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ORIGINAL PAPER

Salinicoccus salitudinis sp. nov., a new moderately halophilic bacterium isolated from a saline soil sample

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Abstract A novel pale-yellow-pigmented, moderately halophilic, facultatively alkaliphilic, non-motile, non-spore-forming, catalase- and oxidase-positive, obligately aerobic Gram-positive coccus, strain YIM-C678^T was isolated from a saline soil sample collected from a hypersaline habitat in the Qaidam basin, northwest China. The organism grew at 4–37°C and pH 6.0–11.0, with optimum growth at 25°C and pH 8.0. Strain YIM-C678^T grew optimally in the presence of 10–12% (w/v) NaCl and growth was observed in 1–25% (w/v) NaCl. The cell wall murein type was L-Lys-Gly_5. Major cellular fatty acids were anteiso-$C_{15:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$ and $C_{16:0}$. Menaquinone 6 (MK-6) was the major respiratory quinone. The DNA G + C content was 46.5 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the strain YIM-C678^T belonged to the family *Staphylococcaceae* and was most closely related to the eight described species of the genus *Salinicoccus* with sequence similarities from 92.2 (*S. luteus* YIM 70202^T) to 97.5% (*S. kunmingensis* YIM Y15^T). The DNA–DNA relatedness between strain YIM-C678^T and *S. kunmingensis* YIM Y15^T was 35.4%. Chemotaxonomic data and 16S rRNA gene sequence analysis supported the affiliation of strain YIM-C678^T with the genus *Salinicoccus*. The combination of phylogenetic analysis, phenotypic characteristics, chemotaxonomic differences and DNA–DNA hybridization data supported the view that the bacterium represents a novel species of the genus *Salinicoccus*, for which the name *Salinicoccus salitudinis* sp. nov. is proposed, with YIM-C678^T (=DSM 17846 = CGMCC 1.6299) as the type strain.

Keywords *Salinicoccus salitudinis* sp. nov. · Halophilic · Hypersaline soil

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Introduction

The genus *Salinicoccus* was proposed by Ventosa et al. (1990) which was defined as Gram-positive, moderately halophilic, non-motile and non-spore-forming coccus. Currently there were eight species in the genus *Salinicoccus* (Ventosa et al. 1990; Marquez et al. 1990; Ventosa et al. 1992; Zhang et al. 2002; Franca et al. 2006; Aslam et al. 2007; Zhang et al. 2007; Pakdeeto et al. 2007; Chen et al. 2007). In the recent study of the microbial diversity of the Qaidam basin, northwest China (Cui et al. 2004; Schumann et al. 2004; Li et al. 2005, 2006; Zhang et al. 2005, 2006, 2007)), a *Salinicoccus*-like strain YIM-C678^T was isolated from a hypersaline soil sample. The strain grew optimally in media that contained 10–12% (w/v) NaCl. Based on the results of a polyphasic taxonomic study, this strain was proposed to represent a novel species of the genus *Salinicoccus*.

Materials and methods

Strains and culture condition

Strain YIM-C678^T was isolated from a hypersaline soil sample by plating 1:10 serial dilutions of the sample on Difco marine agar 2216 (MA) + NaCl (w/v) 10% (named MA10 in this paper) at 28°C for 2 weeks. The sample was collected from the Qaidam basin, northwest China, where there are numerous saline lakes with pH values of 7.0–11.5. The strain was maintained both on MA10 slant at 4°C and in Difco marine broth 2216 (MB) supplemented with 20% (v/v) glycerol at −80°C. Four type strains of the genus *Salinicoccus*, *S. kunmingensis* YIM Y15^T, *S. alkaliphilus* AS 1.2691^T, *S. roseus* DSM 5351^T and *S. hispanicus* DSM 5352 ^{T}, were used in some parallel tests (Table 1). Unless otherwise indicated, morphological, biochemical, chemotaxonomic and molecular systematic studies were performed with cells grown on/in media containing 10% (w/v) NaCl at 25°C and pH 8.0.

Phenotypic characterization

Cell morphology was examined by light microscopy (model BH 2; Olympus). Gram staining was carried out using the standard Gram reaction combined with the KOH lysis test method (Gregersen 1978). Anaerobic growth was determined using the GasPak Anaerobic Systems (BBL, USA) according to the manufacturer's instructions. Motility was observed as described previously (Chen et al. 2007).

Optimum growth was tested at different temperatures (4–50°C) on MA10 and at different pH values (5.0–11.0) in MB supplemented with 10% (w/v) NaCl. For pH tolerance experiments, the buffer solutions described by Chen et al. (2004) were used. Tolerance/requirement of chlorides of sodium, potassium and magnesium [at concentrations of 0–30% (w/v)] was tested on MA, in MB and on some other media as controls, i.e. nutrient agar, tryptic soy broth agar (TSBA; BBL) and ISP 2 agar (Shirling and Gottlieb 1966).

Catalase, oxidase and urease activities, hydrolysis of polymers, nitrate reducing, tests of Voges–Proskauer and methyl red and antibiotic resistance were determined as described previously (Gerhardt et al. 1981; Ventosa et al. 1982; Atlas 1993; Chen et al. 2007). Substrate utilization as sole carbon and energy source, activities of constitutive enzymes and other physiological characteristics were carried out using API 20E, API 20NE, API 50CH (with API 50 CH B/E medium), API ZYM strips (BioMérieux) and BioLog GP2 MicroPlates (BioLog Inc.) according to the manufacturer's instructions. All suspension media were supplemented with 10% (w/v) NaCl.

Peptidoglycan, lipoquinone and fatty acid composition

Isolation of cell wall fraction and preparation of the hydrolysate of the cell wall were carried out using the method of Schleifer (1985). Amino acid composition of the cell wall hydrolysate was determined using the previously described method (Staneck and Roberts 1974). Lipoquinones were purified by TLC and analysed by HPLC as described by Hiraishi 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 on MA10 at 25°C for 3 days.

Determination of G + C content of DNA, 16S rRNA gene sequencing and phylogenetic analysis, and DNA–DNA hybridization

The DNA for the determination of G + C content was isolated according to Hopwood et al. (1985) and its G + C content was determined by the thermal denaturation method (Mandel and Marmur 1968) with a Shimadzu UV–visible spectrophotometer (UV1601). The extraction of genomic DNA for 16S rRNA gene sequence determination, PCR amplification of the 16S rRNA gene and sequencing of the purified PCR products were carried out as described previously (Cui et al. 2001). Phylogenetic analysis was performed using the software packages MEGA version 2.1 (Kumar et al. 2001) after multiple alignment of sequence data by CLUSTAL_X (Thompson et al. 1997). Distances (corrected by Kimura's 2-parameter model; Kimura 1980) were calculated and clustering was performed with the neighbour-joining method (Saitou and Nei 1987). Maximum-likelihood (Felsenstein 1981) and parsimony (Kluge and Farris 1969) trees (not shown) were generated using the treeing algorithms contained in the PHYLIP package (Felsenstein 1993). Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by means of 1,000 re-samplings (Felsenstein, 1985). DNA–DNA hybridization was performed using the optical renaturation method (De Ley et al. 1970; Huß et al. 1983; Jahnke 1992).

Results and discussion

Morphological and biochemical characteristics

Colonies of strain YIM-C678^T were pale yellow-coloured, circular, convex, non-translucent with entire margins, 1–1.5 mm in diameter after incubation for 5 days at 25°C on MA10. No diffusible pigments were produced on any media tested. Cells were Gram-positive cocci, approximately 0.9–1.5 μm in diameter, occurring singly, in pairs,

Table 1 Differentiating characteristics of strain YIM-C678^T from its closest phylogenetic relatives

Characteristics	1	2	3	4	5	6	7	8	9
Colony pigmentation	Pale yellow	Yellow	Pinkish	Pink–red	Reddish orange	Orange	Pink–red	Orange	Orange
Temperature for growth (°C)									
Range	4–37	4–45	10–49	15–37	15–40	20–30	20–45	15–45	4–45
Optimum	25	37	32	37	37	30	37	37	30
NaCl for growth (%; w/v)									
Range	1–25	0.5–25	0–25	0.5–25	0.9–25	0.5–15	0–22	1.5–25	1–25
Optimum	10–12	8–10	10	10	10	5	4	10	10
pH for growth									
Range	6.0–11.0	6.0–10.0	6.5–11.5	5.0–9.0	6.0–9.0	6.5–11.0	6.5–9.5	6–9	7.0–11.0
Optimum	8.0	8.0	9.5	7.5	8.0	7.0	8.0	8.5	8.0–9.0
Hydrolysis of [a]									
Esculin	+	+	−[b]	−	+	ND	−	−	+
Casein	−	−	−	+	−	−	+	−	−
Gelatin	−	−	−	+	+	−	+	−	−
Starch	−	+	−	+	−	−	−	−	−
Tween 80	−	+	−	+	−	−	ND	−	−
H_2S production [a]	−	−	−	−	−	+	ND	ND	−
Indole production [a]	−	−	−	−	−	+	ND	−	−
Methyl red test [a]	−	−	−	−	+/−[b]	+	−	−	−
Nitrate reduction [a]	+	+	+	−	+	+	+	−	+
Urease [a]	−	−	+/−[b]	−	+/−[b]	−	−	−	−
Acid production from									
D-Galactose	−	−	−	−	+	−	−	−	−
D-Glucose	+	−	+	−	+	+	+	+	+
D-Fructose	−	+	−	−	+	+	+	+	−
Glycerol	−	−	−	−	+	+	+	+	ND
D-Maltose	−	−	−	−	+	+	+	−	+
D-Mannitol	−	+	ND	−	+	−	−	−	ND
Sucrose	+	+	−	−	+	−	−	−	+
MK-7[a]	+(3.8%)	+(1.2%)	−	+(0.8%)[b]	tr[b]	tr	ND	ND	+
DNA G + C content (mol%)	46.5	46.2	49.6	51.2	45.7	47.0	46.2	46.0	49.7

Strains: *1 Salinicoccus salitudinis* YIM-C678^T (data from this study), *2 S. kunmingensis* YIM Y15^T (Chen et al. 2007), *3 S. alkaliphilus* JCM 11311^T (Zhang et al. 2002), *4 S. roseus* DSM 5351^T (Ventosa et al. 1990), *5 S. hispanicus* DSM 5352^T (Marquez et al. 1990; Ventosa et al. 1992), *6 S. jeotgali* KCTC 13030^T (Aslam et al. 2007), *7 S. salsiraiae* LMG 22840^T (Franca et al. 2006), *8 S. siamensis* JCM 12822^T (Pakdeeto et al. 2007), *9 S. luteus* YIM 70202^T (Zhang et al 2007)

tr trace amount

ND no data

+ positive

− negative

All strains are positive for Gram-staining and catalase- and oxidase-activities, negative for Voges-Proskauer test, spore formation and motility. All strains possess menaquinone 6 (MK-6) as their predominant respiratory quinones

[a] Unless otherwise indicated, results of these tests for strains *S. kunmingensis* YIM Y15^T, *S. alkaliphilus* AS 1.2691^T, *S. roseus* DSM 5351^T and *S. hispanicus* DSM 5352^T were confirmed in the parallel tests with strain YIM-C678^T under identical conditions

[b] Results from this study

tetrads or clumps and strictly aerobic, non-motile and non-sporulating.

Strain YIM-C678^T was found to be catalase- and oxidase-positive, with a growth temperature range of 4–37°C (optimum 25°C). It was moderately halophilic and the optimum NaCl concentration (w/v) for growth was between 10 and 12%, with a growth NaCl concentration range of 1–25% (Kushner 1993). NaCl could not be

replaced with KCl or $MgCl{\cdot}6H_2O$ in the media. No growth occurred on MA without NaCl, TSBA, nutrient agar or ISP medium 2 agar. Strain YIM-C678^T was a facultatively alkaliphilic organism with a growth range of pH 6.0–11.0 and optimum growth was observed at pH 8.0. The results of other phenotypic tests are listed in the species description and in Table 1.

Chemotaxonomic characteristics

Chemotaxonomic data of strain YIM-C678^T are compatible with its assignment to the genus *Salinicoccus*. The major amino acid constituents of the cell wall composition were glycine and lysine, which is compatible with the type of L-Lys-Gly_5 peptidoglycan described for the genus *Salinicoccus* (Ventosa et al. 1990). The major fatty acids are iso- and anteiso-branched fatty acids, which are consistent with the assignment of strain YIM-C678^T to the genus *Salinicoccus* (Table 2). The strain studied had menaquinone 6 (MK-6; 96.2%) and menaquinone 7 (MK-7; 3.8%) as its respiratory quinones.

DNA G + C content, phylogenetic analysis based on 16S rRNA gene sequence comparison and DNA–DNA relatedness

The DNA G + C content of the strain YIM-C678^T was 46.5 mol%. The almost complete 16S rRNA gene sequence (1491 bp; EF590121) of the strain YIM-C678^T was determined. Preliminary comparison of the sequence against the GenBank database indicated that the isolate was closely related to the members of the genus *Salinicoccus* (Ventosa et al. 1990) and phylogenetically most closely related to *S. kunmingensis* YIM Y15^T with a sequence similarity of 97.5%. The lower levels of 16S rRNA gene sequence similarities between the strain studied and the type strains of the other seven species of the genus *Salinicoccus*, i.e. *S. alkaliphilus* AS 1.2691^T (95.3%), *S. roseus* DSM 5351^T (94.5%), *S. jeotgali* KCTC 13030^T (94.6%), *S. salsiraiae* LMG 22840^T (94.5%), *S. hispanicus* DSM 5352^T (94.2%), *S. siamensis* JCM 12822^T (94.3%) and *S. luteus* YIM 70202^T (92.2%), revealed that strain YIM-C678^T did not belong to any one of these seven *Salinicoccus* species (Stackebrandt and Goebel 1994). A neighbour-joining tree (Fig. 1) showed that the strain YIM-C678^T and the eight type strains of the genus *Salinicoccus* formed a distinct clade in the phylogenetic tree with high bootstrap support (97%), in which strain YIM-C678^T and *Salinicoccus kunmingensis* YIM Y15^T formed a distinct sub-branch with significant bootstrap support (100%) (Fig. 1). To establish the precise taxonomic position of strain YIM-C678^T, DNA–DNA hybridizations were performed between strain YIM-C678^T and the type strain *S. kunmingensis* YIM Y15^T and the level of DNA–DNA relatedness between them was 35.4%. It has been suggested that bacterial strains with a DNA–DNA relatedness level of <70% are not members of the same species (Wayne et al. 1987). It is therefore evident based on the phylogenetic analysis and the DNA–DNA hybridization data that strain YIM-C678^T represents a novel species of the genus *Salinicoccus*.

On the basis of the polyphasic taxonomic results presented above, we propose strain YIM-C678^T to represent a novel species of the genus *Salinicoccus*, *Salinicoccus salitudinis* sp. nov.

Description of *Salinicoccus salitudinis*

Salinicoccus salitudinis (sa.li.tu' di.nis. L. gen. fem.n. *salitudinis*, of/from salinity, saltiness). Cells are Gram-positive, non-motile, non-sporulating, catalase- and oxidase-positive and obligately aerobic cocci (0.9–1.5 μm) that occur singly, in pairs, tetrads or clumps. After 5 days on MA supplemented with 10% (w/v) NaCl at pH 8.0 and 25°C, colonies are circular, convex, pale yellow and non-translucent with entire margins, 1–1.5 mm in diameter. Diffusible pigment is not produced. Growth occurs at pH 6.0–11.0 (optimum at 8.0), at temperatures of 4–37°C (optimum at 25°C) and in media supplemented with 1–25% (w/v) NaCl (optimum, 10–12% NaCl). NaCl cannot be replaced with KCl or $MgCl_2{\cdot}6H_2O$ in media. Esculin is hydrolyzed, whereas casein, cellulose, chitin, starch, gelatin, Tween 20 or Tween 80 are not. Nitrate is reduced to nitrite. H_2S production, indole, methyl red and Voges–Proskauer tests are negative. Cells are resistant to gentamicin (10 μg), kanamycin (30 μg), polymixin B (30 μg), streptomycin (10 μg) and tetracycline (30 μg), but ampicillin (30 μg), chloramphenicol (30 μg), licomycin (2 μg), nalidixic acid (20 μg), novobiocin (30 μg) and rifampicin (5 μg) inhibited the growth. Acid is produced from amygdaline, inositol, potassium 5-ketogluconate, D-saccharose (sucrose) and D-sorbitol. The following compounds are utilized as sole carbon and energy sources: amygdalin, D-arabinose, D-cellobiose, glycerol, *myo*-inositol, D-mannitol, D-mannose, D-raffinose, L-rhamnose, D-ribose, D-sorbitol, D-sucrose and D-trehalose. The following compounds are utilized as sole nitrogen sources: polypeptone, L-leucine and L-tyrosine. Constitutive enzymes expressed by YIM-C678^T are catalase, cytochrome oxidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, (α-chymotrypsin, acid phosphatase, trypsin, naphthol-AS-BI-phosphohydrolase and valine arylamidase; but arginine dihydrolase,

Table 2 Fatty acid composition of strain YIM-C678^T and members of the genus *Salinicoccus*

Fatty acid (%)	1[a]	2[a]	3	4[a]	5[a]	6	7[a]	8	9
Saturated fatty acids									
$C_{14:0}$	2.6	1.9	1.6	1.2 ± 0.3	5.3 ± 0.4	4.4	0.8 ± 0.2	0.5	ND
$C_{15:0}$	0.7	0.7	–	2.1 ± 0.6	5.4 ± 0.8	–	–	–	ND
$C_{16:0}$	5.2	3.2	1.5	3.0 ± 0.7	10.3 ± 2.3	10.3	2.9 ± 0.4	2.4	ND
$C_{17:0}$	0.2	–	–	0.5 ± 0.2	0.9 ± 0.3	–	–	ND	ND
$C_{18:0}$	2.9	2.1	–	1.1 ± 0.2	2.4 ± 0.8	–	0.7 ± 0.1	ND	ND
$C_{19:0}$	–	–	–	1.0 ± 0.3	0.6 ± 0.1	–	–	ND	ND
$C_{20:0}$	0.1	0.2	1.3	1.5 ± 0.4	2.2 ± 0.2	–	2.1 ± 0.5	ND	ND
Unsaturated fatty acids									
$C_{16:1}$ ω7c alcohol	2.7	3.4	5.8	0.8 ± 0.3	1.6 ± 0.1	4.1	–	–	ND
$C_{16:1}$ ω7c	1.8	–	–	ND	ND	–	ND	ND	ND
$C_{16:1}$ ω11c	1.7	2.7	2.2	–	2.0 ± 0.2	3.6	–	ND	ND
$C_{18:1}$ ω7c	1.8	0.4	–	ND	ND	–	ND	ND	ND
$C_{18:1}$ ω9c	1.4	0.5	–	ND	ND	–	ND	ND	ND
Branched fatty acids									
iso-$C_{13:0}$ 3-OH	–	1.0	–	–	–	–	ND		ND
iso-$C_{14:0}$	3.7	2.9	4.4	2.1 ± 0.3	5.8 ± 1.3	3.2	1.0 ± 0.1	2.4	ND
iso-$C_{15:0}$	22.3	23.1	22.3	21.4 ± 1.2	25.9 ± 3.4	22.8	26.6 ± 2.2	14.2	34.2
anteiso-$C_{15:0}$	28.1	28.4	27.6	36.0 ± 2.3	15.6 ± 3.6	32.4	35.3 ± 0.7	38.7	30.6
iso-$C_{16:0}$	7.6	5.8	10.1	5.3 ± 0.8	5.5 ± 1.0	4.1	3.5 ± 0.6	12.1	ND
iso-$C_{17:0}$	4.5	5.7	3.3	5.6 ± 0.3	4.6 ± 0.9	–	9.5 ± 0.8	4.9	ND
iso-$C_{17:1}$ ω10c	2.7	4.7	4.0	1.5 ± 0.4	0.7 ± 0.1	–	3 ± 0.3	4.5	ND
anteiso-$C_{17:0}$	4.9	5.8	8.9	8.0 ± 1.0	1.6 ± 0.7	6.2	8.5 ± 0.6	12.1	ND
iso-$C_{18:0}$	0.5	0.5	–	ND	ND	–	ND	ND	ND
iso-$C_{19:0}$	0.7	0.7	2.8	4.0 ± 0.3	1.1 ± 0.3	–	3.0 ± 0.1	0.4	ND
anteiso-$C_{19:0}$	0.7	1.0	1.9	2.3 ± 0.5	–	–	1.0 ± 0.2	ND	ND
iso-$C_{20:0}$	–	–	1.5	0.6 ± 0.1	–	–	–	ND	ND
Unknown[b]	1.6	2.8	0.9	2.0 ± 0.4	8.2 ± 2.3	4.3	2.3 ± 0.3	3.4	ND

Strains: *1 Salinicoccus salitudinis* YIM-C678^T (data from this study), *2 S. kunmingensis* YIM Y15^T (Chen *et al.* 2007), *3 S. alkaliphilus* AS 1.2691^T (Zhang et al. 2002), 4 *S. roseus* DSM 5351^T, *5 S. hispanicus* DSM 5352^T, *6 S. jeotgali* KCTC 13030^T (Aslam et al. 2007), *7 S. salsiraiae* LMG 22840^T (data for strains 4, 5 and 7 from Franca et al. 2006), *8 S. siamensis* JCM 12822^T (Pakdeeto et al. 2007), *9 S. luteus* YIM 70202^T (Zhang et al. 2007)

Values are percentages of total fatty acids

– not detected

ND no data available

[a] Values for fatty acids present at levels of <0.5% are not shown

[b] Unknown fatty acid with equivalent chain lengths 15.665 for strain 3, 15.669 for strains 1, 2 and 6, and 15.670 for strains 4, 5 and 7

(α-galactosidase, (β-galactosidase, α-glucosidase, β-glucosidase, α-mannosidase, gelatinase, β-glucuronidase, lipase (C14), ornithine decarboxylase, *N*-acetyl-β-glucosaminidase, β-galactosidase/ortho nitrophenyl-βD-galactopyranosidase (ONPG), lysine decarboxylase, β-galactosidase/para-nitrophenyl-βD-galactopyranosidase (PNPG), tryptophane deaminase, (α-fucosidase or urease are not produced. Menaquinone 6 is the predominant respiratory quinone, with menaquinone seven present in minor amounts. The major cellular fatty acids are anteiso-$C_{15:0}$ (28.1%), iso-$C_{15:0}$ (22.3%), iso-$C_{16:0}$ (7.6%) and $C_{16:0}$ (5.2%). The assumed cell-wall murein type is L-Lys-Gly_5. The DNA G + C content is 46.5 mol%.

The type strain YIM-C678^T was isolated from a saline soil sample collected from a hypersaline habitat in the Qaidam basin, northwest China. Strain YIM-C678^T has been deposited in the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstraße 7B, d-38124 Braunschweig, Germany) as strain DSM 17846 and the China General

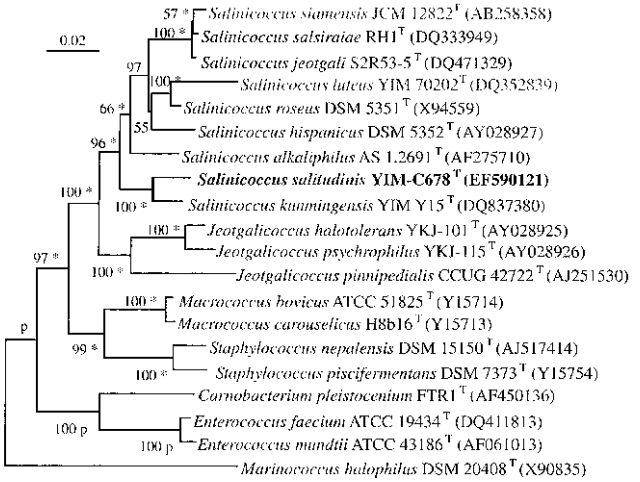

Fig. 1 Phylogenetic dendrogram based on 16S rRNA gene sequence analysis and constructed using the neighbour-joining method showing the phylogenetic positions of strains YIM-C678 ^{T} and related taxa. The *p* labels indicate branches that were also found with the maximum-parsimony (Kluge and Farris 1969) algorithm; *asterisks* indicate branches that were also recovered using the maximum-likelihood (Felsenstein 1981) and parsimony algorithms. *Numbers at nodes* indicate bootstrap values (>50%) based on neighbour-joining analyses of 1,000 re-sampled datasets. *Bar* 2 substitutions per 100 nucleotides

Microbiological Culture Collection Center, Beijing, China as CGMCC 1.6299.

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References

Aslam Z, Lim JH, Im WT, Yasir M, Chung YR, Lee ST (2007) *Salinicoccus jeotgali* sp. nov., isolated from jeotgal, a traditional Korean fermented seafood. Int J Syst Evol Microbiol 57: 633–638

Atlas RM (1993) Handbook of microbiological media. Parks LC (Ed.) CRC, Boca Raton

Chen HH, Li WJ, Tang SK, Kroppenstedt RM, Stackbrandt E, Xu LH, Jiang CL (2004) *Corynebacterium halotolerans* sp. nov., isolated from saline soil in the west of China. Int J Syst Evol Microbiol 54:779–782

Chen YG, Cui XL, Pukall R, Li HM, Yang YL, Xu LH, Wen ML, Peng Q, Jiang CL (2007) *Salinicoccus kunmingensis* sp. nov., a moderately halophilic bacterium isolated from a salt mine in Yunnan, southwest China. Int J Syst Evol Microbiol 57 (in press)

Cui XL, Mao PH, Zeng M, Li WJ, Zhang LP, Xu LH, Jiang CL (2001) *Streptomonospora salina* gen. nov., sp. nov., a new member of the family Nocardiopsaceae. Int J Syst Evol Microbiol 51:357–363

Cui XL, Schumann P, Stackebrandt E, Kroppenstedt RM, Pukall R, Xu LH, Rohde M, Jiang CL (2004) *Myceligenerans xiligouense* gen. nov., sp. nov., a novel hyphae-forming member of the family *Promicromonosporaceae*. Int J Syst Evol Microbiol 54:1287–1293

De Ley J, Cattoir H, Reynaerts A (1970) The quantitative measurement of DNA hybridization from renaturation rates. Eur J Biochem 12:133–142

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

Felsenstein J (1985) Confidence limits on phylogenics: An approach using the bootstap. Evolution 39:783–791

Felsenstein J (1993) PHYLIP (phylogenetic inference package), version 3.5. Distributed by the author. Department of Genetics, University of Washington, Seattle, USA

Franca L, Rainey FA, Nobre MF, Costa MS (2006) *Salinicoccus salsiraiae* sp. nov.: a new moderately halophilic gram-positive bacterium isolated from salted skate. Extremophiles 10:531–536

Gerhardt P, Murray RGE, Costilow RN, Nester EW, Wood WA, Krieg NR, Philips GB (1981) Manual of methods for general bacteriology. Am Soc Microbiol, Washington, DC

Gregersen T (1978) Rapid method for distinction of Gram-negative from Gram-positive bacteria. Eur J Appl Microbiol Biotechnol 5:123–127

Hiraishi A, Ueda Y, Ishihara J, Mori T (1996) Comparative lipoquinone analysis of influent sewage and activated sludge by highperformance liquid chromatography and photodiode array detection. J Gen Appl Microbiol 42:457–469

Hopwood DA, Bibb MJ, Chater KF, Kieser T, Bruton CJ, Kieser HM, Lydiate DJ, Smith CP, Ward JM (1985) Preparation of Chromosomal, plasmid and phage DNA. In Genetic manipulation of Streptomyces: a laboratory manual, F. Crowe and Sons, Norwich, pp 79–80

Huß VAR, Festl H, Schleifer KH (1983) Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. Syst Appl Microbiol 4:184–192

Jahnke KD (1992) BASIC computer program for evaluation of spectroscopic DNA renaturation data from Gilford System 2600 spectrophotometer on a PC/XT/AT type personal computer. J Microbiol Methods 15:61–73

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Kluge AG, Farris FS (1969) Quantitative phyletics and the evolution of anurans. Syst Zool 18:1–32

Kumar S, Tamura K, Jakobsen IB, Nei M (2001) MEGA2: molecular evolutionary genetics analysis software. Bioinformatics 17:1244–1245

Kushner DJ (1993) Growth and nutrition of halophilic bacteria. In: Vreeland RH, Hochstein LI (eds) The biology of halophilic bacteria. CRC, Boca Raton, pp 87–103

Li WJ, Zhang YQ, Schumann P, Tian XP, Zhang YQ, Xu LH, Jiang CL (2006) *Sinococcus qinghaiensis* gen. nov., sp. nov., a novel member of the order *Bacillales* from a saline soil in China. Int J Syst Evol Microbiol 56:1189–1192

Li WJ, Schumann P, Zhang YQ, Xu P, Chen GZ, Xu LH, Stackebrandt E, Jiang CL (2005) Proposal of Yaniaceae fam. nov. and Yania flava sp. nov. and emended description of the genus Yania. Int J Syst Evol Microbiol 55:1933–1938

Mandel M, Marmur J (1968) Use of ultraviolet absorbance temperature profile for determining the guanine plus cytosine content of DNA. Methods Enzymol 12B:195–206

Marquez MC, Ventosa A, Ruiz-Berraquero F (1990) *Marinococcus hispanicus*, a new species of moderately halophilic Gram-positive cocci. Int J Syst Bacteriol 40:165–169

Pakdeeto A, Tanasupawat S, Thawai C, Moonmangmee S, Kudo T, Itoh T (2007) *Salinicoccus siamensis* sp. nov., isolated from fermented shrimp paste in Thailand. Int J Syst Evol Microbiol 57:2004–2008

Saitou N, Nei M (1987) The neighbour-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4(4):406–425

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. USFCC Newsl 20:16

Schleifer KH (1985) Analysis of the chemical composition and primary structure of murein. Methods Microbiol 18:123–156

Schumann P, Cui XL, Stackebrandt E, Kroppenstedt RM, Xu LH, Jiang CL (2004) *Jonesia quinghaiensis* sp. nov., a new member of the suborder *Micrococcineae*. Int J Syst Evol Microbiol 54:2181–2184

Shirling EB, Gottlieb D (1966) Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol 16:313–340

Staneck JL, Roberts GD (1974) Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. Appl Microbiol 28:226–231

Stackebrandt E, Goebel BM (1994) Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Bacteriol 44:846–849

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Ventosa A, Quesada E, Rodriguez-Valera F, Ruiz-Berraquero F, Ramos-Cormenzana A, Kocur M (1982) Numerical taxonomy of moderately halophilic Gram-negative rods. J Gen Microbiol 128:1959–1968

Ventosa A, Marquez MC, Ruiz-Berraquero F, Kocur M (1990) *Salinicoccus roseus* gen. nov., sp. nov., a new moderately halophilic Gram-positive coccus. Syst Appl Microbiol 13:29–33

Ventosa A, Marquez MC, Weiss N, Tindall BJ (1992) Transfer of *Marinococcus hispanicus* to the genus *Salinicoccus* as *Salinicoccus hispanicus* comb. nov. Syst Appl Microbiol 15:530–534

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, other authors (1987) International committee on systematic bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464

Zhang YQ, Yu LY, Liu HY, Zhang YQ, Xu LH, Li WJ (2007) *Salinicoccus luteus* sp. nov., isolated from a desert soil. Int J Syst Evol Microbiol 57:1901–1905

Zhang YQ, Schumann P, Yu LY, Liu HY, Zhang YQ, Xu LH, Stackebrandt E, Jiang CL, Li WJ (2006) *Zhihengliuella halotolerans* gen. nov., sp. nov., a novel member of the family *Micrococcaceae*. Int J Syst Evol Microbiol 56:1018–1023

Zhang YQ, Schumann P, Li WJ, Chen GZ, Tian XP, Stackebrandt E, Xu LH, Jiang CL (2005) *Isoptericola halotolerans* sp. nov., a novel actinobacterium isolated from saline soil from Qinghai province, northwest China. Int J Syst Evol Microbiol 55:1867–1870

Zhang W, Xue Y, Ma Y, Zhou P, Ventosa A, Grant WD (2002) *Salinicoccus alkaliphilus* sp. nov., a novel alkaliphile and moderate halophile from Baer Soda lake in inner Mongolia autonomous region, China. Int J Syst Evol Microbiol 52:789–793