

Salinicoccus halodurans sp. nov., a moderate halophile from saline soil in China

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A moderately halophilic, Gram-positive coccus, designated strain W24^T, was isolated from saline soil in Qinghai province, China. The isolate was able to grow at salinities of 0–24% (w/v) NaCl (optimally at 8%, w/v), at pH 5.5–9.0 (optimally at pH 7.5) and at 8–43 °C (optimally at 28 °C). The genomic DNA G + C content of strain W24^T was 45.8 mol%. The predominant isoprenoid quinone was MK-6 and the cell wall contained lysine and glycine as diagnostic diamino acids. The polar lipids were diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid. The major cellular fatty acids were iso-C_{15:0}, anteiso-C_{15:0} and C_{16:0}. Based on 16S rRNA gene sequence analysis, strain W24^T was found to be a member of the genus *Salinicoccus* and was related most closely to *Salinicoccus hispanicus* DSM 5352^T (96.5% sequence similarity). Based on data from the current polyphasic study, strain W24^T is considered to represent a novel species of the genus *Salinicoccus*, for which the name *Salinicoccus halodurans* sp. nov. is proposed. The type strain is W24^T (=CGMCC 1.6501^T=DSM 19336^T).

The genus *Salinicoccus* was proposed by Ventosa *et al.* (1990) to accommodate a moderately halophilic, Gram-positive coccus, *Salinicoccus roseus*. At the time of writing, eight species of the genus have been described: *S. roseus* and *S. hispanicus* isolated from solar salterns (Ventosa *et al.*, 1990, 1992; Marquez *et al.*, 1990), *S. alkaliphilus* from a soda lake (Zhang *et al.*, 2002), *S. salsiraiiae* from salted skate (França *et al.*, 2006), *S. jeotgali* from jeotgal (Aslam *et al.*, 2007), *S. luteus* from desert soil (Zhang *et al.*, 2007), *S. siamensis* from fermented shrimp paste (Pakdeeto *et al.*, 2007) and *S. kunmingensis* from a salt mine (Chen *et al.*, 2007). During the course of a study of micro-organisms present in the Chaerhan salt lakes (37° 03' 50" N 94° 43' 41" E) of Qinghai province, China, various halophilic strains were isolated. Based on preliminary 16S rRNA gene sequence analysis, most of the moderately halophilic isolates were found to be related to the genera *Bacillus*, *Halobacillus*, *Virgibacillus*, *Marinobacter*, *Idiomarina* and *Halomonas*. Here we describe the phenotypic and genotypic characteristics of a moderately halophilic coccus, strain W24^T, which appeared to represent a novel species of the genus *Salinicoccus*.

Strain W24^T was isolated from a saline soil sample taken around the Chaerhan salt lakes by enrichment in liquid medium at 37 °C and subsequent plating on the same medium with added agar until a pure culture colony was obtained. The medium contained the following (per litre): 7.5 g Casamino acids (Difco), 10 g yeast extract (Difco), 1.0 g sodium glutamate, 3.0 g trisodium citrate, 20 g MgSO₄·7H₂O, 2.0 g KCl, 150 g NaCl, 0.036 g FeSO₄·7H₂O and 0.36 mg MnCl₂·4H₂O. The pH was adjusted to 7.2 with 1 M NaOH before autoclaving at 121 °C for 20 min. Strain W24^T was maintained in this medium supplemented with 30% (v/v) glycerol at –80 °C for long-term preservation. Strain W24^T was routinely grown on GMH medium containing the following (per litre): 5.0 g Casamino acids, 5.0 g yeast extract, 4.0 g MgSO₄·7H₂O, 2.0 g KCl, 80 g NaCl, 0.036 g FeSO₄·7H₂O and 0.36 mg MnCl₂·4H₂O (pH 7.2).

Cell morphology and motility of strain W24^T were examined using light microscopy and transmission electron microscopy. Gram-type was determined according to the staining method of Doetsch (1981) and was confirmed by using the KOH lysis method (Gregersen, 1978). The range of salinity for growth was tested in GMH medium with NaCl concentrations between 0 and 340 g l⁻¹. The pH range for growth was tested at pH intervals of 0.5 in GMH liquid medium buffered with 20 mM MES (pH 5.0–6.0), PIPES (pH 6.5–7.5), HEPES (pH 7.0–8.0), Tricine (pH 7.5–9.0) and CHES (pH 9.0–10.0). Growth temperature

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain W24^T is DQ989633.

Transmission electron micrographs of cells of strain W24^T occurring singly, in pairs and in tetrads are available as a supplementary figure with the online version of this paper.

was determined in liquid GMH medium between 4 and 50 °C by using a temperature gradient incubator (model TN-3; ADVANTEC). General biochemical tests (including nitrate reduction, urease activity, H₂S production, catalase and oxidase activities, citrate utilization, indole production, Voges–Proskauer reaction, methyl red test, acid production from different sugars and biopolymer hydrolysis) were performed according to previously described methods (Harrigan & McCance, 1966; Gerhardt *et al.*, 1994; Hezayen *et al.*, 2001). Unless otherwise indicated, all tests were carried out in triplicate in medium containing 8% (w/v) NaCl and incubated at 37 °C. Growth was monitored based on turbidity at OD₆₀₀. Susceptibility to antibiotics was determined on agar medium plates by using absorbent paper discs impregnated with antibiotic. Tests for use of different substrates as sole carbon and energy sources were performed in modified GMH medium containing 1 g yeast extract l⁻¹ but without Casamino acids. Substrates in sterile stock solutions were each added to the medium at a final concentration of 10 g l⁻¹. API 50 CH test strips (bioMérieux) were also used to examine the assimilation of carbohydrates and to examine the production of acid as recommended by the manufacturer, but with a modification that all suspension media supplied by bioMérieux were supplemented with 8% NaCl (w/v) to resuspend cells of strain W24^T.

Cells of strain W24^T were aerobic cocci (see Supplementary Fig. S1 available in IJSEM Online). Colonies of strain W24^T on GMH agar medium were circular, white, opaque and slightly convex after cultivation at 37 °C for 2 days. Strain W24^T was able to grow at 8–43 °C, at pH 5.5–9.0 and at a salinity of 0–24% (w/v) NaCl. Other phenotypic properties are given in the species description below.

Preparation of the cell wall and determination of peptidoglycan structure were performed according to the methods described by Schleifer & Kandler (1972) with the modification that TLC on cellulose sheets was used instead of paper chromatography. The amino acid composition of the cell-wall hydrolysate was determined by using one-dimensional descending chromatography on cellulose paper. Respiratory quinones were extracted according to the method of Collins *et al.* (1977) and were analysed by reversed-phase HPLC (Groth *et al.*, 1996). Polar lipids were extracted and identified by using one-dimensional TLC followed by spraying with appropriate detection reagents (Kates, 1986). Fatty acids were extracted, methylated and analysed by GC with the standard Sherlock MIDI (Microbial Identification) system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The genomic DNA G+C content was determined by using the thermal denaturation method according to Marmur & Doty (1962). The major amino acid constituents of the cell wall of strain W24^T were glycine and lysine, which is consistent with the L-Lys–Gly₅ cell-wall type described for the genus *Salinicoccus* (Ventosa *et al.*, 1990). The major lipoquinone of strain W24^T was MK-6. The polar lipid extract contained diphosphatidylglycerol, phosphatidylglycerol and an unknown glycolipid.

The major cellular fatty acids of strain W24^T were iso-C_{15:0} (21.85%), anteiso-C_{15:0} (17.89%), C_{16:0} (14.33%), iso-C_{16:0} (8.82%) and iso-C_{14:0} (8.62%). This profile is similar to that of species of the genus *Salinicoccus* (Zhang *et al.*, 2007). The genomic DNA G+C content of strain W24^T was 45.8 mol%. The above chemotaxonomic data are in agreement with those described for the genus *Salinicoccus*.

The 16S rRNA gene of strain W24^T was amplified by PCR by using the universal primers described by Duckworth *et al.* (1996). The almost-complete nucleotide sequence (1467 bp) was determined by direct sequencing and was compared with available 16S rRNA gene sequences in the GenBank database by using the BLAST program. Multiple alignment with sequences of closely related species was performed by using the CLUSTAL_X program (Thompson *et al.*, 1997). Ambiguous and unalignable bases were manually omitted and a phylogenetic tree was constructed from the evolutionary distance matrix by using the neighbour-joining (Saitou & Nei, 1987) method in the MEGA program (version 3.1; Kumar *et al.*, 2004). Robustness of the resultant tree topology was evaluated by bootstrap resampling analysis with 1000 replicates (Felsenstein, 1985). Phylogenetic analysis showed that strain W24^T clustered with members of the genus *Salinicoccus* and formed a distinct branch within the genus *Salinicoccus* cluster (Fig. 1). Strain W24^T was related most closely to *S. hispanicus* DSM 5352^T (96.5% 16S rRNA gene sequence similarity), *S. kunmingensis* YIM Y15^T (96.3%), *S. salsiraiiae* RH1^T (96.2%) and *S. jeotgali* S2R53-5^T (96.1%), and showed levels of similarity of 93.4–95.9% to the type strains of other recognized species of the genus *Salinicoccus*. These high levels of sequence divergence (>3.5%) with recognized members of the genus *Salinicoccus* clearly indicated that strain W24^T represents a novel species (Stackebrandt & Goebel, 1994).

The characteristics that differentiate strain W24^T from related *Salinicoccus* species are summarized in Table 1. Differences in several characteristics, such as pigmentation, range and optimal salt concentration, pH and temperature for growth, hydrolysis of casein, gelatin, Tween 80 and starch, as well as genomic DNA G+C content and fatty acid composition, can be used to distinguish this novel strain from phylogenetically related taxa (Table 1). Therefore, on the basis of data from this polyphasic study, strain W24^T is considered to represent a novel species of the genus *Salinicoccus*, for which the name *Salinicoccus halodurans* sp. nov. is proposed.

Description of *Salinicoccus halodurans* sp. nov.

Salinicoccus halodurans (ha.lo.du'rans. Gr. n. *hals* salt; L. pres. part. *durans* enduring; N.L. part. adj. *halodurans* salt-enduring).

Cells of strain W24^T are Gram-positive, non-motile, non-spore-forming cocci, 0.65–0.90 µm in diameter, occurring singly, in pairs, tetrads or irregular clumps. Colonies on GMH medium are circular, white, opaque and slightly

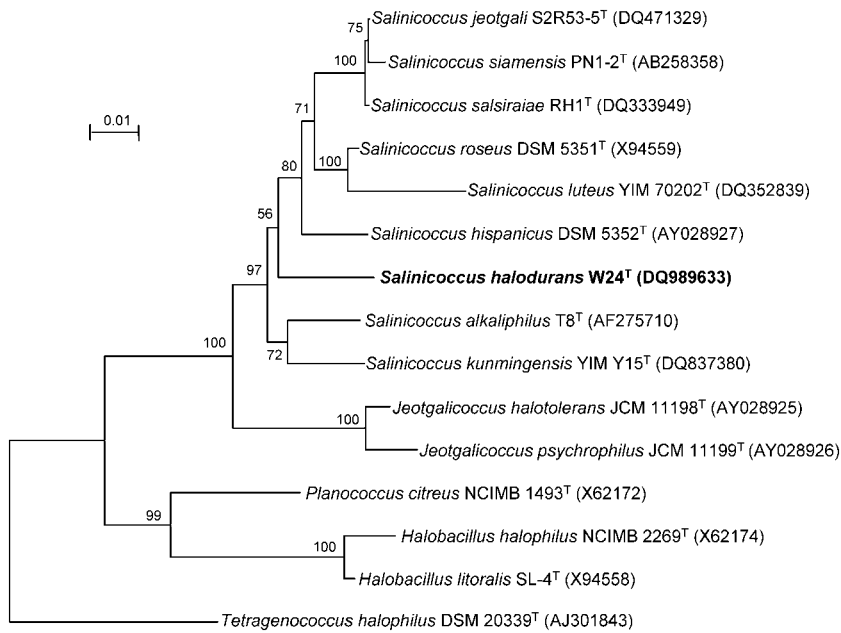


Fig. 1. Phylogenetic tree showing the relationship between strain W24^T, related species of the genus *Salinicoccus* and of related genera based on comparative 16S rRNA gene sequence analysis. Numbers at nodes represent levels of bootstrap support (%), based on a neighbour-joining analysis of 1000 resampled datasets. Bar, 1 % sequence divergence.

Table 1. Characteristics useful in distinguishing between strain W24^T and closely related species of the genus *Salinicoccus*

Strains: 1, W24^T (*Salinicoccus halodurans* sp. nov.); 2, *S. hispanicus* DSM 5352^T (data from Marquez *et al.*, 1990); 3, *S. roseus* DSM 5351^T (Ventosa *et al.*, 1990); 4, *S. salsiraiiae* RH1^T (França *et al.*, 2006); 5, *S. jeotgali* S2R53-5^T (Aslam *et al.*, 2007); 6, *S. siamensis* PN1-2^T (Pakdeeto *et al.*, 2007). +, Positive; -, negative; d, differs among strains; ND, no data available. All are positive for Gram-staining reaction, and catalase and oxidase activities, but are negative for spore formation and motility.

Characteristic	1	2	3	4	5	6
Cell diameter (µm)	0.6–0.9	1.0–2.0	1.0–2.5	1.0–2.5	1.0–2.0	0.6–0.8
Pigmentation	White	Reddish orange	Pink–red	Pink–red	Orange	Orange
pH for growth						
Range	5.5–9.0	5.0–9.0	6.0–9.0	6.5–9.5	6.5–11.0	6.0–9.0
Optimum	7.5	7.5	8.0	8.0	7.0	8.5
Temperature for growth (°C)						
Range	8–43	15–37	15–40	20–45	20–30	15–45
Optimum	28	37	37	37	30	37
Salinity for growth (% w/v)						
Range	0–24	0.5–25	0.9–25	0–22	0.5–15	1.5–25
Optimum	8	10	10	4	5	10
Acid production from:						
D-Glucose	+	+	–	+	+	+
Sucrose	+	d	–	–	–	d
Maltose	+	d	–	+	+	–
D-Mannitol	+	+	–	–	–	–
Trehalose	+	–	–	+	+	+
D-Galactose	–	+	–	–	–	–
Hydrolysis of:						
Casein	–	–	+	+	–	–
Gelatin	+	+	+	+	–	–
Tween 80	–	–	+	ND	–	–
Starch	–	–	+	–	–	–
Nitrate reduction	+	+	–	+	+	–
DNA G + C content (mol%)	45.8	45.6–49.3	51.2	46.2	47.0	44.5–47.5

convex, reaching 1.4–2.8 mm in diameter after cultivation at 37 °C for 2 days. The temperature range for growth is 8–43 °C, and the optimum temperature is approximately 28 °C. The pH range for growth is 5.5–9.0, and maximum growth occurs at pH 7.5. Grows well in media without NaCl. Also able to grow in the presence of NaCl up to a concentration of 24 % (w/v), with optimum growth at 8 % (w/v) NaCl. Positive for oxidase, catalase, NH₃ production, methyl red reaction, nitrate reduction, H₂S production, citrate utilization, urease production, and hydrolysis of gelatin and aesculin, but negative for indole production, Voges–Proskauer reaction, and hydrolysis of starch, casein, cellulose, and Tweens 20, 40, 60 and 80. Utilizes D-mannitol, erythritol, sorbitol, D-glucose, D-fructose, D-mannose, maltose, D-lactose, sucrose, trehalose, cellobiose and L-rhamnose as sole carbon and energy sources, but not lactate, creatine, dodecanoic acids, acetone, ethanol, propanol, pentanol, butanol, iso-amyl alcohol or isopropyl alcohol. Produces acid in the API 50 CH gallery from D-sorbitol, D-mannitol, erythritol, glycerol, D-ribose, D-glucose, D-fructose, D-mannose, maltose, cellobiose, D-lactose, sucrose, trehalose, turanose, N-acetylglucosamine and 5-ketogluconate, but not from D-arabinose, L-arabinose, D-xylose, L-xylose, L-rhamnose, D-galactose, L-sorbose, melibiose, melezitose, gentiobiose, D-lyxose, D-tagatose, D-fucose, L-fucose, raffinose, D-adonitol, dulcitol, inositol, xylitol, D-arabitol, L-arabitol, amygdalin, arbutin, salicin, inulin, glycogen, gluconate, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, methyl β -D-xylopyranoside or 2-ketogluconate. Susceptible to chloramphenicol (30 μ g), kanamycin (30 μ g), erythromycin (15 μ g), tetracycline (30 μ g), ampicillin (10 μ g), novobiocin (5 μ g), rifandin (5 μ g), vancomycin (30 μ g), penicillin (5 μ g) and tetracycline (30 μ g), but resistant to norfloxacin (10 μ g), bacitracin (0.04 U), ciprofloxacin (5 μ g) and streptomycin (5 μ g). Major cellular fatty acids are iso-C_{15:0} (21.85 %), anteiso-C_{15:0} (17.89 %), C_{16:0} (14.33 %), iso-C_{16:0} (8.82 %) and iso-C_{14:0} (8.62 %). Major cellular polar lipids are diphosphatidylglycerol, phosphatidylglycerol and an unknown glycolipid. The major lipoquinone is MK-6. The cell-wall murein is of L-Lys–Gly₅ type. The genomic DNA G+C content of the type strain is 45.8 mol% (T_m).

The type strain, W24^T (=CGMCC 1.6501^T=DSM 19336^T), was isolated from a saline soil sample around the Chaerhan salt lakes of Qinghai province, China.

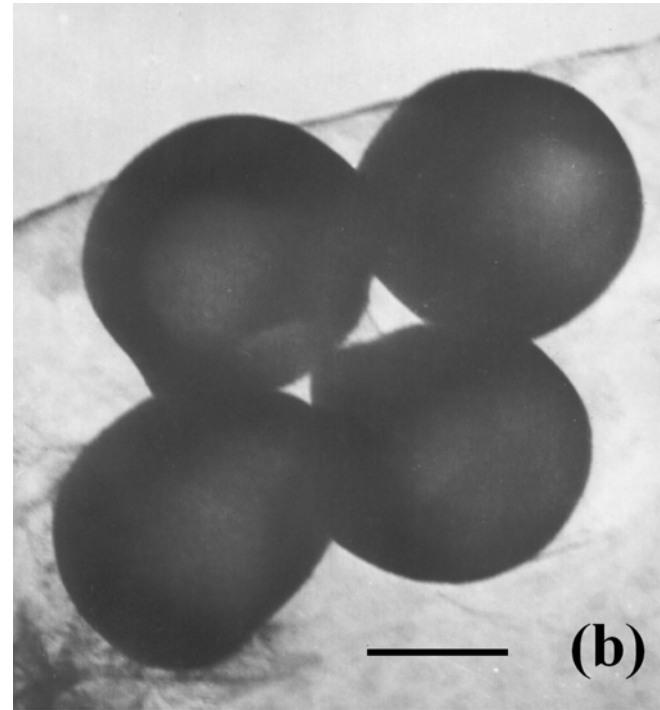
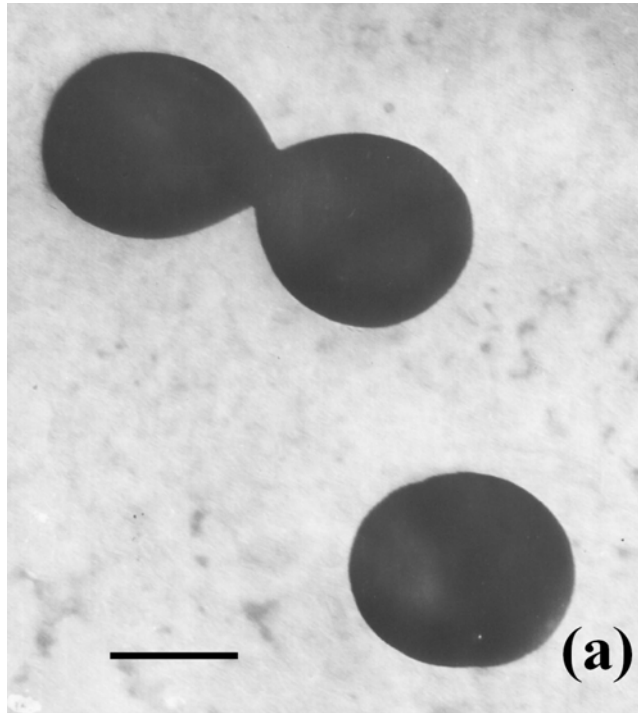
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Supplementary Fig. S1. Transmission electron micrographs of cells of strain W24^T occurring singly and in pairs (a), and tetrads (b) in exponential growth phase at 37 °C. Bars, 0.43 μm (a) and 0.35 μm (b).

Wang, X., Xue, Y., Yuan, S., Zhou, C. & Ma, Y. (2008). *Salinicoccus halodurans* sp. nov., a moderate halophile from saline soil in China. *Int J Syst Evol Microbiol* **58**, 1537–1541.