species were lower than 94.6%. The overall topology of the ML and MP trees were essentially the same as that of...
Major fatty acids are iso-C15 : 1, iso-C15 : 0, anteiso-C15 : 0, iso-C16 : 0, C15 : 0, iso-C17 : 0, summed feature 3 (comprising iso-C15 : 0, anteiso-C15 : 0, iso-C15 : 0, iso-C16 : 0, summed feature 3 (comprising iso-C15 : 0, anteiso-C15 : 0, iso-C15 : 0, iso-C16 : 0, 2-OH and/or C16 : 1ω7c), iso-C17 : 0 3-OH and C17 : 0 2-OH. The DNA G+C content is 42.1–44.4 mol% (HPLC). Phylogenetically, the genus belongs to the family Flavobacteriaceae. The type species is Salinimicrobium catena.

Description of Salinimicrobium catena (Ying et al. 2007) comb. nov.

Salinimicrobium catena (ca.te'na. L. n. catena chain, referring to the fact that cells frequently occur in chains).

Basonym: Salegentibacter catena Ying et al. 2007.

The description is given by Ying et al. (2007). The type strain is HY1T (=CGMCC 1.6101T=JCM 14015T).

Description of Salinimicrobium xinjiangense sp. nov.

Salinimicrobium xinjiangense (xin.ji.ang.en'se. N.L. neut. adj. xinjiangense of Xinjiang, a region of China).

Displays the following properties in addition to those given in the genus description. Cells are 0.6–1.0 μm wide and 1.2–2.4 μm long, occurring in chains, but do not produce appendages in older cultures. Colonies are circular, smooth, glistening and convex. Facultatively aerobic.

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**Table 1.** Phenotypic characteristics of strain BH206T and other related type strains

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tbody>
<tr>
<td>Oxidase</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<td>Nitrate reduction</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Growth at: 37 °C</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>15 % NaCl</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Appendages</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
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<tr>
<td>Casein</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>DNA</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Starch</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Urea</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Acid from:</td>
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<tr>
<td>Arabinose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Galactose</td>
<td>W</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>–</td>
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<td>Glucose</td>
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<td>+</td>
<td>ND</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Lactose</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Maltose</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>H2S production</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Major fatty acids</td>
<td>iso-C15 : 0, iso-C16 : 0, C15 : 0, iso-C16 : 0</td>
<td>iso-C15 : 0, iso-C16 : 0, iso-C15 : 0, iso-C16 : 0</td>
<td>iso-C15 : 1, iso-C15 : 0, iso-C16 : 0, iso-C15 : 0, iso-C16 : 0, summed feature 3, iso-C15 : 0, iso-C16 : 0, iso-C17 : 0, 3-OH, iso-C16 : 0</td>
<td>32–34</td>
<td></td>
<td></td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>42.1</td>
<td>44.4</td>
<td>40.4</td>
<td>37–38</td>
<td>36.4</td>
<td>32–34</td>
</tr>
</tbody>
</table>

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The NJ tree (data not shown). The chemotaxonomic and molecular characteristics and phylogenetic properties described here showed that strain BH206T and [Salegentibacter] catena should be described as members of the same genus in the family Flavobacteriaceae, and that they are distinguishable from other closely related genera (Bernardet et al., 2002; Van Trappen et al., 2004; Ying et al., 2007). Therefore, we propose the reclassification of [Salegentibacter] catena to the genus Salinimicrobium as Salinimicrobium catena gen. nov., comb. nov. In addition, strain BH206T represents a novel species in the genus Salinimicrobium, for which the name Salinimicrobium xinjiangense sp. nov. is proposed.
Growth occurs at 10–48 °C (optimum: 32–35 °C), pH 6.0–9.0 (optimum: pH 7.5–8.0) and 0.5–10 % (w/v) NaCl (optimum: 2–3 %). No growth is observed without NaCl. API ZYM gives positive results for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase and N-acetyl-β-glucosaminidase, but negative results for lipase (C14), trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase. Aesculin, casein, starch and L-tyrosine are hydrolysed, but cellulose (CM-cellulose), hypoxanthine, Tween 80, xanthine and urea are not. Acid is produced from galactose, D-glucose, lactose, maltose and mannose, but not from L-arabinose, D-fructose, glycerol, D-mannitol, melibiose, raffinose, salicin or sucrose. Major cellular fatty acids (>1 % of the total fatty acids) are iso-C14:0 (1.47 %), iso-C15:0(1.20 %), iso-C16:0 (16.17 %), anteiso-C15:0 (11.98 %), C15:0 (4.81 %), iso-C16:1 (1.93 %), iso-C16:0 (10.21 %), summed feature 3 (comprising iso-C15:0 2-OH and/or C16:1ω7c 6.78 %), C16:0 (1.07 %), iso-C15:0 3-OH (1.70 %), C15:0 2-OH (2.23 %), iso-C17:0ω9c (8.66 %), anteiso-C17:0ω9c (5.86 %), iso-C17:0 (1.03 %), anteiso-C17:0 (1.26 %), C17:1ω6c (2.10 %), iso-C16:0 3-OH (1.86 %), iso-C17:0 3-OH (8.18 %) and C17:0 2-OH (6.17 %).

The type strain is BH206T (= KCTC 12883T =DSM 19287T) isolated from a salt lake in China.

Acknowledgements

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