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 L54-1-50T, which was isolated from surface seawater of the South China Sea. Cells of strain L54-1-50T 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 L54-1-50T was a member of the genus Oceanibaculum, related to Oceanibaculum indicum P24T (98.8 %) and Oceanibaculum pacificum MC2UP-L3T (97.7 %). The digital DNA–DNA hybridization values between strain L54-1-50T and the two type strains O. indicum P24T and O. pacificum MC2UP-L3T were 35.4±2.5 and 23.7±2.5, respectively. The average nucleotide identity values between strain L54-1-50T and two type strains were 79.7 and 88.3, respectively. The major cellular fatty acids were summed feature 8 (C18:1ω7c and/or C18:1ω6c), C16:0 and C18:1 2-OH. The respiratory quinone was Q-10. The polar lipids comprised diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositolmonomethylethanolamine, 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 L54-1-50T represents a novel species of the genus Oceanibaculum, for which the name Oceanibaculum nanhaiense sp. nov. is proposed, with the type strain L54-1-50T (=KCTC 52312T=MCCC 1A05150T).

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, L54-1-50T, 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 L54-1-50T was carried out using a polyphasic approach.

Strain L54-1-50T 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 P24T (MCCC 1A02083T) and O. pacificum MC2UP-L3T (MCCC 1A02656T), 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.

Author affiliation: State Key Laboratory Breeding Base of Marine Genetic Resources; Key Laboratory of Marine Genetic Resources, Third Institute of Oceanography, SOA; Fujian Collaborative Innovation Center for Exploitation and Utilization of Marine Biological Resources; Fujian Key Laboratory of Marine Genetic Resources, Xiamen 361005, PR China.

*Correspondence: Zongze Shao, shaozz@163.com

Keyword: Oceanibaculum nanhaiense sp. nov.

Abbreviation: MCCC, Marine Culture Collection of China.

The GenBank accession numbers for the 16S rRNA and genome sequences of Oceanibaculum nanhaiense L54-1-50T are KX870046 and MPOB00000000, 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\(^T\) was determined. It was same as that obtained from the draft genome sequence. The closest species was *O. indicum* P24\(^T\) (98.8 %), followed by *O. pacificum* MC2UP-L3\(^T\) (97.7 %), other species shared <93.7 % sequence similarity in different genera. Strain L54-1-50\(^T\) formed a cluster with *O. indicum* P24\(^T\) and *O. pacificum* MC2UP-L3\(^T\) in the phylogenetic tree (Fig. 1). The similar phylogenetic position of strain L54-1-50\(^T\) 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\(^T\) was sequenced by Shanghai Major Bio-pharm Technology (Shanghai, PR China). A total of 1 Gbp clean data of strain L54-1-50\(^T\) 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 genome sequences of *MC2UP-L3* were obtained from the GenBank database under accession number AMRL00000000. The genome sequences of *LPXN00000000* (>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\(^T\) 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\(^T\) and the two type strains (*O. indicum* P24\(^T\) and *O. pacificum* MC2UP-L3\(^T\)) 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\(^T\) 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\(^T\) represents a novel species of the genus *Oceanibaculum*.

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strain L54-1-50\(^T\) 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\(_{\text{sub}}\)) units.
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 6.0B). The fatty acids were analysed by gas chromatography (Agilent Technologies 6850) and identified by using the TSBA6.0 database of the Microbial Identification System [17]. The fatty acid profiles of strain L54-1-50<sup>T</sup> 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 L54-1-50<sup>T</sup> were identified as summed feature 8 (C<sub>16:1ω7c</sub> and/or C<sub>18:1ω6c</sub>, 55.3%), C<sub>16:0</sub> (8.3%) and C<sub>18:1</sub> 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 <i>O. indicum</i> P24<sup>T</sup> and <i>O. pacificum</i> MC2UP-L3<sup>T</sup>, significant differences were observed in their percentage of C<sub>16:1ω7c</sub> 2-OH and summed feature 8 (Table S1). The respiratory quinone of the strain L54-1-50<sup>T</sup> 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 L54-1-50<sup>T</sup> comprised diphosphatidylglycerol, phosphatidyl ethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol, two unidentified phospholipids and three unidentified lipids, as shown in Fig. S1.

Strain L54-1-50<sup>T</sup> 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 L54-1-50<sup>T</sup> and the two type strains of genus <i>Oceanibaculum</i> 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 L54-1-50<sup>T</sup> should be placed into a new species of genus <i>Oceanibaculum</i>, for which the name <i>Oceanibaculum nanhaiense</i> sp. nov. is proposed.

### Table 1. Different characteristics of strain L54-1-50<sup>T</sup> and related species of the genus <i>Oceanibaculum</i>

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

*Data from draft genome sequence.

**DESCRIPTION OF OCEANIBACULUM NANHAIENCE SP. NOV.**

<i>Oceanibaculum nanhaiense</i> (nan.hai.en’se. N.L. neut. adj. <i>nanhaiense</i> 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 L54-1-50<sup>T</sup> 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 activities; weakly positive for gelatin, esterase (C4) and cystine aminopeptidase activities; and negative for lipase (C14), α-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase, α-fucosidase, esterase lipase (C8), leucine aminopeptidase, naphtol-AS-Bl-phosphoamidase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, 6-N-acetyl-β-glucosaminidase, α-mannosidase or α-fucosidase. Characteristics are scored as: w, weak; +, positive; –, negative.

**Du et al., Int J Syst Evol Microbiol 2017;67:4842–4845**

Downloaded from www.microbiologyresearch.org by IP: 54.70.40.11 On: Sat, 15 Dec 2018 11:10:08
ferment glucose, mannitol, inositol, sorbitol, rhhamnose, sucrose, melibiose, amygdalin and arabinose. Nitrate is reduced to nitrite. Denitrification and utilization of adipic acid and malic acid are positive. Activity of arginine aesculin hydrolysis is negative. No growth occurs on D-glucose, phenylacetic acid, capric acid, D-mannose, N-acetyl-glucosamine, maltose, potassium gluconate, L-arabinose, D-mannitol and trisodium citrate as sole carbon sources. The following compounds are utilized for respiration (Biolog): acetic acid, β-hydroxy butyric acid, α-keto glutaric acid, α-keto valeric acid, D,L-lactic acid, D-saccharic acid, succinic acid, succinamic acid, L-glutamic acid and glycyl-L-glutamic acid; glycyl-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, C18:0, C16:0 3-OH, summed feature 2 (C14:0 3-OH and/or iso-C15:0 1I), C16:1ω5c, C14:0, C13:0 2-OH, C17:1ω7c, iso-C15:0 1I, C16:1ω9c, C18:0 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=MCC11A05150T), was isolated from surface seawater from the South-West Pacific Ocean. Int J Syst Evol Microbiol 2010;60:219–222.


