Jeotgalicoccus marinus sp. nov., a marine bacterium isolated from a sea urchin

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A novel non-sporulating, non-motile, catalase- and oxidase-positive, facultatively anaerobic, moderately halophilic, Gram-positive coccus, designated JSM 076033T, was isolated from a sea urchin (Hemicentrotus pulcherrimus) collected from the South China Sea. Strain JSM 076033T was able to grow in the presence of 0.5–25.0 % (w/v) total salts and at pH 6.0–10.0 and 10–45 °C; optimum growth was observed with 5.0–10.0 % (w/v) total salts and at pH 7.0–8.0 and 25–30 °C. The major amino acid constituents of the cell wall were glycine, lysine and alanine. The major cellular fatty acids were anteiso-C15 : 0, iso-C15 : 0 and anteiso-C17 : 0. The respiratory quinones were MK-7 (60.7 %) and MK-6 (39.3 %) and the polar lipids were diphosphatidylglycerol, phosphatidylglycerol and an unidentified phospholipid. The DNA G+C content was 40.3 mol%. A phylogenetic analysis based on 16S rRNA gene sequence comparisons revealed that strain JSM 076033T should be assigned to the genus Jeotgalicoccus. The sequence similarities between the novel isolate and the type strains of recognized Jeotgalicoccus species were in the range 95.2–97.2 %. The results of the phylogenetic analysis, combined with DNA–DNA relatedness data, phenotypic characteristics and chemotaxonomic information, support the view that strain JSM 076033T represents a novel species of the genus Jeotgalicoccus, for which the name Jeotgalicoccus marinus sp. nov. is proposed. The type strain is JSM 076033T (=CCTCC AA 207028T =DSM 19772T =KCTC 13189T).

The genus Jeotgalicoccus was originally proposed by Yoon et al. (2003) with two species, Jeotgalicoccus halotolerans and Jeotgalicoccus psychrophilus; a third species, Jeotgalicoccus pinnipedialis, was described subsequently (Hoyles et al., 2004). The genus was defined as comprising Gram-positive, catalase- and oxidase-positive, facultatively anaerobic, non-motile, non-spore-forming cocci with MK-7 as the predominant isoprenoid quinone and a cell-wall peptidoglycan of the A3γ type [based on L-Lys–Gly3–4–L-Ala(Gly)] (Yoon et al., 2003). During an investigation of the diversity of the microbial population of invertebrates inhabiting the South China Sea, we isolated a novel moderately halophilic bacterium, designated JSM 076033T, from a sea urchin (Hemicentrotus pulcherrimus) collected from Leizhou Bay, China. On the basis of the results of a polyphasic taxonomic analysis, this strain represents a novel species of the genus Jeotgalicoccus.

For strain isolation, serial dilutions (1:10) of homogenates of the sea urchin were plated on Difco marine agar 2216 (pH 7.5), supplemented with 0–20 % (w/v) NaCl, at 28 °C for 7–28 days. A creamy-white-pigmented colony, designated strain JSM 076033T, was picked from a plate of MA supplemented with 10 % (w/v) NaCl. It was maintained both on slants of MA supplemented with 5 % (w/v) NaCl (referred to as MA5) at 4 °C and in Difco marine...
broth 2216 (MB) supplemented with 20 % (v/v) glycerol at -80 °C. The reference strain *J. halotolerans* DSM 17274^T^ was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). Unless otherwise indicated, morphological and physiological studies were performed using cells grown on MA5 (pH 7.5) at 28°C. Cell morphology was examined by using light microscopy (model BH2; Olympus). Gram staining was carried out by using the standard Gram reaction (Doetsch, 1981) combined with the KOH lysis test (Gregersen, 1978). Growth was tested at various temperatures in the range 5–55°C (using increments of 5°C) on MA5 and at different pH values in the range 5.0–11.0 (using increments of 0.5 pH units) in MB supplemented with 5 % (w/v) NaCl. Tolerance of, and requirements for, salts were investigated on MA, prepared according to the formula of Atlas (1993), at different salt contents [0, 0.1 and 0.5 % (w/v) and 1–30 % (w/v), using increments of 1 %]. Growth under anaerobic conditions was determined on MA5 by using GasPak anaerobic systems (BBL) according to the manufacturer’s instructions. Urease activity and hydrolysis of casein, DNA, hypoxanthine, starch, Tweens 20, 40, 60 and 80, L-tyrosine and xanthine were determined as described by Cowan & Steel (1965). Motility, antibiotic susceptibility, catalase activity and oxidase activity were determined as described previously (Chen et al., 2007). Other enzymic activities were also assayed by using API ZYM strips (bioMérieux) according to the manufacturer’s instructions. Acid production from carbohydrates was determined by employing API 50CH systems (bioMérieux) according to the manufacturer’s instructions. All suspension media were supplemented with 5 % (w/v) NaCl and were incubated at 28 °C. Nutritional assays were performed as recommended by Ventosa et al. (1982) in a modified Koser medium (Koser, 1923) [containing (l^-1^-): 50 g NaCl, 2 g KCl, 0.2 g MgSO_4_, 7H_2O, 1 g KNO_3, 1 g (NH_4)_2HPO_4 and 0.5 g KH_2PO_4]. When amino acids were used as substrates, the basal medium contained neither KNO_3 nor (NH_4)_2HPO_4. Organic compounds tested as sole sources of carbon and energy or sole sources of carbon, nitrogen and energy were used at 0.2 % (w/v). The results of the phenotypic tests are given in the species description and in Table 1.

DNA was isolated according to the method of Hopwood et al. (1985) and the G+C content was determined by using the HPLC method (Mesbah et al., 1989). Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of PCR products were performed as described by Cui et al. (2001). Pairwise sequence similarity was calculated by using a global alignment algorithm, which was implemented at the EzTaxon server (http://www.eztaxon.org/; Chun et al., 2007). Phylogenetic analysis was performed by using the software package MEGA 3.1 (Kumar et al., 2004) after multiple alignment of the sequence data with CLUSTAL_X (Thompson et al., 1997). Distances were calculated by using distance options.

Table 1. Characteristics that serve to differentiate strain JSM 076033^T^ from type strains of recognized *Jeotgalicoccus* species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>JSM 076033^T^</th>
<th><em>J. halotolerans</em> YKJ-101^T^</th>
<th><em>J. psychrophilus</em> YKJ-115^T^</th>
<th><em>J. pinnipedialis</em> CCUG 42722^T^</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony pigmentation</td>
<td>Creamy white</td>
<td>Light yellow</td>
<td>Light yellow</td>
<td>Buff or fawn</td>
</tr>
<tr>
<td>Growth at/with:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45 °C</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>25 % (w/v) salts</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellobirose</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Menaquinones (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MK-6</td>
<td>39.3</td>
<td>13.5*</td>
<td>ND</td>
<td>5</td>
</tr>
<tr>
<td>MK-7</td>
<td>60.7</td>
<td>82.1*</td>
<td>80</td>
<td>89</td>
</tr>
<tr>
<td>MK-8</td>
<td>0</td>
<td>4.5*</td>
<td>ND</td>
<td>3</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>40.3</td>
<td>42</td>
<td>42</td>
<td>38.6</td>
</tr>
</tbody>
</table>

*Data from the present study.
according to Kimura’s two-parameter model (Kimura, 1980) and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). Maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) trees (not shown) were generated by using the treeing algorithms contained in the PHYLIP package (Felsenstein, 2002). Bootstrap analysis (based on 1000 resamplings) was used to evaluate the tree topology of the neighbour-joining data (Felsenstein, 1985). DNA–DNA hybridization was carried out by using photobiotin-labelled probes in microplate wells, as described by Ezaki et al. (1989). A microplate spectrofluorometer (Genius XPS; Molecular Devices) was employed for the fluorescence measurements.

The DNA G+C content of strain JSM 076033T was 40.3 mol%. The almost-complete 16S rRNA gene sequence (1459 bp) of the organism was determined. Sequence comparisons with 16S rRNA gene sequences from the EzTaxon database (Chun et al., 2007) revealed that the novel strain had highest similarity to J. halotolerans YKJ-101T (97.2 %), followed by J. psychrophilus YKJ-115T (96.3 %), J. pinnipedialis CCUG 42722T (95.2 %), Salinicoccus kummin- gensis YIM Y15T (94.6 %; Chen et al., 2007), Salinicoccus alkaliphilus T8T (94.5 %; Zhang et al., 2002) and Nosocomicoccus ampullae TRS-1T (94.1 %; Alves et al., 2008). Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain JSM 076033T was related most closely to the type strains of the three recognized species of the genus Jeotgalicoccus, and that the organism formed a distinct clade with the type strains of J. halotolerans and J. psychrophilus within the phylogenetic tree (with 100 % bootstrap support for all three methods) (Fig. 1). DNA–DNA hybridization was employed to clarify the relatedness between the novel isolate and the type strain of J. halotolerans: the value obtained was 10.8 %, which is considerably below the threshold value (70 %) recommended by Wayne et al. (1987) for assigning strains to the same species. It is therefore evident, on the basis of the phylogenetic and DNA–DNA hybridization data, that strain JSM 076033T represents a novel species of the genus Jeotgalicoccus.

Isolation of the cell-wall fraction and preparation of cell-wall hydrolysates were carried out by using the method of Schleifer (1985). The amino acid composition of the cell-wall hydrolysates was detected by using TLC, as described by Stanek & Roberts (1974). Polar lipids were extracted according to the method of Minnikin et al. (1979) and were identified by two-dimensional TLC and spraying with the appropriate detection reagents (Collins & Jones, 1980). Isoprenoid quinones were analysed by means of HPLC, as described by Groth et al. (1996). Fatty acid compositions were determined as described by Sasser (1990) by using the Microbial Identification system (MIDI; Microbial ID) with cells grown in MB supplemented with 5 % (w/v) NaCl (pH 7.5) in flasks on a rotary shaker (with shaking at 200 r.p.m.) at 28 °C for 3 days. In the chemotaxonomic studies, reference strain J. halotolerans DSM 17274T was employed for comparative purposes.

The chemotaxonomic data obtained for JSM 076033T were consistent with the assignment of the strain to the genus Jeotgalicoccus (Yoon et al., 2003). The major amino acid constituents of the cell-wall hydrolysates were glycine, lysine and alanine, which corresponds with the A3 peptidoglycan type, based on L-Lys–Gly3–4–L-Ala(Gly), described for that genus. The fatty acid profile of strain JSM 076033T was similar to those of type strains of the recognized Jeotgalicoccus species (see Supplementary Table S1, available in IJSEM Online). The major fatty acids of this strain were anteiso-C15 : 0 (37.3 %), iso-C15 : 0 (25.9 %) and anteiso-C17 : 0 (11.1 %). MK-7 (60.7 %) and MK-6 (39.3 %) were the respiratory quinones. The polar lipids of the strain consisted of diphosphatidylglycerol, phosphatidylglycerol and an unidentified phospholipid.

The results of the phylogenetic and chemotaxonomic analyses supported the view that strain JSM 076033T should be assigned to the genus Jeotgalicoccus. However, the creamy-white pigmentation and the ability to tolerate up to 25 % (w/v) total salts, together with the significant amount of MK-6 (39.3 %) and the ability to produce acids from cellobiose, D-fructose, D-galactose, D-glucose and D-
mannose, served to differentiate strain JSM 076033<sup>T</sup> markedly from the three recognized *Jeotgalicoccus* species (Table 1). In conclusion, the phylogenetic analysis based on 16S rRNA gene sequences, the DNA–DNA relatedness results and the phenotypic and chemotaxonomic data presented here indicate that strain JSM 076033<sup>T</sup> represents a novel species of the genus *Jeotgalicoccus*, for which the name *Jeotgalicoccus marinus* sp. nov. is proposed.

**Description of *Jeotgalicoccus marinus* sp. nov.**

*Jeotgalicoccus marinus* (ma'ri.nus. L. masc. adj. marinus of the sea).

Cells are Gram-positive, non-motile, non-sporulating, catalase- and oxidase-positive, coccus-shaped, 0.7–1.1 μm in diameter and occur singly or in pairs, tetrads or clumps. Facultatively anaerobic. Flagella are not observed. Colonies are creamy-white, circular, somewhat convex and non-translucent, have glistening surfaces and entire margins and are 2–3 mm in diameter after incubation at 28 °C for 3–5 days on MA5. No diffusible pigments are produced. Growth occurs at 10–45 °C (optimally at 25–30 °C) and pH 6.0–10.0 (optimally at pH 7.0–8.0). Moderately halophilic, with growth at salinities of 0.5–25.0 % (w/v) total salts (optimally at 5.0–25.0 % (w/v) total salts (optimally at 5.0–10.0 %). Negative for hydrolysis of aesculin, casein, DNA, gelatin, hypoxanthine, starch, Tween 20, 40, 60 and 80, L-tyrosine and xanthine. H₂S and indole are not produced. The Voges–Proskauer test is positive, but the methyl red test is negative. Nitrate is not reduced to nitrite. The following compounds are utilized as sole sources of carbon and energy or sole sources of carbon, nitrogen and energy: l-arabinose, D-fructose, D-glucose, maltose, D-sorbitol, butyrate, citrate, gluconate, malate, succinate, L-alanine, L-arginine, L-asparagine, L-glutamic acid, L-glycine, L-serine and L-valine. The following substances are not utilized as sole sources of carbon and energy or sole sources of carbon, nitrogen and energy: cellobiose, D-galactose, glycogen, D-lactose, D-mannose, D-mannitol, melezitose, melibiose, raffinose, L-rhamnose, D-ribose, salicin, sucrose, trehalose, D-xylene, adonitol, glycerol, *myo*-inositol, acetate, formate, fumarate, malonate, propionate, L-histidine, hydroxy-2-proline, L-isoleucine, L-leucine, L-methionine and L-proline. Produces mycolic acid (20 μg), polymyxin B (30 μg), streptomycin (10 μg) or tetracycline (30 μg). The major amino acid constituents of the cell wall are glycine, lysine and alanine. Possesses menaquinones MK-7 (60.7 %) and MK-6 (39.3 %). The polar lipids consist of diphasphatidylglycerol, phosphatidyglycerol and an unidentified phospholipid. The major cellular fatty acids are anteiso-C<sub>15:0</sub>, iso-C<sub>15:0</sub> and anteiso-C<sub>17:0</sub>. The DNA G+C content of the type strain is 40.3 mol% (HPLC method).

The type strain, JSM 076033<sup>T</sup> (=CCTCC AA 207028<sup>T</sup> =DSM 19772<sup>T</sup> =KCTC 13189<sup>T</sup>), was isolated from a sea urchin (*Hemicentrotus pulcherrimus*) collected from Leizhou Bay, northern South China Sea, China.

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