**Jeotgalicoccus halophilus** sp. nov., isolated from salt lakes

Wen-Yan Liu,1,2 Lin-Lin Jiang,3 Chun-Jing Guo3 and Su Sheng Yang1

1Department of Microbiology and Immunology, College of Biological Sciences, China Agricultural University, Key Laboratory for Agro-Microbial Resource and Application, Ministry of Agriculture, Beijing 100193, PR China
2National Engineering Lab. of Biohydrometallurgy, General Research Institute for Nonferrous Metals, Beijing 100088, PR China
3Northeast Agricultural University, Haerbin 150030, PR China

Two slightly halophilic bacterial strains, C1-52T 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–Gly3–4–L-Ala(Gly). The menaquinones were MK-7 (83.2 %) and MK-6 (16.8 %). The major fatty acids (>10 %) were anteiso-C15 : 0 and iso-C15 : 0. The DNA G+C content of strains C1-52T and YD-9 was 41.2 and 41.0 mol%, respectively. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strains C1-52T and YD-9 were closely related to *Jeotgalicoccus psychrophilus* YKJ-115T (98.0 and 97.1 % 16S rRNA gene sequence similarity, respectively), followed by *Jeotgalicoccus halotolerans* YKJ-101T (97.1 and 96.8 %). Strains C1-52T and YD-9 shared, respectively, 20 and 11 % DNA–DNA relatedness with *J. psychrophilus* JCM 11198T and 8 and 13 % with *J. halotolerans* JCM 11199T. DNA–DNA relatedness between the isolates was 91 %. On the basis of phenotypic and phylogenetic distinctiveness, strains C1-52T 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 C1-52T (=CGMCC 1.8911T =NBRC 105788T).

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-C15 : 0 and iso-C15 : 0, the DNA G+C content is 36.8–42 mol% and the cell-wall peptidoglycan is L-Lys–Gly3–4–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, C1-52T 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 %; 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 microscopy (JEM-1010; JEOL, Tokyo, Japan) at 80 kV. The peptidoglycan type was L-Lys–Gly3–4–L-Ala(Gly) (Yoon et al., 2003).
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<sup>T</sup> and *J. psychrophilus* JCM 11199<sup>T</sup> 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 CO<sub>2</sub> incubator on anaerobically prepared medium. Catalase activity was detected by adding 3% H<sub>2</sub>O<sub>2</sub> 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 H<sub>2</sub>S 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<sup>−1</sup>): 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<sup>T</sup> and *J. psychrophilus* JCM 11199<sup>T</sup> (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<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> and *J. halotolerans* JCM 11198<sup>T</sup> 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<sup>T</sup>) for 1 day. The major amino acids of the cell-wall hydrolysates were glycine, lysine and alanine, which corresponds to the A3<sub>x</sub> peptidoglycan type, based on L-Lys–Gly<sub>3</sub>–4–L-Ala(Gly), described for the genus *Jeotgalicoccus*. The quinones of strain C1-52<sup>T</sup> were MK-7 (83.2%) and MK-6 (16.8%). The major cellular fatty acids in strains C1-52<sup>T</sup> and YD-9 were, respectively, anteiso-C<sub>15</sub>:0 (52.3 and 54.2%), iso-C<sub>15</sub>:0 (26.1 and 22.1%) and iso-C<sub>17</sub>:1<i>ω</i>10c (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<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> 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<sup>T</sup> (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
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).

The phenotypic and chemotaxonomic characteristics of the isolates were also consistent with the genus *Jeotgalicoccus*. For example, the major fatty acids iso-C\textsubscript{15}:0 and anteiso-C\textsubscript{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\textsuperscript{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\textsuperscript{T} and YD-9 were not related to *J. pinnipedialis*, *J. psychrophilus*, *J. halotolerans*, or the lowest DNA G+C content (<46 mol\%); Ventosa et al., 1990, 1993).

Table 1. Differential characteristics of strains C1-52\textsuperscript{T} and YD-9 and members of the genus *Jeotgalicoccus*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain C1-52\textsuperscript{T}</th>
<th>YD-9</th>
<th>Strain J. halotolerans\textsuperscript{3}</th>
<th>Strain J. halotolerans\textsuperscript{4}</th>
<th>Strain J. psychrophilus\textsuperscript{5}</th>
<th>Strain J. psychrophilus\textsuperscript{6}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigment</td>
<td>Light pink</td>
<td>Light pink*</td>
<td>Light pink*</td>
<td>Buff or fawn</td>
<td>White</td>
<td>Creamy white</td>
</tr>
<tr>
<td>NaCl for growth (w/v, %)</td>
<td>0.1–16</td>
<td>0–20</td>
<td>&gt;0–14</td>
<td>&gt;0, &lt;14</td>
<td>0–23</td>
<td>0.5–25</td>
</tr>
<tr>
<td>Optimum</td>
<td>2–3</td>
<td>2–5</td>
<td>2–5</td>
<td>2–5</td>
<td>3–8</td>
<td>5–10</td>
</tr>
<tr>
<td>Temperature for growth (°C)</td>
<td>4–40</td>
<td>4–42</td>
<td>4–34</td>
<td>&gt;4–42</td>
<td>4–43</td>
<td>10–45</td>
</tr>
<tr>
<td>pH for growth</td>
<td>5.5–10</td>
<td>&gt;6.5</td>
<td>&gt;5.5</td>
<td>&gt;5</td>
<td>&gt;4.5</td>
<td>6–10</td>
</tr>
<tr>
<td>Optimum</td>
<td>7.5</td>
<td>7–8</td>
<td>7–8</td>
<td>7–8</td>
<td>6.5–10</td>
<td>7–8</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>–*</td>
<td>–*</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
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<tr>
<td>Cascin</td>
<td>+</td>
<td>–*</td>
<td>–*</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>–*</td>
<td>–*</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Tyrosine</td>
<td>+</td>
<td>+*</td>
<td>+*</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Xanthine</td>
<td>–</td>
<td>–*</td>
<td>–*</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Arabinose</td>
<td>–</td>
<td>+*</td>
<td>–*</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
<td>–</td>
<td>–*</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>d-Glucose</td>
<td>+</td>
<td>–</td>
<td>–*</td>
<td>w</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>d-Mannitol</td>
<td>+/–</td>
<td>+*</td>
<td>–*</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>–</td>
<td>w</td>
<td>w</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>41.2*!/41.0</td>
<td>42</td>
<td>42</td>
<td>38.6</td>
<td>36.8</td>
<td>40.3</td>
</tr>
</tbody>
</table>

*Data from this study and are the same as those reported previously.
†Data for strain C1-52\textsuperscript{T}.
sonication (Braun Melsungen) at 50 W for three periods of 10 s and renaturation was performed in 2×SSC at 69 °C. Levels of DNA–DNA relatedness were estimated spectro-

photometrically (De Ley et al., 1970; Huß et al., 1983).

Strains C1-52T and YD-9 shared, respectively, 20 and 11 % DNA–DNA relatedness with J. halotolerans JCM 11198T and 8 and 13 % with J. psychrophilus JCM 11199T. DNA–DNA relatedness between strains C1-52T 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 C1-52T 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 C1-52T 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 coccii (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, lecinthinase 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–L-Ala (Gly). The major cellular fatty acids (>5 % of the total) are anteiso-C15 : 0, iso-C15 : 0 and iso-C17 : 1ω10c. The DNA G+C content is 41.0–41.2 mol%.

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

**Acknowledgements**

This work was supported financially by the Chinese International Science and Technology Cooperation (grant number 2006DFA31060). We are grateful to Y. Zhou (China General Microbiological Culture
Collection Center, Institute of Microbiology, Chinese Academy of Sciences), W.-J. Li (The Key Laboratory for Microbial Resources of the Ministry of Education, PR China, and Laboratory for Conservation and Utilization of Bio-Resources, Yunnan Institute of Microbiology, Yunnan University) and J.-D. Jiang (Key Laboratory for Microbiological Engineering of the Agricultural Environment, Ministry of Agriculture, Nanjing Agricultural University) for providing biological samples.

References


