Jeotgalicoccus halophilus sp. nov., isolated from salt lakes

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Two slightly halophilic bacterial strains, C1-52^T and YD-9, were isolated from Daban and Aiding salt lakes in Xinjiang, China, respectively. The isolates were Gram-positive, non-endospore-forming, non-motile, facultatively anaerobic cocci. Colonies were pale yellow, and a light pink, diffusible pigment was produced after a few additional days of incubation. The isolates grew optimally with 2–3 % (w/v) NaCl, at pH 7.5 and at 30–35 °C. The peptidoglycan type was L-Lys–Gly_{3–4}–L-Ala(Gly). The menaquinones were MK-7 (83.2 %) and MK-6 (16.8 %). The major fatty acids (>10 %) were anteiso-C_{15:0} and iso-C_{15:0}. The DNA G + C content of strains C1-52^T and YD-9 was 41.2 and 41.0 mol%, respectively. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strains C1-52^T and YD-9 were closely related to *Jeotgalicoccus psychrophilus* YKJ-115^T (98.0 and 97.1 % 16S rRNA gene sequence similarity, respectively), followed by *Jeotgalicoccus halotolerans* YKJ-101^T (97.1 and 96.8 %). Strains C1-52^T and YD-9 shared, respectively, 20 and 11 % DNA–DNA relatedness with *J. halotolerans* JCM 11198^T and 8 and 13 % with *J. psychrophilus* JCM 11199^T. DNA–DNA relatedness between the isolates was 91 %. On the basis of phenotypic and phylogenetic distinctiveness, strains C1-52^T and YD-9 belonged to the same species, which should be placed in the genus *Jeotgalicoccus* as a novel species. The name *Jeotgalicoccus halophilus* sp. nov. is proposed, with the type strain C1-52^T (=CGMCC 1.8911^T =NBRC 105788^T).

The genus *Jeotgalicoccus* was originally proposed by Yoon *et al.* (2003) with two species, *Jeotgalicoccus halotolerans* and *Jeotgalicoccus psychrophilus*, which were isolated from a traditional Korean food, jeotgal. A third species, *Jeotgalicoccus pinnipedialis*, from the oral cavity of a southern elephant seal, was subsequently described by Hoyles *et al.* (2004). To date, two more species have been described within this genus: *Jeotgalicoccus marinus* (Chen *et al.*, 2009), from a sea urchin collected from the South China Sea, and *Jeotgalicoccus huakuii* (Guo *et al.*, 2010), from a seaside soil sample in Shandong province of China. The main characteristics of this genus are Gram-positive, non-motile, non-endospore-forming, catalase- and oxidase-positive cocci. The predominant isoprenoid quinone is MK-7, the major fatty acids are anteiso-C_{15:0} and iso-C_{15:0}, the DNA

G + C content is 36.8–42 mol% and the cell-wall peptidoglycan is L-Lys–Gly_{3–4}–L-Ala(Gly) (Yoon *et al.*, 2003).

In the course of investigating the microbial diversity of salt lakes in Xinjiang, we isolated two *Jeotgalicoccus*-like organisms, C1-52^T and YD-9, from the sediments of Daban salt lake (43° 21' 00"–25° 25' N 88° 03' 53"–12° 15' E) and Aiding salt lake (42° 32' 10"–49° 13' N 89° 10' 32"–54° 32' E), respectively. At the time of sampling, the NaCl concentration and pH of sediments collected from Daban salt lake were 14.7–16.9 % and pH 7.6–7.8 and those of sediments from Aiding salt lake were 12.1–15.4 % and pH 7.1–7.3. For isolation, the samples were suspended in sterilized water with 2 % (w/v) NaCl, serially diluted and spread on improved Gibbson medium (containing 1^{–1}: 5 g tryptone, 10 g yeast extract, 5 g casein, 2 g KCl, 20 g MgSO₄·7H₂O, 20 g NaCl, 3 g trisodium citrate; pH 7.4; Xu *et al.*, 1995). The isolation medium was also used for maintenance of the cultures.

Cell morphology was examined by phase-contrast microscopy (Eclipse 50i; Nikon) and transmission electron

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains C1-52^T and YD-9 are FJ386517 and GQ304892, respectively.

A supplementary figure and table are available with the online version of this paper.

microscopy (JEM 1230; JEOL) after 16 h. Motility was examined by phase-contrast microscopy. Colony morphology was examined after 3 days at 33 °C (pH 7.5). Gram reaction was determined according to the methods described by Gregersen (1978) and Doetsch (1981). Cells were Gram-positive, non-motile cocci (0.5–0.8 µm; Supplementary Fig. S1, available in IJSEM Online). Colonies were circular, smooth, entire, slightly raised, pale yellow and 1–2 mm in diameter after 3 days. The two isolates and the reference strains *J. halotolerans* JCM 11198^T and *J. psychrophilus* JCM 11199^T produced a light pink, diffusible pigment after a few additional days of incubation.

Physiological and biochemical tests were performed according to the recently published notes on the characterization of prokaryotic strains for taxonomic purposes (Tindall *et al.*, 2010). Unless otherwise indicated, all tests were performed in test tubes in triplicate at 33 °C using maintenance medium containing 5 % (w/v) NaCl and uninoculated tubes were used as negative controls. Growth at 4 and 5–55 °C (in increments of 5 °C) at pH 7.5 and at pH 5.0–11.0 (in increments of 0.5 pH units) at 33 °C was estimated in liquid medium by monitoring the increase in optical density at 600 nm. For the pH experiments, the buffers described by Chen *et al.* (2007) were used. Growth with 0–0.5 % NaCl (in increments of 0.1 %) and 1–25 % (w/v) NaCl (in increments of 1 %) at 33 °C and pH 7.5 was estimated in liquid MM63 medium (Larsen *et al.*, 1987), which is a minimal medium with sucrose as the carbon source. Growth under anaerobic conditions was determined after incubation in a CO₂ incubator on anaerobically prepared medium. Catalase activity was detected by adding 3 % H₂O₂ to culture plates. The oxidase reaction was performed on filter paper moistened with 1 % (w/v) aqueous solution of tetramethyl-*p*-phenylenediamine. Urease, arginine dihydrolase, phosphatase and lecithinase activities and hydrolysis of casein, aesculin, hypoxanthine, tyrosine, xanthine, starch, Tween 80 and gelatin were determined as described by Cowan & Steel (1965) and Lányi (1987). The methyl red and Voges–Proskauer tests and tests for indole and H₂S production, nitrate reduction, phenylalanine deaminase and lysine and ornithine decarboxylases were performed as recommended by Smibert & Krieg (1994) and Dong & Cai (2001). Antibiotic susceptibility was determined on plates containing the following (µg ml^{−1}): rifampicin (5), streptomycin (100), ampicillin (30), gentamicin (40), chloramphenicol (20), kanamycin (100), erythromycin (50), tetracycline (20), spectinomycin (50) and nalidixic acid (30). Tests for utilization of various substrates as sole carbon and energy sources were performed according to the methods described by Shirling & Gottlieb (1966) and acid production from carbohydrates was determined as described by Leifson (1963), using substrate concentrations of 0.5 % (w/v). The biochemical tests were also performed with the reference strains *J. halotolerans* JCM 11198^T and *J. psychrophilus* JCM 11199^T (Table 1). The biochemical properties of the

reference strains determined in this study were the same as those reported previously. The detailed physiological and biochemical characteristics of strains C1-52^T and YD-9 are given in the species description and the phenotypic differences between the isolates and the type strains of species of the genus *Jeotgalicoccus* are given in Table 1.

For analysis of the cell-wall peptidoglycan type and menaquinones, biomass of strain C1-52^T was harvested after incubation on maintenance medium supplemented with 2 % (w/v) NaCl (pH 7.5) at 33 °C for 18 h. Cell walls were prepared using the methods described by Schleifer & Kandler (1972) and the determination of the major amino acid was carried out by using Amino-acid Analysers (A300, membraPure). Menaquinones were analysed as described by Collins (1985) using reversed-phase HPLC (HP 1050; Hewlett Packard), using *Arthrobacter nicotinovorans* CGMCC 1.1933^T and *J. halotolerans* JCM 11198^T as the reference strains. Fatty acids of the isolates and the reference strains were analysed according to the Microbial Identification System (MIDI) using cells grown in maintenance medium supplemented with 2 % (w/v) NaCl (pH 7.5) in Erlenmeyer flasks on a rotary shaker (150 r.p.m.) at 33 °C (25 °C for *J. psychrophilus* JCM 11199^T) for 1 day. The major amino acids of the cell-wall hydrolysates were glycine, lysine and alanine, which corresponds to the A3α peptidoglycan type, based on L-Lys–Gly_{3–4}–L-Ala(Gly), described for the genus *Jeotgalicoccus*. The quinones of strain C1-52^T were MK-7 (83.2 %) and MK-6 (16.8 %). The major cellular fatty acids in strains C1-52^T and YD-9 were, respectively, anteiso-C_{15:0} (52.3 and 54.2 %), iso-C_{15:0} (26.1 and 22.1 %) and iso-C_{17:1ω10c} (7.3 and 5.9 %). The major cellular fatty acid compositions obtained for the two reference strains were similar to the original descriptions. Detailed information on the cellular fatty acid compositions of strains C1-52^T and YD-9 is provided in Supplementary Table S1.

Chromosomal DNA was extracted and purified according to standard methods (Marmur, 1961). The determination of DNA G+C content was carried out by the thermal denaturation method using a BIO-20 UV spectrophotometer according to Marmur & Doty (1962), with *Escherichia coli* K-12 as the standard. The DNA G+C content of strains C1-52^T and YD-9 was 41.2 and 41.0 mol%, respectively. These values are within the range for the genus *Jeotgalicoccus* (Yoon *et al.*, 2003).

The 16S rRNA gene sequences of strains C1-52^T and YD-9 were amplified as described by Duckworth *et al.* (1996) and the amplification products were sequenced with an automated DNA sequencer (373A; Applied Biosystems). The almost complete 16S rRNA gene sequences of strains C1-52^T (1457 bp) and YD-9 (1457 bp) were compared with sequences in public databases using BLAST via the National Center for Biotechnology Information. Pairwise sequence similarities were calculated using BioEdit (Hall, 1999). A multiple sequence alignment was created using CLUSTAL X (Thompson *et al.*, 1997). The continuous stretches of nucleotides in the alignment, without any

Table 1. Differential characteristics of strains C1-52^T and YD-9 and members of the genus *Jeotgalicoccus*

Strains: 1, *Jeotgalicoccus halophilus* sp. nov. C1-52^T and YD-9 (data from this study); 2, *J. halotolerans* JCM 11198^T; 3, *J. psychrophilus* JCM 11199^T (data in columns 2 and 3 from Yoon *et al.*, 2003 unless otherwise stated); 4, *J. pinnipedialis* CIP 107946^T (Hoyles *et al.*, 2004); 5, *J. huakuui* JCM 15687^T (Guo *et al.*, 2010); 6, *J. marinus* DSM 19772^T (Chen *et al.*, 2009). +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4	5	6
Pigment	Light pink	Light pink*	Light pink*	Buff or fawn	White	Creamy white
NaCl for growth (w/v, %)						
Range	0.1–16	0–20	>0–14	>0, <14	0–23	0.5–25
Optimum	2–3	2–5	2–5	2–5	3–8	5–10
Temperature for growth (°C)						
Range	4–40	4–42	4–34	>4–42	4–43	10–45
Optimum	30–35	30–35	20–25	28–35	28–37	25–30
pH for growth						
Range	5.5–10	>5.5	>5.5	>5	>4.5	6–10
Optimum	7.5	7–8	7–8	7–8	6.5–10	7–8
Urease	+	–*	–*	–	–	–
Hydrolysis of:						
Casein	+	–*	–*	+	–	–
Starch	+	–*	–*	–	–	–
Tyrosine	+	+	+	–	+	–
Xanthine	–	–*	–*	+	–	–
Acid production from:						
Arabinose	–	+	–*	–	+	+
Fructose	+	–	–*	–	+	+
D-Glucose	+	–	–*	w	–	+
D-Mannitol	+†/–	+	–*	–	–	+
Sucrose	+	–	w	w	–	–
DNA G + C content (mol%)	41.2†/41.0	42	42	38.6	36.8	40.3

*Data from this study and are the same as those reported previously.

†Data for strain C1-52^T.

ambiguous bases or gaps, were used to reconstruct phylogenetic trees using MEGA version 3.1 (Kumar *et al.* 2004). Distances were calculated according to Kimura's two-parameter model (Kimura, 1980) and clustering was performed using the neighbour-joining, maximum-parsimony and minimum-evolution methods. The stability of the relationships was assessed using bootstrap analysis with 1000 replications. Because of the highly similar tree topologies obtained with the different clustering methods, only the neighbour-joining phylogenetic tree is shown (Fig. 1). 16S rRNA gene sequence comparisons revealed that strains C1-52^T and YD-9 were most similar to *J. psychrophilus* YKJ-115^T (98.0 and 97.1 % 16S rRNA gene sequence similarity, respectively), *J. halotolerans* YKJ-101^T (97.1 and 96.8 %), *J. huakuui* NY-2^T (94.8 and 95.2 %), *J. marinus* JSM 076033^T (94.6 and 95.7 %), *J. pinnipedialis* CCUG 42722^T (92.2 and 91.7 %), *Salinicoccus siamensis* PN1-2^T (91.8 and 91.7 %), *Salinicoccus roseus* DSM 5351^T (91.7 and 91.5 %) and *Salinicoccus alkaliphilus* T8^T (91.6 and 91.4 %). 16S rRNA gene sequence similarity between the isolates was 99.1 %. The phylogenetic analysis indicated that strains C1-52^T and YD-9 and the type strains of the five recognized species of the genus *Jeotgalicoccus* were most closely related and formed a distinct clade in the

phylogenetic tree with significant bootstrap support (99 %), within which the isolates formed a distinct subcluster (Fig. 1).

The phenotypic and chemotaxonomic characteristics of the isolates were also consistent with the genus *Jeotgalicoccus*. For example, the major fatty acids iso-C_{15:0} and anteiso-C_{15:0}, the predominant menaquinone MK-7 and the low DNA G + C content (41.0–41.2 mol%) were similar to those of members of the genus *Jeotgalicoccus*. By contrast, strains C1-52^T and YD-9 could be distinguished from members of the genus *Salinicoccus*, which possess MK-6 as the major menaquinone and have a higher DNA G + C content (46–51 mol%; Ventosa *et al.*, 1990, 1993).

It has been suggested that bacterial strains with <97 % 16S rRNA gene sequence similarity should be classified in different genomic species (Stackebrandt & Goebel, 1994). Therefore, the phylogenetic data in this study showed that strains C1-52^T and YD-9 were not related to *J. pinnipedialis* CCUG 42722^T, *J. huakuui* NY-2^T or *J. marinus* JSM 076033^T at the species level. DNA–DNA hybridization was performed between the isolates and their closest phylogenetic neighbours, *J. halotolerans* JCM 11198^T and *J. psychrophilus* JCM 11199^T. DNA was sheared by

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strains C1-52^T and YD-9 and type strains of species of the genus *Jeotgalicoccus* and species of other closely related genera. Bootstrap values (>50%) based on 1000 replications are shown at branch nodes. Bar, 0.02 substitutions per site.

sonication (Braun Melsungen) at 50 W for three periods of 10 s and renaturation was performed in $2 \times \text{SSC}$ at 69 °C. Levels of DNA–DNA relatedness were estimated spectrophotometrically (De Ley *et al.*, 1970; Huß *et al.*, 1983). Strains C1-52^T and YD-9 shared, respectively, 20 and 11 % DNA–DNA relatedness with *J. halotolerans* JCM 11198^T and 8 and 13 % with *J. psychrophilus* JCM 11199^T. DNA–DNA relatedness between strains C1-52^T and YD-9 was 91 %. Because <70 % DNA–DNA relatedness is a key marker for the identification of a novel species (Wayne *et al.*, 1987), it is clear that the isolates belong to the same species and represent a previously unknown species of the genus *Jeotgalicoccus*.

The phylogenetic and chemotaxonomic analyses suggested that strains C1-52^T and YD-9 belong to the genus *Jeotgalicoccus*. Moreover, phenotypic features, DNA–DNA relatedness, DNA base composition and phylogenetic analysis clearly revealed that the isolates belong to a species that is different from *J. halotolerans*, *J. psychrophilus*, *J. marinus*, *J. huakuii* and *J. pinnipedialis*. In conclusion, it is proposed to place strains C1-52^T and YD-9 in a novel species of the genus *Jeotgalicoccus*, for which the name *Jeotgalicoccus halophilus* sp. nov. is proposed.

Description of *Jeotgalicoccus halophilus* sp. nov.

Jeotgalicoccus halophilus (ha.lo.phi'lus. Gr. n. *hals halos* salt; Gr. adj. *philos* loving; N.L. masc. adj. *halophilus* salt-loving).

The species description is based on two isolates. Cells are cocci (0.5–0.8 µm), Gram-positive and non-motile. Colonies are circular, smooth, entire, slightly raised, pale yellow and 1–2 mm in diameter after 3 days. A light pink, diffusible pigment is produced after a few additional days

of incubation. Grows with 0.1–16 % (w/v) NaCl (optimum 2–3 % NaCl), at 4–40 °C (optimum 30–35 °C) and at pH 5.5–10 (optimum pH 7.5). Catalase, oxidase, urease and phenylalanine deaminase are produced, but phosphatase, lecithinase and arginine dihydrolase are not. Produces acid from D-fructose, D-glucose, D-mannitol (type strain) and sucrose, but not from maltose, D-xylose, trehalose, lactose, arabinose or D-galactose. Hydrolyses starch, casein and tyrosine, but not aesculin, gelatin, xanthine, hypoxanthine or Tween 80. As sole sources of carbon and energy, utilizes D-mannitol (type strain), sucrose, D-fructose, D-glucose and maltose, but not cellobiose, D-galactose, glycogen, lactose, D-mannose, L-arabinose, trehalose or D-xylose. Voges–Proskauer and methyl red tests are negative. Nitrate is not reduced to nitrite. H₂S and indole are not produced. Susceptible to (µg ml^{−1}) rifampicin (5), ampicillin (30) and gentamicin (40), but not to streptomycin (100), chloramphenicol (20), kanamycin (100), erythromycin (50), tetracycline (20), spectinomycin (50) or nalidixic acid (30). The menaquinones and peptidoglycan type of the type strain are MK-7 and MK-6 and L-Lys–Gly_{3–4}–L-Ala(Gly). The major cellular fatty acids (>5 % of the total) are anteiso-C_{15:0}, iso-C_{15:0} and iso-C_{17:1}ω10c. The DNA G + C content is 41.0–41.2 mol%.

The type strain is C1-52^T (=CGMCC 1.8911^T =NBRC 105788^T), isolated from Daban salt lake in Xinjiang, China. A strain has also been isolated from Aiding salt lake.

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