Mucilaginibacter kameinonensis sp., nov., isolated from garden soil

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An extracellular polysaccharide-producing bacterium, strain SCK^T, was isolated from a soil sample taken from Kameino, Fujisawa, Japan. The isolate was Gram-negative and cells were non-motile, irregular-shaped rods that grew optimally at 25 °C and grew between pH 5 and 8. Strain SCK^T contained MK-7 as the major isoprenoid quinone, iso-C_{15:0} and C_{16:1}ω7c and/or iso-C_{15:0} 2-OH as the major fatty acids and sphingolipids, with d-17:0 as the main dihydrosphingosine. Flexirubin-type pigments were also present. The DNA G+C content was 43.7 mol%. On the basis of 16S rRNA gene sequence analysis, strain SCK^T was shown to belong to the genus *Mucilaginibacter*. The 16S rRNA gene sequence similarity between strain SCK^T and the two type strains of *Mucilaginibacter* was 93%. The results of physiological and biochemical tests allowed phenotypic differentiation of the strain from published *Mucilaginibacter* species. Therefore, strain SCK^T represents a novel species, for which the name *Mucilaginibacter kameinonensis* sp. nov. is proposed. The type strain is SCK^T (=NBRC 102645^T =KCTC 22227^T).

It has been reported that some bacterial strains belonging to the genus *Rhodococcus* produce extracellular polysaccharides (EPS), including fatty acids containing EPS, and that these EPS have a variety of functions, including protection of bacterial cells from the toxicity of hydrocarbons (Aizawa *et al.*, 2005; Iwabuchi *et al.*, 2000), emulsification, moisture absorption (Urai *et al.*, 2002, 2004) and stimulation of degradation of polyaromatic hydrocarbons (PAH) spilled in marine environments, the latter probably occurring via stimulation of marine PAH-assimilating bacteria (Iwabuchi *et al.*, 2002). These EPS are acidic, high-molecular-mass polysaccharides containing uronic acids (Urai *et al.*, 2006a, b, 2007a, b). During the screening for micro-organisms that were able to assimilate the EPS produced by *Rhodococcus rhodochrous* S-2 (S-2 EPS), strain SCK^T was isolated from a soil sample taken

from a garden on our campus at Kameino, Fujisawa, Japan. The strain formed mucoid colonies and produced a large amount of EPS. On the basis of phenotypic, genotypic, chemotaxonomic and phylogenetic analyses, it was concluded that strain SCK^T was affiliated with the genus *Mucilaginibacter* and represents a novel species of this genus.

The genus *Mucilaginibacter* was established recently and belongs to the family *Sphingobacteriaceae*; it currently includes two species, *Mucilaginibacter gracilis* and *Mucilaginibacter paludis*, which were isolated from an acidic *Sphagnum* peat bog as pectin-, xylan- and laminarin-degrading bacteria (Pankratov *et al.*, 2007).

After a series of three enrichments in MU liquid medium [0.2 g MgCl₂·7H₂O, 0.1 g CaCl₂·2H₂O, 0.1 g NaCl, 0.02 g FeCl₂·6H₂O, 0.5 g (NH₄)₂SO₄, 1 g S-2 EPS l⁻¹; pH 7.2], strain SCK^T was isolated on MU agar plates (solidified with 15 g agar l⁻¹) at 28 °C under aerobic conditions. The strain formed mucoid colonies on MU agar plates as well as on tryptic soy agar (TSA) and IB agar (Sunairi *et al.*, 1997) plates but showed little or no growth on MacConkey agar. After cultivation of the strain in IB liquid medium at 28 °C for 1 week, EPS was detected in the culture supernatant at

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Abbreviations: EPS, extracellular polysaccharide; PAH, polyaromatic hydrocarbon.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SCK^T is AB330392.

0.5 g l⁻¹, which was calculated as 0.7 g EPS (g dry cells)⁻¹. This EPS contained D-galactose, D-glucose, D-mannose and L-rhamnose in equimolar amounts. The chemical structure and functional aspects of the EPS produced by strain SCK^T will be described elsewhere. The strain showed good growth on TSA at 5–30 °C, with an optimum at 25 °C, but did not grow at 45 °C. Strain SCK^T showed good growth in tryptic soy broth (TSB) over the pH range 4–9, with optimum growth at pH 5–8, but showed little or no growth below pH 3 or above pH 10, when grown at 30 °C for 5 days. The strain showed good growth in the presence of 0.5 % (w/v) NaCl in TSB, but little or no growth occurred in the presence of 1 % (w/v) NaCl or higher. The strain was Gram-negative (Ryu, 1938) and was non-endospore-forming and aerobic. Motility was not observed. Cellular morphology was observed by scanning electron microscopy. After critical-point drying, specimens were sputter-coated with gold and palladium. The cells appeared as irregular rods, 0.3–0.7 µm wide and 0.8–3.5 µm long, when the organisms were cultured on IB agar plates at 25 °C for 2 days (Fig. 1).

The 16S rRNA gene of the strain was amplified by PCR using universal primers (Tamura & Hatano, 2001), and the nearly complete 16S rRNA gene nucleotide sequence (1451 bp) was determined. By sequence match analysis using the Ribosome Database Project II, the sequence of strain SCK^T showed high similarities to sequences of the type strains of species belonging to the family *Sphingobacteriaceae*, i.e. the genera *Mucilaginibacter*, *Olivibacter*, *Parapedobacter*, *Pedobacter* and *Sphingobacterium*. The highest sequence similarities were found with the type strains of *M. gracilis* and *M. paludis* (93 % similarity). The 16S rRNA gene sequences of all other species of the family *Sphingobacteriaceae* had <91 % similarity with that of strain SCK^T. The phylogenetic

relationship between strain SCK^T and closely related species was determined by using MEGA version 4 (Tamura *et al.*, 2007) after multiple alignments of the data performed by using CLUSTAL_X (Thompson *et al.*, 1997). Evolutionary distances were computed as described by Jukes & Cantor (1969). Phylogenetic trees were constructed by using the maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) methods. The reliabilities of these tree topologies were evaluated by bootstrap analysis with 1000 replicates (Felsenstein, 1985). The phylogenetic trees constructed by the two methods were topologically similar and showed that strain SCK^T belonged to the genus *Mucilaginibacter* and formed a separate line of descent in the phylogenetic cluster of the genus (Fig. 2). As pointed out by Stackebrandt & Goebel (1994), a low degree of 16S rRNA gene sequence similarity (below 97 %) is of value for differentiating species, and DNA–DNA hybridization studies are not needed. These data suggest that strain SCK^T represents a novel species of the genus *Mucilaginibacter*.

Analyses of menaquinones, DNA G+C content and cellular fatty acids were performed as described previously (Tamura *et al.*, 1994). The major isoprenoid quinone of the strain was MK-7. The DNA G+C content was 43.7 mol%. The fatty acid profile of strain SCK^T (>1.0 % of total fatty acids) included straight-chain fatty acids C_{16:0} (7.6 %), C_{14:0} (2.0 %), and C_{15:0} (1.2 %), branched fatty acids iso-C_{15:0} (25.9 %) and iso-C_{17:1}ω9c (1.4 %), summed feature 3 (comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) (49.2 %), hydroxy fatty acids iso-C_{17:0} 3-OH (5.6 %), C_{16:0} 3-OH (2.7 %) and iso-C_{15:0} 3-OH (1.1 %) and unsaturated fatty acid C_{16:1}ω5c (3.3 %).

By the bathochromic shift test with 20 % (w/v) KOH (Fautz & Reichenbach, 1980), flexirubin-type pigments were detected. The long-chain acyl components of cellular sphingolipids were analysed as described previously (Yano *et al.*, 1982). Strain SCK^T contained sphingolipids with d-17:0 as the main dihydrosphingosine.

Standard physiological tests were carried out according to the methods of Smibert & Krieg (1994). Acid production from carbon sources was assessed by using the API 50 CH system and enzyme activities with the API 20 E and API ZYM systems according to the manufacturer's instructions (bioMérieux) (incubation times of up to 7 days). Oxidation of various substrates was tested using Biolog GN2 Microplates (Biolog) in accordance with the manufacturer's instructions. Heparinase activity was tested according to Zimmermann *et al.* (1990). Strain SCK^T could be distinguished from the other species of *Mucilaginibacter* on the basis of physiological characteristics (Table 1). Resistance to antibiotics was examined by using the API VET system (bioMérieux) in accordance with the manufacturer's instructions.

Based on phenotypic and phylogenetic characterization, strain SCK^T was concluded to represent a novel species, for which the name *Mucilaginibacter kameinonensis* sp. nov. is

Fig. 1. Scanning electron micrograph of cells of strain SCK^T. Cells were grown on IB agar plates at 25 °C for 2 days. After critical-point drying, specimens were sputter-coated with gold/palladium and observed with a scanning electron microscope (S-3500N; Hitachi). Bar, 2 µm.

Fig. 2. Neighbour-joining tree (Saitou & Nei, 1987) based on nearly complete 16S rRNA gene sequences (positions 56–1396 of the *Escherichia coli* 16S rRNA gene), showing the position of the strain SCK^T among its phylogenetic neighbours. Numbers at branch nodes are percentages of bootstrap support based on 1000 resamplings; only values over 50 % are given. The sequence of *Flexibacter flexilis* IFO 15060^T was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

proposed. As some properties of strain SCK^T are not compatible with the original description of the genus *Mucilaginibacter* (Pankratov *et al.*, 2007), an emended description of the genus is also given.

Emended description of the genus *Mucilaginibacter* Pankratov *et al.* 2007

In contrast to the genus description given by Pankratov *et al.* (2007), this study shows that strains belonging to the genus *Mucilaginibacter* are variable for the presence of flexirubin-type pigments, oxidase and the utilization of melibiose.

Description of *Mucilaginibacter kameinonensis* sp. nov.

Mucilaginibacter kameinonensis (ka.me.i.no.nen'sis. N.L. masc. adj. *kameinonensis* pertaining to Kameino, the location from which the organism was first isolated).

Gram-negative, non-endospore-forming, non-motile, aerobic, irregular rods, 0.3–0.7 µm wide and 0.8–3.5 µm long. Growth occurs on TSA and IB agar, but not on MacConkey agar. Colonies are pale pink, mucoid, convex and round with entire margins on TSA and IB agar. Growth occurs between 5 and 30 °C with an optimum at 25 °C. The pH range for growth is pH 4–9, with optimum growth at pH 5–8. Growth occurs in the absence of NaCl and in the presence of 0.5 % (w/v) NaCl, but not in the presence of 1 % (w/v) NaCl or higher. Positive for hydrolysis of aesculin and Tweens 20 and 60, but negative for casein and Tweens 40 and 80. Positive for activities of *N*-acetyl-β-glucosaminidase, acid and alkaline phosphatases, catalase, cystine arylamidase, DNase, esterase (C4), esterase lipase (C8), α-fucosidase, α-galactosidase, β-galactosidase, gelatinase, α-glucosidase, β-glucosidase, β-glucuronidase, leucine arylamidase, α-mannosidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase, but negative for activities of arginine dihydrolase, α-chymotrypsin, heparinase, lipase (C14), lysine decarboxylase, ornithine decarboxylase, oxidase, tryptophan deaminase and urease, as determined with the API ZYM system.

Positive for the Voges–Proskauer test, but negative for hydrogen sulphide and indole production and nitrate reduction. Positive for acid production from *N*-acetylglucosamine, amygdalin, D- and L-arabinose, cellobiose, aesculin, D-fructose, L-fucose, D-galactose, gentiobiose, D-glucose, glycogen, inulin, D-lactose, maltose, D-mannose, melezitose, melibiose, methyl β-D-xylopyranoside, potassium 5-ketogluconate, raffinose, salicin, starch, sucrose, trehalose, turanose and D-xylose, but negative for acid production from D-adonitol, D- and L-arabitol, arbutin, dulcitol, erythritol, D-fucose, glycerol, inositol, D-lyxose, D-mannitol, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, potassium gluconate, potassium 2-ketogluconate, L-rhamnose, D-ribose, D-sorbitol, L-sorbose, D-tagatose, xylitol and L-xylose, as determined with the API 50CH system. Positive for Biolog GN2 MicroPlate substrates cellobiose, α-cyclodextrin, dextrin, D-fructose, D-galactose, gentiobiose, α-D-glucose, glycerol, glycogen, lactulose, maltose, D-mannose, melibiose, methyl β-D-glucoside, L-proline, raffinose, sucrose, trehalose and turanose, but negative for acetic acid, *N*-acetyl-D-galactosamine, *cis*-aconitic acid, adonitol, L-alaninamide, D- and L-alanine, L-alanylglycine, γ-aminobutyric acid, 2-aminoethanol, L-arabinose, D-arabitol, L-asparagine, L-aspartic acid, bromosuccinic acid, 2,3-butanediol, D- and L-carnitine, citrate, D-erythritol, formic acid, L-fucose, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, α-D-glucose 1-phosphate, D-glucose 6-phosphate, glucuronamide, D-glucuronic acid, L-glutamic acid, D- and L-α-glycerol phosphate, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, α-, β- and γ-hydroxybutyric acids, *p*-hydroxyphenylacetic acid, hydroxy-L-proline, inosine, *myo*-inositol, itaconic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, D- and L-lactic acid, L-leucine, malate, D-mannitol, L-ornithine, L-phenylalanine, phenylethylamine, propionic acid, D-psicose, putrescine, L-pyrogutamic acid, pyruvic acid methyl ester, quinic acid, D-saccharic acid, sebacic acid, D- and L-serine, D-sorbitol, succinamic acid, succinic acid, succinic acid monomethyl ester, L-threonine, thymidine, Tweens 40 and 80, uridine, urocanic acid and xylitol.

Table 1. Physiological characteristics of strain SCK^T and type strains of *Mucilagibacter* species

Data for *M. paludis* and *M. gracilis* were taken from Pankratov *et al.* (2007). +, Positive; –, negative; w, weakly positive. All strains had the following characteristics: positive for assimilation of cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, maltose, sucrose and trehalose; positive for acid production from cellobiose, D-fructose, D-galactose, D-glucose, maltose, sucrose and D-xylose; positive for catalase activity; negative for assimilation of acetic acid, adonitol, D-arabitol, citric acid, malic acid, D-mannitol, propionic acid, D-sorbitol and succinic acid; negative for Gram stain, motility, H₂S production, indole production and heparinase activity.

Characteristic	Strain SCK ^T	<i>M. paludis</i> TPT56 ^T	<i>M. gracilis</i> TPT18 ^T
Ranges for growth			
Temperature (°C)	5–30	2–33	2–33
pH	4–9	4.2–8.2	4.2–8.2
DNA G + C content (mol%)	43.7	46.1	42.4
Quinone(s)	MK-7	MK-7, MK-6	MK-7, MK-6
Flexirubin pigments	+	–	–
Oxidase activity	–	+	+
Hydrolysis of aesculin	+	–	–
Acid production from:			
D-Mannose	+	+	–
Melezitose	+	–	–
Melibiose	+	–	–
Trehalose	+	–	+
Assimilation of:			
D-Galacturonic acid	–	+	–
D-Gluconic acid	–	+	–
D-Mannose	+	+	–
Melibiose	+	–	–
Raffinose	+	–	+
myo-Inositol	–	+	–
N-Acetyl-D-glucosamine	w	+	–

Resistant to apramycin, cefoperazone, cephalothin, chloramphenicol, colistin, enrofloxacin, erythromycin, flumequine, gentamicin, kanamycin, lincomycin, metronidazole, nitrofurantoin, oxacillin, oxolinic acid, penicillin, rifampicin and streptomycin, but sensitive to amoxicillin, augmentin, cotrimoxazole, doxycycline, fusidic acid, pristinamycin, spectinomycin, sulphamethizole, tetracycline and tylosin (API VET system). The major isoprenoid quinone is MK-7 and the major fatty acids (>10 % of total fatty acids) are iso-C_{15:0} (25.9 %) and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) (49.2 %). Sphingolipids are present, with d-17:0 as the main dihydrosphingosine. Flexirubin-type pigments are present. The DNA G + C content of the type strain is 43.7 mol%.

The type strain, SCK^T (=NBRC 102645^T =KCTC 22227^T), was isolated from a soil sample from the campus of the College of Bioresource Sciences, Nihon University, at Kameino, Fujisawa, Kanagawa, Japan.

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