Oceanitalea nanhaiensis gen. nov., sp. nov., an actinobacterium isolated from seawater

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A Gram-positive, motile, short-rod-shaped bacterium, designated strain JLT1488T, was isolated from the South China Sea and investigated in a taxonomic study using a polyphasic approach. The peptidoglycan type determined for strain JLT1488T was A4α with lysine as the diagnostic cell-wall diamino acid and an interpeptide bridge of L-Lys–L-Glu. The polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides, an unknown glycolipid and an unknown phospholipid. The only detected menaquinone was MK-8(H4), and the major fatty acids were summed feature 8 (C18:1ω7c/C18:1ω6c), C16:0 and summed feature 3 (C16:1ω7c/C16:1ω6c); significant amounts of C12:0 3-OH, C10:0 and C19:0 cyclo ω8c were also present. The G+C content of the genomic DNA was 62.3 mol%. Comparison of the 16S rRNA gene sequence of strain JLT1488T with those of related type strains demonstrated that it represented a novel lineage within the family Bogoriellaceae, suborder Micrococccineae, being closely related to species of the genera Georgenia, Bogoriella and Cellulomonas (94.6–96.8 % sequence similarity). These results demonstrate that strain JLT1488T is a member of a new genus, for which the name Oceanitalea nanhaiensis gen. nov., sp. nov. is proposed. The type strain of the type species is JLT1488T (=JCM 17755T=CGMCC 1.10826T).

Strain JLT1488T was isolated from a seawater sample from the South China Sea, following incubation on a rich organic (RO) medium (Yurkov et al., 1999) at 28 °C for 10 d. The isolate was routinely cultured on RO medium and maintained as glycerol suspensions (15 %, v/v) at −80 °C. Strain JLT1488T was characterized by means of physiological, biochemical and chemotaxonomic traits as well as analyses of partial 16S rRNA gene sequences, and was identified as a member of the class Actinobacteria (Altenburger et al., 1996; Wieser et al., 1999). On the basis of our results, it is suggested that strain JLT1488T represents a novel species in a new genus.

All physiological and biochemical tests were performed at 28 °C. Colony morphology was determined after 3 days' incubation at 28 °C on RO medium and TSA medium [3 % (w/v) trypticase soy broth (BBL); 1.5 % (w/v) Bacto agar (Difco)]. Colour determination was achieved using colour chips from the ISCC–NBS colour charts (standard sample No. 2106) (Kelly, 1964). Gram staining was carried out using the standard Gram reaction and examined using a light microscope (BX61; Olympus). Cell motility was assessed based on the presence of turbidity throughout a tube of semisolid medium (Leifson, 1960). Cellular morphology was examined by transmission electron microscopy (JEM-1230; JEOL) using cells from exponentially growing cultures (28 °C, 12 h). Oxidase activity was determined using a 1 % (w/v) solution of tetramethyl-p-phenylenediamine (Kovács, 1956). Catalase activity was determined by assessing the production of bubbles following the addition of a drop of 3 % (v/v) H2O2. Hydrolysis of casein, chitin, cellulose, dextrin, gelatin, starch and Tween 20, 40, 60 and 80 was determined as described by Cowan & Steel (1965). Growth parameters (temperature, pH and NaCl concentration) were investigated by determining the turbidity (optical density at 610 nm) of cultures in test tubes containing 5 ml RO medium after 1–4 days' incubation. The optimum temperature and range for growth were tested at 4–50 °C. The pH range and optimum pH for growth, NaCl tolerance and susceptibility to antibiotics were examined as described by Xu et al. (2005). The incubation temperature for the pH, NaCl, phenol and antibiotic tests was 28 °C.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of JLT1488T is HQ638978.
utilization was tested using GP2 microplates (Biolog). Fatty acid methyl esters were prepared, separated, and identified with the Sherlock Microbial Identification System (MIS), produced by MIDI, Inc. (Sasser, 1990; MIDI Sherlock version 6.0, MIDI database TSBA40).

The cells of strain JLT1488T were short-rod-shaped (1.1–1.3 μm long and 0.5–0.6 μm wide) and motile. The cells were Gram-stain-positive and chemoorganotrophic, with a respiratory type of metabolism. Endospores and poly-

Table 1. Differential characteristics of Oceaitalea nanhaiensis gen. nov. and related taxa

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell morphology</td>
<td>Short rods</td>
<td>Rod–coccus cycle</td>
<td>Irregular rods, coccolid</td>
<td>Short rods</td>
<td>Rod</td>
<td>Rod</td>
<td>Branched rods</td>
</tr>
<tr>
<td>Major fatty acid(s)</td>
<td>Summed feature 8 (C_{18:1ω7c}/C_{18:0ω6c}), C_{16:0} summed feature 3 (C_{16:1ω7c}/C_{16:1ω6c})</td>
<td>ai-C_{15:0}, ai-C_{15:1}, i-C_{14:0} ai-C_{15:0}, ai-C_{17:0} ai-C_{15:0}, i-C_{15:0}, C_{14:0} ai-C_{15:0}, C_{16:0}, i-C_{16:0}, ai-C_{17:0} ai-C_{15:0}, C_{16:0}</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Major menaquinone</td>
<td>MK-8(H_4)</td>
<td>MK-8(H_4)</td>
<td>MK-8(H_4)</td>
<td>MK-10 type</td>
<td>MK-9(H_4)</td>
<td>MK-9(H_4)</td>
<td>MK-9(H_4)</td>
</tr>
<tr>
<td>Polar lipid composition</td>
<td>DPG, PG, PI, PIM, GL, PL</td>
<td>DPG, PG, PI, 2PI, GL</td>
<td>DPG, PG, PI, 1PI, 2PI, GL</td>
<td>Unknown</td>
<td>DPG, PG, PI</td>
<td>Unknown</td>
<td></td>
</tr>
<tr>
<td>Interpeptide bridge</td>
<td>L-Lys ←→ L-Glu</td>
<td>L-Lys ←→ L-Glu</td>
<td>L-Lys ←→ L-Ala ←→ L-Glu</td>
<td>Unknown</td>
<td>L-Thr ←→ D-Asp or L-Thr ←→ D-Glu</td>
<td>L-Orn ←→ D-Orn ←→ D-Glu</td>
<td></td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>62.3</td>
<td>70–73</td>
<td>70</td>
<td>63–72</td>
<td>70–72</td>
<td>70.5–75.0</td>
<td>72–76</td>
</tr>
</tbody>
</table>

The results of the other physiological and biochemical analyses are summarized in the species description. The peptidoglycan of cells of strain JLT1488T was hydrolysed for amino acid and sugar analyses as described by Schleifer (1985). The cell wall chemical properties of strain JLT1488T are given in Table 1 and in the species description. The peptidoglycan of cells of strain JLT1488T was isolated after disruption of the cells by shaking with glass beads and subsequent trypsin digestion, according to the method of Schleifer (1985). The cell wall was hydrolysed for amino acid and sugar analyses as described by Schleifer & Kandler (1972). Amino acids in cell-wall hydrolysates were analysed by HPLC using precolumn derivatization with o-phthaldialdehyde (Tang et al., 2009a, b). Whole-cell sugars were detected by HPLC using precolumn derivatization with 1-phenyl-3-methyl-5-pyrazolone (Tang et al., 2009b).

Polar lipids were extracted from cultures grown in TSB [per litre: 15.0 g tryptone; 5.0 g soya peptone; 5.0 g NaCl; 15.0 g agar; pH 7.3 ± 0.2], examined by two-dimensional TLC and identified using published procedures (Minnikin et al., 1979; Collins & Jones, 1980). Menaquinones were isolated using the method of Collins et al. (1977) and were analysed by HPLC (Groth et al., 1997b). Analysis of the whole-cell fatty acid pattern was performed using previously described methods involving the MIDI system (Microbial ID) (Kroppenstedt, 1985; Meier et al., 1993). The G+C content of the DNA was determined by reversed-phase HPLC of nucleosides according to Mesbah et al. (1989).

The polar lipids were diphasphatidylglycerol, phosphatidylglycerol, phosphatidylglycerol, phosphatidylglycerol, mannolipid, unknown glycolipid and an unknown phospholipid. The only detected menaquinone was MK-8(H_4). The major fatty acids were summed feature 8 (C_{18:1ω7c}/C_{18:0ω6c}) (41.48 %), C_{16:0} (29.63 %) and summed feature 3 (C_{16:1ω7c}/C_{16:1ω6c}) (8.40 %); significant amounts of C_{12:0} 3-OH (6.31 %), C_{10:0} (2.0 %) and C_{19:0} cyclo o8c (1.78 %) were also present. The G+C content of genomic DNA of strain JLT1488T was 62.3 mol%.

Genomic DNA for PCR amplification was prepared from cells lysed by microwaves: a small amount of biomass was transferred from solid medium to a new Eppendorf tube. The cells were washed with 1 ml PBS (137 mM NaCl; 2.7 mM KCl; 4.3 mM Na_2HPO_4; 1.4 mM KH_2PO_4;
pH 8.0) and 1 ml washing buffer [50 mM Tris/HCl, pH 7.7; 25 mM EDTA; 0.1 % (w/v) SDS; 0.1 % (w/v) polyvinylpyrrolidone (PVP)], resuspended in 50 µl lysis buffer [50 mM Tris/HCl, pH 8.0; 25 mM EDTA; 3 % (w/v) SDS; 1.2 % (w/v) PVP], and heated at 700 W in a microwave oven for 45 s. 400 µl warm extraction buffer [10 mM

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence (1448 bp) comparisons indicating the phylogenetic position of strain JLT1488T within the suborder Micrococcineae. Brevibacterium linens DSM 20425T (Genbank accession no. X77451) was included as an outgroup. Bar, 1 substitution per 100 nt.
Displays the following characteristics in addition to those of the genus. Colonies are 1.1–1.3 mm in diameter, circular, slightly convex, opaque and yellow on RO agar after 3 days at 28°C. Cells are short–rod-shaped (1.1–1.3 μm long and 0.5–0.6 μm wide). Chemoorganotrophic, with a respiratory type metabolism. The temperature range for growth is 4–40°C (optimum, 35°C). NaCl is required for growth and tolerated at 1–10% (w/v). The pH range for growth is 4.0–10.0 (optimum, pH 8.0). Negative result in tests for hydrolysis of gelatin, production of melanin, H₂S and indole, resistance to KCN, and peptonization of milk. Nitrate is reduced to nitrite. Utilizes L-arabinose, D-ribose, D-mannitol, amygdalin, salicin, trehalose and glycogen as sole carbon and energy sources. Hydrolyses starch and Tween 20 and 80. Does not degrade urea. Positive result in tests for lipase, N-acetyl-β-glucosaminidase, lipase esterase and alkaline phosphatase enzymes, but negative result in tests for β-glucosidase, β-galactosidase, α-galactosidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphoric acid hydrolyse and β-glucuronidase. Resistant to (μg) gentamicin (10), aztreonam (30) and sulfafurazole (300), but sensitive to penicillin G (10), vancomycin (30), polymyxin B (30), erythromycin (15), aureomytin (30), streptomycin sulfate (10), ofloxacin (5), tetracycline (30), kanamycin (30) and chloramphenicol (30).

The type strain, JLT1488ᵀ (≡CGMCC 17755ᵀ=CGMCC 1.10826ᵀ), was isolated from the South China Sea. The DNA G+C content of the type strain is 62.3 mol%.

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References


