Oceanibaculum nanhaiense sp. nov., isolated from surface seawater

Yaping Du, Xiupian Liu, Qiliang Lai, Weiwei Li, Fengqin Sun and Zongze Shao*

Abstract

A taxonomic study was carried out on strain L54-1-50\textsuperscript{T}, which was isolated from surface seawater of the South China Sea. Cells of strain L54-1-50\textsuperscript{T} were Gram-stain-negative, rod-shaped, oxidase-positive and catalase-positive. Growth was observed at salinities from 0 to 9 % (optimum 2 %, w/v), at pH 6.0–10.0 (optimum 8.0–9.0) and at temperatures from 10 to 45 °C (optimum 25–37 °C), but not at 4 or 50 °C. The 16S rRNA gene sequence analysis indicated that strain L54-1-50\textsuperscript{T} was a member of the genus Oceanibaculum, related to Oceanibaculum indicum P24\textsuperscript{T} (98.8 %) and Oceanibaculum pacificum MC2UP-L3\textsuperscript{T} (97.7 %). The digital DNA–DNA hybridization values between strain L54-1-50\textsuperscript{T} and the two type strains O. indicum P24\textsuperscript{T} and O. pacificum MC2UP-L3\textsuperscript{T} were 35.4±2.5 and 23.7±2.5 %, respectively. The average nucleotide identity values between strain L54-1-50\textsuperscript{T} and two type strains were 79.7 and 88.3 %, respectively. The major cellular fatty acids were summed feature 8 (C\textsubscript{18:1}ω7c and/or C\textsubscript{18:1}ω6c), C\textsubscript{16:0} and C\textsubscript{18:1} 2-OH. The respiratory quinone was Q-10. The polar lipids comprised diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol, two unidentified phospholipids and three unidentified lipids. The G+C content of the chromosomal DNA was 65.1 mol%. The combined genotypic and phenotypic data showed that strain L54-1-50\textsuperscript{T} represents a novel species of the genus Oceanibaculum, for which the name Oceanibaculum nanhaiense sp. nov. is proposed, with the type strain L54-1-50\textsuperscript{T} (=KCTC 52312\textsuperscript{T}=MCCC 1A05150\textsuperscript{T}).

The genus Oceanibaculum belongs to the family Rhodospirillaceae [1] and currently comprises two recognized species, Oceanibaculum indicum [2] and Oceanibaculum pacificum [3], both of which were isolated from polycyclic aromatic hydrocarbon-degrading consortia. In this study, we described a novel strain, L54-1-50\textsuperscript{T}, which was isolated from surface seawater of the South China Sea. It was related to members of the genus Oceanibaculum. Characterization and classification of strain L54-1-50\textsuperscript{T} was carried out using a polyphasic approach.

Strain L54-1-50\textsuperscript{T} was isolated from surface seawater of the South China Sea (111° E, 18°16′ N) in October 2006. The surface seawater was diluted and spread on marine agar 2216 medium (MA; BD Difco). After 1 week aerobic incubation at 25 °C, the colonies were picked out. Purity was confirmed by the uniformity of cell morphology after restreaking. The strain was preserved in a 20 % (v/v) glycerol suspension at −80 °C. The routine cultivation of the strain and phenotypic tests were carried out on MA unless otherwise indicated. Two type strains, O. indicum P24\textsuperscript{T} (MCCC 1A02083\textsuperscript{T}) and O. pacificum MC2UP-L3\textsuperscript{T} (MCCC 1A02656\textsuperscript{T}), obtained from MCCC were used as references in this study.

The genomic DNA was prepared according to the described method by Ausubel et al. [4] and the 16S rRNA gene was amplified by PCR using primers described previously [5] and then sequenced by the ABI3730xl platform (Shanghai Majorbio Bio-pharm Technology). The related taxa sequences were obtained from the GenBank database. Sequence similarity was determined using the EzBioCloud server [6]. Phylogenetic analysis was performed using the MEGA version 5.05 software [7]. Distances (distance options according to the Kimura two-parameter model) and clustering with the methods of neighbour-joining (NJ) [8], maximum-likelihood (ML) [9] and minimum-evolution (ME) [10] were determined by using bootstrap values based on 1000 replications.

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Abbreviation: MCCC, Marine Culture Collection of China.

The GenBank accession numbers for the 16S rRNA and genome sequences of Oceanibaculum nanhaiense L54-1-50\textsuperscript{T} are KX870046 and MPOB000000000, respectively.

One supplementary table and two supplementary figures are available with the online Supplementary Material.
A nearly full-length 16S rRNA gene sequence (1451 nt) of strain L54-1-50 was determined. It was same as that obtained from the draft genome sequence. The closest species was *O. indicum* P24<sup>T</sup> (98.8 %), followed by *O. pacificum* MC2UP-L3<sup>T</sup> (97.7 %), other species shared <93.7 % sequence similarity in different genera. Strain L54-1-50 formed a cluster with *O. indicum* P24<sup>T</sup> and *O. pacificum* MC2UP-L3<sup>T</sup> in the phylogenetic tree (Fig. 1). The similar phylogenetic position of strain L54-1-50<sup>T</sup> was revealed in the ME and ML trees, which were integrated into the NJ tree (Fig. 1).

The draft genome sequence of strain L54-1-50<sup>T</sup> was sequenced by Shanghai Major Bio-pharm Technology (Shanghai, PR China). A total of 1 Gbp clean data of strain L54-1-50<sup>T</sup> was generated to reach about 200-fold depth of coverage using an Illumina/Solexa Genome Analyzer IIX. The clean data were assembled using SOAPdenovo2 [11]. The draft genome sequence of strain L54-1-50<sup>T</sup> was deposited in the GenBank database under accession number MPOB00000000. The genome sequences of *O. indicum* P24<sup>T</sup> (AMRL00000000) and *O. pacificum* MC2UP-L3<sup>T</sup> (LPXN00000000) were obtained from the GenBank database. The G+C content of the chromosomal DNA was calculated using genome sequencing. The digital DNA–DNA hybridization (dDDH) values were calculated using the Genome-to-Genome Distance Calculator (GGDC 2.0) [12]. The average nucleotide identity (ANI) values were calculated using the algorithm of Goris et al. [13] by using the EzGenome web service.

The genome of strain L54-1-50<sup>T</sup> consisted of 40 contigs (>200 bp) of 3 845 221 bp and has the shortest contig size 311 bp and the longest contig size 628 419 bp. From the draft genome sequence, the G+C content of strain L54-1-50<sup>T</sup> was calculated to be 65.1 mol%, which is in accordance with reported values for *Oceanibaculum* species (65.5–65.7 mol%). The dDDH values between strain L54-1-50<sup>T</sup> and the two type strains (*O. indicum* P24<sup>T</sup> and *O. pacificum* MC2UP-L3<sup>T</sup>) were, respectively, 35.4±2.5 % (using the result of recommended formula 2) and 23.7±2.5 %, which are far below the 70 % cut-off value generally recommended for species differentiation [14, 15]. The ANI values between strain L54-1-50<sup>T</sup> and the two type strains were, respectively, 79.7 and 88.3 %, which was below the 95–96 % value recommended as the ANI criterion for interspecies identity [15]. These results indicated that strain L54-1-50<sup>T</sup> represents a novel species of the genus *Oceanibaculum*.

Gram-staining, catalase and oxidase activity, hydrolysis of aesculin and starch, general cell morphology, and electron microscopy were studied as previously described [16]. The growth temperature was determined over a range of 4–55 °C in marine broth 2216 medium (MB; BD Difco). The pH range for growth was determined in the MB medium adjusted to pH 2.0–10.0 (at 1 pH unit intervals) with citrate/phosphate (pH 2.0–7.0), Tris/HCl (pH 8.0–9.0), or sodium carbonate/sodium bicarbonate (pH 10.0) buffers. Tolerance to NaCl was tested using a modified MB medium formula without NaCl, and with NaCl concentrations of 0, 0.5, 1, 2, 3, 4, 5, 7, 9, 12, 15 and 18 (w/v, %). Anaerobic growth was examined on MA supplemented with nitrate (1 g l<sup>−1</sup>) incubated in a jar with the Anoxomat Mark II Anaerobic System (Mart Microbiology). Other biochemical tests were carried out using API 20E, API 20NE and API ZYM strips (bio-Mérieux) and Biolog GN2 according to the manufacturer’s instructions. *O. indicum* P24<sup>T</sup> and *O. pacificum* MC2UP-L3<sup>T</sup> were tested at the same time under the same conditions.

**Fig. 1.** Neighbour-joining tree showing the phylogenetic positions of strain L54-1-50<sup>T</sup> and related genera of the family *Rhodospirillaceae*, based on 16S rRNA gene sequences. Filled circles indicate nodes that were also recovered in maximum-likelihood and minimum evolution trees based on the same sequences. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch points. Bar, 0.01 nucleotide substitution rate (K<sub>sub</sub>) units.

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These results are given in the species description and Table 1.

Fatty acids in whole cells grown on MA at 28 °C for 48 h, were saponified, methylated and extracted using the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.0). The fatty acids were analysed by gas chromatography (Agilent Technologies 6890) and identified by using the TSBA6.0 database of the Microbial Identification System [17]. The fatty acid profiles of strain L54-1-50T and the two type strains were done in parallel with this study when the cells of three strains reached the exponential stage. The results of the three strains are shown in Table S1 (available in the online Supplementary Material). The predominant fatty acids (>5% of) strain L54-1-50T were identified as summed feature 8 (C16:1ω7c and/or C18:1ω6c, 55.3%), C16:0 (8.3%) and C18:1 2-OH (7.4%), which accounted for 71.0% of the total fatty acids. Although most of the detected fatty acids were also present in O. indicum P24T and O. pacificum MC2UP-L3T, significant differences were observed in their percentage of C16:1ω7c 2-OH and summed feature 8 (Table S1). The respiratory quinone of the strain L54-1-50T was determined to be Q-10 by high-performance liquid chromatography analysis according to the described method by Collins [18]. Polar lipids were extracted using a chloroform/methanol system and analysed by using two-dimensional thin-layer chromatography, as described previously [19]. The polar lipids of strain L54-1-50T comprised diphosphatidylglycerol, phosphatidyl-ethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol, two unidentified phospholipids and three unidentified lipids, as shown in Fig. S1.

Strain L54-1-50T was Gram-stain-negative, rod-shaped and motile by a single polar flagellum (see Fig. S2). The differences in physiological, biochemical and chemotaxonomic characteristics between strain L54-1-50T and the two type strains of genus Oceanibaculum are listed in Table 1. On the basis of morphological, physiological and chemotaxonomic characteristics, together with data from DDH and ANI comparison described above, strain L54-1-50T should be placed into a new species of genus Oceanibaculum, for which the name Oceanibaculum nanhaiense sp. nov. is proposed.

### DESCRIPTION OF OCEANIBACULUM NANHAIENSE SP. NOV.

**Oceanibaculum nanhaiense** (nan.hai.en’s e. N.L. neut. adj. nanhaiense pertaining to Nanhai, the Chinese name for the South China Sea, where the type strain was isolated).

Cells are rods, about 1.5–2.5 μm long and 0.8–1.0 μm wide, motile by a single polar flagellum, positive for catalase and oxidase. On MA medium, strain L54-1-50T produces smooth grey colonies with regular edges that are 1 mm in diameter and slightly raised in the centre after 4 days at 28 °C. Grows in 0–9% NaCl (optimum 2%, w/v), at 10–45 °C (optimum 25–37 °C), but not at 4 or 50 °C within a week. Positive results in tests for alkaline phosphatase, valine aminopeptidase and acid phosphatase; weak positive for esterase (C4); negative for trypsin, esterase lipase (C8), lipase (C14), cystine aminopeptidase, trypsin, α-chymotrypsin, naphthol-AS-Bl-phosphoamidase, α-galactosidase, β-galactosidase, β-glucoronidase, α-glucosidase, β-glucosidase, N-acyctyl-β-glucosaminidase, α-mannosidase or α-fucosidase. Characteristics are scored as: +, positive; −, negative.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td><strong>Cells (µm)</strong></td>
<td>0.8–1.0×1.5–2.5</td>
<td>0.6–1.5×2.3–2.5</td>
<td>0.5–0.7×1.2–2.1</td>
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<tr>
<td>Temperature</td>
<td>10–45</td>
<td>10–42</td>
<td>10–45</td>
</tr>
<tr>
<td><strong>pH (optimum)</strong></td>
<td>6–10 (8–9)</td>
<td>6–11</td>
<td>ND</td>
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<tr>
<td></td>
<td>(7–9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NaCl (optimum, %, w/v)</strong></td>
<td>0–9 (2)</td>
<td>0–9</td>
<td>0–9</td>
</tr>
<tr>
<td></td>
<td>(0.5–7)</td>
<td>(1.5–5)</td>
<td></td>
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<tr>
<td><strong>API 20E</strong></td>
<td>Citrate utilization</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>Urease</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Gelatinase</td>
<td>−</td>
<td>W</td>
</tr>
<tr>
<td><strong>API 20NE</strong></td>
<td>Reduction of nitrate</td>
<td>+</td>
<td>−</td>
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<tr>
<td></td>
<td>Denitrification</td>
<td>+</td>
<td>−</td>
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<tr>
<td></td>
<td>Urease</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Gelatin hydrolysis</td>
<td>−</td>
<td>W</td>
</tr>
<tr>
<td><strong>API ZYM</strong></td>
<td>Leucine aminopeptidase</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td><strong>DNA G+C content (mol%)</strong></td>
<td>65.1</td>
<td>65.5</td>
<td>65.7</td>
</tr>
</tbody>
</table>

*Data from draft genome sequence.*
ferment glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose. Nitrate is reduced to nitrite. Denitrification and utilization of adipic acid and malic acid are positive. Activity of arginine amines is negative. Growth occurs on D-glucose, phenylacetic acid, caprylic acid, D-mannose, N-acetyl-glucosamine, maltose, potassium gluconate, L-arabinose, D-mannotol and trisodium citrate as sole carbon sources. The following compounds are utilized for respiration (Biolog): acetic acid, succinic acid, valeric acid, α-keto glutaric acid, α-keto valeric acid, D,L-lactic acid, D-saccharic acid, succinic acid, succinamic acid, D-glutamic acid and glycol-L-glutamic acid; glycyrl-1-aspartic acid and L-proline (weak). The principal fatty acids (>5%) are summed feature 8 (C18:1ω7c and/or C18:1ω6c), C16:0 and C18:1 2-OH, with minor amounts of C19:0 cyclo ω8c, summed feature 3 (C16:1ω7c and/or C16:1ω6c), C18:1ω9c, C16:1ω7c, C18:0 and C16:0, summed feature 2 (C14:0 3-OH and/or isomer C16:1 I), C16:1ω9c, C14:0, C18:1ω7c, C17:1ω7c, iso-C15:1 G, C18:1ω7c, C16:1ω7c, iso-C16:1 G, C18:1ω7c, iso-C15:1 G, C18:0 3-OH, C16:0 3-OH, summed feature 7 (unknown 18.846 and/or C19:1ω6c), C11:0 3-OH and iso-C11:0. The quinone is Q-10. The polar lipids comprise diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol, two unidentified phospholipids and three unidentified lipids. The G+C content of the DNA is 65.1 mol%.

The type strain, L54-1-50T (=KCTC 52312T=MCCC 1A05150T), was isolated from surface seawater from the South-West Pacific Ocean.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References