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-C338T, 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 C16 : 0, C18 : 0, C16 : 1v7c, C18 : 1v7c, iso-C13 : 03-OH and iso-C15 : 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-C338T was a member of the genus Salinimicrobium, having sequence similarities of 97.3 and 94.0 % with respect to Salinimicrobium xinjiangense BH206T and Salinimicrobium catena HY1T. The combination of the phylogenetic data, phenotypic characteristics and chemotaxonomic differences supported the view that strain YIM-C338T represents a novel species of the genus Salinimicrobium, for which the name Salinimicrobium terrae sp. nov. is proposed. The type strain is YIM-C338T (=DSM 17865T =CGMCC 1.6308T). 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-C338T, 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-C338T 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-C338T 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.
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 RNA 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-C338T was 42.8 mol%. The almost-complete 16S RNA gene sequence (1467 bp) was determined. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain YIM-C338T 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-C338T was phylogenetically most closely related to Salinimicrobium xinjiangense BH206T (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 between strain YIM-C338T and Salinimicrobium catena HY1T (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-C338T 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 (Gregersen, 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 physiologival characteristics were examined using API 20E, API 50 CH (with API 50 CH B/E medium) and API ZYM 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-C338T from *Salinimicrobium* type strains

<table>
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<tr>
<th>Characteristic</th>
<th>1</th>
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<tr>
<td>Facultatively anaerobic</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Acid production from:</td>
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<tr>
<td>Arabinose</td>
<td>+</td>
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<td>Glucose</td>
<td>−</td>
<td>+</td>
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<td>Lactose</td>
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<td>Maltose</td>
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<td>+</td>
<td>−</td>
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<td>Growth at/with:</td>
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<tr>
<td>4 °C</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td>40 °C</td>
<td>−</td>
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<td>10 % NaCl</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<td>Hydrolysis of:</td>
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<tr>
<td>Casein</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Gelatin</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Tween 20</td>
<td>−</td>
<td>ND</td>
<td>+</td>
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<tr>
<td>Tween 80</td>
<td>+</td>
<td>−</td>
<td>ND</td>
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<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td>H₂S production</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>+</td>
<td>+</td>
<td>−</td>
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</table>

Strains: 1, strain YIM-C338T (data from this study); 2, *Salinimicrobium xinjiangense* BH206T (Lim et al., 2008); 3, *Salinimicrobium catena* HY1T (Ying et al., 2007). +, Positive; −, negative; ND, no data available.

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-C338T and related type strains are given in Supplementary Table S1 (available in IJSEM Online). The major fatty acids of this strain were C₁₆:0, C₁₈:0, C₁₆:1ω7c, C₁₈:1ω7c, iso-C₁₃:0 3-OH and iso-C₁₅:0 3-OH. The strain produced carotenoid pigments with absorbance peaks at 455 and 480 nm. Flexirubin-type pigments were absent.

Strain YIM-C338T 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-C338T 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-C338T differed from its closest phylogenetic neighbour, *Salinimicrobium xinjiangense* BH206T, 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 demonstrates that strain YIM-C338T 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, cellubiose, α-cyclodextrin, dextrin, glycen, Tweens 40 and 80, D-fructose, D-galactose, gentiobiose, x-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-glucic acid, D-glucuronic acid, D-hydroxybutyric acid, β-hydroxybutyric acid, itaconic acid, x-ketobutyric acid, x-ketoglutaric acid, D-lactic acid, malonic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinic acid, glucuronamide, L-alaninamide, D-alanine, L-alanylglycine, L-asparagine, L-glutamic acid, glycol-L-glutamic acid, L-leucine, L-proline, L-propyglutamic acid, L-threonine, D-lactinovase, γ-aminobutyric acid, inosine, glycerol, x-D-glucose 1-phosphate and D-glucose 6-phosphate. The constitutive enzymes expressed by strain
YIM-C338\textsuperscript{T} are N-acetyl-\(\beta\)-glucosaminidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, \(\alpha\)-glucosidase, \(\beta\)-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\(_{16:1}\)t, C\(_{18:1}\)\text{\textregistered}T, iso-C\(_{13:0}\)\text{3-OH} and iso-C\(_{15:1}\)\text{\textregistered}. The DNA G+C content of the type strain is 42.8\% mol\%.

The type strain, YIM-C338\textsuperscript{T} (=DSM 17865\textsuperscript{T} =CGMCC 1.6308\textsuperscript{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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**References**


