Salinicoccus luteus sp. nov., isolated from a desert soil

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A moderately halophilic bacterium, strain YIM 70202^T, was isolated from a desert soil sample collected from Egypt and was subjected to a taxonomic investigation. In a phylogenetic dendrogram based on 16S rRNA gene sequence analysis, strain YIM 70202^T was affiliated to the *Salinicoccus* clade, showing 94.5–96.8% 16S rRNA gene sequence similarity to the recognized species of the genus *Salinicoccus*, in which *Salinicoccus roseus* CCM 3516^T was the nearest neighbour. The DNA–DNA relatedness value of the novel isolate with *S. roseus* CCM 3516^T was 12.7%. The novel isolate grew at temperatures between 4 and 45 °C and at pH values ranging from 7.0 to 11.0, with an optimum of 30 °C and pH 8.0–9.0, respectively. Strain YIM 70202^T grew optimally in the presence of 10% NaCl (w/v) and growth was observed at NaCl concentrations in the range 1–25% (w/v). Chemotaxonomic data revealed that strain YIM 70202^T contained MK-6 as the predominant respiratory quinone, possessed L-Lys–Gly₅ as the cell-wall peptidoglycan, had phosphatidylglycerol, diphosphatidylglycerol and an unknown glycolipid as the polar lipids and contained i-C_{15:0} and ai-C_{15:0} as the predominant fatty acids. The DNA G + C content was 49.7 mol%. The biochemical and chemotaxonomic properties demonstrate that strain YIM 70202^T represents a novel species of the genus *Salinicoccus*. The name *Salinicoccus luteus* sp. nov. is proposed with strain YIM 70202^T (=CGMCC 1.6511^T=KCTC 3941^T) as the type strain.

The genus *Salinicoccus* was first described by Ventosa *et al.* (1990) and at present the genus comprises only five species with validly published names (Ventosa *et al.*, 1990, 1992; Zhang *et al.*, 2002; Franca *et al.*, 2006; Aslam *et al.*, 2007). Members of the genus *Salinicoccus* are moderately halophilic, aerobic Gram-positive cocci, which are chemotaxonomically characterized by having menaquinone-6 (MK-6) as the predominant isoprenoid quinone, a cell-wall peptidoglycan type based on L-Lys–Gly₅ and a DNA G + C content of 46–51 mol% (Ventosa *et al.*, 1992). In this paper, we present our studies on a new isolate affiliated to the genus *Salinicoccus*.

Strain YIM 70202^T was isolated from a desert soil sample collected from Wadi Sannur by using the dilution plating method on marine agar (Difco 2216; MA), supplemented with 15% NaCl (w/v). Wadi Sannur is one of the most famous wadis in the Eastern Desert of Egypt where the mean monthly air temperature ranges from 12.2 °C during January to 29.1 °C during July. The pH value of the

medium was adjusted to 8.0 using Na₂CO₃/NaHCO₃ buffer. The strain was maintained on MA slants containing 10% NaCl at 4 °C and as 20% (w/v) glycerol suspensions at –20 °C. Biomass for chemical and molecular studies was obtained by cultivation in shaken flasks (about 150 r.p.m.) using marine broth (Difco 2216; MB) supplemented with 10% NaCl (w/v) at pH 8.0 and 30 °C for 1 week.

Gram staining and the KOH lysis test were carried out according to Gram (1884) and Cerny (1978), respectively. Morphology and motility were examined by light microscopy (model BH 2; Olympus) and electron microscopy (JEM-1010) using cells from exponentially growing cultures. For transmission electron microscopy (TEM) observation, cells were negatively stained with 1% (w/v) phosphotungstic acid after air-drying. Colony morphology was observed on MA or ISP5 medium (Shirling & Gottlieb, 1966) containing 10% NaCl and trypticase soy agar (TSA) containing 10% NaCl after incubation at 30 °C for 2 days. The colony colour was determined with the ISCC-NBS colour charts (Kelly, 1964). The growth temperature was tested at 4, 10, 28, 30, 37, 40, 45 and 55 °C using MB supplemented with 10% NaCl. The ability of the strain to

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 70202^T is DQ352839.

grow at different NaCl concentrations was examined on TSA and MA. To determine the optimum pH for growth, the following buffer solutions were used (with intervals of 0.5 pH units): pH 5.0–8.5: NaOH/KH₂PO₄; pH 9.0–9.5: borax/boric acid; pH 10.0–10.5: borax/NaOH; pH 11.0–11.5: Na₂HPO₄/NaOH; pH 12.0–13.0: KCl/NaOH. Metabolic properties were determined using API ID 32E, API Coryne and API ZYM test kits (bioMérieux) according to the manufacturer's instructions, except that the cell suspension to inoculate the systems was prepared by using sterilized artificial seawater supplemented with 10 % (w/v) NaCl. Most metabolic properties (listed in Table 1) were also checked as described by Shirling & Gottlieb (1966).

Strain YIM 70202^T comprised aerobic cocci that stained Gram-positive. No flagella or spores formed. Smooth-surfaced orange colonies with a maximum diameter of about 1 mm formed on MA or TSA containing 10 % NaCl

after incubation for 48 h at 30 °C. Strain YIM 70202^T grew well at 30 °C, but very slowly at 4 °C and 45 °C. Growth was observed at initial pH values between 7.0 and 11.0 and on TSA containing 1–25 % NaCl. The strain grew optimally at pH 8.0–9.0 and in the presence of 10 % NaCl. The detailed physiological and biochemical characteristics of the strain are given in Table 1 and in the species description.

A purified cell-wall preparation was obtained after disruption of cells by shaking with glass beads and subsequent trypsin digestion by the method of Schleifer (1985). Amino acids and peptides in cell-wall hydrolysates were analysed by two-dimensional ascending TLC on cellulose plates using the solvent systems of Schleifer & Kandler (1972). Respiratory quinones were isolated according to the method of Collins *et al.* (1977) and analysed by HPLC (Groth *et al.*, 1997). Polar lipids were extracted, examined

Table 1. Phenotypic characteristics that differentiate strain YIM 70202^T from all the other recognized species of the genus *Salinicoccus*

Species: 1, *S. luteus* sp. nov. YIM 70202^T; 2, *S. roseus* CCM 3516^T; 3, *Salinicoccus hispanicus* CCM 4148^T; 4, *Salinicoccus alkaliphilus* AS 1.2691^T; 5, *Salinicoccus jeotgali* S2R53-5^T; 6, *Salinicoccus salsiraiiae* RH1^T (data from Franca *et al.*, 2006). All data are from this study unless otherwise indicated. +, Positive; –, negative; ND, not determined.

Characteristic	1	2	3	4	5	6
Colony pigmentation (MA medium)	Orange	Pink–red	Reddish orange	Pinkish	Orange	Pink–red
pH for growth:						
Range	7.0–11.0	5.0–9.0	6.0–9.0	6.5–11.5	6.0–11.0	6.5–9.5
Optimum	8.0–9.0	7.5	8.0	9.5	7.0	8.0
NaCl tolerance for growth (% w/v)	1–25	0.5–25.0	1.0–25.0	0.0–25.0	0.5–15.0	0–22
Temperature for growth (°C):						
Range	4–45	15–37	15–40	10–49	20–30	20–45
Optimum	30	37	37	32	30	37
Acid production from:						
Fructose	–	–	+	–	+	+
Galactose	–	–	+	–	–	–
D-Glucose	+	–	+	+	+	+
Maltose	+	–	+	–	+	+
Sucrose	+	–	–	–	–	–
Hydrolysis of:						
Aesculin	+	–	+	+	+	–
Casein	–	+	–	–	–	+
Gelatin	–	+	+	+	+	+
Starch	–	+	–	+	–	ND
Tween 20	+	–	–	–	–	ND
Tween 80	–	+	–	–	+	ND
Nitrate reduction	+	–	+	+	+	+
Methyl red test	–	–	+	–	–	ND
Voges–Proskauer test	–	–	–	+	+	ND
Urease	–	–	+	+	+	ND
H ₂ S production	–	–	–	–	+	ND
Major fatty acids:						
i-C _{15:0}	34.2	25.8*	27.9*	22.3*	22.8*	26.6 ± 2.2
ai-C _{15:0}	30.6	36.4*	18.66*	27.6*	32.4*	35.3 ± 0.7
DNA G + C content (mol%)	49.7	51.2*	45.6–49.3*	49.6*	47.0*	46.0

*Data from Ventosa *et al.*, 1990, 1992, Zhang *et al.*, 2002 and Aslam *et al.*, 2007.

by two-dimensional TLC and identified using previously published procedures (Minnikin *et al.*, 1979; Collins & Jones, 1980). Analysis of the whole-cell fatty acid pattern followed the instructions of the MIDI system (Kroppenstedt, 1985) by using exponential phase cultures.

The cell-wall peptidoglycan structure of strain YIM 70202^T was L-Lys–Gly₅. The predominant respiratory quinone was MK-6 and minor amounts of MK-7 were also detected. The polar lipids were phosphatidylglycerol, diphosphatidylglycerol and an unknown glycolipid. The major cellular fatty acids were i-C_{15:0} (34.2 %) and ai-C_{15:0} (30.6 %).

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were performed as described by Xu *et al.* (2003). Multiple alignments with sequences of the most closely related moderately halophilic species and calculations of levels of sequence similarity were carried out using CLUSTAL_X (Thompson *et al.*, 1997). A phylogenetic tree was constructed using the neighbour-joining method of Saitou & Nei (1987) from *K*_{nuc} values (Kimura, 1980) using MEGA version 2.1 (Kumar *et al.*, 2001). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The DNA base composition of strain YIM 70202^T was determined by reverse-phase HPLC of nucleosides according to Mesbah *et al.* (1989). DNA–DNA hybridizations were carried out between strain YIM 70202^T and *Salinicoccus roseus* CCM 3516^T applying the optical renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992).

The nearly complete 16S rRNA gene (1507 bp) was sequenced for strain YIM 70202^T. 16S rRNA gene sequence comparative analysis with sequences available from the NCBI online databases revealed that strain YIM 70202^T was much closer to species of the genus *Salinicoccus* (94.5–96.8 % 16S rRNA gene sequence similarity) than to other moderately halophilic species. The closest neighbour of

strain YIM 70202^T was *S. roseus* CCM 3516^T, showing 96.8 % gene sequence similarity. The phylogenetic tree (Fig. 1) depicts the phylogenetic position of strain YIM 70202^T within the radiation of type strains of all recognized species of the genus *Salinicoccus*.

In the phylogenetic tree based on the 16S rRNA gene sequence, strain YIM 70202^T and *S. roseus* CCM 3516^T formed a stable clade (supported by very high bootstrap value, see Fig. 1) within the genus *Salinicoccus*. The chemotaxonomic characteristics of strain YIM70202^T, containing MK-6 as the predominant respiratory quinone, L-Lys–Gly₅ as the cell-wall peptidoglycan, i-C_{15:0} (34.2 %) and ai-C_{15:0} (30.6 %) as the predominant fatty acids and a DNA G + C content of 49.7 mol%, are all consistent with those of the genus *Salinicoccus* (Ventosa *et al.*, 1990, 1992). Comparative analysis of the 16S rRNA gene sequence of strain YIM 70202^T with all recognized species of the genus *Salinicoccus* were lower than 97.0 % suggesting that strain YIM 70202^T represents a novel species (Stackebrandt & Goebel, 1994). Phenotypic characteristics (see Table 1) also differentiate strain YIM 70202^T from all of the recognized species of the genus *Salinicoccus*. Furthermore, the DNA–DNA relatedness value of 12.7 % between strain YIM 70202^T and *S. roseus* CCM 3516^T is far lower than 70 %, which is considered to be the threshold value for the delineation of genomic species (Wayne *et al.*, 1987). Thus, we propose that strain YIM 70202^T be classified in the genus *Salinicoccus* as representing the type strain of a novel species, *Salinicoccus luteus* sp. nov.

Description of *Salinicoccus luteus* sp. nov.

Salinicoccus luteus (lu.te'us. L. masc. adj. *luteus* orange-coloured).

Aerobic, Gram-positive, non-spore-forming, non-motile cocci with a diameter of about 0.9 µm. The colony colour on most media tested is orange. Colonies are circular,

Fig. 1. Phylogenetic dendrogram obtained by distance-matrix analysis of 16S rRNA gene sequences showing the position of strain YIM 70202^T among phylogenetic neighbours. Numbers on branch nodes are bootstrap values (1000 resamplings). The sequence of *Nesterenkonia xinjiangensis* YIM 70097^T (GenBank accession number AY226510) was used as the root. Bar, 2 % sequence divergence.

opaque and approximately 1.0 mm in diameter after 48 h at 30 °C. The optimum concentration of NaCl, optimum pH and temperature for growth are 10 % (w/v), 8.0–9.0 and 30 °C, respectively. The concentration range of NaCl and pH and temperature ranges for growth are 1–25 % (w/v), 7.0–11.0, and 4–45 °C, respectively. Gives a positive reaction in tests for catalase, oxidase, ornithine decarboxylase, arginine dihydrolase, lysine decarboxylase, lipase, β -glucosidase, nitrate reduction and Tween 20 hydrolysis, but is negative in tests for *N*-acetylglucosaminidase, β -glucuronidase, β -galactosidase, α -galactosidase, gelatin liquefaction, indole and ammonia production, methyl red and Voges–Proskauer tests, milk peptonization and coagulation, growth on cellulose, H₂S and melanin production, casein, starch, and Tween 80 hydrolysis. The following substrates are utilized: maltose, mannitol, glucose, adonitol, arabinose, arabitol, mannose, inositol, sorbitol, fructose, cellobiose, salicin, acetamide, galactose, xylose and dextrin, while rhamnose and starch are not utilized. Acid is produced from maltose, D-glucose, sucrose, malonate and *N*-acetylglucosamine. The cell-wall peptidoglycan structure is L-Lys–Gly₅. The predominant respiratory quinone is MK-6. Polar lipids include phosphatidylglycerol, diphosphatidylglycerol and an unknown glycolipid. The major fatty acids are i-C_{15:0} and ai-C_{15:0}. The DNA G+C content is 49.7 mol% (HPLC method).

The type strain, YIM 70202^T (=CGMCC 1.6511^T=KCTC 3941^T), was isolated from a desert soil sample collected from Egypt.

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