Terriglobus aquaticus sp. nov., isolated from an artificial reservoir

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A pink-pigmented, chemo-organotrophic bacterium, designated strain 03SUJ4T, was isolated from the freshwater of Juam reservoir, Republic of Korea (35° 03′ 43″ N 127° 14′ 15″ E). Cells were aerobic, Gram-reaction-negative and non-motile rods. Strain 03SUJ4T grew at pH 6–7 (optimum, pH 6) and at 15–30°C (optimum, 25°C). Phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate belonged to the genus Terriglobus, showing sequence similarities of 97.09% and 96.82% to Terriglobus roseus DSM 18391T and Terriglobus saanensis SP1PR4T, respectively. Low rpoB gene sequence similarity with members of the genus Terriglobus and different fingerprints with the repetitive primers BOX, ERIC and REP indicated that the isolate represented a novel species of the genus Terriglobus. The major cellular fatty acids were iso-C15:0, C16:0, C20:1ω9c, C14:0 and summed feature 3 (C16:1ω7c/C16:1ω6c). The DNA G+C content of strain 03SUJ4T was 63.2 ± 0.1 mol% (mean ± SD of three determinations). The predominant menaquinone was MK-8. The major polar lipids were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and two unidentified phospholipids. Several phenotypic characteristics served to differentiate the novel isolate from recognized members of the genus Terriglobus. On the basis of the evidence presented in this study, a novel species, Terriglobus aquaticus sp. nov. is proposed for strain 03SUJ4T (KCTC 23332T = JCM 17517T).

The genus Terriglobus belonging to the family Acidobacteria was created by Eichorst et al. (2007) to encompass moderate acidophilic soil bacteria. At the time of writing, the genus Terriglobus comprises two species with validly published names: Terriglobus roseus (Eichorst et al., 2007) and Terriglobus saanensis (Männistö et al., 2011). Members of the genus Terriglobus are Gram-negative, aerobic, non-fermentative, non-spore-forming, pigmented rods. In this study, we report the description of a novel species of the genus Terriglobus, which showed low 16S rRNA gene sequence similarity to all recognized species of this genus.

Strain 03SUJ4T was isolated from a freshwater sample collected from the intake tower of Juam artificial reservoir (35° 03′ 43″ N 127° 14′ 15″ E) located in the Jeonam, Republic of Korea in June 2011. The reservoir was constructed in 1992 to supply mainly drinking water, and its capacity was 350 Mt. At the time of water sampling, water quality was good for drinking. Isolation was achieved with the standard dilution plating technique using R2A agar (Becton Dickinson) at 25°C for 10 days. The isolate was routinely cultured on R2A agar and preserved at −80°C as a suspension in distilled water containing 20% (w/v) glycerol. Reference strains Terriglobus roseus DSM 18391T and T.
**Terriglobus aquaticus sp. nov.**

The 16S rRNA gene sequence of strain 03SUJ4\(^T\) was a continuous stretch of 1448 nt. Sequence comparisons with 16S rRNA gene sequences held in the GenBank database indicated that the isolate was closely related to the genus *Terriglobus*. Strain 03SUJ4\(^T\) showed the highest level of 16S rRNA gene sequence similarity to *Terriglobus roseus* DSM 18391\(^T\) (97.09 %) and *Terriglobus saanensis* SP1PR4\(^T\) (96.82 %). The neighbour-joining tree showed that the isolate formed a robust clade with members of the genus *Terriglobus*, but formed a distinct phyletic line. This relationship was confirmed also by the other two trees (Fig. 1).

The rpoB gene sequence of strain 03SUJ4\(^T\) was a continuous stretch of 882 nt. Sequence comparisons with rpoB gene sequences held in the GenBank database indicated that the isolate was closely related to the genus *Terriglobus*. Strain 03SUJ4\(^T\) showed low rpoB gene sequence similarities to *Terriglobus roseus* DSM 18391\(^T\) (87.46 %) and *Terriglobus saanensis* SP1PR4\(^T\) (83.07 %). These similarity values were lower than the recommended cut-off value of 97.7 % for separating species (Adékambi *et al.*, 2008).

PCR amplifications using repetitive primers were performed to fingerprint the genomes of strain 03SUJ4\(^T\), *T. roseus* DSM 18391\(^T\) and *T. saanensis* DSM 23119\(^T\) using TaKaRa LA Taq polymerase (Takara Bio) and primers

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REPR1-I, REP2-I, ERIC1R, ERIC2 and BOXA1R (Louws et al., 1994). The banding patterns from these PCR experiments clearly showed differences between the strain 03SUJ4T, T. roseus DSM 18391T and T. saanensis DSM 23119T (Fig. S1, available in IJSEM Online).

All the growth media were adjusted to pH 6 and test conditions were set at pH 6 unless otherwise stated. Cells of strain 03SUJ4T and reference strains grown on R2A agar at 25 °C for 5–7 days were used for the physiological and biochemical tests. Growth on various standard bacteriological media was tested by using nutrient agar (NA), plate-count agar (PCA), marine agar 2216 (MA), and tryptic soy agar (TSA) according to the instructions of manufacturer (Becton Dickinson). The Gram reaction test of cells grown on R2A agar at 25 °C for 3–14 days was performed using the bioMérieux Gram stain kit according to the manufacturer’s instructions and the Ryu non-staining KOH method (Powers, 1995). Motility was examined by observing the cells grown in wet mounts using phase-contrast microscopy (TMS-F; Nikon). Flagellation was determined by transmission electron microscopy (CM-20; Philips) using cells cultured for 5 days in R2A broth. Growth at various NaCl concentrations (0–10 %, w/v, using increments of 1.0 %) was investigated in R2A broth prepared according to the formula of R2A agar medium except that no NaCl was used. The pH range for growth was determined under aerobic conditions at 25 °C in R2A broth medium that was adjusted to pH 4 and 5 using glacial acetic acid, to pH 6 with MES, pH 7 with MOPS, pH 8 with HEPES, pH 9 with Tris, pH 10 with CHES, and pH 11 with CAPS. Growth was monitored by measuring the increase in OD600 using a UV/vis spectrophotometer (Ultraspec2100 pro; GE Healthcare Life Sciences). The optimal temperature and temperature range for growth was tested on R2A agar at 4 °C, 10–50 °C (at 5 °C intervals), 37 °C and 42 °C. Anaerobic growth was tested on R2A agar in a jar containing the AnaeroPack-Anaero (Mitsubishi Gas Chemical), which works as oxygen absorber and carbon dioxide generator, for up to 10 days. Catalase and oxidase activities were tested in 3 % (v/v) hydrogen peroxide solution (Hanker & Rabin, 1975) and 1 % (w/v) p-tetramethyl phenylenediamine (bioMérieux), respectively. Acid production from sugars was tested as described by Yamaguchi & Yokoe (2000). Nitrate reduction was determined with the disc diffusion method (Hanker & Rabin, 1975) and by spraying with appropriate detection reagents (Embley & Wait, 1994). For DNA G+C content calculations, the DNA sample was prepared in triplicate and determined by the thermal denaturation method of Marmur & Doty (1962). Respiratory quinones were extracted from 300 mg freeze-dried cells (strain 03SUJ4T and T. roseus DSM 18391T), purified according to the method of Minnikin et al. (1984) and analysed by using an ultra-performance liquid chromatography system (AQUITY UPLC; Waters).

Similar to other species of the genus Terriglobus, the fatty acid profile of strain 03SUJ4T contained mainly saturated and monounsaturated fatty acids: iso-C15:0 (44.5 %), or after flooding with adequate staining solutions (Smibert & Krieg, 1994). Hydrolysis of aesculin (0.01 %, aesculin, w/v; 0.05 %, ferric citrate, w/v), gelatin (12 %, w/v) and arginine dihydrolase activity was tested as described by Tindall et al. (2007) using R2A agar as the basal medium. Decomposition of tyrosine (0.5 %, w/v) was tested using R2A agar as the basal medium (Barrow & Feltham, 1993). DNase activity was determined with DNase test agar (Becton Dickinson). Some physiological characteristics and enzyme activities were determined using API 20NE and API ZYM kits (bioMérieux) and GN2 MicroPlate (Biolog) prepared according to the instructions of the manufacturers with the modification of incubation period, i.e. 7 days for API 20NE and GN2 MicroPlate. Antibiotic resistance was determined with the disc diffusion method (Bauer et al., 1966) using commercial antibiotic-impregnated discs (Becton Dickinson). After 10 days of incubation at 25 °C on R2A agar, the results were interpreted according to the guidelines set forth by the CLSI (2009).

For analyses of fatty acids and polar lipids, strain 03SUJ4T and the two reference strains were grown on R2A agar at 25 °C for 5 days (T. roseus DSM 18391T and T. saanensis DSM 23119T) and 7 days (strain 03SUJ4T). The fatty acid methyl esters were analysed by GLC (HP 6890; Hewlett Packard) and the MIDI aerobe method (Sherlock version 6.1) according to the instructions of the Microbial Identification System (Sasser, 1990; TSBA6 database). Polar lipids were analysed by using standard procedures (Minnikin et al., 1984). Extracted lipids were separated by two-dimensional TLC (Minnikin et al., 1977) and identified by spraying with appropriate detection reagents (Embley & Wait, 1994). For DNA G+C content calculations, the DNA sample was prepared in triplicate and determined by the thermal denaturation method of Marmur & Doty (1962). Respiratory quinones were extracted from 300 mg freeze-dried cells (strain 03SUJ4T and T. roseus DSM 18391T), purified according to the method of Minnikin et al. (1984) and analysed by using an ultra-performance liquid chromatography system (AQUITY UPLC; Waters).
Table 1. Phenotypic characteristics of strains 03SUJ4T, *Terriglobus roseus* DSM 18391T and *T. saanensis* DSM 23119T.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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</thead>
<tbody>
<tr>
<td><strong>Source</strong></td>
<td>Freshwater</td>
<td>Agricultural soil</td>
<td>Tundra soil</td>
</tr>
<tr>
<td>Pigment</td>
<td>Pink</td>
<td>Pink</td>
<td>Light pink</td>
</tr>
<tr>
<td>pH range for growth (optimum)</td>
<td>6–7 (6)</td>
<td>5–7 (6)*</td>
<td>4.5–7.5 (6)†</td>
</tr>
<tr>
<td>Hydrolysis of starch</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Urease activity</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Utilization (BIOLOG except otherwise indicated)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose (API 20NE)</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>L-Asparagine</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L-Aspartic acid</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>L-Ketoglutaric acid</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Maltose (API 20NE)</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Enzyme activity (API ZYM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Chymotrypsin</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Trypsin</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>63.2</td>
<td>59.8*</td>
<td>57.3†</td>
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</tbody>
</table>

*Data from Eichorst et al. (2007).
†Data from Männistö et al. (2011).

summed feature 3 (C_{16:1}ω7c/C_{16:1}ω6c: 22.5%), C_{16:0} (9.1%), C_{20:1}ω9c (9.0%) and C_{14:0} (6.8%). The small amount of iso-C_{13:0} and large amount of C_{20:1}ω9c in strain 03SUJ4T differed from the amounts in the two other species of the genus *Terriglobus*. A comparison of the cellular fatty acid composition of strain 03SUJ4T with related species is given in Table 2. Phosphatidylethanolamine and diphosphatidylglycerol were found as major polar lipids in all the members of the genus *Terriglobus*. In addition to the common polar lipids, strain 03SUJ4T contained phosphatidylethanolamine and two unidentified phospholipids (Fig. S4). The DNA G+C content of strain 03SUJ4T was 63.2 ± 0.1 mol% (mean ± SD of three determinations). The only isoprenoid quinone of strain 03SUJ4T and *T. roseus* DSM 18391T was menaquinone with eight isoprene units (MK-8).

On the basis of data from the polyphasic study presented here, it is evident that strain 03SUJ4T represents a novel species in the genus *Terriglobus*, for which the name *Terriglobus aquaticus* sp. nov. is proposed.

### Description of *Terriglobus aquaticus* sp. nov.

*Terriglobus aquaticus* (aqua’ticus. L. masc. adj. aquaticus living, growing, or found in or by water, aquatic).

Cells are Gram-reaction-negative, aerobic, oxidase-negative, catalase-positive and non-motile rods (0.6–0.8 x 1.4–2.0 μm in size). Cells grow best on media such as R2A agar;
not on MA, NA, PCA and TSA. Colonies on R2A agar are convex, circular, smooth, opaque with entire margins, pink-pigmented and approximately 1.0 mm in diameter after 7 days at 25 °C (pH 6). Growth occurs at NaCl concentrations of up to 1.0% (w/v). Growth occurs at pH 6–7 (optimum, pH 6) and at 15–30 °C (optimum, 25 °C). Nitrate is not reduced to nitrite. Negative result for arginine dihydrolase activity. Does not produce H₂S or indole. Aesculin, Tween 20, Tween 80 and urea are hydrolysed, but casein, chitin, DNA, gelatin and starch are not. Does not decompose tyrosine. Positive reactions are obtained for the assimilation of N-acetylglucosamine and glucose. Negative reactions are obtained for the assimilation (API 20NE) of adipate, arabinose, caprate, citrate, gluconate, malate, maltose, mannitol, mannose and phenylacetate. In the API ZYM gallery, there are positive reactions for N-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, z-fucosidase, z- and β-galactosidase, z- and β-glucosidase, β-glucuronidase, z-mannosidase and naphthol-AS-BI-phosphohydrolase activities; weakly positive reaction for leucine arylamidase activity; and negative reactions are for z-chymotrypsin, cysteine arylamidase, esterase (C4), esterase lipase (C8), lipase (C14), trypsin and valine arylamidase activities. In the GN2 Microplate (Biolog) system, the following substrates are assimilated: l-arabinose, l-asparagine, l-aspartic acid, d-fructose, d-glucose, l-glutamic acid, z-ketoglutaric acid, DL-lactic acid, succinamic acid and succinic acid; the following substrates are weakly assimilated: dextrin, cellobiose, maltose, z-lactose and trehalose; all other substrates are not assimilated. Cells are sensitive to (μg per disc, unless otherwise indicated): amoxicillin/clavulanic acid (30), meropenem (10), cefotaxin (30), doxycycline (30), tetracycline (30) and vancomycin (30), but resistant to amikacin (30), ampicillin (10), ampicillin/subbactam (20), aztreonam (30), cefaclor (30), ceftriaxone (30), ceftazidime (30), cephazolin (30), chloramphenicol (30), ciprofloxacin (5), erythromycin (15), gentamicin (10), kanamycin (30), levofloxacin (5), nalidixic acid (30), penicillin (10 IU), polymyxin B (300 IU), streptomycin (10) and sulphamethazine (25). Major fatty acids are iso-C₁⁵:₀ (6.5%), summed feature 3 (C₁₆:₁ω7c/C₁₆:₁ω6c), C₁₆:₀, C₂₀:₁ω9c and C₁₄:₀; complete fatty acid composition is given in Table 2. Major polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and two unidentified phospholipids. The predominant menaquinone is MK-8.

The type strain, 03SUJ4T (=KCTC 23332T=JCM 17517T), was isolated from the freshwater of Juam reservoir, Republic of Korea. The DNA G+C content of the type strain is 63.2 ± 0.1 mol% (mean ± SD of three determinations).

Acknowledgements

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (no. 2010-006681). This work was also supported by the National Research Foundation of Korea grant funded by the Korea Government (no. 2012R1A2A2A01015706), and the project on survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR) under the Ministry of Environment, Republic of Korea.

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