Salinimicrobium terrae sp. nov., isolated from saline soil, and emended description of the genus *Salinimicrobium*

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A novel yellow-pigmented, Gram-negative, slightly halophilic, catalase-positive, oxidase-negative, obligately aerobic bacterium, designated strain YIM-C338^T, was isolated from saline soil from the Qaidam Basin in north-west China. Cells were non-sporulating, non-motile, short rods, predominantly occurring singly. Coccoid bodies and slightly curved rod-shaped cells of varying length developed in older cultures. Growth occurred with 0.5–8% (w/v) NaCl (optimally with 1–3%, w/v), at pH 6.0–9.0 (optimally at pH 7.0) and at 4–37 °C (optimally at 28 °C). The major cellular fatty acids were C_{16:0}, C_{18:0}, C_{16:1ω7c}, C_{18:1ω7c}, iso-C_{13:0} 3-OH and iso-C_{15:1}. MK-6 was the only respiratory quinone. Non-diffusible carotenoid pigments were produced. Flexirubin-type pigments were absent. The genomic DNA G+C content was 42.8 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain YIM-C338^T was a member of the genus *Salinimicrobium*, having sequence similarities of 97.3 and 94.0% with respect to *Salinimicrobium xinjiangense* BH206^T and *Salinimicrobium catena* HY1^T. The combination of the phylogenetic data, phenotypic characteristics and chemotaxonomic differences supported the view that strain YIM-C338^T represents a novel species of the genus *Salinimicrobium*, for which the name *Salinimicrobium terrae* sp. nov. is proposed. The type strain is YIM-C338^T (=DSM 17865^T =CGMCC 1.6308^T). An emended description of the genus *Salinimicrobium* is also provided.

The genus *Salinimicrobium*, belonging to the family *Flavobacteriaceae*, was recently proposed by Lim *et al.* (2008) with the transfer of *Salegentibacter catena* (Ying *et al.*, 2007) to *Salinimicrobium catena*, as well as the description of *Salinimicrobium xinjiangense*. In a recent study of the microbial diversity of the Qaidam Basin in Qinghai Province, north-west China, a novel heterotrophic, aerobic, yellow-pigmented, Gram-negative bacterial strain, designated YIM-C338^T, was isolated from a

saline soil sample collected from the north-west bank of the Chaka salt lake (36° 18'–36° 55' N 99° 02'–99° 12' E). On the basis of the distinctiveness of strain YIM-C338^T with respect to molecular systematics, fatty acid composition and phenotypic features, we propose that it represents a novel species of the genus *Salinimicrobium*.

Strain YIM-C338^T was isolated from a saline soil sample by plating 1:10 serial dilutions of the sample on marine agar 2216 (MA; Difco) (pH 7.2) for cultivation at 28 °C for 14 days. After primary isolation and purification, the isolate was preserved both on MA slants at 4 °C and in marine broth 2216 (MB; Difco) supplemented with 20% (v/v) glycerol at –80 °C. For chemotaxonomic and molecular systematic studies, the organism was grown in MB in flasks on a rotary shaker at 200 r.p.m. and 28 °C.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM-C338^T is EU135614.

A table showing the whole-cell fatty acid compositions (%) of YIM-C338^T and strains of the genus *Salinimicrobium* is available as supplementary material with the online version of this paper.

Biomass was harvested by centrifugation and was then washed twice with distilled water and freeze-dried.

DNA was isolated as described by Hopwood *et al.* (1985) and the G+C content was determined using the thermal denaturation method (Mandel & Marmur, 1968) with a UV-visible spectrophotometer (UV1601; Shimadzu). Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of PCR products were done as described previously (Cui *et al.*, 2001). Phylogenetic analysis was performed using the software package MEGA, version 3.1 (Kumar *et al.*, 2004), after multiple alignment of the sequence data by using CLUSTAL_X (Thompson *et al.*, 1997). Distances (corrected using Kimura's two-parameter model; Kimura, 1980) were calculated and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis (based on 1000 resamplings) was used to evaluate the tree topology of the neighbour-joining data (Felsenstein, 1985).

The DNA G+C content of strain YIM-C338^T was 42.8 mol%. The almost-complete 16S rRNA gene sequence (1467 bp) was determined. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain YIM-C338^T was closely related to the type strains of the two recognized members of the genus *Salinimicrobium*. The three strains formed a distinct clade in the phylogenetic tree, in which strain YIM-C338^T was phylogenetically most closely related to *Salinimicrobium xinjiangense* BH206^T (Lim *et al.*, 2008) with a 16S rRNA gene sequence similarity of 97.3 %. The two strains formed a distinct subclade with significant bootstrap support (100 %) (Fig. 1). The sequence similarity

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic position of strain YIM-C338^T and related taxa. *Capnocytophaga ochracea* ATCC 27872^T was used as the outgroup. Numbers at nodes indicate bootstrap percentages (>50%) based on a neighbour-joining analysis of 1000 resampled datasets. Bar, 1 substitution per 100 nucleotide positions.

between strain YIM-C338^T and *Salinimicrobium catena* HY1^T (Ying *et al.*, 2007) was 94.0 %. Stackebrandt & Ebers (2006) recommended an increased 16S rRNA gene sequence similarity threshold of 98.7–99 % above which DNA–DNA hybridization is necessary to demonstrate the uniqueness of a novel isolate, provided that these data are supported by the presence of clear phenotypic differences. Taking this view into consideration, the results of our phylogenetic analysis strongly suggested that strain YIM-C338^T did not belong to either of the recognized *Salinimicrobium* species.

Cell morphology was examined using light microscopy (model BH 2; Olympus). Gram staining was carried out using the standard Gram reaction combined with the KOH lysis test (Gegersen, 1978). Motility was observed both on half-strength MB solidified with 0.3 % agar under high-moisture conditions and in a hanging-drop preparation according to the methods of Bernardet *et al.* (2002).

Growth was tested at 4, 10, 15, 20, 28, 37, 40 and 45 °C on MA and at pH 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 in MB. The buffer solutions described by Chen *et al.* (2007) were used to adjust the pH of the medium. Tolerance of, and any requirement for, salts was determined on MA supplemented with 0–20 % (w/v) NaCl, MgCl₂ or KCl and on some other media as controls, i.e. nutrient agar, tryptic soy agar (BBL) and ISP 2 agar (Shirling & Gottlieb, 1966). NaCl-free MA was prepared as described by Atlas (1993). Hydrolysis of polymers, urease activity, nitrate reduction and Voges–Proskauer and methyl red tests were performed as described previously (Gerhardt *et al.*, 1981; Ventosa *et al.*, 1982; Atlas, 1993). Growth under anaerobic conditions, resistance to antibiotics and catalase and oxidase activities were detected as described by Chen *et al.* (2007). The utilization of various substrates as sole carbon and energy sources, the activity of constitutive enzymes and other physiological characteristics were examined using API 20E, API 50 CH (with API 50 CH B/E medium) and API ZYM strips (all from bioMérieux) and Biolog GN2 microplates according to the manufacturer's instructions. All suspension media were supplemented with 2 % (w/v) NaCl and all commercial systems were incubated at 28 °C. The results of the phenotypic tests are given in the species description and in Table 1.

Isoprenoid quinones were analysed using HPLC as described by Groth *et al.* (1996). The fatty acid composition was determined as described by Sasser (1990), using the Microbial Identification System (MIDI; Microbial ID) with cells grown in MB in flasks on a rotary shaker at 200 r.p.m. at 28 °C for 3 days. The bathochromic shift test with 10 % (w/v) KOH was used to detect flexirubin-type pigments. The test was performed on a small mass of cells collected with a loop and deposited on a glass slide placed on a white background; another similar mass of cells was deposited on the slide as a control (Bernardet *et al.*, 2002). Carotenoid pigments were extracted using methanol, as described by Schmit *et al.* (1994). Absorption spectra were

Table 1. Characteristics that serve to differentiate strain YIM-C338^T from *Salinimicrobium* type strains

Strains: 1, strain YIM-C338^T (data from this study); 2, *Salinimicrobium xinjiangense* BH206^T (Lim *et al.*, 2008); 3, *Salinimicrobium catena* HY1^T (Ying *et al.*, 2007). +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3
Facultatively anaerobic	–	+	–
Acid production from:			
Arabinose	+	–	–
Glucose	–	+	+
Lactose	–	+	–
Maltose	+	+	–
Growth at/with:			
4 °C	+	–	–
40 °C	–	+	+
10 % NaCl	–	+	+
Hydrolysis of:			
Casein	–	+	+
Gelatin	–	+	+
Tween 20	–	ND	+
Tween 80	+	–	ND
Nitrate reduction	+	–	–
H ₂ S production	–	–	+
N-Acetyl-β-glucosaminidase	+	+	–

determined between 250 and 700 nm using a UV-visible spectrophotometer (UV1601; Shimadzu).

MK-6 was the only respiratory quinone detected. The fatty acid profiles of strain YIM-C338^T and related type strains are given in Supplementary Table S1 (available in IJSEM Online). The major fatty acids of this strain were C_{16:0}, C_{18:0}, C_{16:1ω7c}, C_{18:1ω7c}, iso-C_{13:0} 3-OH and iso-C_{15:1}. The strain produced carotenoid pigments with absorbance peaks at 455 and 480 nm. Flexirubin-type pigments were absent.

Strain YIM-C338^T shared many phenotypic characteristics with the two members of the genus *Salinimicrobium*. However, it could be distinguished on the basis of a number of morphological, physiological and biochemical properties (Table 1) and by its very different fatty acid composition (Supplementary Table S1), although the latter may result partly from different culture conditions. The ability of YIM-C338^T to produce acid from arabinose, to reduce nitrate to nitrite and to grow at 4 °C, together with its inability to produce acid from glucose or lactose and its inability to hydrolyse casein or gelatin clearly differentiated this strain from the two recognized *Salinimicrobium* species. In addition, YIM-C338^T differed from its closest phylogenetic neighbour, *Salinimicrobium xinjiangense* BH206^T, in terms of its ability to hydrolyse Tween 80, its inability to produce acid from lactose and its inability to grow under anaerobic conditions (Table 1).

Hence, the combination of phylogenetic distinctiveness and phenotypic differences presented above clearly demon-

strates that strain YIM-C338^T represents a novel species of the genus *Salinimicrobium*, for which the name *Salinimicrobium terrae* sp. nov. is proposed. Additional data obtained in this study also allow an emendation of the description of the genus *Salinimicrobium*.

Emended description of the genus *Salinimicrobium* Lim *et al.* 2008

The description is as given by Lim *et al.* (2008) but with the following changes. Aerobic or facultatively anaerobic. Coccoid bodies and slightly curved rod-shaped cells of varying length may develop in older cultures. Na⁺ ions are required for growth. Nitrate may be reduced to nitrite.

Description of *Salinimicrobium terrae* sp. nov.

Salinimicrobium terrae (ter'rae. L. gen. n. *terrae* of the soil).

Cells are Gram-negative rods approximately 0.6–1 µm wide and 2–4 µm long, predominantly occurring singly. Coccoid bodies and slightly curved rod-shaped cells of varying length develop in older cultures. Endospores are not formed. Devoid of flagellar and gliding types of motility. Catalase-positive, oxidase-negative and strictly aerobic. Colonies are circular, convex, smooth, yellow-pigmented, non-translucent with entire margins and 1–1.5 mm in diameter after incubation for 5 days at 28 °C on MA. Non-diffusible yellow carotenoid pigments are produced. Flexirubin-type pigments are absent. Growth occurs at 4–37 °C (optimally at 28 °C) and pH 6.0–9.0 (optimally at pH 7.0). Slightly halophilic; growth occurs with 0.5–8 % (w/v) NaCl (optimally with 1–3 %, w/v). NaCl cannot be replaced by MgCl₂ or KCl. No growth occurs on nutrient agar, ISP 2 agar or tryptic soy agar. Aesculin, starch and Tweens 20 and 80 are hydrolysed, but casein, cellulose (CM-cellulose and filter paper), chitin, DNA, elastin and gelatin are not hydrolysed. Nitrate is reduced to nitrite. H₂S and indole are not produced. Methyl red and Voges–Proskauer tests give negative results. Acids are produced from D-arabinose, amygdalin, gentiobiose, inositol, maltose, potassium 5-ketogluconate, D-ribose and starch (API 50 CH). The following carbon sources are utilized in Biolog GN2 microplates: L-arabinose, D-arabitol, cellobiose, α-cyclodextrin, dextrin, glycogen, Tweens 40 and 80, D-fructose, D-galactose, gentiobiose, α-D-glucose, *myo*-inositol, maltose, D-mannitol, D-mannose, D-psicose, sucrose, trehalose, pyruvic acid methyl ester, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, DL-lactic acid, malonic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, D-alanine, L-alanyl glycine, L-asparagine, L-glutamic acid, glycyl L-glutamic acid, L-leucine, L-proline, L-pyrogutamic acid, L-threonine, DL-carnitine, γ-aminobutyric acid, inosine, glycerol, α-D-glucose 1-phosphate and D-glucose 6-phosphate. The constitutive enzymes expressed by strain

YIM-C338^T are *N*-acetyl- β -glucosaminidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, α -glucosidase, β -glucosidase, naphthol-AS-BI-phosphohydrolase and tryptophan deaminase (API ZYM, API 20E). The major fatty acids (making up 77.2% of the total in the type strain) are C_{16:0}, C_{18:0}, C_{16:1 ω 7c}, C_{18:1 ω 7c}, iso-C_{13:0} 3-OH and iso-C_{15:1}. The DNA G+C content of the type strain is 42.8 mol%.

The type strain, YIM-C338^T (=DSM 17865^T =CGMCC 1.6308^T), was isolated from a sample of saline soil collected from the bank of the Chaka salt lake in the Qaidam Basin, Qinghai Province, north-west China.

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