Mucilaginibacter antarcticus sp. nov., isolated from tundra soil

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The novel, pale yellow bacterial strain, designated S14-88T, was isolated from a tundra soil near Antarctic Peninsula, South Shetland Islands, and its taxonomic position was investigated by a genotypic and phenotypic analysis. Cells were facultatively anaerobic, Gram-stain-negative, non-motile and rod-shaped. Growth occurred at 4–28 °C (optimum at 15 °C), at pH 7.0–8.0 (optimum at 7.0) and with 0–0.6 % (w/v) NaCl (optimum, no NaCl). Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain S14-88T formed a lineage within the genus Mucilaginibacter. The 16S rRNA gene sequence similarity between strain S14-88T and the type strains of related species ranged from 92.2 to 96.5 %, and the 16S rRNA gene sequence of S14-88T showed highest similarity of 96.5 % to Mucilaginibacter soyangensis HME6664T. The major cellular fatty acids of strain S14-88T were iso-C15:0 and summed feature 3 (C16:1ω7c and/or C16:1ω6c). The major respiratory quinone was menaquinone MK-7, and the main polar lipid was phosphatidylethanolamine. The DNA G+C content of strain S14-88T was 42.3 mol%. On the basis of the evidence presented in this study, strain S14-88T is considered to represent a novel species of the genus Mucilaginibacter, for which the name Mucilaginibacter antarcticus sp. nov. is proposed. The type strain is S14-88T (=CCTCC AB 2015321T=KCTC 52232T).

The genus Mucilaginibacter, which belongs to the family Sphingobacteriaceae, accommodates Gram-negative, chemooorganotrophic and aerobic or facultatively anaerobic bacteria (Urai et al., 2008). It contains menaquinone-7 (MK-7) as the major respiratory quinone and iso-C15:0 as the major fatty acid. The DNA G+C content of this genus ranges from 40.8 to 47.0 mol% (Baik et al., 2010; Joung et al., 2014a). At the time of writing, the genus Mucilaginibacter comprises at least 38 species with validly published names, including nine recently described species: Mucilaginibacter aquae dulcis (Joung et al., 2015), M. auburnensis (Kämpfer et al., 2014), M. defluvii (Hwang et al., 2014), M. flavus (Joung et al., 2014b), M. gotjawali (Lee et al., 2015), M. koreensis (Park et al., 2014), M. pineti (Paiva et al., 2014), M. polytrichastri (Chen et al., 2014) and M. soyangensis (Joung et al., 2014a). In this study, an Antarctic bacterial isolate was subjected to a polyphasic analysis and identified as a novel member of the genus Mucilaginibacter.

A bacterial strain, designated S14-88T, was isolated from a soil sample collected from a low-altitude tundra in the South Shetland Islands of Antarctica (62° 22' 34" S 59° 42' 34" W). Isolation was carried out by the standard dilution plating method on R2A agar (BD) at 4 °C; single colonies were purified by the streak plate method, and transferred onto fresh plates of R2A agar followed by further incubation. Strain S14-88T was routinely cultured for 5 days on R2A agar at 15 °C and stored by lyophilization. Mucilaginibacter ximonomensis CCTCC AB207094T, Mucilaginibacter dorajii CCTCC AB2011078T and Mucilaginibacter paludis CCTCC AB2010405T were obtained from the China Center for Type Culture Collection (CCTCC), and Mucilaginibacter soyangensis KCTC 23261T was obtained from the Korean Collection for Type Cultures (KCTC). The four strains were used as reference strains based on 16S rRNA gene sequence similarities and phylogenetic analysis. The reference strains were routinely cultured on R2A agar at 15 °C for 5 days.

The 16S rRNA gene of strain S14-88T was amplified by PCR with the universal primers 27F (5'-GAGTTTGATCCTGGA
The 16S rRNA gene sequence similarity values between strain S14-88 and other *Mucilaginibacter* species ranged from 96.5 to 92.2 % (<97 %). Strain S14-88 showed highest similarity to *M. soyangensis* HME6664T (96.5 %), and the next highest similarity to *M. soyangensis* 3C003531T (96.3 %). Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain S14-88 was affiliated to the genus *Mucilaginibacter* in the family *Sphingobacteriaceae*, and formed a robust clade with *M. ximonensis* XM-003 in the neighbour-joining tree, maximum-likelihood tree and minimum-evolution tree (Fig. 1). Based on data from the phylogenetic analysis, strain S14-88 could not be assigned to any recognized species, which suggested that strain S14-88 could be regarded as representing a novel species in the genus *Mucilaginibacter*.

**Fig. 1.** Neighbour-joining tree based on 16S rRNA gene sequences, showing the relationship between strain S14-88 and members of the genus *Mucilaginibacter* and neighbouring genera. Numbers at nodes are percentages of bootstrap support (1000 replications, ≥70 %) based on neighbour-joining analyses. The sequence of *Flexibacterium aquatile* LMG 4008T was used as an outgroup. Bar, 0.02 substitutions per nucleotide position. Filled circles indicate branches also recovered in trees generated with the maximum-parsimony and maximum-likelihood methods.
culturing the isolate on R2A agar supplemented with KNO₃ (0.1 %) in a GasPak (BBL) jar for 1 month. Oxidase activity was determined via the oxidation of 1 % (w/v) tetramethyl-p-phenylenediamine (Fluka). Catalase activity was tested based on bubble production after the application of a 3 % (v/v) hydrogen peroxide (Hushi) solution. The ability to hydrolyse casein and l-tyrosine was accessed after culture on R2A agar plates supplemented with 1 % (w/v) colloidal chitin, 3 % (w/v) casein and 0.5 % (w/v) l-tyrosine. Hydrolysis of starch (1.0 %, w/v) was tested using R2A agar as the basal medium, after which the plate was stained with iodine solution. For CM-cellulose hydrolysis activity, the isolate was cultured on an R2A agar plate containing 1 % (w/v) CM-cellulose, after which the plate was stained with a solution of Congo red. A clear zone around bacterial colonies indicated positive activity. Other physiological and biochemical characteristics of the isolate were tested using the API kits (API ZYM, API 20E, API 20NE, API 50CH; bioMérieux) and the GN2 MicroPlate (Biolog) according to the manufacturer’s instructions. Antibiotic sensitivity was tested by using the disc diffusion method (Doetsch et al., 1981) with discs impregnated with the following antibiotics (concentration per disc): chloramphenicol (30 µg), kanamycin (30 µg), vancomycin (30 µg), penicillin (10 µg), neomycin (30 µg), amikacin (30 µg), cephalixin (30 µg), tetracycline (30 µg), ampicillin (10 µg), gentamicin (10 µg), polymixin B (300 IU) and ampicillin/sublactam (10 µg). Any sign of growth inhibition was determined as sensitivity to that antibiotic. Resistance to an antimicrobial drug was indicated if no inhibition zone was observed.

Ultrathin sections of cells revealed the presence of numerous outer-membrane vesicles which was also detected in M. paludis DSM 18603T (Pankratov et al., 2007) (Fig. S1, available in the online Supplementary Material). Flexirubin-type pigment was present. Other phenotypic characteristics of strain S14-88T are given in the species description and Table 1.

The DNA G+C content of strain S14-88T was determined by using reversed-phase HPLC (UltiMate 3000; Dionex) as described by Mesbah et al. (1989). The DNA G+C content of strain S14-88T was 42.3 mol%, which is in the range reported for the genus Mucilaginibacter (40.8–47.0 mol%).

For the analysis of cellular fatty acids, strain S14-88T and the four reference strains, M. dorajii CCTCC AB2011078T, M. soyangensis KCTC 23261T, M. paludis CCTCC AB2010405T and M. ximonensis CCTCC AB207094T, were grown on R2A agar plates at 15 °C and cells from late-exponential phase growth were used in this study. Celluar fatty acid methyl esters were prepared as described by Sasser (1990) and were analysed by using a gas chromatograph (6890N; Hewlett Packard) with the MIDI Sherlock TSBA6 (version of the database). The major cellular fatty acids of strain S14-88T were iso-C₁₅:₀ (26.5 %) and summed feature 3 (C₁₆:₁ω7c and/or C₁₆:₁ω6c, 24.5 %). The fatty acid profile of strain S14-88T was similar to those of the type strains of the four reference species of the genus Mucilaginibacter under the same growth conditions, but differed in terms of proportions. Detailed fatty acid composition data are shown in Table 2.

Respiratory quinones were extracted and identified by HPLC as described by Xie & Yokota (2003). Strain S14-88T contained MK-7 as the predominant respiratory quinone. The quinone profile was in good agreement with that expected for a member of the genus Mucilaginibacter.

The polar lipids were extracted from 150 mg of dried cells of strain S14-88T as described by Minnikin et al. (1984) and

<table>
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<td>+</td>
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<td>40.8†</td>
<td>42.6‡</td>
<td>46.1§</td>
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*Data from Luo et al. (2009).
†Data from Joung et al. (2014a).
‡Data from Kim et al. (2010).
§Data from Pankratov et al. (2007).
were separated by two-dimensional TLC (silica gel plates, layer thickness 0.2 mm; Merck). The first direction was developed in chloroform/methanol/water (65:25:4; by vol.), and the second in chloroform/acetic acid/methanol/water (80:15:12:4; by vol.). The spots were identified by spraying appropriate detection reagents (molybdatophosphoric acid, ninhydrin and molybdenum blue) according to the method of Tindall (1990). The major polar lipid was phosphatidylethanolamine, and three unknown phospholipids, three unknown lipids, two unknown aminolipids and two unknown aminophospholipids were also detected (Fig. S2).

Combining the above data presented, isolate S14-88T should be classified as a member of a novel species of the genus *Mucilaginibacter*, for which the name *Mucilaginibacter antarcticus* sp. nov. is proposed.

### Table 2. Cellular fatty acid composition of strain S14-88T and the type strains of phylogenetically related species of the genus *Mucilaginibacter*

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<td>TR</td>
<td>TR</td>
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*As indicated by Montero-Calasanz et al. (2013), summed features are groups of two or three fatty acids that are treated together for the purpose of evaluation in the MIDI system and include both peaks with discrete ECLS as well as those where the ECLS are not reported separately. Summed feature 3 was listed as C<sub>16</sub>:<i>ω</i>7c and/or C<sub>16</sub>:<i>ω</i>6c; summed feature 8 was listed as C<sub>16</sub>:<i>ω</i>7cC<sub>15</sub>:<i>ω</i>7c; and summed feature 9 was listed as iso-C<sub>17</sub>:<i>ω</i>9c.*

**Description of *Mucilaginibacter antarcticus* sp. nov.**

*Mucilaginibacter antarcticus* (ant.arc’ti.cus. L. masc. adj. ant-arcticus of the Antarctic, the environment from where the type strain was isolated).

Cells are Gram-stain-negative, facultatively anaerobic, non-motile, non-spore-forming rods approximately 0.2–0.3 µm wide and 0.3–0.8 µm long. Colonies are pale yellow, circular, smooth and convex after 5 days at 15°C on R2A agar. Growth occurs at 15°C on R2A agar, NA and MacConkey agar, but not on TSB agar or MB agar. The range of temperature for growth is 4–28°C (optimum, 15°C) and range of pH is 7.0–8.0 (optimum, pH 7.0). The range of NaCl for growth is 0–0.6% (w/v); optimum growth occurs without NaCl. Oxidase- and catalase-positive. Negative in tests for hydrolysis of TWEENs 20, 40 and 80, chitin, CM-cellulose, casein, starch and tyrosine, and positive in the test for hydrolysis of TWEEN60. In the API ZYM test, there are positive reactions for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, N-acetyl-β-glucosaminidase and β-fucosidase, weakly positive reactions for valine arylamidase and cystine arylamidase, and negative reactions for esterase (C4), lipase (C14), trypsin, α-chymotrypsin, β-gluconoridase, α-glucosidase, β-glucosidase and α-mannosidase. On API 20E and 20NE strips, there are positive results in tests for aesculin hydrolysis and β-glucosidase, but negative reactions for β-galactosidase, trypthphan deaminase, arginine dihydrase, lysine decarboxylase, ornithine decarboxylase, urease and gelatinase activities; utilization of citrate; production of H<sub>2</sub>S; indole and acetoin; nitrate reduction; fermentation of glucose; assimilation of glucose, arabinose, mannose, mannotol, N-acetylgalactosamine, malate, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate and phenylacetic acid; and oxidation of mannotol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose. In the API 50CH test, acid is produced from D-arabinose, D-galactose, glucose, D-fructose, D-mannose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylgalactosamine, aesculin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, raffinose, gentiobiose, turanose and L-fucose; acid is produced weakly from D-xylene, but other substrates do not produce acid in the API 50CH test. In the Biolog GN2 MicroPlate system, there are positive oxidation reactions for L-arabinose and bromosuccininc acid, but other carbon sources give a negative reaction. Sensitive to tetracycline, vancomycin, cephaloridine, sodium/sulbactam, ampicillin and cefalaxin, but resistant to gentamicin, penicillin, polymyxin B, kanamycin, chloromycetin, amikacin and neomycin. The predominant respiratory quinone is MK-7 and the major cellular fatty acids are iso-C<sub>15</sub>:0 and summed feature 3 (C<sub>16</sub>:<i>ω</i>7c and/or C<sub>16</sub>:<i>ω</i>6c). The major polar lipid
is phosphatidylethanolamine, and three unknown phospholipids, three unknown lipids, two unknown aminolipids and two unknown aminophospholipids are also detected. The type strain, S14-88T (=CCTCC AB2015321T=KCTC 52232T), was isolated from soil in the South Shetland Islands of Antarctica, and the DNA G+C content of the type strain is 42.3 mol%.

Acknowledgements

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References


