Mucilaginibacter daejeonensis sp. nov., isolated from dried rice straw

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A novel strain, designated Jip 10^T, isolated from dried rice straw, was characterized by a polyphasic approach to clarify its taxonomic position. The isolate was Gram-negative, facultatively aerobic, heterotrophic, non-motile, non-spore-forming and rod-shaped. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate clustered with the genus *Mucilaginibacter* in the phylum *Bacteroidetes*. 16S rRNA gene sequence similarities between strain Jip 10^T and the type strains of *Mucilaginibacter gracilis* and *Mucilaginibacter paludis* were 93.7 and 93.6 %, respectively. The G + C content of the genomic DNA was 48.1 mol%. Chemotaxonomic data [major menaquinone MK-7 and major fatty acids iso-C_{15:0}, iso-C_{17:0} 3-OH and summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c)] supported the affiliation of strain Jip 10^T to the genus *Mucilaginibacter*. However, the results of physiological and biochemical tests allowed phenotypic differentiation of strain Jip 10^T from other *Mucilaginibacter* species with validly published names. Therefore, strain Jip 10^T (=KCTC 12639^T =LMG 23488^T) was classified in the genus *Mucilaginibacter* as the type strain of a novel species, for which the name *Mucilaginibacter daejeonensis* sp. nov. is proposed.

The genus *Mucilaginibacter* was proposed by Pankratov *et al.* (2007) to accommodate species characterized by Gram-negative, non-motile rods that are facultatively aerobic, produce large amounts of extracellular polymeric substances (EPS), are capable of fermenting glucose and sucrose, hydrolyse pectin, xylan, laminarin, chondroitin 6-sulfate, gellan gum, pullulan and starch, are negative for H₂S production from thiosulfate or indole from tryptophan and contain iso-C_{15:0}, iso-C_{17:0} 3-OH and summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c) as major fatty acids and MK-7 as major menaquinone. At the time of writing, it comprises two recognized species, *Mucilaginibacter paludis* and *Mucilaginibacter gracilis* (Pankratov *et al.*, 2007). In this study, we have characterized the novel *Mucilaginibacter*-like strain, Jip 10^T, which was isolated from dried rice straw in a field in Daejeon city in South Korea.

Strain Jip 10^T was isolated from dried rice straw via direct plating onto R2A agar (Difco). Single colonies on these

plates were purified by transferring them onto new plates and subjecting them to additional incubation for 3 days at 30 °C. The purified colonies were tentatively identified by using partial 16S rRNA gene sequences and maintained as a glycerol suspension (20 %, w/v) at –70 °C.

The Gram reaction was performed by the non-staining method as described by Buck (1982). Cell morphology was observed at ×1000 magnification with a light microscope (Nikon) using cells grown for 3 days at 30 °C on R2A agar. Catalase activity was determined by bubble production in 3 % (v/v) H₂O₂ and oxidase activity was determined using 1 % (w/v) tetramethyl *p*-phenylenediamine. Carbon-source utilization and enzyme activities were tested by using the API 20NE, ID 32 GN, API 50 CH and API ZYM test kits (bioMérieux). The ability to use nitrate as an alternative terminal electron acceptor was tested in serum bottles by adding sodium thioglycolate (1 g l^{–1}) to R2A broth and replacing the upper air layer with nitrogen gas. The anaerobic nitrate reduction test to determine the final electron acceptor was performed in serum bottles by adding sodium thioglycolate (1 g l^{–1}) and 10 mM KNO₃ to R2A broth, under nitrogen gas. Growth at different

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temperatures (4, 15, 20, 25, 30, 37 and 42 °C) and at various pH values (pH 5.0–10.0 at intervals of 0.5 pH units) was assessed after 5 days of incubation. Salt tolerance was tested on R2A agar supplemented with 1–10 % (w/v) NaCl after 5 days of incubation. Growth on nutrient agar, trypticase soy agar (TSA) and MacConkey agar was also evaluated at 30 °C.

Cells of strain Jip 10^T were facultatively aerobic, Gram-negative, heterotrophic, non-motile, non-spore-forming rods. The strain did not grow on nutrient agar or MacConkey agar. The ability to reduce nitrate was absent. Other physiological characteristics of strain Jip 10^T are summarized in the species description and a comparison of selective characteristics with closely related species is shown in Table 1.

For the measurement of G + C content of the chromosomal DNA, genomic DNA of the novel strain was extracted and purified as described by Moore & Dowhan (1995), degraded enzymically into nucleosides and analysed as described by Mesbah *et al.* (1989) using reversed-phase HPLC. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum conditions and re-extracted in n-hexane/water (1:1, v/v). The crude n-hexane-quinone solution was purified using Sep-Pak Vac silica cartridges (Waters) and subsequently analysed by HPLC as described by Hiraishi *et al.* (1996). Cellular fatty acid profiles were determined from biomass harvested from one-tenth-strength TSA (Difco) after 3 days of incubation. The cellular fatty acids were

saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System. The fatty acids were analysed by a gas chromatograph (Hewlett Packard 6890) and identified by the Microbial Identification software package (Sasser, 1990).

The G + C content of genomic DNA of strain Jip 10^T was 48.1 mol%. MK-7 was the major respiratory quinone and MK-6 was a minor component. As shown in Table 2, the major fatty acids of this strain were iso-C_{15:0} (26.5 %), C_{16:0} (9.3 %), iso-C_{17:0} 3-OH (7.1 %) and summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c) (39.1 %). These results supported the affiliation of strain Jip 10^T to the genus *Mucilaginibacter*.

Extraction of genomic DNA was performed with a commercial genomic DNA extraction kit (Solgent) and PCR amplification of the 16S rRNA gene and sequencing of the purified PCR product were performed according to Kim *et al.* (2005). The full sequence of the 16S rRNA gene was compiled using DNASTAR. The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed by using the CLUSTAL x program (Thompson *et al.*, 1997). Gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983) and phylogenetic trees were constructed by using the neighbour-joining method (Saitou & Nei, 1987) using the MEGA 3 program (Kumar *et al.*, 2004) with

Table 1. Biochemical characteristics of strain Jip 10^T and the type strains of *Mucilaginibacter* species

Strains: 1, strain Jip 10^T; 2, *M. gracilis* TPT18^T; 3, *M. paludis* TPT56^T. Data for reference strains were taken from Pankratov *et al.* (2007). All three strains are positive for copious EPS production, acid production from D-fructose, D-galactose, D-glucose and maltose and catalase and oxidase activities. All strains are negative for indole production. All three strains assimilate D-glucose, sucrose and maltose, but not acetate, citrate, malate, mannitol, melibiose, propionate or valerate.

Characteristic	1	2	3
Growth at/in:			
37 °C	+	–	–
>1 % NaCl	+	–	–
Acid production from:			
L-Rhamnose	+	+	–
D-Mannose	+	–	+
Trehalose	+	–	+
D-Xylose	–	+	+
Assimilation of:			
Mannose	+	–	+
Gluconate	–	–	+
Salicin	+	–	+
N-Acetyl-D-glucosamine	+	+	–
G + C content (mol%)	40.4	47.2	48.9

Table 2. Fatty acid profiles of strain Jip 10^T and the type strains of *Mucilaginibacter* species

Strains: 1, strain Jip 10^T; 2, *M. gracilis* TPT18^T; 3, *M. paludis* TPT56^T. Data for reference strains were taken from Pankratov *et al.* (2007). –, <1.0 % of the total/not reported.

Fatty acid	1	2	3
Saturated			
C _{15:0}	–	3.2	4.5
C _{16:0}	9.3	3.1	–
C _{18:0}	1.7	–	–
Unsaturated			
C _{15:1} ω6c	–	–	1.2
C _{16:1} ω5c	5.9	5.8	2.6
C _{18:3} ω6c	1.3	–	–
Branched-chain			
iso-C _{15:0}	26.5	15.7	32.2
iso-C _{15:0} 3-OH	2.2	1.8	3.2
iso-C _{16:0}	–	1.5	–
iso-C _{17:0} 3-OH	7.1	8.9	13.1
iso-C _{17:1} ω9c	3.0	–	2.7
anteiso-C _{15:0}	1.8	21.1	5.8
anteiso-C _{17:0}	–	4.5	–
Hydroxy			
C _{16:0} 3-OH	2.3	–	–
Summed feature 3*	39.1	24.7	21.5

*Summed feature 3 comprises iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

The nearly complete 16S rRNA gene sequence (1469 bp) of strain Jip 10^T was determined and subjected to comparative analysis. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate clustered with *Mucilaginibacter* in the phylum *Bacteroidetes* (Fig. 1). 16S rRNA gene sequence similarities between strain Jip 10^T and the type strains of *M. gracilis* and *M. paludis* were 93.7 and 93.6 %, respectively. These values (<97 %) were low enough to place strain Jip 10^T in a novel species in the genus *Mucilaginibacter* according to Stackebrandt & Goebel (1994).

Description of *Mucilaginibacter daejeonensis* sp. nov.

Cells are Gram-negative, facultatively aerobic, non-motile, non-spore-forming and rod-shaped, 0.6–0.8 µm in

diameter and 1.3–1.6 μm long after 2 days on R2A agar. Colonies are opaque, circular, convex and orange in colour after 2 days on R2A agar. On R2A agar, grows at 15–37 $^{\circ}\text{C}$, but not at 4 or 42 $^{\circ}\text{C}$. Growth occurs at pH 4.5–8.5 and with up to 3% NaCl. Optimal growth occurs at 30 $^{\circ}\text{C}$, pH 7.0 and without NaCl. Substrate utilization, enzyme production and other physiological characteristics are indicated in Table 1. In addition to the properties listed in Table 1, L-arabinose and rhamnose are utilized as sole carbon sources. Adipate, L-alanine, 4-hydroxybenzoate, caprate, L-fucose, glycogen, histidine, 3-hydroxybenzoate, 3-hydroxybutyrate, inositol, itaconate, 2-ketogluconate, 5-ketogluconate, lactate, malonate, phenylacetate, L-proline, D-ribose, L-serine and suberate are not utilized as sole carbon sources. In API 50 CH tests, acid is produced from *N*-acetylglucosamine, aesculin ferric citrate, amygdalin, DL-arabinose, cellobiose, gentiobiose, lactose, melibiose, methyl α -glucopyranoside, starch, sucrose and turanose, but acid is not produced from adonitol, DL-arabitol, arbutin, dulcitol, erythritol, DL-fucose, glycerol, glycogen, inositol, inulin, D-lyxose, D-mannitol, melezitose, methyl α -D-mannopyranoside, methyl α -D-xyloside, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate, raffinose, D-ribose, D-sorbitol, L-sorbose, salicin, D-tagatose, xylitol or L-xylose. According to the API ZYM gallery, positive for *N*-acetyl- α -glucosaminidase, acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), α -galactosidase, α -galactosidase, leucine arylamidase and valine arylamidase activities, weakly positive for α -glucosidase and negative for chymotrypsin, α -fucosidase, α -glucosidase, α -glucuronidase, lipase (C14), α -mannosidase, naphthol-AS-BI-phosphohydrolase and trypsin. According to the API 20NE gallery, positive for arginine dihydrolase, urease, α -glucosidase, protease, α -galactosidase and glucose fermentation, but negative for nitrate reduction. The predominant respiratory quinone is MK-7. The major fatty acids are iso-C_{15:0}, C_{16:0}, iso-C_{17:0} 3-OH and summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c). The DNA G + C content of the type strain is 40.4 mol%.

The type strain, strain Jip 10^T (=KCTC 12639^T =LMG 23488^T), was isolated from dried rice straw in a field in Daejeon city in South Korea.

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Fig. 1. Rooted phylogenetic tree based on the 16S rRNA gene sequences of strain Jip 10^T and related bacteria in the phylum *Bacteroidetes*, made using the neighbour-joining method (Saitou & Nei, 1987) with a distance matrix generated using the two-parameter model of Kimura (1983) and pairwise deletion. Bootstrap values (expressed as percentages of 1000 replications) greater than 70 % are shown at branch points. Bar, 2 substitutions per 100 nucleotides.

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