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 BH206^T, 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* HY1^T with 95.8% 16S rRNA gene sequence similarity, and formed a tight phyletic group with [*Salegentibacter*] *catena* HY1^T with a bootstrap value of 99% within the family *Flavobacteriaceae*. However, strain BH206^T and [*Salegentibacter*] *catena* HY1^T 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%. 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 HY1^T=CGMCC 1.6101^T=JCM 14015^T) and that strain BH206^T 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 BH206^T (=KCTC 12883^T=DSM 19287^T).

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 BH206^T, was isolated from soil sediment of a salt lake. Comparative analysis of 16S rRNA gene sequences indicated that the closest relative of strain BH206^T was [*Salegentibacter*] *catena* HY1^T, with 95.8% 16S rRNA gene sequence similarity, and strain HY1^T should be reclassified as a new genus on the basis of phylogenetic and phenotypic characteristics.

Strain BH206^T was isolated on marine agar 2216 (MA; Difco) with the addition of 8% (w/v) NaCl [final

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

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BH206^T is EF520007.

A transmission electron micrograph of cells of strain BH206^T and a table showing the cellular fatty acid composition of strain BH206^T 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 °C 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, aesculin, 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 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 aesculin, 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 °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 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-C_{15:0} (16.17 %), anteiso-C_{15:0} (11.98 %), iso-C_{16:0} (10.21 %), iso-C_{17:1ω9c} (8.66 %), iso-C_{17:0} 3-OH (8.18 %) and summed feature 3 (6.78 %), comprising C_{16:1ω7c} and/or iso-C_{15:0} 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 phosphatidylethanolamine (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 [*Salagentibacter*] *catena* HY1^T and allow the differentiation of strain BH206^T and [*Salagentibacter*] *catena* HY1^T from *Salagentibacter* 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 [*Salagentibacter*] *catena* HY1^T, with 95.8 % 16S rRNA gene sequence similarity, and formed a tight phyletic group with [*Salagentibacter*] *catena* HY1^T with 99 % bootstrap value within the family *Flavobacteriaceae* (Fig. 1). However, strain BH206^T and [*Salagentibacter*] *catena* HY1^T formed a phyletic lineage distinct from other *Salagentibacter* 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

Table 1. Phenotypic characteristics of strain BH206^T and other related type strains

Strains: 1, BH206^T; 2, [*Salegentibacter*] *catena* HY1^T (data from Ying *et al.*, 2007); 3, *Salegentibacter flavus* KMM 6000^T (Ivanova *et al.*, 2006); 4, *Salegentibacter salegens* DSM 5424^T (Dobson *et al.*, 1993); 5, *Gillisia mitskevichiae* KCTC 12261^T (Nedashkovskaya *et al.*, 2005b); 6, *Mesonium algae* KCTC 12089^T (Nedashkovskaya *et al.*, 2003). All strains are positive for catalase activity and hydrolysis of gelatin and negative for flexirubin pigments and gliding motility. +, Positive; –, negative; w, weak; ND, no data.

Characteristic	1	2	3	4	5	6
Oxidase	–	–	+	+	+	+
Nitrate reduction	–	–	–	+	–	–
Growth at:						
37 °C	+	+	–	–	–	–
15 % NaCl	–	–	–	+	–	+
Appendages	–	+	–	–	ND	ND
Hydrolysis of:						
Casein	+	+	–	–	+	+
DNA	–	–	ND	+	+	–
Starch	+	+	+	+	–	–
Urea	–	–	+	–	+	–
Acid from:						
Arabinose	–	–	–	+	–	–
Galactose	w	–	ND	ND	–	–
Glucose	+	+	ND	+	+	–
Lactose	+	–	ND	–	–	–
Maltose	+	–	ND	+	–	–
H ₂ S production	–	+	–	–	–	+
Major fatty acids	iso-C _{15:0} , anteiso-C _{15:0} , iso-C _{16:0}	iso-C _{16:0} , C _{15:0}	anteiso-C _{15:0} , C _{15:0} , iso-C _{16:0}	iso-C _{15:1} , iso-C _{15:0} , anteiso-C _{15:0}	iso-C _{15:1} , summed feature 3, iso-C _{15:0}	iso-C _{15:0} , iso-C _{17:0} 3-OH, iso-C _{16:0}
DNA G + C content (mol%)	42.1	44.4	40.4	37–38	36.4	32–34

the NJ tree (data not shown). The chemotaxonomic and molecular characteristics and phylogenetic properties described here showed that strain BH206^T 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 BH206^T represents a novel species in the genus *Salinimicrobium*, for which the name *Salinimicrobium xinjiangense* sp. nov. is proposed.

Description of *Salinimicrobium* gen. nov.

Salinimicrobium (Sa.li.ni.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(ω7c)}, 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 HY1^T (=CGMCC 1.6101^T=JCM 14015^T).

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.

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 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-C_{14:0} (1.47 %), iso-C_{15:1} (1.20 %), iso-C_{15:0} (16.17 %), anteiso-C_{15:0} (11.98 %), C_{15:0} (4.81 %), iso-C_{16:1} (1.93 %), iso-C_{16:0} (10.21 %), summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c; 6.78 %), C_{16:0} (1.07 %), iso-C_{15:0} 3-OH (1.70 %), C_{15:0} 2-OH (2.23 %), iso-C_{17:1} ω 9c (8.66 %), anteiso-C_{17:1} ω 9c (5.86 %), iso-C_{17:0} (1.03 %), anteiso-C_{17:0} (1.26 %), C_{17:1} ω 6c (2.10 %), iso-C_{16:0} 3-OH (1.86 %), iso-C_{17:0} 3-OH (8.18 %) and C_{17:0} 2-OH (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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