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Mucilaginibacter oryzae sp. nov., isolated from soil of a rice paddy

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A Gram-negative-staining, non-spore-forming bacterium devoid of flagella, designated strain B9^T, was isolated from rice paddy soil associated with the roots of *Oryza sativa* collected from Jinju, South Korea. Cells were straight rods, were catalase- and oxidase-positive and were able to hydrolyse pectin, xylan and laminarin. Growth of strain B9^T was observed between 15 and 35 °C (optimum 25–30 °C) and between pH 5.0 and 8.0 (optimum pH 6.5–7.5). Strain B9^T contained menaquinone-7 (MK-7) as a major isoprenoid quinone and summed feature 3 ($C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH), iso-$C_{15:0}$ and $C_{16:0}$ as major fatty acids. The G+C content of the genomic DNA was 44.4 mol%. Comparative 16S rRNA gene sequence analysis showed that strain B9^T belonged to the genus *Mucilaginibacter*, a member of the family *Sphingobacteriaceae*, and was most closely related to *Mucilaginibacter kameinonensis* SCK^T (95.9 % sequence similarity). On the basis of chemotaxonomic data and molecular properties, strain B9^T represents a novel species of the genus *Mucilaginibacter*, for which the name *Mucilaginibacter oryzae* sp. nov. is proposed. The type strain is B9^T (=KACC 12816^T =DSM 19975^T).

The genus *Mucilaginibacter*, a member of the family *Sphingobacteriaceae* (phylum *Bacteroidetes*) was first established by Pankratov *et al.* (2007). The genus initially contained two species, *Mucilaginibacter paludis* and *M. gracilis*, which were both isolated from acidic *Sphagnum* peat bogs. Recently, another species, *Mucilaginibacter kameinonensis*, isolated from garden soil, was added to the genus (Urai *et al.*, 2008). Typical features of the genus *Mucilaginibacter* are the production of exopolysaccharide (EPS), the synthesis of sphingolipids, the ability to hydrolyse pectin, xylan and laminarin, the presence of menaquinone-7 (MK-7) and the synthesis of iso-$C_{15:0}$ and summed feature 3 ($C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH) as the major fatty acids. In this study, we report on the taxonomic characterization of a bacterial strain, B9^T, isolated from a rice paddy, which is phylogenetically related to the genus *Mucilaginibacter*.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain B9^T is EU109722.

A transmission electron micrograph of cells of strain B9^T is available as supplementary material with the online version of this paper.

Strain B9^T was isolated from rice paddy soil associated with the roots of *Oryza sativa* collected from Jinju, South Korea. We sampled rice roots from a rice paddy in September and removed soil debris from the roots by tapping. The roots were washed and the washings were serially diluted using 0.9 % (w/v) saline. The diluted solution was spread on R2A agar (Difco) and incubated at 25 °C for 5 days. Strain B9^T was subsequently obtained from the primary isolation plates. Except where indicated otherwise, the isolate was routinely grown aerobically on R2A agar at 30 °C for 3 days.

Sequencing of the 16S rRNA gene of strain B9^T was carried out as described by Lane (1991). The resulting 16S rRNA gene sequence (1484 nt) was compared with 16S rRNA gene sequences available from GenBank using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) to determine an approximate phylogenetic affiliation and gene sequences were aligned with those of closely related species using the CLUSTAL W software program (Thompson *et al.*, 1994). Phylogenetic trees were constructed using three different algorithms, neighbour-joining, maximum-likelihood and maximum-parsimony, available in PHYLIP, version 3.6 (Felsenstein, 2002). The resulting tree topologies

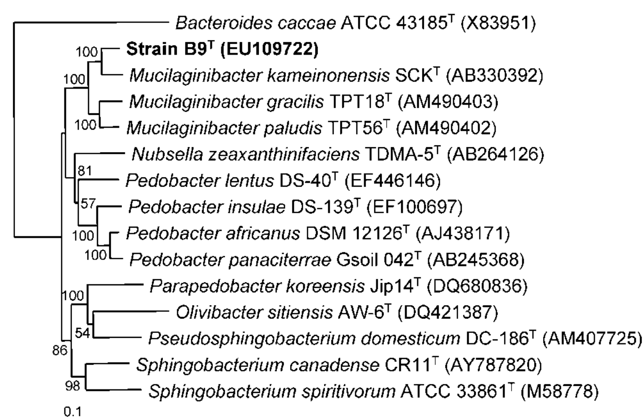

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the phylogenetic relationships of strain B9^T and related taxa. Bootstrap values (< 50 %) based on 1000 resamplings are shown at branch nodes. *Bacteroides caccae* ATCC 43185^T was used as the outgroup. Bar, 0.1 substitutions per nucleotide position.

were evaluated by bootstrap analysis based on 1000 resamplings. Sequence similarity values between the novel strain and other related bacteria were computed using the nucleotide similarity search program FASTA (http://www.ebi.ac.uk/fasta33/nucleotide.html; Pearson & Lipman, 1988). Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain B9^T formed a tight phyletic lineage with the type strains of the genus *Mucilaginibacter* (Fig. 1). The overall topology of the neighbour-joining tree was supported by the trees built using the maximum-likelihood and maximum-parsimony algorithms (not shown). Comparative 16S rRNA gene sequence analysis showed that the isolate was most closely related to *M. kameinonensis* SCK^T, *M. paludis* TPT56^T and *M. gracilis* TPT18^T, with sequence similarities of 95.9, 95.1 and 94.8 %, respectively.

Growth was tested at 30 °C on several bacteriological media: R2A agar (Difco), laboratory-prepared Luria–Bertani (LB) agar, tryptic soy agar (TSA; Difco) and nutrient agar (NA; Difco). Physiological characteristics of strain B9^T were examined by growing the isolate at different temperatures (5–40 °C at 5 °C intervals) on R2A agar and at different pH values (pH 4.0–9.0 at 0.5 pH unit intervals) in R2A broth. R2A broth was adjusted to different pH values as described by Gomori (1955). Gram staining was performed using a bioMérieux Gram stain kit according to the instructions of the manufacturer. Cell morphology, the presence of flagella and gliding motility were studied using phase-contrast microscopy and transmission electron microscopy (JEM-1010; JEOL) as described elsewhere (Bernardet *et al.*, 2002; Jeon *et al.*, 2004). Salt tolerance was tested in R2A broth supplemented with 0–3 % (w/v) NaCl (at 0.5 % intervals) for 5 days at 30 °C.

Oxidase activity was tested by oxidation of 1 % (w/v) tetramethyl *p*-phenylenediamine (Merck) and catalase activity was evaluated by the production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution. The production of flexirubin-type pigments was investigated by using the bathochromic shift test with 20 % KOH (Bernardet *et al.*, 2002). Hydrolysis of aesculin, casein, pectin, xylan, laminarin, CM-cellulose, Tweens 80 and 20, urea, tyrosine, gelatin and starch was investigated on R2A agar after 7 days of incubation according to Lányí (1987) and Smibert & Krieg (1994). Nitrate reduction was performed according to Lányí (1987) and acid production from carbohydrates was tested as described by Leifson (1963). Antibiotic susceptibility tests were performed in duplicate using filter-paper discs (diameter, 8 mm) containing the following antibiotics (μg per disc unless otherwise stated): ampicillin (10), polymyxin B (100 U), streptomycin (50), penicillin G (10 IU), gentamicin (30), chloramphenicol (100), tetracycline (30), kanamycin (30), lincomycin (15), oleandomycin (15), carbenicillin (100) and novobiocin (50). The oxidation of various substrates was assessed using GN2 MicroPlates (Biolog) at 30 °C in accordance with the manufacturer's instructions. Additional enzyme activities were determined by using the API 20E and API ZYM systems (bioMérieux) at 30 °C based on the manufacturer's instructions (incubation up to 7 days). Anaerobic growth was assessed on R2A agar under anaerobic (with 4–10 % CO_2) conditions using the GasPak Plus system (BBL) at 30 °C for 20 days.

Strain B9^T grew well at 30 °C on R2A agar, LB agar, TSA and NA. Growth of the strain was observed on R2A agar at temperatures between 15 and 35 °C, with an optimum temperature of 25–30 °C, and in R2A broth over a range of pH 5.0–8.0, with an optimum of pH 6.5–7.5. Bacterial cells were Gram-negative-staining, non-spore-forming, non-motile, aerobic single rods, 0.5–0.7 μm wide and 1.2–2.8 μm long (Supplementary Fig. S1, in IJSEM Online). The isolate grew optimally in R2A broth without the addition of NaCl and growth was severely inhibited in R2A broth containing more than 2 % (w/v) NaCl. Phenotypic features of strain B9^T are presented in Table 1 and in the species description. Some of them are in accordance with characteristics of members of the genus *Mucilaginibacter*, whereas others allow the differentiation of strain B9^T from closely related *Mucilaginibacter* species (Table 1).

Isoprenoid quinones were analysed using an HPLC (model LC-20A; Shimadzu) equipped with a diode array detector (SPD-M20A; Shimadzu) and a reversed-phase column (250 × 4.6 mm, Kromasil; Akzo Nobel) as described by Komagata & Suzuki (1987). For analysis of fatty acid methyl esters, cells of strain B9^T were harvested from agar plates after incubation at 30 °C for 3 days on TSA. Analysis of fatty acid methyl esters was performed according to the instructions of the Microbial Identification System (Microbial ID, Inc.). The DNA G + C content of strain B9^T was determined using an HPLC fitted with a reversed-phase column (GROM-SIL

Table 1. Phenotypic characteristics of strain B9^T and related *Mucilaginibacter* species

Strains: 1, strain B9^T (data from this study); 2, *M. kameinonensis* SCK^T (Urai *et al.*, 2008); 3, *M. paludis* TPT56^T (Pankratov *et al.*, 2007); 4, *M. gracilis* TPT18^T (Pankratov *et al.*, 2007). All strains are positive for the following characteristics: catalase production, acid production from cellobiose, D-galactose, D-fructose, D-glucose, maltose, sucrose and lactose and assimilation of cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, sucrose and trehalose. All strains are negative for the following characteristics: Gram staining, motility, H_2S production, indole production, sporulation and assimilation of adonitol, D-arabitol, citric acid, malic acid, D-mannitol, propionic acid, D-sorbitol and succinic acid. All strains are resistant to ampicillin, gentamicin and kanamycin. +, Positive; −, negative; W, weakly positive; ND, no data available.

Characteristic	1	2	3	4
Oxidase	+	−	+	+
Growth with 1 % NaCl	+	−	−	−
Quinone(s)	MK-7	MK-7	MK-7, MK-6	MK-7, MK-6
Flexirubin-type pigment	−	+	−	−
Hydrolysis of:				
Aesculin	+	+	−	−
CM-cellulose	+	ND	−	−
Laminarin	+	ND	+	+
Pectin	W	ND	+	+
Starch	−	ND	+	+
Xylan	W	ND	+	+
Acid production from:				
D-Mannose	+	+	+	−
Melibiose	+	+	−	−
Trehalose	+	+	−	−
Assimilation of:				
N-Acetyl D-glucosamine	+	W	+	−
D-Galacturonic acid	−	−	+	+
D-Gluconic acid	+	−	+	−
myo-Inositol	−	−	+	−
D-Mannose	+	+	+	−
Melibiose	+	+	−	−
Raffinose	+	+	−	+
Susceptibility to:				
Chloramphenicol	−	−	+	−
Lincomycin	−	−	−	+
Streptomycin	−	−	+	+
DNA G+C content (mol%)	44.4	43.7	46.1	42.4

100 ODS-2FE; Grom) according to the method of Tamaoka & Komagata (1984). The major respiratory lipoquinone of strain B9^T was menaquinone-7 (MK-7). The major cellular fatty acids were summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH; 43.7 %), iso-$C_{15:0}$ (23.9 %), $C_{16:0}$ (8.5 %), iso-$C_{17:0}$ 3-OH (5.4 %), $C_{16:0}$ 3-OH (4.6 %), iso-$C_{15:0}$ 3-OH (2.2 %), iso-$C_{17:1}$ $\omega9c$ (1.9 %), $C_{14:0}$ (1.7 %), $C_{15:0}$ (1.6 %) and $C_{16:1}\omega5c$ (1.4 %). Traces (<1 %) of the following fatty acids were also present: unknown 13.565 (0.7 %), $C_{14:0}$ 2-OH (0.6 %), summed feature 2 (any combination of $C_{12:0}$ aldehyde, an unknown fatty acid of equivalent chain-length 10.928, iso-$C_{16:1}$ I and $C_{14:0}$ 3-OH; 0.4 %), $C_{15:1}\omega6c$ (0.4 %), iso-$C_{17:0}$ (0.3 %), $C_{15:0}$ 2-OH (0.3 %), unknown 16.582 (0.3 %), $C_{17:1}\omega8c$ (0.3 %), $C_{17:1}\omega8c$ (0.3 %), iso-$C_{16:0}$ 3-OH (0.2 %), $C_{16:0}$ 2-OH (0.2 %), iso-$C_{14:0}$ 3-OH (0.2 %), summed feature 1 (iso-$C_{15:1}$ I and/or $C_{13:0}$ 3-OH; 0.2 %), $C_{13:1}$ AT 12–13 (0.2 %), anteiso-$C_{15:0}$ (0.2 %), $C_{18:0}$ 3-OH (0.1 %), iso-$C_{11:0}$ (0.1 %) and $C_{18:1}\omega9c$ (0.1 %). This profile is similar to those of phylogenetically related species but differs from them in the proportions of some components (Pankratov *et al.*, 2007; Urai *et al.*, 2008). The G+C content of strain B9^T was 44.4 mol%. The major lipoquinone, major fatty acids and G+C content were in accordance with those of members of the genus *Mucilaginibacter* (Pankratov *et al.*, 2007; Urai *et al.*, 2008). Therefore, the physiological, biochemical and phylogenetic properties of strain B9^T support the description of a novel species within the genus *Mucilaginibacter*, for which the name *Mucilaginibacter oryzae* sp. nov. is proposed.

Description of *Mucilaginibacter oryzae* sp. nov.

Mucilaginibacter oryzae (o.ry′zae. L. gen. fem. n. *oryzae* of rice, referring to the rice paddy fields where the type strain was isolated).

Cells are Gram-negative-staining, non-motile, aerobic rods, 0.5–0.8 μm wide and 1.5–2.8 μm long. Colonies on R2A agar are pale pink, convex and round with entire margins. Growth occurs at 15–35 °C (optimum 25–30 °C) and at pH 5.0–8.0 (optimum pH 6.5–7.5). Catalase- and oxidase-positive. Nitrate is not reduced to nitrite. Cells produce large amounts of EPS. Casein, urea, pectin, xylan, laminarin, CM-cellulose, aesculin, gelatin and tyrosine are hydrolysed, but starch and Tweens 20 and 80 are not. Flexirubin-type pigments are not produced. Acid is produced from raffinose, *myo*-inositol, lactose, L-arabinose, melibiose, D-fructose, salicin, D-mannose, D-galactose, D-mannitol, maltose, trehalose, cellobiose, sucrose, but not from arbutin. Positive for Voges–Proskauer test, but negative for indole production, H_2S production, ornithine decarboxylase, tryptophan deaminase, citrate utilization and lysine decarboxylase (API 20E). Produces alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, α-mannose, β-glucuronidase and *N*-acetyl-β-glucosaminidase activities, but not trypsin or α-chymotrypsin (API ZYM). Weak activities are observed for lipase (C14), β-glucuronidase and α-fucosidase (API ZYM). Positive for oxidization of Biolog GN2 MicroPlate substrates α-cyclodextrin, dextrin, glycogen, *N*-acetyl D-glucosamine, L-arabinose, cellobiose, D-fructose, D-galactose, gentiobiose, α-D-glucose, lactose, lactulose, maltose, melibiose, methyl β-D-glucoside, D-psicose, raffinose, sucrose, trehalose, turanose, acetic acid, D-gluconic acid, L-alaninamide, L-alanine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-ornithine, L-proline, L-serine, L-threonine, α-D-glucose 1-phosphate and D-glucose 6-phosphate. Other organic substrates included in the Biolog GN2 MicroPlate are not oxidized. Resistant to polymyxin B, gentamicin, kanamycin, oleandomycin, ampicillin, streptomycin, penicillin G, lincomycin, chloramphenicol, tetracycline, carbenicillin and novobiocin. The major isoprenoid quinone is MK-7. The major cellular fatty acids are summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH), iso-$C_{15:0}$ and $C_{16:0}$. The DNA G+C content of the type strain is 44.4 mol% (HPLC).

The type strain is $B9^T$ (=KACC 12816^T =DSM 19975^T), which was isolated from rice paddy soil associated with the roots of *Oryza sativa* in Jinju, South Korea.

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