NOTE

***Salinicoccus alkaliphilus* sp. nov., a novel alkaliphile and moderate halophile from Baer Soda Lake in Inner Mongolia Autonomous Region, China**

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A novel alkaliphilic and moderately halophilic Gram-positive coccus, designated strain T8^T, was isolated from Baer Soda Lake in Inner Mongolia Autonomous Region, China. Strain T8^T grew in the presence of 0–25% (w/v) NaCl and at pH 6.5–11.5, with optimum growth at 10% (w/v) NaCl and pH 9.0. It grew at 10.0–46.0 °C, with an optimum growth temperature of 32.0 °C. The organism was strictly aerobic, non-motile, non-sporulating and catalase- and oxidase-positive. The DNA G+C content was 49.6 mol%. The cell wall contained Lys and Gly. The major isoprenoid quinone was menaquinone 6 (MK-6). Phylogenetic analyses based on 16S rDNA sequence comparisons indicate that strain T8^T is a member of the genus *Salinicoccus*. DNA–DNA relatedness of less than 50% with the described species of *Salinicoccus* supported the view that this organism represents a novel species of the genus *Salinicoccus*. The name *Salinicoccus alkaliphilus* sp. nov. is proposed for this novel species. The type strain is T8^T (= AS 1.2691^T = JCM 11311^T).

Keywords: *Salinicoccus alkaliphilus* sp. nov., moderately halophilic bacteria, alkaliphile, 16S rDNA sequence

Moderately halophilic bacteria are micro-organisms that grow optimally in media containing between 3 and 15% (w/v) salt (Kushner & Kamekura, 1988). Their physiological adaptations to highly saline conditions and their ecology have attracted the interest of researchers. They are represented by a heterogeneous group of micro-organisms included in many different genera (Ventosa *et al.*, 1998). With respect to moderately halophilic Gram-positive cocci, there are only seven moderate halophiles currently recognized as valid species: *Marinococcus albus* and *Marinococcus halophilus* (Hao *et al.*, 1984), *Salinicoccus roseus* (Ventosa *et al.*, 1990) and *Salinicoccus hispanicus* (Ventosa *et al.*, 1992), *Nesterenkonia halobia* (Stackebrandt *et al.*, 1995), *Halobacillus halophilus* (Spring *et al.*, 1996) and *Tetragenococcus muriaticus* (Satomi *et al.*, 1997). These species show optimum growth in environments with neutral pH values and, up to now, aerobic

Gram-positive cocci have not been identified as both moderately halophilic and alkaliphilic micro-organisms.

Soda lakes are highly alkaline extreme environments with salt concentrations ranging from 5% (w/v) up to saturation (Jones *et al.*, 1998), suitable biotopes for the growth of alkaliphilic and halophilic micro-organisms. In this paper, an alkaliphilic, moderately halophilic, aerobic Gram-positive coccus designated strain T8^T, isolated from a soda lake in China, is described. Based on the results of 16S rDNA sequence comparisons, as well as phenotypic and chemotaxonomic features, strain T8^T is proposed as a novel species of the genus *Salinicoccus*, *Salinicoccus alkaliphilus* sp. nov.

Strain T8^T was isolated from a water sample collected on 10 October 2000 from Baer Soda Lake, Inner Mongolia Autonomous Region, China (49° 24' N and 118° 08' E). The pH value of the water of the lake was 9.5, determined with a Beckman Φ71 pH meter, and the salt content was 18% (w/v), determined with a

The GenBank accession number for the 16S rRNA gene sequence of strain T8^T is AF275710.

Fig. 1. Phylogenetic tree of strain T8^T and other related moderately halophilic microorganisms based on their 16S rDNA sequences. Evolutionary distances were calculated by the neighbour-joining method and Kimura's two-parameter calculation model. Numbers represent the confidence level from 100 replicate bootstrap samplings. Bar, 0.1 substitutions per nucleotide.

WYY-1 salimeter. Enrichment and isolation were performed according to Horikoshi (1971). The strain was grown aerobically at 37 °C in a complex medium with the following composition (g l⁻¹): glucose, 10; polypeptone, 5; yeast extract, 5; K₂HPO₄, 1; MgSO₄ · 7H₂O, 0.2; NaCl, 100; and Na₂CO₃, 10. When required, the medium was solidified with 20 g agar l⁻¹. Cellular morphology was examined by light microscopy either without fixation under phase-contrast microscopy or with acetic acid fixation (Dussault, 1955). The Gram reaction was determined by the KOH lysis method of Gregersen (1978). The methods used for physiological studies were described previously (Gerhardt *et al.*, 1981; Ventosa *et al.*, 1982; Quesada *et al.*, 1984). Hydrolysis of urea was tested according to Spanka & Fritze (1993). Unless otherwise indicated, all tests were carried out in media with 10% (w/v) NaCl at pH 9.0 and incubated at 37 °C. Growth was monitored by turbidity at OD₆₀₀. Preparation and hydrolysis of the cell wall was carried out using the method of Schleifer (1985). The amino acid composition of the cell wall hydrolysate was determined using one-dimensional descending film chromatography on micro-cellulose paper with methanol/water/6 M HCl/pyridine (80:26:4:10, by vol.) and amino acids were visualized with ninhydrin. Quinones were extracted and purified from freeze-dried cells using the method of Stackebrandt *et al.* (1995) and determined by HPLC (Lu, 1999). Fatty acid analysis was performed using standard methods and compared to the database of fatty acids in the MIDI Sherlock Microbial Identification system (Microbial ID). DNA was isolated and purified as described previously (Zhou *et al.*, 1994). The G + C content of the DNA was determined by the thermal denaturation method (Marmur & Doty, 1962). DNA–DNA hybridization was carried out as described by Tindall *et al.* (1984) with a minor modification: DNA fragments were labelled with [α -³²P]dATP using a nick-translation kit (Boehringer Mannheim). 16S rDNA was amplified from strain T8^T genomic DNA by PCR with primers

5'-GAGAGTTTGATCCTGGCTCAG-3' and 5'-AAGGAGGTGATCCAGCCGCA-3' (Hain *et al.*, 1997), which correspond to positions 7–27 and 1541–1522 in the 16S rRNA (*Escherichia coli* numbering; Brosius *et al.*, 1978). PCR products were purified with the Wizard PCR Preps DNA purification system (Promega) and sequenced directly using the DYEnamic ET terminator cycle sequencing premix kit (Pharmacia), as directed in the manufacturer's protocol, on an ABI 373S DNA sequencer (Applied Biosystems).

The raw sequence dataset included nearly complete sequences and was used for phylogenetic analysis (1459 bp). Multiple sequence alignments were performed using CLUSTAL W version 1.8 (Thompson *et al.*, 1994). Phylogenetic analysis for multiple sequence alignments was done with TREECONW version 1.2 (Van de Peer & De Wachter, 1994). The phylogenetic tree was built using the neighbour-joining method with Kimura's two-parameter calculation model in TREECONW version 1.2. 16S rRNA gene sequences used for phylogenetic comparisons were obtained from the GenBank database and their strain designations and accession numbers are shown in Fig. 1.

The novel strain, designated T8^T, was an alkaliphilic, moderately halophilic coccus, occurring singly, in pairs, tetrads or clumps. The strain grew on nutrient agar at pH 9.5 with 1% (w/v) K₂CO₃, in the absence of NaCl or Na₂CO₃. At 32.0 °C, the strain grew optimally in the presence of 10% (w/v) NaCl at pH 9.5 and could grow on liquid medium of pH 6.5–11.5. The novel strain could be distinguished easily from the other moderately halophilic Gram-positive cocci by its ability to grow in media in the absence of NaCl and with a wide pH range.

Chemotaxonomic data for the test strain are consistent with its assignment to the genus *Salinicoccus* (Ventosa *et al.*, 1990, 1992). The major lipoquinone of strain T8^T is MK-6. The major amino acid constituents of the cell

Table 1. Fatty acid composition of cells of strain T8^T

Values are percentages of total fatty acids.

Fatty acid	Content
14:0 iso	4.39
14:0	1.56
15:0 iso	22.28
15:0 anteiso	27.61
16:1 ω 7c alcohol	5.81
16:0 iso	10.13
Unknown (15:665)	0.86
16:1 ω 11c	2.19
16:0	1.51
17:1 ω 10c iso	4.01
17:0 iso	3.33
17:0 anteiso	8.91
19:0 iso	2.83
19:0 anteiso	1.86
20:0 iso	1.47
20:0	1.26

wall are glycine and lysine, which is compatible with the type of L-Lys–Gly₅ cell wall described for the genus *Salinicoccus* (Ventosa *et al.*, 1990). The fatty acid

composition is shown in Table 1. The G + C content of the DNA of strain T8^T was 49.6 mol %. These data are compatible with the assignment of strain T8^T to the genus *Salinicoccus*.

The characteristics of this organism are shown in Table 2 and in the description of the species. A combination of phenotypic properties can distinguish this strain from representatives of both validly published *Salinicoccus* species.

The almost complete 16S rDNA sequence (1455 bp) of strain T8^T was determined. Alignments of this sequence with sequences available from the GenBank database showed that the closest relative of strain T8^T was *S. roseus*, with a sequence similarity of 95 %. The level of 16S rDNA sequence similarity between strain T8^T and other Gram-positive cocci was less than 91 %. The phylogenetic tree (Fig. 1), constructed with K_{nuc} values from the new sequence and other known relevant sequences in GenBank, clearly indicates that strain T8^T and the other known species of genus *Salinicoccus* grouped into the same lineage. The 16S rDNA sequence of *S. hispanicus* has not been deposited in GenBank, so it could not be included in the analysis. Based on both sequence dissimilarity values (> 3 %) and placement in the phylogenetic tree, the isolate did not belong to any other described species and was

Table 2. Comparison of strain T8^T with members of the genus *Salinicoccus*

+, Positive; –, negative; D, differs among strains.

Characteristic	Strain T8 ^T	<i>S. hispanicus</i>	<i>S. roseus</i>
Diameter of cells (μm)	0.5–0.8	1.0–2.5	1.0–2.5
Colony pigmentation	Pinkish	Reddish orange	Pink-red
pH for growth:			
Range	6.5–11.5	6.0–9.0	5.0–9.0
Optimum	9.5	8.0	7.5
Salt range for growth (%)	0.0–25.0	0.9–25.0	0.5–25.0
Temperature for growth (°C):			
Range	10.0–49.0	15.0–40.0	15.0–37.0
Optimum	32.0	37.0	37.0
Acid production from:			
Maltose	–	+	–
Galactose	–	+	–
D-Glucose	+	+	–
Fructose	–	+	–
Sucrose	–	D	–
Hydrolysis of:			
Casein	–	–	+
Aesculin	+	+	–
Gelatin	–	+	+
Tween 80	–	–	+
Starch	–	–	+
Nitrate reduction	+	+	–
Methyl red test	–	+	–
Urease	+	+	–
G + C content (mol %)	49.6	45.6–49.3	51.2

likely to represent a novel *Salinicoccus* species (Stackebrandt & Goebel, 1994).

DNA–DNA reassociation was studied to confirm the species status of strain T8^T. The results of DNA–DNA reassociation studies showed that the relatedness values of strain T8^T to *S. roseus* and *S. hispanicus* were respectively 44.1 and 48.1%. According to Wayne *et al.* (1987), less than 70% DNA–DNA relatedness is considered to be the threshold value for the delineation of genospecies, so the values are low enough to separate strain T8^T from the other two species.

Chemical, genotypic and phenotypic data show that strain T8^T merits species status in the genus *Salinicoccus*. Therefore, it is proposed that this organism be classified in the genus *Salinicoccus* as the type strain of a novel species, *Salinicoccus alkaliphilus* sp. nov.

Description of *Salinicoccus alkaliphilus* sp. nov.

Salinicoccus alkaliphilus (N.L. fem. n. *alkali* alkali; Gr. adj. *philos* loving; N.L. masc. adj. *alkaliphilus* liking alkaline media).

Cells are cocci, 0.5–0.8 µm in diameter, occurring singly, in pairs, tetrads or clumps. Non-motile. Endospores are not formed. Gram-positive and strictly aerobic. Colonies are round, smooth and slightly convex with pinkish colour and no diffusible pigment. Growth occurs at pH 6.5–11.5 (optimum at 9.5), at temperatures of 10.0–49.0 °C (optimum at 32 °C) and in media with 0–25.0% (w/v) NaCl [optimum at 10% (w/v) NaCl]. Catalase, oxidase and urease are positive. H₂S production, indole, methyl red and Voges–Proskauer tests are negative. Aesculin is hydrolysed, whereas casein, starch, gelatin and Tweens 80, 60 and 20 are not. Utilizes D-arabinose, D(+)-melibiose, creatinine, aesculin, lactose, D-xylose, D-fructose, amylose, sorbose, D(+)-α-melezitose, erythritol, inulin, *meso*-erythritol, trehalose, D-mannose, D-sorbitol, stachyose, ribose, mannitol, D-galactose, adonitol, D-cellobiose, glycogen, dulcitol and α-L-rhamnose as sole carbon and energy sources. Acid is produced from D-glucose, but not from maltose, galactose, fructose, sucrose, D-arabinose, glycerol or D-xylose. Nitrate is reduced. The following compounds are utilized as sole nitrogen sources: polypeptone, yeast extract, DL-tryptophan, bacto-peptone, casitone, urea, L-proline, L-methionine, L-serine, L-arginine, L-alanine, L-threonine, L-histidine, sodium L-glutamate, L-ornithine, L-leucine, L-glycine, L-glutamic acid and DL-lysine. Amino acids that are not utilized as sole nitrogen sources are L-asparagine, L-tyrosine, L-isoleucine and L-cysteine. The DNA G+C content is 49.6 mol% (determined by thermal denaturation). The predominant lipoquinone is MK-6; no other quinones are detected. The assumed cell wall murein type is L-Lys–Gly₅. Isolated from Baer Soda Lake, China. The type strain is strain T8^T (= AS 1.2691^T = JCM 11311^T).

Acknowledgements

This work was partially supported by grants from Chinese Academy of Sciences.

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