Myroides indicus sp. nov., isolated from garden soil

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A novel aerobic, non-motile, rod-shaped, catalase- and oxidase-positive bacterial strain, designated UKS3T, was isolated from garden soil, and subjected to polyphasic taxonomic analysis. Strain UKS3T formed whitish, viscous colonies on nutrient agar and was Gram-staining negative. Phylogenetic analysis, based on 16S rRNA gene sequence, showed that maximum pairwise similarity occurs with representatives of the genus Myroides. The most closely related species include Myroides marinus JS-08T (92.7 % sequence similarity), Myroides phaeus MY15T (92.7 %), Myroides odoratus DSM 2801T (91.5 %) and Myroides odoratimimus CCUG 39352T (91.4 %). Strain UKS3T contained menaquinone-6 (MK-6) as the major respiratory quinone and iso-C15 : 0 (40.2 %), anteiso-C15 : 0 (9.4 %) and iso-C17 : 0 3-OH (8.5 %) as major fatty acids. Phosphatidylethanolamine, phospholipids and three aminolipids were the major polar lipids. The DNA G + C content of strain UKS3T was 36.8±2.0 mol%. On the basis of phenotypic, chemotaxonomic and molecular analysis, strain UKS3T represents a novel species of the genus Myroides, for which the name Myroides indicus sp. nov., is proposed. The type strain is UKS3T (= DSM 28213T = NCIM 5555T).

The genus Myroides was created as a consequence of the reclassification of Flavobacterium odoratum (Stutzer & Kwaschnina, 1929; Vancanneyt et al., 1996). The genus Myroides is a member of the family Flavobacteriaceae (Bernardet et al., 2002). At the time of writing this manuscript, the genus Myroides comprises species isolated from different habitats, including Myroides odoratus and Myroides odoratimimus from clinical samples (Holmes et al., 1977; Vancanneyt et al., 1996), Myroides pelagicus and Myroides marinus from seawater (Yoon et al., 2006; Cho et al., 2011), Myroides profundi from deep-sea sediment (Zhang et al., 2008), Myroides phaeus from human saliva (Yan et al., 2012), Myroides guano- nis from a prehistoric guano painting (Tomova et al., 2013), Myroides xuanwuensis from forest soil (Zhang et al., 2014), ‘Myroides gitamensis’ from slaughter house soil (Talluri et al., 2014) and Myroides injenensis from human urine (Paek et al., 2015). The range of habitats shows the wide distribution of strains representing the genus.

In the present study, the taxonomic position of the novel bacterial strain UKS3T was investigated on the basis of its morphological, biochemical, physiological and phylogenetic characteristics. The strain was isolated from a garden soil sample collected from the Department of Botany, University of Delhi (North Campus), India.

The soil sample was suspended in sterile saline solution (0.9 %, w/v, NaCl) and was spread on to nutrient agar (HiMedia) plates followed by incubation at 37 °C for 48 h. Among the different colonies growing on nutrient agar, one whitish, viscous colony of strain UKS3T was selected and subsequently streaked three consecutive times on the same medium for purification. Strain UKS3T was preserved at 4 °C on nutrient agar and at −80 °C in nutrient broth (HiMedia) containing 15 % (v/v) glycerol.

The morphology of colonies was determined on nutrient agar after incubation of pure culture at 30 °C for 24 h. Gram staining of strain UKS3T was performed using the standard protocol of Popescu & Doyle (1996). The cell morphology was assessed using optical and scanning electron microscopy (Fig. S1 available in the online Supplementary Material).

Catalase activity was detected in the broth culture of strain UKS3T using 10 % (v/v) H2O2. Oxidase activity was detected by observing the colour change after rubbing the culture on a small piece of filter paper treated with Kovács oxidase reagent (1 % tetramethyl-p-phenylenediamine). The motility of strain UKS3T was assessed by stabbing a loopful of culture
up to 1 cm in motility test medium (beef extract, 3.0 g; pancreatic digest of casein, 10 g; sodium chloride, 5.0 g; and agar, 4.0 g; supplemented with 5 ml 1 % 2,3,5-triphenyltetrazolium chloride). The occurrence of a red, turbid area extending away from the line of inoculation was observed after 48 h. Anaerobic growth of strain UKS3\(^T\) was assessed in an anaerobic candle jar through incubating streaked nutrient agar plates for 72 h (Ye et al., 2013). The influence of a wide range of temperature and pH on the growth of strain UKS3\(^T\) was determined spectroscopically by measuring the turbidity at 600 nm after incubation for 24 h (Yoon et al., 2006). The pH range for growth was examined in nutrient broth with starting pH of 5.0–9.0 (using 0.5 M citrate, phosphate and glycine buffers) adjusted at intervals of 0.5 pH unit. The temperature range for growth was determined in nutrient broth at 5, 10, 15, 20, 25, 30, 35, 40 and 45 °C. Hydrolysis of lipid, gelatin, starch and urea were assessed using specific media (Clarke & Cowan, 1952; McDade & Weaver, 1959; Kouker & Jaeger, 1987). Growth of strain UKS3\(^T\) in the presence of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 % NaCl was investigated on nutrient agar plates after incubation under optimum conditions for 5 days. The ability to utilize different carbohydrates was determined using a KB009 Hi-Carbohydrate kit (Hi-Media). The kit includes a standardized colorimetric identification system for 35 different carbohydrates based on the principle of pH change due to metabolic activity during bacterial growth. The kit results were confirmed manually for the following 15 different carbohydrates: sucrose, trehalose, maltose, D-xylose, raffinose, diethylaminoethyl cellulose, cellobiose, mannitol, glucose, D-fructose, D-glucose, lactose monohydrate, D-sorbitol, D-galactose and melibiose using a minimal salts medium (g l\(^{-1}\): (NH\(_4\))\(_2\)HPO\(_4\), 1.0; KCL, 0.2; MgSO\(_4\), 0.1; thiamine, 0.0001; and bromothymol blue, 0.00015 (Tomova et al., 2013). The enzyme activities of strain UKS3\(^T\) were assessed using API ZYM (bioMérieux) according to manufacturer’s instructions.

Sensitivity of strain UKS3\(^T\) to different antibiotics, namely streptomycin (10 μg disc\(^{-1}\)), tetracycline (10 μg disc\(^{-1}\)), penicillin-G (2 units disc\(^{-1}\)), gentamicin (10 μg disc\(^{-1}\)), ampicillin (10 μg disc\(^{-1}\)), chloramphenicol (30 μg disc\(^{-1}\)), carbencillin (100 μg disc\(^{-1}\)), kanamycin (5 μg disc\(^{-1}\)), erythromycin (10 μg disc\(^{-1}\)) and rifampicin (30 μg disc\(^{-1}\)), was tested on nutrient agar plates using the disc diffusion method, and a zone of inhibition of bacterial growth was determined after 24 h of incubation under optimal conditions (Bisht et al., 2013). Analysis of fatty acid profiles of strain UKS3\(^T\), M. marinus JS-08\(^T\) and M. phaeus MY15\(^T\) was performed using the method described in the Sherlock Microbial Identification System manual (version 4.0; MIDI) (Table 1). The major fatty acids in strain UKS3\(^T\) were iso-(45.1 %) and anteiso-branched saturated acids (9.42 %). The major respiratory quinone of strain UKS3\(^T\) was determined by following the protocol described by Collins et al. (1977). Polar lipids analysis of strain UKS3\(^T\) was carried out by two-dimensional TLC (Bligh & Dyer, 1959). Total polar lipids were detected by spraying with 1 % aqueous primuline (Jindal et al., 2013) dissolved in acetone, followed by air-drying. For genomic DNA G+C content analysis, thermal denaturation of 5 μg DNA was carried out in 0.1 × standard saline citrate (De Ley et al., 1970). SYBR green at

**Table 1. Cellular fatty acid contents of strain UKS3\(^T\) and strains of recognized species of the genus Myroides**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<tbody>
<tr>
<td>Straight-chain saturated</td>
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<tr>
<td>C(_{12}) : 0</td>
<td>1.01</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>C(_{16}) : 0</td>
<td>3.23</td>
<td>2.31</td>
<td>1.15</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.0</td>
<td>2.9</td>
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<tr>
<td>Branched saturated</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>iso-C(_{14}) : 0</td>
<td>2.13</td>
<td>3.12</td>
<td>2.24</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.2</td>
</tr>
<tr>
<td>iso-C(_{15}) : 0</td>
<td>40.16</td>
<td>27.90</td>
<td>44.71</td>
<td>45.5</td>
<td>49.1</td>
<td>49.9</td>
<td>42.8</td>
<td>34.2</td>
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<tr>
<td>anteiso-C(_{15}) : 0</td>
<td>9.42</td>
<td>5.39</td>
<td>1.07</td>
<td>2.0</td>
<td>0.9</td>
<td>1.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>iso-C(_{16}) : 0</td>
<td>2.83</td>
<td>4.74</td>
<td>1.13</td>
<td>–</td>
<td>0.5</td>
<td>–</td>
<td>–</td>
<td>4.7</td>
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<tr>
<td>Hydroxy</td>
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<tr>
<td>iso-C(_{15}) : 0 3-OH</td>
<td>4.46</td>
<td>3.45</td>
<td>4.94</td>
<td>5.8</td>
<td>3.8</td>
<td>6.1</td>
<td>4.6</td>
<td>4.2</td>
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<tr>
<td>C(_{16}) : 0 3-OH</td>
<td>2.50</td>
<td>3.64</td>
<td>3.44</td>
<td>1.5</td>
<td>0.8</td>
<td>2.8</td>
<td>3.6</td>
<td>6.3</td>
</tr>
<tr>
<td>iso-C(_{17}) : 0 3-OH</td>
<td>8.50</td>
<td>5.85</td>
<td>5.16</td>
<td>21.2</td>
<td>14.0</td>
<td>10.1</td>
<td>10.9</td>
<td>12.5</td>
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<tr>
<td>Summed features</td>
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<tr>
<td>3</td>
<td>7.47</td>
<td>14.76</td>
<td>6.63</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>11.4</td>
</tr>
<tr>
<td>9</td>
<td>10.61</td>
<td>12.51</td>
<td>15.88</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>20.1</td>
<td>8.9</td>
</tr>
</tbody>
</table>

Strains: 1, UKS3\(^T\); 2, M. marinus KCTC 23023\(^T\) (Cho et al., 2011); 3, M. phaeus JCM 17139 (Yan et al., 2012); 4, M. odoratus DSM 2801\(^T\) (Holmes et al., 1977; Vancanneyt et al., 1996); 5, M. guanunis IM13\(^T\) (Tomova et al., 2013); 6, M. pelagicus SM1\(^T\) (Yoon et al., 2006); 7, M. profundus D25\(^T\) (Zhang et al., 2008); 8, M. xianwuensis TH-19\(^T\) (Zhang et al., 2014).
1 : 100 000 concentration was used. A Step One Plus Real-Time PCR system (Applied Biosystems) was used to perform DNA G+C analysis basis on DNA melt curve and \( T_m \) (Gonzalez & Saiz-Jimenez, 2002).

Genomic DNA of strain UKS\(^T\) was extracted and purified using a Fermentas GeneJET Genomic DNA Purification kit. The 16S rRNA gene was amplified using PCR with universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') (Weisburg et al., 1991), and the amplified region was sequenced using a Big Dye terminator cycle sequencing kit (Applied BioSystems). The sequences thus obtained were resolved using an Applied Biosystems model 3730XL automated DNA sequencing system. The nearly complete 16S rRNA gene sequence (1388 bp) of UKS\(^T\) was analysed using Eztaxon server software (Kim et al., 2012) to determine the most closely related taxa. Maximum pairwise similarity was observed between strain UKS\(^T\) and M. phaeus MY15\(^T\) (92.7 %), M. marinus JS-08\(^T\) (92.7 %), M. odoratus DSM 2801\(^T\) (91.5 %) and M. odoratimimus CCUG 39352\(^T\) (91.4 %). The 16S rRNA gene sequence of strain UKS\(^T\) was aligned with sequences retrieved for the closest recognized species, and a neighbour-joining phylogenetic tree was reconstructed using MEGA 5.2 software (Fig. 1) (Tamura et al., 2011). The neighbour-joining tree was topologically similar to the maximum-likelihood and maximum-parsimony trees (data not shown).

Strain UKS\(^T\) showed similarity in its shape (rod-shaped), Gram staining (negative) and enzyme activity in the API ZYM kit with its closest phylogenetic neighbours. It differed from them in having a narrow pH range for growth, and being able to utilize galactose and glucose but not xylose as a carbon source.

Colonies of strain UKS\(^T\) were white with a viscous consistency on nutrient agar. Cells were Gram-stain-negative, short rods (1.4–1.8 mm long and 0.5 mm wide) and non-motile. Strain UKS\(^T\) could grow at 10–40 °C, pH 5.5–8.5 and with 0–6 % (w/v) NaCl, but optimum growth was observed at 30 °C, pH 6.5 and 0–4 % (w/v) NaCl. No growth was observed below 10 °C and pH 5.5 or above 6 % (w/v) NaCl. Detailed

**Fig. 1.** Neighbour-joining tree based on almost-complete 16S rRNA gene sequences showing the relationship of strain UKS\(^T\) with other representative type species of the genera *Myroides* and *Flavobacterium*. Numbers at nodes are bootstrap values, only those >50 % are given. Asterisks indicate that the corresponding nodes (groupings) are also recovered in maximum-parsimony and maximum-likelihood trees. Bar, 0.01 substitutions per nucleotide. *Aquimarina gracilis* PSC32\(^T\) (HM998909) was used as an outgroup.
Table 2. Differential characteristics of strain UKS3\textsuperscript{T} compared with other species of the genus Myroides

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Morphology</td>
<td>Short rods</td>
<td>Short rods</td>
<td>Straight rods</td>
<td>Rods</td>
<td>Rods</td>
<td>Rods</td>
<td>Spindle rods</td>
<td>Rods</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>Whitish</td>
<td>Yellow-orange</td>
<td>Yellow-bROWN</td>
<td>Pale yellow</td>
<td>Yellow</td>
<td>Yellow-orange</td>
<td>Pale yellow</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>pH range for growth</td>
<td>5.5–8.5</td>
<td>5.0–8.5</td>
<td>5.0–8.5</td>
<td>6–9</td>
<td>7.0–9.5</td>
<td>5–9</td>
<td>5–9</td>
<td>4–10</td>
</tr>
<tr>
<td>Optimum pH</td>
<td>6.5</td>
<td>7.0</td>
<td>7.0</td>
<td>ND</td>
<td>7.5</td>
<td>5–9</td>
<td>ND</td>
<td>6–7</td>
</tr>
<tr>
<td>Temperature range for growth (°C)</td>
<td>10–40</td>
<td>10–37</td>
<td>6–37</td>
<td>18–37</td>
<td>5–35</td>
<td>10–37</td>
<td>8–42</td>
<td>10–42</td>
</tr>
<tr>
<td>Optimum temp. (°C)</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>ND</td>
<td>30</td>
<td>30</td>
<td>ND</td>
<td>30</td>
</tr>
<tr>
<td>NaCl range for growth (%)</td>
<td>0–6.0</td>
<td>0–5.0</td>
<td>0–6.0</td>
<td>0–5</td>
<td>0–4.0</td>
<td>0–9</td>
<td>0–8</td>
<td>0–6</td>
</tr>
<tr>
<td>Substrate utilization</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Galactose</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Xylose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Glucose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>36.8</td>
<td>34.2</td>
<td>34.3</td>
<td>36–38</td>
<td>33.5</td>
<td>33.6</td>
<td>33</td>
<td>37.2</td>
</tr>
<tr>
<td>Source of isolation</td>
<td>Garden soil</td>
<td>Seawater</td>
<td>Human saliva</td>
<td>Clinical sample</td>
<td>Guano painting</td>
<td>Seawater</td>
<td>Deep-sea sediment</td>
<td>Forest soil</td>
</tr>
</tbody>
</table>

The type strain is UKS3\textsuperscript{T} = DSM 28213\textsuperscript{T} = NCIM 5555\textsuperscript{T}.

The DNA G+C content of the type strain is 37 mol%.

The type strain was isolated from a garden soil sample of the Department of Botany, University of Delhi (North Campus), Delhi, India.

The DNA G+C content of the type strain is 37 mol%.

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Acknowledgements

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References


