Stakelama sediminis sp. nov., isolated from tidal flat sediment

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A novel bacterial strain designated CJ70T was isolated from tidal flat sediment in Korea. A polyphasic approach was used to identify this strain taxonomically. The isolate was Gram-stain-negative, strictly aerobic, yellow-pigmented, rod-shaped and non-motile. Phylogenetic analysis of the 16S rRNA gene sequence revealed that strain CJ70T was related most closely to Stakelama pacifica JLT832T with 95.7% similarity and formed an independent phyletic line from recognized species of the genus Sphingomonas, comprising a clade with Stakelama pacifica, which is the only recognized species of the genus Stakelama. The predominant cellular fatty acids of strain CJ70T were C18:1ω7c (60.0%), C16:0 (21.2%) and C14:0 2-OH (5.8%). The major isoprenoid quinone was ubiquinone-10. The G+C content of the genomic DNA was 61.4 mol%. The results obtained from this study suggested that strain CJ70T represents a novel species of the genus Stakelama, for which the name Stakelama sediminis sp. nov. is proposed. The type strain is CJ70T (=KACC 16559T =JCM 18079T).

The family Sphingomonadaceae was first proposed by Kosako et al. (2000). The original genus Sphingomonas was first established by Yabuuchi et al. (1990) and was subsequently divided into four genera, Sphingomonas, Sphingobium, Novosphingobium and Sphingopyxis, based on phylogenetic and chemotaxonomic differences (Takeuchi et al., 2001). Recently, Chen et al. (2010) proposed a new genus, Stakelama, within the family Sphingomonadaceae.

A bacterial strain designated CJ70T was isolated from tidal flat sediment in Korea. Isolation was carried out using a standard dilution plating method on R2A agar (Conda) at 30 °C. The isolate was routinely cultured on R2A agar and maintained at −80 °C as a suspension in R2A broth supplemented with glycerol (20%, v/v). Growth was tested on various media, i.e. R2A agar, marine agar (MA; Difco), Luria–Bertani (LB; Conda) agar, nutrient agar (NA; Difco) and trypticase soy agar (TSA; Difco). Growth occurred on R2A agar, MA, LB agar and NA but not on TSA.

The 16S rRNA gene was amplified from a single colony by PCR using AccuPower PCR Premix (Bioneer) and primers 27F and 1492R (Lane, 1991). The PCR product was purified by using the AccuPrep PCR purification kit (Bioneer) and sequencing of the 16S rRNA gene was performed with an Applied Biosystems automatic sequencer (ABI 3730 XL) at Macrogen. Pairwise 16S rRNA gene sequence similarity comparison was achieved by using the EzTaxon e-server (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012). Phylogenetic analyses were performed by the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods. Evolutionary distance matrices for the neighbour-joining method were generated according to the model of Jukes & Cantor (1969). The neighbour-joining tree topology was evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. The phylogenetic tree was constructed using the MEGA5 software (Tamura et al., 2011). Initial similarity analyses indicated that strain CJ70T formed a distinct phylogenetic lineage within the family Sphingomonadaceae (Fig. 1). Strain CJ70T exhibited the highest 16S rRNA gene sequence similarity to Stakelama pacifica JLT832T (95.7%), followed by Sphingomonas desicabilis CP1D5T (95.6%).

Gram-staining was performed as described by Smibert & Krieg (1994). Anaerobic growth test was carried out using a BD GasPak pouch (Becton Dickinson) on R2A agar at 30 °C. Growth was tested at different temperatures (4–45 °C) on R2A agar and pH (4.0–11.0, adjusted by HCl or NaOH) in R2A broth. Salt tolerance test was carried out in R2A broth supplemented with 0–9% (w/v) NaCl (1% intervals). Motility was tested on semisolid R2A media containing 0.4% agar (Smibert & Krieg, 1994). Growth occurred at 20–37 °C and at pH 5.0–10.0. Optimal growth temperature and pH of strain CJ70T were 30 °C and pH 6. Growth occurred in the presence of 0–5% NaCl with an optimum of 0–1%. Catalase activity was evaluated by assessing the production of O2 bubbles in a 3% (v/v) aqueous H2O2 solution. Oxidase activity was tested by assessing the oxidation of 1% (w/v) tetramethyl-p-phenylenediamine (Merck). Citrate utilization...
was tested by using Simmons’ citrate agar. Hydrolysis of starch (1%, w/v) and casein (10%, w/v, skimmed milk) was tested on R2A agar as the basal medium. Carbon source utilization, enzyme activities and other physiological properties were determined by using API 50CH, API ZYM, API 20E and API 20NE galleries (bioMe`rieux), and the GN2 MicroPlate system (Biolog) according to the instructions of the manufacturers. The results of biochemical and physiological properties are presented in Table 1 and in the species description.

Chemotaxonomic characteristics were determined from cells grown on R2A agar at 30°C for 3 days. Isoprenoid quinones were extracted and purified as described by Minnikin et al. (1984), and analysed by HPLC (Varian) as described by Collins (1985). Determination of DNA G+C content was carried out in triplicate by HPLC analysis of genomic DNA as described by Mesbah et al. (1989), using a reversed-phase column (Supelcosil LC-18 S; Supelco). Fatty acid methyl ester analysis was done by GLC according to the instructions of the Microbial Identification System (MIDI) version 6.1 and the RTSBA6 6.10 database (Microbial ID). The predominant cellular fatty acids of strain CJ70T were C18:1ω7c (60.0%), C16:0 (21.2%) and C14:0 2-OH (5.8%). The fatty acid profile of strain CJ70T and related species of Sphingomonadaceae are presented in Table 2. Strain CJ70T displayed a profile that was more similar to Stakelama pacifica JLT832T than to Sphingomonas desiccabilis CP1DT, although all three strains shared major fatty acids. The presence of C19:1ω8c distinguished strain CJ70T from related species. The major respiratory quinone was ubiquinone-10. Polar lipids were extracted from freeze-dried cells by the method of Tindall (1990a, b) and separated by two-dimensional silica-gel TLC. Total lipids and functional

Fig. 1. Neighbour-joining phylogenetic tree, based on almost-complete 16S rRNA gene sequences, showing the relationships between strain CJ70T and representative members of the family Sphingomonadaceae. Bootstrap percentages of greater than 50% (based on neighbour-joining analyses of 1000 resampled datasets) are shown at nodes. Solid circles indicate that the corresponding nodes are also recovered in the tree generated by the maximum-likelihood algorithm. Rhodobacter sphaeroides ATH 2.4.1T (CP000143) was used as an outgroup. Bar, 0.01 nt substitutions per position.
Table 1. Differential characteristics of strain CJ70T and related members of the family Sphingomonadaceae

Strains: 1, Stakelama sediminis sp. nov. CJ70T; 2, Stakelama pacifica JLT832T; 3, Sphingomonas desiccabilis CP1D. All data were obtained from this study except DNA G+C content of Stakelama pacifica JLT832T (Chen et al., 2010). +, Positive; -, negative.

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>3</th>
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<tbody>
<tr>
<td>Motility</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Optimum growth pH</td>
<td>6.0</td>
<td>7.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>β-Galactosidase (PNPG)</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Acid production from:

- L-Xylose: − − +
- Methyl β-D-xlyopyranoside: − − +
- D-Galactose: − + +
- D-Fructose: − − −
- D-Mannose: − + +
- L-Sorbose: − − −
- L-Rhamnose: − + +
- Methyl α-D-mannopyranoside: − − −
- Methyl α-D-glucopyranoside: − + +
- D-Lactose: − + +
- Melibiose: − + +
- Sucrose: − + +
- Melezitose: − + +
- Raffinose: − − −
- Starch: + - −
- D-Lyxoros: + − −
- Potassium gluconate: − − −

Utilization as a sole carbon source:

- Dextrin: + − −
- Glycogen: − + −
- Tween 80: + − −
- D-Fructose: + + −
- α-D-Lactose: − + +
- D-Mannose: + + +
- Melibiose: − + +
- Methyl β-D-glucose: + + +
- L-Rhamnose: − + −
- Sucrose: − + +
- Turanose: + + −
- Succinate acid monomethyl ester: + + −
- D-Gluconic acid: − + −
- D-Glucuronic acid: + − −
- α-Ketobutyric acid: − − −
- DL-Lactic acid: + − −
- Succinic acid: + − −
- Bromosuccinic acid: + − −
- α-Alanine: + + −
- l-Alanine: − + −
- l-Alanyl glycine: + − −
- l-Glutamic acid: + − −

DNA G+C content (mol%)


<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Glycyl l-glutamic acid</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Hydroxy-l-proline</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>l-Proline</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>l-Serine</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>61.4</td>
<td>66.0</td>
<td>64.0</td>
</tr>
</tbody>
</table>

groups were detected by using molybedosphoric acid, molybdenum blue spray, ninhydrin and z-naphthol as described previously (Minnikin et al. 1984). Chromatograms of the total lipids of strain CJ70T and related members of the family Sphingomonadaceae are shown in Fig. S1 (available in IJSEM Online). The results showed that all strains have similar total lipid profiles, including sphingoglycolipid which is distinctive amongst the family Sphingomonadaceae. However, the presence of a sphingoglycolipid (SGL2), an unidentified aminophospholipid (APL) and an unidentified lipid (L2) in the polar lipid profile of strain CJ70T distinguished it from those of related species (Fig. S1). The pigments of strain CJ70T were analysed spectrophotometrically as described by Ohta et al. (2004). The acetone extract showed peaks at 455 and 485 nm.

As shown by the 16S rRNA gene sequence analysis, strain CJ70T formed an independent phyletic line with recognized species of the genus Sphingomonas, comprising an identical clade with the genus Stakelama. Based on a polyphasic taxonomic approach, the low level of 16S rRNA gene sequence similarity and several phenotypic characteristics, including the distinctive polar lipid profile, strain CJ70T can be differentiated from Stakelama pacifica JLT832T, the only recognized species of the genus Stakelama. Therefore,

Table 2. Cellular fatty acid composition (% of strain CJ70T and the type strains of related organisms in the family Sphingomonadaceae

Strains: 1, CJ70T; 2, Stakelama pacifica JLT832T; 3, Sphingomonas desiccabilis CP1D. All data were obtained from this study. Cellular fatty acids were determined from cells grown on R2A agar at 30 °C for 3 days. tr, Trace amounts (<1% of the total). ND, Not detected.

<table>
<thead>
<tr>
<th>Fatty acid</th>
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<tbody>
<tr>
<td>C16 : 0</td>
<td>21.2</td>
<td>11.2</td>
<td>14.1</td>
</tr>
<tr>
<td>C17 : 0:06</td>
<td>2.5</td>
<td>1.0</td>
<td>7.9</td>
</tr>
<tr>
<td>C17 : 0:08</td>
<td>tr</td>
<td>tr</td>
<td>1.2</td>
</tr>
<tr>
<td>C18 : 0:07</td>
<td>60.0</td>
<td>71.7</td>
<td>53.3</td>
</tr>
<tr>
<td>C19 : 1: cyclo 0:08</td>
<td>3.9</td>
<td>tr</td>
<td>tr</td>
</tr>
<tr>
<td>11-Methyl C18 : 1:07c</td>
<td>tr</td>
<td>ND</td>
<td>3.9</td>
</tr>
<tr>
<td>C14 : 0 2-OH</td>
<td>5.8</td>
<td>9.1</td>
<td>7.0</td>
</tr>
<tr>
<td>C15 : 0 2-OH</td>
<td>tr</td>
<td>tr</td>
<td>1.6</td>
</tr>
<tr>
<td>Summed feature 3*</td>
<td>1.0</td>
<td>1.5</td>
<td>5.6</td>
</tr>
</tbody>
</table>

*Summed feature 3 contains C16 : 0:06 and/or C16 : 1:07c.
we propose that strain CJ70T be classified as the type strain of a novel species in the genus Stakelama, for which the name Stakelama sediminis sp. nov. is proposed.

**Description of Stakelama sediminis sp. nov.**

Stakelama sediminis (se.di.mi.nis. L. gen. n. sediminis of sediment, referring to the source of isolation of the type strain).

Cells are Gram-stain-negative, rod-shaped and non-motile. Growth occurs only under aerobic conditions. Colonies on R2A agar are circular, small, smooth and golden yellow. Grows at 20–37 °C (optimum 30 °C) and pH 5.0–10.0 (optimum pH 6). Growth occurs in the presence of 0–5 % NaCl (optimum 0–1 %). Positive for catalase and oxidase. Citrate is not utilized. Nitrate is reduced to nitrite. Indole and H2S are not produced. Aesculin is hydrolysed but casein, starch and gelatin are not. Acid is produced from the following substrates: L-arabinose, D-xylose, D-glucose, N-acetylglucosamine, amygdalin, arbutin, aescin, ferric citrate, salicin, cellobiose, maltose, trehalose, starch, gentiobiose, turanose, D-fucose and potassium 5-ketogluconate. Acid is not produced from the following substrates: glycerol, erythritol, D-ribose, L-xyllose, D-adonitol, methyl β-D-xylpyranoside, D-galactose, D-fructose, D-mannose, L-sorbitose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-glycopyranoside, D-lactose, melibiose, sucrose, inulin, melezitose, raffinose, glycojen, xylitol, D-lyxose, D-tagatose, L-fucose, D-arabitol, L-arabitol, potassium glutonate and potassium 2-ketogluconate. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine aminopeptidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, β-glucuronidase and urease activities are present but lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, α-mannosidase and α-fucosidase activities are absent. The following substrates are utilized as a sole carbon source: Tween 80, L-arabinose, cellobiose, D-fructose, D-galactose, gentiobiose, α-D-glucose, maltose, D-mannose, methyl β-D-glucoside, trehalose, turanose, pyruvic acid methyl ester, succinic acid monomethyl ester, D-glucuronic acid, β-hydroxybutyric acid, DL-lactic acid, bromosuccinamic acid, L-α-laninamidase, L-alanine, L-α-lalyn glycine, L-glutamic acid, glycol L-glutamic acid, L-proline, L-α-serine, α-D-glucose 1-phosphate and D-glucose 5-phosphate. The following substrates are not utilized as a sole carbon source: Tween 40, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, D-arabitol, i-erythritol, L-fucose, myo-inositol, α-D-lactose, lactulose, D-mannitol, melibiose, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, xylitol, acetic acid, cis-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, α-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylactic acid, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, succinimide acid, glucuronamide, D-alanine, L-asparagine, L-aspartic acid, glycy l L-aspartic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-pyrogulamic acid, D-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminooethanol, 2,3-butanediol, glycerol and DL-α-glycerol phosphate. The major isoprenoid quinone is ubiquinone-10. The predominant cellular fatty acids are C18:1ω7c, C16:0 and C14:0 2-OH. Acetone-soluble pigment is characterized by λ_{max} at 455 and 485 nm.

The type strain, CJ70T (=KACC 16559^T=JCM 18079^T), was isolated from tidal flat sediment in Korea. The DNA G+C content of the type strain is 61.4 mol%.

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**References**


