Pedobacter boryungensis sp. nov., isolated from soil

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A Gram-negative, non-sporulating, non-flagellated rod, designated BR-9^T, was isolated from soil collected on the Korean peninsula. Strain BR-9^T grew optimally at pH 6.0–7.0, at 30 °C and in the absence of NaCl. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain BR-9^T belonged to the genus *Pedobacter* and clustered with *Pedobacter insulae* DS-139^T and *Pedobacter koreensis* WPCB189^T. Strain BR-9^T exhibited 98.2 and 97.5 % 16S rRNA gene sequence similarity with *P. insulae* DS-139^T and *P. koreensis* WPCB189^T, respectively, and <96.7 % sequence similarity with the type strains of other species in the genus *Pedobacter*. Strain BR-9^T contained MK-7 as the predominant menaquinone and iso-C_{15:0} and summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) as the major fatty acids. The DNA G + C content of strain BR-9^T was 38.5 mol%. DNA–DNA relatedness between strain BR-9^T and *P. insulae* DS-139^T and *P. koreensis* KCTC 12536^T was 3.4–4.2 %, which indicated that the isolate was genetically distinct from these type strains. Strain BR-9^T was also distinguishable by differences in phenotypic properties. On the basis of the data presented, strain BR-9^T is considered to represent a novel species of the genus *Pedobacter*, for which the name *Pedobacter boryungensis* sp. nov. is proposed. The type strain is BR-9^T (=KCTC 23344^T =CCUG 60024^T).

The genus *Pedobacter* was proposed by Steyn *et al.* (1998) with the reclassification of two *Sphingobacterium* species and the description of two novel species. Recently, the number of described *Pedobacter* species has increased considerably. Interestingly, many novel *Pedobacter* species have been isolated from various regions of Korea (Hwang *et al.*, 2006; Ten *et al.*, 2006; Yoon *et al.*, 2006, 2007a, b, c, d; Baik *et al.*, 2007; Kwon *et al.*, 2007; Roh *et al.*, 2008; An *et al.*, 2009; Lee *et al.*, 2009; Jeon *et al.*, 2009). At the time of writing, the genus *Pedobacter* comprised 32 species with validly published names (Euzéby, 1997, 2010). In this study, we report the taxonomic characterization of a *Pedobacter*-like bacterial strain, BR-9^T, which was isolated from soil in Korea.

The standard dilution-plating technique was used for isolation of bacterial strains from surface soil samples collected from Boryung city. Strain BR-9^T was isolated on R2A agar (Difco) at 25 °C and routinely cultivated on R2A agar at 30 °C. The type strains of two species of the genus *Pedobacter* were used as reference strains for DNA–DNA hybridization, phenotypic characterization and fatty acid

analysis. *Pedobacter insulae* DS-139^T was obtained in our previous study (Yoon *et al.*, 2007b) and *Pedobacter koreensis* KCTC 12536^T was obtained from the KCTC.

Cell morphology was examined using light microscopy (E600; Nikon) and transmission electron microscopy (CM-20; Philips). Flagellation was investigated using cells from an exponentially growing culture. For this purpose, 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 investigated using the bioMérieux Gram-stain kit, according to the manufacturer's instructions. Growth under anaerobic conditions was determined by incubation in a Forma anaerobic chamber on R2A agar and R2A agar supplemented with 0.1 % (w/v) potassium nitrate, both prepared under a nitrogen atmosphere. Growth at 4, 10, 20, 25, 30, 35 and 37 °C was measured on R2A agar. Growth at pH 4.5–10.5 (in increments of 0.5 pH units) was investigated in R2 broth (MBcell) after adjusting the pH with HCl or Na₂CO₃. The pH was verified after autoclaving. Growth with 0, 0.5, 1.0 and 2.0 % (w/v) NaCl was investigated using trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BR-9^T is HM640986.

omitted. Catalase and oxidase activities and hydrolysis of casein, gelatin, hypoxanthine, starch, Tweens 20, 40, 60 and 80, tyrosine, urea and xanthine were determined as described by Cowan & Steel (1965). Aesculin hydrolysis and nitrate reduction were studied as described by Lányi (1987) and H_2S production was tested as described by Bruns *et al.* (2001). Utilization of various substrates, enzyme activities and other physiological and biochemical properties were investigated using the API 20 NE, API 50 CH and API ZYM systems (bioMérieux); to inoculate the API 50 CH system, cells were suspended in AUX medium, according to the manufacturer's instructions. Susceptibility to antibiotics was investigated on R2A agar using antibiotic discs containing the following (μg per disc unless otherwise stated): ampicillin (10), carbenicillin (100), cephalothin (30), chloramphenicol (100), gentamicin (30), kanamycin (30), lincomycin (15), neomycin (30), novobiocin (5), oleandomycin (15), penicillin G (20 U), polymyxin B (100 U), streptomycin (50) and tetracycline (30).

Cell biomass for DNA extraction and isoprenoid quinone analysis was obtained from cultures grown for 2 days in R2A broth at 30 °C. Chromosomal DNA was extracted 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), and the amplification products were purified using a QIAquick PCR purification kit (Qiagen). Sequencing and phylogenetic analysis were performed as described by Yoon *et al.* (2003). Isoprenoid quinones were extracted according to

the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 $\times$ 4.6 mm) column. For cellular fatty acid analysis, cell mass of strain BR-9^T, *P. insulae* DS-139^T and *P. koreensis* KCTC 12536^T was harvested from R2A agar after cultivation for 3 days at 30 °C. Fatty acids were saponified, methylated and extracted using the standard protocol of the Sherlock Microbial Identification System version 4.0 (Microbial ID). The fatty acids were analysed by GC (Hewlett Packard 6890) and identified using the TSBA40 database (Sasser, 1990). 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. DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample; the highest and lowest values obtained for each sample were excluded and the means of the remaining three values are quoted as DNA–DNA relatedness.

The almost-complete 16S rRNA gene sequence of strain BR-9^T obtained in this study comprised 1443 nt and represented approximately 96% of the *Escherichia coli* 16S rRNA gene sequence. In the phylogenetic tree constructed using the neighbour-joining algorithm, strain BR-9^T fell within the genus *Pedobacter* and joined the cluster containing *P. insulae* DS-139^T and *P. koreensis* WPCB189^T (Fig. 1). The topology was supported by a bootstrap resampling value of 99.5% and was recovered in trees reconstructed using the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). Strain BR-9^T exhibited 98.2 and 97.5% 16S rRNA

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strain BR-9^T with members of the genus *Pedobacter* and some other related taxa. Bootstrap values (>70%) based on 1000 replications are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-likelihood and maximum-parsimony algorithms. The open circle indicates that the corresponding node was also recovered in a tree generated with one of these algorithms. The sequence of *Cytophaga hutchinsonii* ATCC 33406^T (GenBank accession no. M58768) was used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.

gene sequence similarity to *P. insulae* DS-139^T and *P. koreensis* WPCB189^T, respectively, and <96.7 % sequence similarity to the type strains of other species of the genus *Pedobacter*. The predominant isoprenoid quinone detected in strain BR-9^T was menaquinone-7 (MK-7), which is in agreement with that of members of the genus *Pedobacter* (Steyn *et al.*, 1998). The fatty acid profile of strain BR-9^T is shown in Table 1, together with those obtained in this study for the reference strains. The fatty acid profiles of the three strains were similar, although there were differences in the proportions of some fatty acids (Table 1). The DNA G+C content of strain BR-9^T was 38.5 mol%, which is similar to those reported for *P. insulae* and *P. koreensis* (39.4 and 38.0 mol%, respectively) and most other *Pedobacter* species.

Strain BR-9^T exhibited 3.4 and 4.2 % DNA–DNA relatedness to *P. insulae* DS-139^T and *P. koreensis* KCTC 12536^T,

Table 1. Cellular fatty acid compositions of strain BR-9^T and its closest phylogenetic neighbours

Strains: 1, *Pedobacter boryungensis* sp. nov. BR-9^T; 2, *P. insulae* DS-139^T; 3, *P. koreensis* KCTC 12536^T. All data were taken from this study. Fatty acids that represented <0.5 % in all strains are omitted. tr, Trace (<0.5 %); –, not detected; ECL, equivalent chain-length.

Fatty acid	1	2	3
Straight-chain			
C _{14:0}	0.8	1.1	3.6
C _{15:0}	6.9	1.7	2.7
C _{16:0}	2.0	3.8	12.1
Unsaturated			
C _{15:1} ω6c	4.8	1.5	0.8
C _{16:1} ω5c	0.9	1.1	2.4
Branched			
iso-C _{14:0}	1.1	–	0.8
iso-C _{15:0}	29.9	26.1	26.3
anteiso-C _{15:0}	1.9	2.6	3.8
iso-C _{16:0}	3.0	3.0	3.3
iso-C _{16:1} H*	1.5	2.2	0.6
iso-C _{17:0}	tr	tr	0.6
iso-C _{17:1} ω9c	1.6	4.4	0.7
anteiso-C _{17:1} ω9c	1.0	2.0	–
Hydroxy			
C _{15:0} 2-OH	1.7	2.0	1.1
iso-C _{15:0} 3-OH	2.5	2.8	1.8
C _{16:0} 3-OH	0.7	0.6	2.6
iso-C _{16:0} 3-OH	5.2	3.5	5.7
C _{17:0} 2-OH	2.2	5.5	4.6
iso-C _{17:0} 3-OH	6.7	8.1	6.1
Summed feature 3†	17.8	24.4	17.4
Unknown ECL 13.565	1.4	1.1	1.0

*The double bond position indicated by the capital letter is unknown.

†Summed features represent groups of two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted of C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

Table 2. Differential phenotypic characteristics of strain BR-9^T and its closest phylogenetic neighbours

Strains: 1, *P. boryungensis* sp. nov. BR-9^T; 2, *P. insulae* DS-139^T; 3, *P. koreensis* KCTC 12536^T. Data were obtained in this study or taken from Yoon *et al.* (2007b) and Baik *et al.* (2007). All strains are positive for catalase, hydrolysis of aesculin, starch, Tweens 20, 40, 60 and 80 and L-tyrosine, assimilation of D-glucose and D-mannose, susceptibility to chloramphenicol, gentamicin, lincomycin, novobiocin and tetracycline and activities of alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl-β-glucosaminidase. All strains are negative for gliding motility, anaerobic growth, Gram staining, nitrate reduction, hydrolysis of casein, gelatin, hypoxanthine, urea and xanthine, assimilation of malate, citrate, phenylacetate, erythritol, D-arabinose, L-xylose, methyl β-D-xyloside, D-fructose, L-sorbose, dulcitol, inositol, D-mannitol, D-sorbitol, inulin, melezitose, raffinose, xylitol, D-tagatose, D- and L-fucose, D- and L-arabitol, 2-ketogluconate and 5-ketogluconate, susceptibility to ampicillin and activities of esterase (C4), esterase lipase (C8), lipase (C14), trypsin, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase. +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3
Optimal temperature (°C)	30	25	25
Oxidase	+	+	–
Assimilation (API 50 CH) of:			
Glycerol	–	+	–
L-Arabinose	–	+	–
D-Ribose	–	–	+
D-Xylose	–	+	+
D-Adonitol	–	+	–
D-Galactose	–	–	+
L-Rhamnose	–	w	–
Methyl α-D-mannoside	–	w	–
Methyl α-D-glucoside	–	+	–
N-Acetylglucosamine	+	+	–
Amygdalin	–	+	–
Arbutin	w	+	–
Salicin	–	+	–
Cellobiose	–	+	+
Maltose	–	+	+
Lactose	–	+	+
Melibiose	–	+	–
Trehalose	–	+	–
Starch	–	+	–
Glycogen	–	+	+
Gentiobiose	–	+	+
Turanose	–	w	–
Susceptibility to:			
Streptomycin	+	+	–
Penicillin G	–	+	–
Enzyme activities (API ZYM)			
Cystine arylamidase	w	–	–
α-Chymotrypsin	–	+	–
α-Galactosidase	+	–	–
β-Galactosidase	–	–	+
α-Glucosidase	+	+	–

respectively. These values indicate that strain BR-9^T represents a genomic species distinct from these two *Pedobacter* species (Wayne *et al.*, 1987). The phenotypic characteristics of strain BR-9^T were compared with those of the reference strains determined using the same methods (Yoon *et al.*, 2007b; Baik *et al.*, 2007; Table 2). Strain BR-9^T could be differentiated from the reference strains by differences in some phenotypic characteristics, including assimilation of various substrates, activity of several enzymes and susceptibility to several antibiotics.

The phylogenetic distinctiveness, together with DNA–DNA relatedness and differential phenotypic properties, is sufficient to allocate strain BR-9^T to a species that is separate from recognized members of the genus *Pedobacter* (Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain BR-9^T is considered to represent a novel species of the genus *Pedobacter*, for which the name *Pedobacter boryungensis* sp. nov. is proposed.

Description of *Pedobacter boryungensis* sp. nov.

Pedobacter boryungensis (bo.ry.un.gen'sis. N.L. masc. adj. *boryungensis* pertaining to Boryung, the Korean city from where the type strain was isolated).

Cells are Gram-negative, non-sporulating, non-flagellated rods (0.2–0.5 × 2.0–5.0 µm). Colonies on R2A agar are circular, convex, smooth, glistening, pale yellow and 0.5–1.0 mm in diameter after incubation for 2 days at 30 °C. Optimal temperature for growth is 30 °C; grows at 4–35 °C, but not at 37 °C. Optimal pH for growth is pH 6.0–7.0; grows at pH 5.5, but not at pH 5.0. Optimal growth occurs without NaCl; grows with 1.0 % (w/v) NaCl, but not with 2.0 % (w/v) NaCl. Anaerobic growth does not occur on R2A agar or R2A agar supplemented with nitrate. H₂S is not produced. Sucrose, D-xylose and gluconate are not utilized as sole carbon and energy sources. Susceptible to cephalothin, chloramphenicol, gentamicin, lincomycin, novobiocin, polymyxin B, streptomycin and tetracycline, but not to ampicillin, carbenicillin, kanamycin, neomycin, penicillin G or oleandomycin. The predominant menaquinone is MK-7. The major fatty acids (>10 % of the total) are iso-C_{15:0} and summed feature 3 (C_{16:1ω7c} and/or iso-C_{15:0} 2-OH). The DNA G + C content of the type strain is 38.5 mol% (HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, BR-9^T (=KCTC 23344^T =CCUG 60024^T), was isolated from soil in Boryung city, on the Korean peninsula.

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