Mucilaginibacter boryungensis sp. nov., isolated from soil

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A Gram-stain-negative, non-motile, non-spore-forming bacterial strain, BDR-9^T, was isolated from soil collected from Boryung on the west coast of the Korean peninsula, and its taxonomic position was investigated by using a polyphasic study. Strain BDR-9^T grew optimally at 25 °C, at pH 6.0–7.5 and in the absence of NaCl. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain BDR-9^T fell within the clade comprising species of the genus *Mucilaginibacter* within the phylum *Bacteroidetes*. 16S rRNA gene sequence similarity values between strain BDR-9^T and the type strains of species of the genus *Mucilaginibacter* were in the range 94.0–95.6 %. Strain BDR-9^T contained MK-7 as the predominant menaquinone and iso-C_{15:0} and C_{16:1}ω7c and/or iso-C_{15:0} 2-OH as the major fatty acids. The DNA G + C content was 44.3 mol%. Differential phenotypic properties and phylogenetic distinctiveness of strain BDR-9^T demonstrated that this strain is distinguishable from species of the genus *Mucilaginibacter*. On the basis of phenotypic, chemotaxonomic and phylogenetic data, strain BDR-9^T is considered to represent a novel species of the genus *Mucilaginibacter*, for which the name *Mucilaginibacter boryungensis* sp. nov. is proposed. The type strain is BDR-9^T (=KCTC 23157^T =CCUG 59599^T).

The genus *Mucilaginibacter* was first proposed by Pankratov *et al.* (2007) through the descriptions of two novel species, *Mucilaginibacter paludis* and *Mucilaginibacter gracilis*. Subsequently, five further species with validly published names, *Mucilaginibacter kameinonensis* (Urai *et al.*, 2008), *Mucilaginibacter daejeonensis* (An *et al.*, 2009), *Mucilaginibacter ximonensis* (Luo *et al.*, 2009), *Mucilaginibacter oryzae* (Jeon *et al.*, 2009) and *Mucilaginibacter rigui* (Baik *et al.*, 2010), were described. Phylogenetic analysis based on 16S rRNA gene sequences showed that the genus *Mucilaginibacter* forms an evolutionary lineage within the phylum *Bacteroidetes* (Pankratov *et al.*, 2007). Members of the genus *Mucilaginibacter* have been isolated from peat bogs, soil, dried rice straw and wetland freshwater (Pankratov *et al.*, 2007; Urai *et al.*, 2008; An *et al.*, 2009; Baik *et al.*, 2010). In the study, we report the taxonomic characterization of a *Mucilaginibacter*-like bacterial strain, BDR-9^T, which was isolated from soil collected from Boryung on the west coast of the Korean peninsula.

Strain BDR-9^T was isolated by means of the standard dilution plating technique at 25 °C on R2A agar (Difco). The type strains of four species of the genus *Mucilaginibacter*

were used as reference strains for phenotypic characterization and fatty acid analysis. *M. paludis* KACC 13450^T and *M. oryzae* KACC 12816^T were obtained from the Korean Agricultural Culture Collection (KACC), Suwon, South Korea. *M. kameinonensis* KCTC 22227^T and *M. ximonensis* KCTC 22437^T were obtained from the Korean Collection for Type Cultures (KCTC), Taejeon, South Korea. The morphological, physiological and biochemical characteristics of strain BDR-9^T were investigated using routine cultivation on R2A agar at 25 °C. Cell morphology was examined by using light microscopy (E600; Nikon) and transmission electron microscopy (CM-20; Philips). Flagellation was determined by using transmission electron microscopy on cells from an exponentially growing culture. For this purpose, the cells were negatively stained with 1 % (w/v) phosphotungstic acid and the grids were examined after being air-dried. Gliding motility was investigated as described by Bowman (2000). The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth in the absence of NaCl and in the presence of 0.5, 1.0, 2.0 and 3.0 % (w/v) NaCl was investigated by using R2A broth prepared according to the formula of the Difco medium except that agar was excluded. Growth at 4, 15, 20, 25, 28, 30, 37 and 40 °C was measured on R2A agar. The pH range for growth was determined in R2A broth adjusted to pH 4.0–9.5 in increments of 0.5 pH units by the addition of HCl or Na₂CO₃. Growth under anaerobic conditions was

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The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain BDR-9^T is HM061614.

Table 1. Differential phenotypic characteristics of strain BDR-9^T and some phylogenetically related species of the genus *Mucilaginibacter*

Strains: 1, *M. boryungensis* sp. nov. BDR-9^T; 2, *M. kameinonensis* KCTC 22227^T; 3, *M. oryzae* KACC 12816^T; 4, *M. ximonensis* KCTC 22437^T. All data from this study except for flexirubin-type pigments, oxidase and DNA G + C content; these data from Urai *et al.* (2008), Jeon *et al.* (2009) and Luo *et al.* (2009). +, Positive reaction; –, negative reaction; w, weakly positive reaction. All strains are Gram-stain-negative and rod-shaped. All strains are positive for hydrolysis of aesculin, utilization of D-xylose, galactose, glucose, mannose and maltose, and activity of leucine arylamidase, acid phosphatase, α -galactosidase (weak for *M. kameinonensis* KCTC 22227^T), β -galactosidase (weak for *M. kameinonensis* KCTC 22227^T) and N-acetyl- β -glucosaminidase. All species are negative for motility, utilization of erythritol, D-arabinose, ribose, L-xylose, sorbose, dulcitol, inositol, mannitol, amygdalin, inulin, xylitol, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, 2-ketogluconate and 5-ketogluconate, and activity of esterase (C4), lipase (C14), cystine arylamidase, trypsin, α -chymotrypsin and β -glucuronidase.

Characteristic	1	2	3	4
Colony colour on R2A agar	Light yellow	Pale yellow	Light pink	Yellow
Flexirubin-type pigments	+	+	–	–
Oxidase	–	–	+	+
Utilization of:				
Glycerol	–	+	–	–
L-Arabinose	+	+	+	–
Adonitol	+	–	–	–
β -Methyl D-xyloside	–	+	+	–
Fructose	–	+	+	+
Rhamnose	+	+	+	–
Sorbitol	–	–	–	+
α -Methyl D-mannoside	–	+	+	+
α -Methyl D-glucoside	–	+	+	+
N-Acetylglucosamine	–	+	+	+
Arbutin	–	+	+	–
Aesculin	–	+	+	+
Salicin	–	+	–	–
Cellobiose	–	+	+	+
Lactose	+	+	+	–
Melibiose	–	+	+	+
Sucrose	–	+	+	+
Trehalose	–	+	+	+
Melezitose	–	+	+	+
Raffinose	–	+	+	+
Starch	–	+	+	–
Glycogen	–	+	+	–
Gentiobiose	+	+	–	–
Turanose	–	+	+	+
L-Fucose	–	+	–	–
Gluconate	–	+	+	–
Enzyme activity (API ZYM)				
Alkaline phosphatase	+	–	+	+
Esterase lipase (C8)	–	w	–	–
Valine arylamidase	–	–	w	–
Naphthol-AS-BI-phosphohydrolase	+	–	–	–
α -Glucosidase	–	+	+	+
β -Glucosidase	–	+	+	–
α -Mannosidase	–	–	–	+
α -Fucosidase	–	–	–	+
DNA G + C content (mol%)	44.3	43.7	44.4	43.4

determined after incubation in a Forma anaerobic chamber on R2A agar and on R2A agar supplemented with potassium nitrate (0.1 %, w/v), both of which had been prepared anaerobically under a nitrogen atmosphere. Catalase and oxidase activities were determined as described by Cowan &

Steel (1965). Hydrolysis of casein, starch, hypoxanthine, tyrosine and xanthine was tested on R2A agar by using the substrate concentrations described by Cowan & Steel (1965). Nitrate reduction and hydrolysis of aesculin, gelatin, Tweens 20, 40, 60 and 80 and urea were investigated as described by

Lányi (1987). H₂S production was tested as described by Bruns *et al.* (2001). Susceptibility to antibiotics was tested on R2A agar plates using antibiotic discs (Advantec) containing the following: polymyxin B (100 U), streptomycin (50 µg), penicillin G (20 U), chloramphenicol (100 µg), ampicillin (10 µg), cephalothin (30 µg), gentamicin (30 µg), novobiocin (5 µg), tetracycline (30 µg), kanamycin (30 µg), lincomycin (15 µg), oleandomycin (15 µg), neomycin (30 µg) and carbenicillin (100 µg). Utilization of various substrates, enzyme activities and other physiological and biochemical properties were tested by using the API 20E, API 50CH and API ZYM systems (bioMérieux); utilization of various substrates was determined by inoculating the API 50CH strip with cells suspended in AUX medium (bioMérieux). The presence of flexirubin-type pigments was investigated as described by Reichenbach (1992). Morphological, cultural, physiological and biochemical characteristics of strain BDR-9^T are given in the species description or in Table 1.

Cell biomass of strain BDR-9^T for DNA extraction and for isoprenoid quinone analysis was obtained from cultures grown for 3 days at 25 °C in R2A broth prepared according to the formula of the Difco medium except that agar was excluded. Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). The almost complete 16S rRNA gene sequence of strain BDR-9^T determined in this study comprised 1445 nt (approx. 96 % of the *Escherichia coli* 16S rRNA sequence). In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain BDR-9^T fell within the cluster

comprising species of the genus *Mucilaginibacter* (Fig. 1). Strain BDR-9^T exhibited 94.0–95.6 % 16S rRNA gene sequence similarity to the type strains of species of the genus *Mucilaginibacter*, including species without validly published names at the time of writing, and less than 91.6 % to the other species used in the phylogenetic analysis. The DNA G+C content was determined by the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. The DNA G+C content of strain BDR-9^T was 44.3 mol%.

Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. The predominant isoprenoid quinone detected in strain BDR-9^T was menaquinone-7 (MK-7), which is the same as that found in members of the genus *Mucilaginibacter* (Pankratov *et al.*, 2007; Urai *et al.*, 2008; An *et al.*, 2009; Luo *et al.*, 2009; Jeon *et al.*, 2009). For fatty acid methyl ester analysis, cell mass of strain BDR-9^T and the type strains of four species of the genus *Mucilaginibacter* was harvested from R2A agar after incubation for 3 days at 25 °C. Fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). Cellular fatty acid profiles of strain BDR-9^T and the type strains of the four species of the genus *Mucilaginibacter* analysed in this study are shown in Table 2. The major fatty acids (>10 % of total fatty acids) found in strain BDR-9^T were iso-C_{15:0} and C_{16:1}ω7c and/or iso-C_{15:0} 2-OH (Table 2). The fatty acid profiles of the five strains were similar, although there were differences in the proportions of some fatty acids (Table 2). The fatty acid profiles of the four reference strains were also similar to those analysed previously (Pankratov *et al.*, 2007; Urai *et al.*, 2008; Luo

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain BDR-9^T, other species of the genus *Mucilaginibacter* and some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) >70 % are shown at branching points. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Flavobacterium aquatile* ATCC 11947^T (GenBank accession no. M62797) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Table 2. Cellular fatty acid compositions (%) of strain BDR-9^T and the type strains of some species of the genus *Mucilaginibacter*

Strains: 1, *M. boryungensis* sp. nov. BDR-9^T; 2, *M. paludis* KACC 13450^T; 3, *M. kameinonensis* KCTC 22227^T; 4, *M. oryzae* KACC 12816^T; 5, *M. ximonensis* KCTC 22437^T. All data from this study. Fatty acids that represented <0.5% in all strains were omitted. —, Not detected.

Fatty acid	1	2	3	4	5
Straight-chain fatty acid					
C _{14:0}	0.4	0.9	2.1	1.3	0.4
C _{15:0}	9.3	6.0	—	3.9	3.7
C _{16:0}	1.1	5.3	11.5	8.0	3.9
Branched fatty acid					
iso-C _{15:0}	28.3	22.6	21.0	18.8	37.9
anteiso-C _{15:0}	1.5	13.5	—	—	0.6
iso-C _{16:0}	1.0	0.4	—	—	0.1
iso-C _{17:0}	0.2	—	—	—	0.9
iso-C _{17:1ω9c}	4.2	0.6	—	2.5	5.0
Unsaturated fatty acid					
C _{15:1ω6c}	5.0	1.3	—	1.1	1.8
C _{16:1ω5c}	1.5	5.1	4.0	4.2	3.5
C _{17:1ω6c}	0.6	0.5	—	—	0.2
C _{17:1ω8c}	2.2	0.7	—	0.9	0.44
Hydroxy fatty acid					
C _{15:0} 2-OH	0.8	1.0	—	—	0.3
C _{15:0} 3-OH	0.6	—	—	—	0.7
C _{16:0} 3-OH	0.9	1.4	11.0	5.7	2.1
C _{17:0} 2-OH	0.4	0.6	—	—	—
C _{17:0} 3-OH	0.8	0.7	—	—	0.2
iso-C _{15:0} 3-OH	2.3	1.5	—	2.2	1.9
iso-C _{17:0} 3-OH	9.1	8.8	10.8	11.4	9.2
Summed features*					
3	24.5	26.8	39.7	39.4	25.2
5	2.3	—	—	—	—
Unknown fatty acids†					
ECL 13.565	0.3	1.0	—	0.8	0.5
ECL 16.582	0.8	0.5	—	—	0.8

*Summed features represent groups of two or three fatty acids which could not be separated by GLC with the MIDI system. Summed feature 3 contained C_{16:1ω7c} and/or iso-C_{15:0} 2-OH. Summed feature 5 contained C_{18:2ω6,9c} and/or anteiso-C_{18:0}.

†ECL, Equivalent chain-length.

et al., 2009; Jeon *et al.*, 2009). The results obtained from chemotaxonomic analyses, showing the properties that are shared by species of the genus *Mucilaginibacter*, were in agreement with the result of the phylogenetic analysis, i.e. that strain BDR-9^T belongs to the genus *Mucilaginibacter* (Pankratov *et al.*, 2007; Urai *et al.*, 2008; An *et al.*, 2009; Luo *et al.*, 2009; Jeon *et al.*, 2009).

Strain BDR-9^T was distinguishable from phylogenetically related species of the genus *Mucilaginibacter* by differences in several phenotypic characteristics, most of which were determined in this study under the same conditions (Table 1). The phylogenetic distinctiveness of strain BDR-9^T, together

with the differential phenotypic properties, is sufficient to demonstrate that this strain is separate from other species of the genus *Mucilaginibacter* (Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain BDR-9^T is considered to represent a novel species of the genus *Mucilaginibacter*, for which the name *Mucilaginibacter boryungensis* sp. nov. is proposed.

Description of *Mucilaginibacter boryungensis* sp. nov.

Mucilaginibacter boryungensis (bo.ry.ung.en'sis. N.L. masc. adj. *boryungensis* pertaining to Boryung, from where the type strain was isolated).

Cells are Gram-staining-negative, non-spore-forming, non-flagellated rods (0.4–0.6 × 1.0–6.0 µm). Colonies on R2A agar are circular to slightly irregular, slightly convex, smooth, glistening, light yellow and 2.0–3.0 mm in diameter after incubation for 5 days at 25 °C. Optimal growth occurs at 25 °C; grows at 4 and 37 (weakly) °C, but not at 40 °C. Optimal pH for growth is between 6.0 and 7.5; grows at pH 4.5 and 8.5, but not at pH 4.0 or 9.0. Optimal growth occurs in the absence of NaCl; growth occurs with 0–0.5% (w/v) NaCl. Growth occurs under anaerobic conditions on R2A and on R2A supplemented with nitrate. Aesculin, starch, tyrosine and Tweens 20, 40, 60 and 80 are hydrolysed, but casein, hypoxanthine, xanthine and urea are not. Flexirubin-type pigments are produced. Susceptible to chloramphenicol, gentamicin, lincomycin, neomycin, oleandomycin, streptomycin and tetracycline, but not to ampicillin, carbenicillin, cephalothin, kanamycin, novobiocin, penicillin G or polymyxin B. The predominant menaquinone is MK-7. The major fatty acids (>10% of total fatty acids) are iso-C_{15:0} and C_{16:1ω7c} and/or iso-C_{15:0} 2-OH. The DNA G + C content is 44.3 mol% (HPLC). Other phenotypic properties are shown in Table 1.

The type strain, BDR-9^T (=KCTC 23157^T =CCUG 59599^T), was isolated from soil around the coast at Boryung, Korea.

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