Hansschlegelia zhihuaiae sp. nov., isolated from a polluted farmland soil

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A novel Gram-negative, aerobic, coccoid-shaped strain designated S 113T was isolated from a polluted-soil sample collected in Jiangsu Province, China. A polyphasic taxonomic study including phylogenetic analysis based on the 16S rRNA gene sequence and determination of phenotypic characteristics was performed on the new isolate. The highest 16S rRNA gene sequence similarity was 96.8%, with Hansschlegelia plantiphila S1T. The predominant respiratory quinone was ubiquinone 10 (Q-10). The major fatty acids were C18:1ω7c and C16:0. The G+C content of the DNA was about 65.7 mol%. DNA–DNA hybridization experiments showed 44.9% relatedness for strain S 113T with its closest relative, H. plantiphila NCIMB 14035T. The dominant phospholipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidyldimonomethylethanolamine and phosphatidylcholine. The results of our polyphasic taxonomic analysis indicate that strain S 113T represents a novel species within the genus Hansschlegelia, for which the name Hansschlegelia zhihuaiae sp. nov. is proposed. The type strain is S 113T (=DSM 18984T =CCTCC AB 206143T =KCTC 12880T).

In the course of an investigation of the bacterial community in a farmland soil that was seriously contaminated by sulfonylurea herbicides in Jiangsu province, China, strain S 113T was isolated and subjected to a polyphasic taxonomic analysis. We propose here that it represents a novel species in the genus Hansschlegelia.

Strain S 113T was isolated using the medium and methods described by Huang et al. (2007) from a sample of polluted farmland soil. The pure culture was preserved in a 25% (v/v) glycerol solution in distilled water at −80°C.

The morphological characteristics of the strain were observed by transmission electron microscopy (model H-7650; Hitachi) after incubation for 3 days at 30°C on YTC agar (0.3% yeast, 0.5% tryptone and 0.001% CaCl2). Electron microscopy preparations were performed as described by Yoon et al. (2003). Growth was tested in liquid mineral medium K in which methanol or methylamine was added at a concentration of 0.5% (v/v) and formaldehyde was added at 0.05% (v/v), as described by Doronina et al. (1998). The method of Yamaguchi & Yokoe (2000) was followed to determine acid production from carbohydrates. Utilization of sole carbon sources was performed as described by Zhou et al. (2007). Growth was tested at 5, 10, 25, 30, 35 and 40°C in YTC broth and pH tolerance was tested using modified YTC broth adjusted to pH 3.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0 and 9.0. Antibiotic susceptibility was examined by placing antibiotic

The family Methylocystaceae was proposed by Bowman (2005, 2006) to harbour the group II methanotrophs, and the type genus is Methylocystis (Bowman et al., 1993). At the time of writing, the family contains six established genera: Allobacter, Methylocystis, Methyloplana, Methylosinus, Pleomorphomonas and Terasakiella. Most members of the family have been isolated from soil, groundwater or other complex environmental samples (Bowman et al., 1993; Doronina et al., 1998, 2001; Satomi et al., 2002; Dedysz et al., 2007). The genus Hansschlegelia, which was described by Ivanova et al. (2007), is also a member of family Methylocystaceae; the name has recently been validly published (Ivanova et al., 2010). Many methanotrophic bacteria represent critical links in the global carbon cycle, act as N2 fixers, degrade a wide array of organic contaminants and display many enzymic functions (Gulledge et al., 2001). 16S rRNA gene sequence analysis has shown that members of the family Methylocystaceae are able to utilize methanol as a carbon source and exhibit the serine or ribulose-bisphosphate pathway for formaldehyde assimilation (Gallego et al., 2005).

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain S 113T is DQ916067.

A supplementary table and a supplementary figure are available with the online version of this paper.
discs on YTC plates as described by Zhou et al. (2007). Gram-staining and other physiological characteristics were assessed according to the methods of Doronina et al. (1998) and Gerhardt et al. (1994). API 20NE strips (bioMérieux) were used to investigate additional biochemical features.

Biomass for molecular systematic and chemotaxonomic tests was obtained using YTC broth or methanol medium K by incubation at 30 °C for 10 days in shaking flasks (about 180 r.p.m.). Respiratory quinones were extracted from lyophilized cells and the sample was purified and analysed by HPLC using the procedures reported by Hu et al. (2001). Cellular fatty acid composition was determined as described by Sasser (1990) using the Microbial Identification System (MID, Inc.). Polar lipid analyses were carried out as described by Minnikin et al. (1984). The predominant respiratory quinone of strain S 113T was ubiquinone 10 (Q-10). The major fatty acids were C18:1ω7c (77 %) and C16:0 (14.3 %); the detailed fatty acid profile is displayed in Supplementary Table S1, available in IJSEM Online. The dominant phospholipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine and phosphatidylcholine.

Genomic DNA for base composition analysis was prepared following the procedure of Marmur (1961). DNA G+C content was determined by reversed-phase HPLC according to Tamaoka & Komagata (1984); the DNA G+C content of S 113T was 65.7 mol\%. DNA–DNA relatedness was determined by the initial renaturation rate method in 2× SSC (De Ley, 1970). Genomic DNA–DNA relatedness between S 113T and Hansschlegelia plantiphila NCIMB 14035T was 44.9 %, which is below the value of 70 % recommended for species definition (Wayne et al., 1987).

Phylogenetic analysis, genomic DNA–DNA hybridization and chemotaxonomy support the inclusion of the new isolate as a member of genus Hansschlegelia. Strain S 113T can be differentiated easily from the only species currently classified in this genus, H. plantiphila, by some important phenotypic properties such as motility, catalase activity, H2S production and NaCl tolerance (Table 1). The type strain of the two species also displayed significant differences in fatty acid and polar lipid compositions (Table 1 and Supplementary Table S1). On the basis of the phylogenetic and chemotaxonomic evidence together with the phenotypic characteristics presented in this study, the newly isolated strain S 113T is assigned to a novel species

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of strain S 113T compared with members of representative genera of the family Methylocystaceae and some non-methylotrophs in the phylum Proteobacteria. Numbers at nodes indicate percentage bootstrap values from 1000 resamplings; values ≥50 % are shown. Bar, 0.02 substitutions per nucleotide position. The maximum-parsimony tree showed essentially the same topology (not shown).
in the genus Hansschlegelia, for which the name Hansschlegelia zhihuaiae sp. nov. is proposed.

**Description of Hansschlegelia zhihuaiae sp. nov.**

Hansschlegelia zhihuaiae [zhi.hua.i"ae. N.L. fem. gen. n. zhihuaiae named after Zhihua Wu (1935–2001), a Chinese pedologist who devoted herself to the study of soil biology].

Cells are Gram-negative, non-spore-forming, aerobic coccoids (0.6–0.9 × 1.0–1.3 μm) (Supplementary Fig. S1). Colonies are circular and white on YTC agar, 1.0–2.0 mm in diameter after 3 days of cultivation at 30 °C. Moderately sensitive to NaCl (<0.5 %). Shows good growth in liquid mineral medium K with 0.5 % (v/v) methanol but not with formaldehyde or methylamine. Good growth occurs at 25–30 °C and at pH 6.5–7.5, but not above pH 9.0 or below pH 6.0. Starch is hydrolysed; casein, cellulose, DNA, gelatin and Tween 80 are not hydrolysed. Acid is produced from adonitol, melezitose and sucrose, but not from D-arabinose, cellobiose, dulcitol, D-fructose, L-fucose, D-galactose, D-glucose, inositol, lactose, maltose, D-mannitol, melibiose, raffinose, L-rhamnose, D-ribose, L-sorbose, D-sorbitol, trehalose or D-xylose. D-Arabinose, glucosamine, pyruvate and starch are utilized. N-Acetylgalactosamine, acetate, asaccharose, cellobiose, dulcitol, dextrin, D-erythrose, D-fructose, D-galactose, gelatin, D-glucose, inulin, inositol, lactose, D-mannose, maltose, melibiose, D-mannitol, mucic acid, malonate, raffinose, D-ribose, ribulose, D-sorbitol, D-sorbitose, salicin, trehalose, Tween 80, turanose, trartrate, xylose and D-xylose are not utilized. Weak oxidase and urease activities are present. Arginine decarboxylase, arginine dihydrolase, catalase, DNase, β-galactosidase, lysine decarboxylase, methyl α-D-glucosidase, ornithine decarboxylase and phenylalanine decarboxylase activities are absent. H₂S is not produced (triple sugar-iron reaction). Reduces nitrates to nitrites. Methyl red and Voges–Proskauer tests are negative. KCN (0.0075 %) is not tolerated. Susceptible to (mg per disc unless indicated) chloramphenicol (30), doxycycline (30), gentamicin (10), kanamycin (30), neomycin (30), polymyxin B (100 U), rifampicin (5), streptomycin (10) and tetracycline (30); resistant to ampicillin (10), erythromycin (15), lincomycin (15), penicillin G (10 U) and vancomycin (30). The major fatty acids are C₁₈₋₁₀7c and C₁₆₋₀. The dominant phospholipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and phosphatidylglycerol. The DNA G+C content of the type strain is 65.7 mol% (HPLC).

The type strain, S 113 T (=DSM 18984 T =CCMT AB 206143 T =KCTC 12880 T), was isolated from the surface layer of a polluted farmland soil from Jiangsu province, China.

**Acknowledgements**

The authors are grateful to the Identification Service of the DSMZ (Braunschweig, Germany) and Dr B. J. Tindall (DSMZ) for polar lipid analyses. This work was supported by grants from the National Natural Science Foundation of China (30830001, 30900044), the Fund for the Doctoral Program of Higher Education (2009009

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**Table 1.** Phenotypic characteristics that differentiate strain S 113 T from its phylogenetically closest neighbour, H. plantiphila NCIMB 14035 T

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain S 113 T</th>
<th>H. plantiphila NCIMB 14035 T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell morphology</td>
<td>Coccoid</td>
<td>Short rod</td>
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<tr>
<td>Flagella</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>NaCl tolerance</td>
<td>&lt;0.5 %</td>
<td>&lt;2 %</td>
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<tr>
<td>H₂S production</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Catalase</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Oxidase</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>Urease</td>
<td>(+)</td>
<td>+</td>
</tr>
<tr>
<td>Carbon source utilization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinate</td>
<td>−</td>
<td>(+)</td>
</tr>
<tr>
<td>Formaldehyde</td>
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<td>+</td>
</tr>
<tr>
<td>Methylamine</td>
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<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Major fatty acids</td>
<td>C₁₈₋₁₀7c, C₁₆₋₀</td>
<td>C₁₆₋₀, C₁₉₋₀ cyclo, C₁₈₋₁₀7c</td>
</tr>
<tr>
<td>Dominant phospholipids*</td>
<td>DPG, PC, PE, PME, PG</td>
<td>DPG, PC, PE</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>65.7</td>
<td>68.5</td>
</tr>
</tbody>
</table>

*DPG, Diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PME, phosphatidylmonomethylethanolamine.
References


