**Mucilaginibacter soli** sp. nov., isolated from Arctic tundra soil

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A novel pale-pink-coloured strain, designated R9-65T, was isolated from a tundra soil near Ny-Ålesund, Svalbard Archipelago, Norway (78° N). The cells were facultatively anaerobic, Gram-staining-negative, non-motile and rod-shaped. Growth occurred at 4–32 °C (optimum, 25–28 °C), at pH 5.0–9.0 (optimum, pH 6.0–7.0) and with 0–1.0 % (w/v) NaCl (optimum, no NaCl). Flexirubin-type pigments were absent. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain R9-65T belonged to the genus *Mucilaginibacter* in the family *Sphingobacteriaceae*. The 16S rRNA gene sequence similarity between strain R9-65T and type strains of related species ranged from 93.4 to 96.6 %. Strain R9-65T contained summed feature 3 (C16 : 1ω7c and/or C16 : 1ω6c, 34.3 %) and iso-C15 : 0 (20.3 %) as major cellular fatty acids, MK-7 as the major respiratory quinone, and phosphatidylethanolamine as the main polar lipid. The DNA G+C content of strain R9-65T was 47.2 mol%. On the basis of phylogenetic, physiological and chemotaxonomic data, strain R9-65T is considered to represent a novel species of the genus *Mucilaginibacter*, for which the name *Mucilaginibacter soli* sp. nov. is proposed.

The type strain is R9-65T (= CCTCC AB 2010331T = NRRL B-59458T).

**Abbreviation:** PE, phosphatidylethanolamine.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain R9-65T is JF701183.

Four supplementary figures are available with the online version of this paper.

The genus *Mucilaginibacter*, belonging to the family *Sphingobacteriaceae* within the phylum *Bacteroidetes*, was first described by Pankratov et al. (2007) and its description was subsequently emended by Urai et al. (2008) and Baik et al. (2010). Members of the genus contain menaquinone-7 (MK-7) as the major respiratory quinone, possess straight- and branched-saturated fatty acids as the major cellular fatty acids, and have DNA G+C contents in the range 42.4–47.0 mol% (Pankratov et al., 2007; Urai et al., 2008; Baik et al., 2010). At the time of writing, the genus *Mucilaginibacter* contained 16 recognized species isolated from various environments such as peat bog, soil, rice straw, rice paddies, freshwater, the rhizosphere and isolated from various environments such as peat bog, soil, freshwater, the rhizosphere and decaying lichen, namely *M. paludis* (the type species) and *M. gracilis* (Pankratov et al., 2007), *M. kameinonensis* (Urai et al., 2008), *M. daejeonensis* (An et al., 2009), *M. ximonensis* (Luo et al., 2009), *M. oryzae* (Jeon et al., 2009), *M. rigui* (Baik et al., 2010), *M. gossypii* and *M. gossypiicola* (Madhaiyan et al., 2010), *M. frigoritolerans* and *M. lappiensis* (Männistö et al., 2010), *M. doraji* (Kim et al., 2010), *M. myungsuensis* (Joung & Joh, 2011), *M. boryungensis* (Kang et al., 2011) and *M. angelicae* (Kim et al., 2012). In this study, an Arctic bacterial isolate was subjected to a polyphasic analysis and identified as a novel member of the genus *Mucilaginibacter*.

A bacterial strain, designated R9-65T, was isolated from a soil sample collected from a high Arctic tundra near the settlement Ny-Álesund (78° 55’S 11° 58’E) in the Svalbard Archipelago, Norway. Isolation was carried out using the standard dilution plating method on 0.3 × R2A agar (BD) at 25 °C. The isolate produced slime, indicative of extracellular polysaccharide, around the colonies when grown on 0.3 × R2A agar at 25 °C for 4 days. The novel strain was routinely cultured on 0.3 × R2A agar and stored by lyophilization.

Sequencing of the 16S rRNA gene of strain R9-65T was carried out as described by Lane (1991). An almost-complete 16S rRNA gene sequence (1426 bp) was obtained and subjected to comparative analysis. Sequence similarity was investigated using NCBI BLAST and pairwise alignment was calculated using the EzTaxon database (Chun et al., 2007). Sequences were aligned using CLUSTAL_X software (Thompson et al., 1997), and phylogenetic trees were constructed by the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) tree-making algorithms using the MEGA version 4.0 (Tamura et al., 2007) and PHYLIP version 3.6 (Felsenstein, 2005) software packages. Evolutionary distances for the neighbour-joining algorithm were
calculated with the Kimura two-parameter model (Kimura, 1980) and close-neighbour-interchange (search level=2, random additions=100) was applied in the maximum-parsimony analysis. In each case, bootstrap values were calculated based on 1000 replications (Felsenstein, 1985).

Phylogenetic analysis based on 16S rRNA gene sequence revealed that strain R9-65\(^T\) was affiliated to the genus *Mucilaginibacter* in the family *Sphingobacteriaceae*, and formed a robust clade with *M. rigui* WPCB133\(^T\) in the neighbour-joining tree (Fig. 1). The same relationship was also found in trees constructed using the maximum-likelihood (Fig. S1, available in IJSEM Online) and maximum-parsimony algorithms (Fig. S2). Levels of 16S rRNA gene sequence similarity between strain R9-65\(^T\) and the type strains of recognized species of the genus *Mucilaginibacter* (93.4–96.6 %) were below the 97.0 % threshold recommended for the discrimination of separate bacterial species (Stackebrandt & Goebel, 1994). Based on data from phylogenetic analysis, strain R9-65\(^T\) could not be assigned to any recognized species and thus represents a novel species within the genus *Mucilaginibacter*.

Growth was evaluated at 25 °C on several standard bacteriological media (all from BD): R2A agar, 0.3 x R2A agar, 0.3 x marine broth 2216 (MB) agar, tryptic soy broth (TSB) agar, nutrient agar (NA) and MacConkey agar. Gram-staining of cells was carried out according to the classical Gram-stain procedure described by Doetsch (1981). Growth at different temperatures (0, 4, 8, 18, 20, 25, 28, 30, 32, 37 and 42 °C) was investigated on R2A agar for up to 2 weeks. The pH range for growth was determined in 0.3 x MB at pH 4.0–11.0 (in increments of 1.0 pH unit). Salt tolerance was tested on 0.3 x R2A agar supplemented with 0, 0.5, 1, 2, 3, 4 and 5 % NaCl (w/v) for 2 weeks at 25 °C. Cell morphology was examined by phase-contrast (BX51; Olympus) and transmission electron (8100; Hitachi) microscopy using cells grown for 2 days at 25 °C on R2A agar. Gliding motility was investigated as described by Bowman (2000). Cell life-cycle was examined using cultures grown on R2A agar for up to 2 weeks. Growth under anaerobic conditions was tested on 0.3 x R2A agar in a GasPak (BBL) jar at 25 °C for 7 days. Oxidase activity was evaluated via the oxidation of 1 % (w/v) tetramethyl-p-phenylenediamine. Catalase activity was determined by measurement of bubble production after the application of 3 % (v/v) hydrogen peroxide solution. The presence of flexirubin-type pigments was tested using the KOH test as described by Bernardet *et al.* (2002). For pectin hydrolysis activity, the isolate was cultured on a 0.3 x R2A agar plate containing 0.3 % (w/v) citric pectin, followed by staining with a solution of 1 % n-hexadecyl-trimethylammonium bromide. Hydrolysis of chitin, CM-cellulose, alginite, casein, starch, tyrosine and xylan was investigated on 0.3 x R2A agar after 1 week of incubation according to Smibert & Krieg (1994). The hydrolysis of Tween 20, 40, 60 and 80 was measured using the formation of an opaque halo of precipitation around the colony (Barrow & Feltham, 1993). Acid production from sugars was examined by using phenol red broth base containing 1 % of each sugar. Antibiotic sensitivity was tested by using filter-paper discs (diameter, 6.35 mm) impregnated with the following antibiotics (concentration per disc): ampicillin (10 μg), aztreonam (30 μg), ceftriaxone (30 μg), cefepime (30 μg), ceftazidime (30 μg), streptomycin (10 μg), kanamycin (30 μg), pipéracillin (100 μg), gentamicin (10 μg), oxacillin (1 μg), penicillin G (10 μg), erythromycin (15 μg), vancomycin (30 μg), clindamycin (2 μg), chloramphenicol (30 μg), amikacin (30 μg), tetracycline (30 μg), ciprofloxacin (5 μg).

**Fig. 1.** Neighbour-joining tree based on 16S rRNA gene sequences, showing the relationship of strain R9-65\(^T\) and related taxa. Percentage bootstrap values (1000 replications) >70 % are shown at nodes. The sequence of *Flexibacter flexilis* NBRC 15060\(^T\) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.
Strains: 1, R9-65<sup>T</sup>; 2, M. rigui NBRC 101115<sup>T</sup>; 3, M. paludis DSM 18603<sup>T</sup>; 4, M. lappiensis LMG 25358<sup>T</sup>; 5, M. dorajii KACC 14556<sup>T</sup>. Data were obtained in this study unless otherwise indicated. +, Positive; (+), weakly positive; −, negative. In API ZYM tests, all strains were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase and N-acetyl-β-glucosaminidase, and were negative for lipase (C14), α-chymotrypsin and β-glucuronidase. In API 50CH tests, all strains produced acid from aesculin, D-galactose, D-fructose, D-mannose, maltose and sucrose, but not from erythritol, D-ribose, D-xylose, D-adonitol, methyl β-D-xylopyranoside, α-L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, melezitose, xylitol, gentiobiose, D-tagatose, D-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate.
(w/v); optimum growth occurred without NaCl. Other physiological characteristics of strain R9-65T are summarized in the species description. Selected characteristics that differentiate strain R9-65T from related species of the genus *Mucilaginibacter* are shown in Table 1.

The DNA G+C content of strain R9-65T was determined by HPLC (UltiMate 3000; Dionex) according to the method of Mesbah et al. (1989). Respiratory quinones were extracted and identified by HPLC as described by Xie & Yokota (2003). For analysis of cellular fatty acids, strain R9-65T and four related strains, *M. rigui* NBRC 101115T, *M. paludis* DSM 18603T, *M. lappiensis* DSM 25538T and *M. dorajii* KACC 14556T were grown on R2A agar plates at 25 °C, and cells from late-exponential phase growth were used in this study. Harvesting, saponification, methylation and extraction of cellular fatty acids were performed following the protocol of the Sherlock Microbial Identification System (MIDI) version 6.0. Separation and identification of fatty acid methyl esters was performed using a gas chromatograph (6890N; Hewlett Packard) with MIDI Sherlock TSBA6 (version of the database) (Sasser, 1990). For sphingolipid and polar lipid analysis, strain R9-65T and the four reference strains were grown on/in R2A agar or broth, respectively, at 25 °C. The sphingolipids were extracted and analysed according to Kato et al. (1995), while the polar lipids were extracted and analysed by two-dimensional TLC (silica gel plates, layer thickness 0.2 mm, Merck) according to Tindall (1990).

The DNA G+C content of strain R9-65T was 47.2 mol%. Strain R9-65T contained MK-7 as the predominant isoprenoid quinone which represented 91.5 % of the total. In addition, small amounts of MK-6 (8.5 %) were detected. The quinone profile was in agreement with that expected for a member of the genus *Mucilaginibacter*. The major cellular fatty acids of strain R9-65T were summed feature 3 (C16:1ω7c and/or C16:1ω6c) (34.3 %) and iso-C15:0 (20.3 %). The fatty acid profile of strain R9-65T was similar to those of species of the genus *Mucilaginibacter*, but differed in the relative amounts of iso-C17:1ω9c. Detailed fatty acid composition data are shown in Table 2. The major polar lipid of strain R9-65T and the four reference strains was phosphatidylethanolamine (PE), and several unknown polar lipids were also detected. In particular, the larger proportions of an unknown phospholipid (PL1) distinguished the novel isolate from the four reference strains. All five strains contained sphingolipid, the characteristic polar lipid of members of the family *Sphingobacteriaceae* (Fig. S4).

On the basis of the data presented, isolate R9-65T should be classified as a member of a novel species of the genus *Mucilaginibacter*, for which the name *Mucilaginibacter soli* sp. nov. is proposed.

**Description of Mucilaginibacter soli sp. nov.**

*Mucilaginibacter soli* (so’li. L. neut. gen. n. soli of soil, the isolation source of the type strain).

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Cells are Gram-staining-negative, facultatively anaerobic, non-motile and non-spor-forming rods, approximately 0.5–0.7 μm wide and 1.0–2.0 μm long. No cyclic cell shape change is noticed during culture. Colonies are pale pink, circular, convex and smooth after growth for 5 days at 25 °C on 0.3 x R2A agar. Growth occurs at 25 °C on R2A agar, 0.3 x R2A agar, 0.3 x MB agar and NA, but not on TSB agar or MacConkey agar. Growth occurs at 4–32 °C (optimum, 25–28 °C), at pH 5.0–9.0 (optimum, pH 6.0–7.0) and in the presence of 0–1.0 % (w/v) NaCl (optimum, no NaCl). Oxidase-positive and catalase-negative. Flexirubin-type pigments are absent. Negative in tests for hydrolysis of pectin, chitin, CM-cellulose, alginate, casein, starch, tyrosine, xylan, Tween 20, 40, 60 and 80. Acid is produced from galactose, maltose, mannose, succrose and glucose, but not from lactose, rhamnose or xylan. In the API ZYM test, there are positive reactions for alkaline phosphatase, esterase (C4), glucosidase, N-acetyl-b-glucosaminidase and x-mannosidase; weakly positive reactions for cystine arylamidase; and negative reactions for lipase (C14), trypsin, z-chymotrypsin, z-galactosidase, b-glucuronidase, b-glucosidase and b-fucosidase. On API 20E and 20NE strips, there are positive results in tests for aesculin hydrolysis; b-glucosidase and gelatinase activities; utilization of citrate; and assimilation of glucose, mannose, N-acetylglucosamine, potassium gluconate, malate and trisodium citrate, but negative reactions for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease and trypthphan deaminase activities; production of H2S, indole and acetoin; nitrate reduction; oxidation of mannitol, inositol, sorbitol, rhamnose, succrose, melibiose,
amygdalin and arabinose; fermentation of glucose; and assimilation of mannitol, capric acid, adipic acid and phenylacetic acid. In the API 50CH test, acid is produced from aesculin, D-galactose, D-fructose, D-mannose, maltose, sucrose, raffinose, starch and glycogen; but not from glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylene, L-xylene, D-adonitol, methyl β-D-xylpyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, arbutin, salicin, cellobiose, lactose, melibiose, trehalose, inulin, melezitose, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium glutonate, potassium 2-ketogluconate or potassium 5-ketogluconate. Positive result for the following Biolog GN2 MicroPlate substrates: D-galactose, sucrose, DL-lactic acid and L-proline, but negative result for α-cyclodextrin, dextrin, glycogen, Tween 40 and Tween 80, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, cellobiose, L-erythritol, D-fructose, L-fucose, gentiobiose, α-D-glucose, myo-inositol, L-xactose, lactulose, maltose, D-mannitol, D-mannose, melibiose, methyl β-D-glucoside, D-psicose, raffinose, L-rhamnose, D-sorbitol, trehalose, turanose, xylitol, pyruvic acid methyl ester, succinic acid, acetic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, γ-hydroxybutyric acids, P-hydroxyphenylacetic acid, itaconic acid, α-κetoacetic acid, 2-κetoacid, α-κetoacetic acid, 2-κetoysalicillic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromo-succinic acid, succinamic acid, glucuronamide, L-alaninate, D- and L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycol L-aspartic acid, glycol L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-pyroglutamic acid, D- and L-serine, L-threonine, D- and L-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butenediol, glycerol, DL-γ-gluceraldehyde, DL-γ-glucose 1-phosphate and DL-γ-glucose 6-phosphate. Sensitive to cefepime, streptomycin, kanamycin, piperacillin, gentamicin, penicillin G, vancomycin, chloramphenicol, amikacin, tetracycline, polymyxin B and nitrofurantoin, but resistant to ampicillin, aztreonam, ceftriaxone, cefazedone, oxacillin, erythromycin and ciprofloxacin. The predominant isoprenoid quinone is MK-7 and the major cellular fatty acids are summed feature 3 (C16:1ω7c and/or C16:1ω6c) and iso-C15:0. The major polar lipid is PE; three unknown phospholipids and four polar lipids are also detected. Sphingolipid is present.

The type strain, R9-65T (=CCTCC AB 2010331=NRRL B-59458T), was isolated from a tundra soil near Ny-Ålesund, Svalbard Archipelago, Norway. The DNA G+C content of the type strain is 47.2 mol%.

Acknowledgements

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References


