Shinella curvata sp. nov., isolated from hydrocarbon-contaminated desert sands

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The taxonomic position of a novel bacterial strain, designated C3T, isolated from hydrocarbon-contaminated desert sands was determined. Strain C3T was a Gram-stain-negative, rod- to curved-rod-shaped and non-motile bacterium. It was able to grow at 4–45 °C (optima, 28–35 °C) and at pH 6.1–8.8 (optima, 6.9–7.7). No added NaCl was required for growth of strain C3T and it tolerated up to 3.5 % (w/v) NaCl with optimal growth with 0.5–1.5 %. Catalase and oxidase were positive. C18:1ω6c/C18:1ω7c, C16:0, C12:0 aldehyde, C14:0 3-OH/iso-C16:1 I and C18:1ω7c 11-methyl were predominant fatty acids. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine were major polar lipids. The genomic DNA G+C content was 65.4 mol%. 16S rRNA gene sequence comparisons indicated that strain C3T represents a member of the genus Shinella within the family Rhizobiaceae of the class Alphaproteobacteria. Strain C3T showed a 16S rRNA gene sequence similarity of 98.39% with Shinella kummerowiae CCBAU 25048T, 98.37 % with Shinella granuli Ch06T, 98.17 % with Shinella zoogloeoidees l-16-M1T, 97.74 % with Shinella fusca DC-196T, 97.46 % with Shinella yambaruensis MS4T and <96.68 % with other members of the family Rhizobiaceae. DNA–DNA hybridization values between strain C3T and the type strains of the nearest species were clearly below the 70 % threshold for species delineation.

Distinct morphological, physiological and genotypic differences from previously described taxa support the classification of strain C3T as a representative of a novel species in the genus Shinella, for which the name Shinella curvata sp. nov. is proposed. The type strain is C3T (=KEMB 2255–446T=JCM 31239T).

The genus Shinella belongs to the family Rhizobiaceae of the class Alphaproteobacteria in the phylum Proteobacteria. Species of the genus have been isolated from different environmental samples such as from a sludge treatment plant (Lee et al., 2011), soil (Matsui et al., 2009), granules from a waste water reactor (An et al., 2006), domestic waste compost (Vaz-Moreira et al., 2010) and root nodules (Lin et al., 2008). Members of the genus are Gram-stain-negative and non-endospore-forming. C18:1ω6c/C18:1ω7c and C16:0 are major fatty acids of species of the genus Shinella (Lin et al., 2008; Matsui et al., 2009; Vaz-Moreira et al., 2010; Lee et al., 2011). At the time of writing, a total of six species names have been validated in this genus. Through this communication we propose to add one more novel species to the genus Shinella.

Strain C3T was isolated from hydrocarbon-contaminated desert sands located near a sea beach in Kuwait (GPS positioning of the sample; 29°37′07″ N 47°48′13″ E) and characterized based on a polyphasic taxonomic approach. Sand samples were collected in December 2014. Sand samples of 1 g were serially diluted up to a 10−7 dilution and 100 µl was spread on a 1.5 % (w/v) agar medium (pH 7.0) which contained (g l−1): KH2PO4 (0.2), NH4Cl (0.25), KCl (0.5), CaCl2.2H2O (0.15), NaCl (1.0), MgCl2.6H2O (0.6), Na2SO4 (2.8), HEPES (2.8), yeast extract (3.0), peptone (3.0), casamino acid (0.5), dextrose (0.5) and sodium pyruvate (3.0; Subhash et al., 2013a). Culture plates were incubated at 30 °C for 5 days. Bacterial isolates were purified by repeated streaking on agar plates and preserved using lyophilization. Purified cultures were grown in conical flasks (500 ml) with shaking (150 r.p.m.) in liquid medium (pH 7.0) as detailed above (Subhash et al., 2013a). For routine culturing and for physiological tests, strain C3T was grown at pH 7.0 and 30 °C.

Genomic DNA was isolated and purified according to the method of Marmur (1961) and the G+C content of strain C3T was determined by HPLC (Mesbah et al., 1989). 16S rRNA gene amplification was done by using universal primers.

Abbreviations: ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain C3T is LT545981.

Two supplementary figures are available with the online Supplementary Material.
Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strain C3 T with members of the genus Shinella. The tree was reconstructed via the NJ method using the MEGA 6 software and rooted by using Phyllobacterium endophyticum PEPV15 T (JN848778) as the out-group. Numbers at nodes represent bootstrap values (based on 1000 resamplings). Bootstrap percentages refer to NJ/ML/MP analysis. The GenBank accession numbers for 16S rRNA gene sequences are shown in parentheses.
Table 1. Differential characteristics between strain C3<sup>T</sup> and related species of the genus *Shinella*

Strains: 1, C3<sup>T</sup>; 2, *S. kummerowiae* KACC 14150<sup>T</sup>; 3, *S. granuli* KACC 11815<sup>T</sup>; 4, *S. zoogloeoides* KACC 15129<sup>T</sup>; 5, *S. fusca* DSM 21319<sup>T</sup>; 6, *S. yambaruensis* KACC 14483<sup>T</sup>. All strains: are positive for oxidase and catalase activity; are negative for DNA, gelatin and chitin hydrolysis; utilize D-glucose, D-mannitol, D-sorbitol, D-galactose, glutamate, gluconate, lactose and pyruvate; do not utilize raffinose; are positive for acid production from D-galactose and sucrose; and are positive for alkaline phosphatase. +, Present/utilized; −, absent/not utilized. Characterization of all strains was done in the present study under identical conditions. Data presented in square brackets are those published by Matsui *et al.* (2009), An *et al.* (2006) and Vaz-Moreira *et al.* (2010).

<table>
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<td>0.2–0.5×3.9–6</td>
<td>0.7–0.8×2.1–3.6</td>
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<td>1–4</td>
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<td>4–40</td>
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<td>15–42</td>
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<td>+</td>
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<td>−</td>
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<td>Esterase (C4)</td>
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<td>Lipase (C14)</td>
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<td>Leucine arylamidase</td>
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<td>−</td>
<td>+</td>
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<td>Valine arylamidase</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
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[aerobically with pyruvate (0.3 %, w/v) as carbon source/ electron donor]. Fermentative growth [anaerobically, in the dark with glucose/fructose/pyruvate (0.3 %, w/v)] could not be demonstrated. Many organic substrates supported the growth of strain C3^T (Table 1). Strain C3 had no requirement for vitamins for growth and grew even in the absence of yeast extract (growth was observed without yeast extract even after five subcultures), although growth yield improved with addition of yeast extract (0.01 %). NaCl was not obligatory for growth of strain C3^T and it tolerated up to 4.5 % (w/v). Growth of strain C3^T occurred at pH 6.1–8.8, with an optimum pH range of 6.9–7.7. Growth optima at different conditions (temperature, NaCl and pH) indicated that strain C3^T is a mesophile. Ammonium chloride was used as a good nitrogen source for growth while diazotrophic growth was not observed. Strain C3^T was sensitive to nalidixic acid (40 µg) and ampicillin (40 µg) but resistant to chloramphenicol (40 µg), penicillin-G (40 µg) and rifampicin (40 µg). Other physiological and biochemical characteristics of strain C3^T and its closest phylogenetic neighbours are given in Tables 1 and 2.

The G+C content of the genomic DNA of strain C3^T was 65.4 mol%. The phylogenetic relationship of strain C3^T was examined by 16S rRNA gene sequence (1423 nt) analysis, and BLAST searches indicated that strain C3^T is a member of the genus Shinella in the family Rhizobiaceae (Fig. 1). Highest 16S rRNA gene sequence similarity was 98.39% with Shinella kummerowiae CCBAU 25048^T, 98.37% with S. granuli Ch06^T, 98.17% with S. zoogloeoides I-16-M^T, 97.74% with S. fusca DC-196^T, 97.46% with S. yambaruensis MS4^T and <96.68% with other members of the family Rhizobiaceae. Strain C3^T showed 45.6±1, 40±2, 39±1, 35±2 and 23±1 % relatedness (based on DNA–DNA hybridization) with Shinella kummerowiae KACC 14150^T (=CCBAU 25048^T), S. granuli KACC 11815^T (=Ch06^T), S. zoogloeoides KACC 15129^T (=I-16-M^T), S. fusca DSM 21319^T (=DC-196^T) and S. yambaruensis KACC 14483^T (=MS4^T), respectively.

Strain C3^T was distinct (Table 1) from its closest phylogenetic neighbours, S. kummerowiae CCBAU 25048^T, S. granuli Ch06^T, S. zoogloeoides I-16-M^T, S. fusca DC-196^T and S. yambaruensis MS4^T, with respect to cell morphology, absence of flagellar motility, organic carbon source utilization, hydrolysis of starch, casein, Tween 20, Tween 80, urea and ascorbic,
Table 2. Cellular fatty acid compositions (% of strain C3T and related species of the genus Shinella

<table>
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<th>Fatty acid</th>
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<td>4.3</td>
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<td>1.8</td>
<td>2.6</td>
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<tr>
<td>C&lt;sub&gt;14:0&lt;/sub&gt; 3-OH/iso-C&lt;sub&gt;16:0&lt;/sub&gt; I</td>
<td>5.5</td>
<td>4.3</td>
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<td>1.6</td>
<td>6.8</td>
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<td>3.2</td>
<td>8.9</td>
<td>6.4</td>
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<td>11.7</td>
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<td></td>
<td>1.7</td>
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<td>1.8</td>
<td>1.3</td>
<td>1.4</td>
<td>0.7</td>
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<td>1.7</td>
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antibiotic susceptibility, growth temperature range, NaCl tolerance, pH range, fatty acids and polar lipid profile. Based on morphological, physiological, chemotaxonomic and genotypic differences with the above reference species (Tables 1 and 2), we suggest a novel species to accommodate strain C3T, with the name Shinella curvata sp. nov.

**Description of Shinella curvata sp. nov.**

Shinella curvata (cur.va’ta. L. adj. curvata curved, referring to the cell shape).

Colonies are glistening, convex with an entire margin, viscous and cream colour on agar medium. Cells are small rod- to curved-rod-shaped, Gram-stain-negative, 0.5–0.6 µm wide and 1.9–2.8 µm long. Cells are non-motile and multiply by binary fission. Mesophilic. Growth occurs at 4–45 °C (optima 28–35 °C) and at pH 6.1–8.8 (optima 6.9–7.7). No added NaCl is required for growth and tolerates 3.5 % (w/v) NaCl (optimum 0.5 %). Casein, starch and Tween 20 are hydrolysed while DNA, gelatin, chitin, urea, Tween 80 and aesculin are not. Positive for catalase and oxidase activity but negative for methyl red, arginine decarboxylase, lysine decarboxylase, Voges–Proskauer and H<sub>2</sub>S production tests. D-Glucose, maltose, fructose, D-mannitol, D-sorbitol, sucrose, aspartate, malate, D-ribose, melibiose, glutamate and pyruvate are utilized for growth while L-rhamnose, cellulose, raffinose and L-arabinose are not. Positive for acid production from D-glucose, D-fructose, melibiose and sucrose while acid is not produced from maltose, cellulose, L-rhamnose, raffinose or L-arabinose. Ammonium chloride is used as a good nitrogen source for growth while diazotrophic growth is not possible. The niFH gene is not present. Sensitive to ampicillin and nalidixic acid but resistant to chloramphenicol, penicillin-G and rifampicin. Indole is not produced from L-tryptophan and negative for denitrification activity. Positive for alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, β-galactosidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-fucosidase but negative for esterase lipase (C8), lipase (C14), trypsin, α-chymotrypsin, naphthol-AS–Bl-phosphohydrolase, α-galactosidase, β-glucuronidase, α-glucosidase and α-mannosidase. C<sub>18:1ω6c/C<sub>18:1ω7c</sub> C<sub>16:0</sub> C<sub>12:0</sub> aldehyde, C<sub>14:0</sub> 3-OH/iso-C<sub>16:0</sub> I and C<sub>18:1ω7c</sub> 11-methyl are predominant fatty acids with minor amounts of C<sub>16:0</sub> 3-OH, iso-C<sub>17:0</sub> 3-OH, C<sub>18:0</sub> C<sub>16:1ω6c/C<sub>16:1ω7c</sub> C<sub>16:0</sub> 3-OH, C<sub>19:0</sub> cyclo ω8c and C<sub>20:2ω6c</sub>.

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References


