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

Abbreviations: NJ, neighbour-joining; ML, maximum-likelihood; MP, maximum-parsimony.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BH206T is EF520007.

A transmission electron micrograph of cells of strain BH206T and a table showing the cellular fatty acid composition of strain BH206T and related type strains are available as supplementary material with the online version of this paper.
concentration: 10.94 % (w/v) NaCl] from a salt lake in Xinjiang province in China. After isolation, the novel strain was cultivated at 32 °C for 2 days on MA and stored at −80 °C in marine broth (Difco) supplemented with 10 % (v/v) glycerol. Except where indicated, the isolate was routinely grown aerobically on MA for 2 days at 32 °C. Requirement for and tolerance of NaCl was determined at 0.5 % increments in MH medium (Carrasco et al., 2006) [0–14 % (w/v) NaCl, 0.7 g MgCl₂, 0.96 g MgSO₄, 0.036 g CaCl₂, 0.2 g KCl, 0.006 g NaHCO₃, 0.0026 g NaBr, 1 g yeast extract (Difco), 0.5 g proteose peptone no. 3 (Difco) and 0.1 g glucose per litre]. Growth was examined at different temperatures (5–55 °C at 5 °C increments) and pH values (5.0–11.0 at 0.5 pH unit increments) in marine broth (Gomori, 1955). Anaerobic growth was determined by incubation in an anaerobic chamber at 32 °C for 5 days on MA. After 2 days incubation at 32 °C on MA, strain BH206⁻ᵀ formed yellow, circular, smooth, glistening and convex colonies. The strain grew in MH medium with the addition of 0.5–10 % (w/v) NaCl and optimum growth occurred at 2–3 % (w/v) NaCl. Growth was observed at temperatures between 10 and 48 °C, with an optimum growth temperature of 32–35 °C, and from pH 6.0 to 9.0 (optimum, pH 7.5–8.0). Anaerobic growth was observed after 5 days at 32 ºC on MA.

Gram-staining was determined using the bioMérieux Gram stain kit according to the manufacturer’s instructions. Oxidase activity was tested using Bactident oxidase strip (Merck) and catalase activity was determined by bubble production in 3 % (v/v) hydrogen peroxide solution. The production of flexirubin-type pigments was investigated using KOH, following the requirements specified in the minimal standards for the description of new taxa in the family Flavobacteriaceae (Bernardet et al., 2002). Nitrate reduction and hydrolysis of starch, asesculin, 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 ºC. 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 BH206⁻ᵀ 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 BH206⁻ᵀ hydrolysed asesculin, casein, starch and L-tyrosine, but hydrolysis of CM-cellulose, hypoxanthine, Tween 80, xanthine and urea was not observed. Other phenotypic features of strain BH206⁻ᵀ are presented in Table 1 and in the description of the novel species.

Whole-cell fatty acids of strain BH206⁻ᵀ were analysed according to the instructions of the Microbial Identification System (MIDI; Microbial ID) after cultivation on MA for 2 days at 32 ºC. 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 BH206⁻ᵀ 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 BH206⁻ᵀ was menaquinone-6 (MK-6). The predominant cellular fatty acids of strain BH206⁻ᵀ were iso-C₁₅:₀ (16.17 %), anteiso-C₁₅:₀ (11.98 %), iso-C₁₆:₀ (10.21 %), iso-C₁₇:₀(9c (8.66 %), iso-C₁₇:₀ 3-OH (8.18 %) and summed feature 3 (6.78 %), comprising C₁₆:₁₀7c 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 BH206⁻ᵀ was 42.1 mol%. A study of the phenotype of strain BH206⁻ᵀ 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 BH206⁻ᵀ was carried out as described previously (Lane, 1991). The resulting 16S rRNA gene sequence (1404 nt) of strain BH206⁻ᵀ 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 CLUSTAL W 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 phyletic group with [Salegentibacter catena HY1ᵀ with 99 % bootstrap value within the family Flavobacteriaceae (Fig. 1). However, strain BH206⁻ᵀ and [Salegentibacter catena HY1ᵀ formed a phyletic lineage distinct from other Salegentibacter species. The 16S rRNA gene sequence similarities of strain BH206⁻ᵀ 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

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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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<td>Oxidase</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<td>Nitrate reduction</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td>−</td>
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<td>Growth at:</td>
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<tr>
<td>37 °C</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td>15% NaCl</td>
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<td>−</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Appendages</td>
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<tr>
<td>Casein</td>
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<td>+</td>
<td>−</td>
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<td>DNA</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>+</td>
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<tr>
<td>Starch</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>Urea</td>
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<td>−</td>
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<td>Acid from:</td>
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<tr>
<td>Galactose</td>
<td>W</td>
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<td>ND</td>
<td>−</td>
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<tr>
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<tr>
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<tr>
<td>Maltose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Major fatty acids</td>
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<tr>
<td>iso-C_{15.0}, anteiso-C_{16.0}, iso-C_{15.0}</td>
<td></td>
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<td></td>
<td></td>
</tr>
<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>

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.

Description of Salinimicrobium gen. nov.

Salinimicrobium (Sal.in.i.mi.cro'bi.um. L. pl. n. salinae salt-works, salt-pits; N.L. neut. n. microbium microbe; N.L. neut. n. Salinimicrobium small, saline microbe).

Cells are Gram-negative, non-spore-forming rods. Catalase-positive and oxidase-negative. Colonies are yellow pigmented. No flexirubins are formed. Devoid of flagellar and gliding motilities. Urease-negative. Nitrate is not reduced to nitrite. Major isoprenoid quinone is MK-6. Major fatty acids are iso-C_{15.1}, iso-C_{15.0}, anteiso-C_{15.0}, C_{15.0}, iso-C_{16.0}, summed feature 3 (comprising iso-C_{15.0} 2-OH and/or C_{16.1(α7)c}, iso-C_{17.0} 3-OH and C_{17.0} 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.
**Flavobacterium denitrificans** ED5\(^{T}\) (AJ18907)

**Strain BH206\(^{T}\) (EF520007)

**Salegentibacter catena** HY\(^{T}\) (DO640642)

**Mesoria algae** KCTC 12099\(^{T}\) (AF536383)

**Marianthromonas ophiurae** NRR 0845\(^{T}\) (AB261012)

**Gramella echniocha LMG 22585\(^{T}\) (AY608409)

**Salegentibacter flavus** FG9\(^{T}\) (AY682200)

**Salegentibacter mitis** KMM 60497\(^{T}\) (AY576553)

**Salegentibacter aggarivorans** KMM 7019\(^{T}\) (DQ191176)

**Salegentibacter salagens** ACAM 46\(^{T}\) (M82279)

**Psychoflexus torquis** ACAM 623\(^{T}\) (U58581)

**Gillisia limnaea** DSM 15749\(^{T}\) (AJ440991)

**Gillisia mitsukurii** KCTC 12261\(^{T}\) (AY576655)

**Gillisia sandarakina** IC148\(^{T}\) (AY940077)

**Gillisia illustris** IC157\(^{T}\) (AY954008)

**Gillea laberoidea** ACAM 188\(^{T}\) (U82913)

**Gelidiobacter algicida** ACAM 536\(^{T}\) (U62914)

**Kordia algicida** KCTC 8814\(^{T}\) (AY195836)

**Stankierella latentera** ATCC 23177\(^{T}\) (M58769)

**Fig. 1.** Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain BH206\(^{T}\) and related taxa. Bootstrap values are shown as percentages of 1000 replicates, when greater than 50%. *Flavobacterium denitrificans* ED5\(^{T}\) was used as an outgroup. Bar, 0.01 changes per nucleotide position.

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 mannos, 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-C\(_{14:0}\) (1.47 %), iso-C\(_{15:0}\) \(\beta\) (1.20 %), iso-C\(_{15:0}\) \(\delta\) (16.17 %), anteiso-C\(_{15:0}\) (11.98 %), C\(_{16:0}\) (4.81 %), iso-C\(_{16:1}\) \(\beta\) (1.93 %), iso-C\(_{16:0}\) (10.21 %), summed feature 3 (comprising iso-C\(_{15:0}\) \(\beta\) and/or C\(_{16:1}\) \(\alpha7\)C) (6.78 %), C\(_{16:0}\) (1.07 %), iso-C\(_{15:0}\) \(\beta\) (1.70 %), C\(_{15:0}\) \(\beta\) (2.23 %), iso-C\(_{17:1}\) \(\alpha9\)C (8.66 %), anteiso-C\(_{17:1}\) \(\alpha9\)C (5.86 %), iso-C\(_{17:0}\) \(\beta\) (1.03 %), anteiso-C\(_{17:0}\) \(\beta\) (1.26 %), C\(_{17:1}\) \(\alpha9\)C (2.10 %), iso-C\(_{16:0}\) \(\beta\) (1.86 %), iso-C\(_{17:0}\) \(\beta\) (8.18 %) and C\(_{17:0}\) \(\beta\) (6.17 %).

The type strain is BH206\(^{T}\) (= KCTC 12883\(^{T}\) = DSM 19287\(^{T}\)), isolated from a salt lake in China.

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