Parapedobacter koreensis gen. nov., sp. nov.

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Strain Jip14^T, a Gram-negative, non-spore-forming, rod-shaped, non-motile bacterium, was isolated from dried rice straw and characterized in order to determine its taxonomic position. 16S rRNA gene sequence analysis revealed that strain Jip14^T belongs to the family *Sphingobacteriaceae*, and the highest degree of sequence similarity was determined to be to *Pedobacter saltans* DSM 12145^T (88.5%), *Pedobacter africanus* DSM 12126^T (87.6%), *Pedobacter heparinus* DSM 2366^T (87.1%) and *Pedobacter caeni* LMG 22862^T (86.9%). Chemotaxonomic data revealed that strain Jip14^T possesses menaquinone MK-7 and the predominant fatty acids C_{15:0} iso, C_{16:0}, C_{16:0} 10-methyl, C_{17:0} iso 3-OH and summed feature 3 (C_{15:0} iso 2-OH/C_{16:1ω7c}). The results of physiological and biochemical tests clearly demonstrated that strain Jip14^T represents a distinct species. Based on these data, Jip14^T should be classified within a novel genus and species, for which the name *Parapedobacter koreensis* gen. nov., sp. nov. is proposed. The type strain of *Parapedobacter koreensis* is Jip14^T (=KCTC 12643^T=LMG 23493^T).

The family *Sphingobacteriaceae* was proposed by Steyn *et al.* (1998). Typical features of the family *Sphingobacteriaceae* are the possession of sphingolipids, the presence of the MK-7 quinone system, a relatively low G+C content (37–44 mol%) and a unique fatty acid profile (including C_{15:0} iso, C_{15:0} iso 2-OH and C_{15:0} iso 3-OH). At present, the family *Sphingobacteriaceae* includes two genera, *Pedobacter* and *Sphingobacterium*. The genus *Sphingobacterium* was established by Yabuuchi *et al.* (1983) with three species, *Sphingobacterium spiritivorum* (originally *Flavobacterium spiritivorum*), *S. mizutaii* and *S. multivorum*. At present, following the reclassification of two species to *Pedobacter*, the genus *Sphingobacterium* includes another four accepted species, *Sphingobacterium antarcticum* (Shivaji *et al.*, 1992), *Sphingobacterium faecium*, *Sphingobacterium* (*Flavobacterium*) *thalpophilum* (Takeuchi & Yokota, 1992) and *Sphingobacterium daejeonense* (Kim *et al.*, 2006). The genus *Pedobacter* was proposed by Steyn *et al.* (1998) with the reclassification of two *Sphingobacterium* species as *Pedobacter heparinus* and *Pedobacter piscium* and the description of two novel species, *Pedobacter africanus* and *Pedobacter saltans*. Subsequently, *Pedobacter cryoconitis* (Margesin *et al.*, 2003), *Pedobacter himalayensis* (Shivaji *et al.*, 2005),

Pedobacter caeni (Vanparrys *et al.*, 2005), *Pedobacter aquatilis* (Gallego *et al.*, 2006), *Pedobacter ginsengisoli* (Ten *et al.*, 2006), *Pedobacter sandarakinus* (Yoon *et al.*, 2006), *Pedobacter roseus* (Hwang *et al.*, 2006), *Pedobacter panaciterrae* (Yoon *et al.*, 2007) and *Pedobacter suwonensis* (Kwon *et al.*, 2007) have been described. Data for these last six recently described species were not available for comparison at the time of writing.

In a series of studies, we attempted to isolate micro-organisms from dried straw in order to investigate the community structure based on a culture-dependent method. In this study, strain Jip14^T was isolated from dried rice straw in a field in Daejeon city in South Korea and characterized by a polyphasic approach, including phylogenetic analysis based on 16S rRNA gene sequences, genomic relatedness and chemotaxonomic and phenotypic properties. The results obtained in this study indicated that strain Jip14^T can be assigned as a member of the family *Sphingobacteriaceae* within a novel genus and species.

Strain Jip14^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 5 days at 30 °C. The purified colonies were tentatively identified by using partial 16S rRNA gene sequences.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain Jip14^T is DQ680836.

Cell morphology and motility were observed with a Nikon light microscope ($\times 1000$ magnification), with the cells being allowed to grow for 3 days at 30 °C on R2A agar. The Gram reaction was examined according to the non-staining method (Buck, 1982). Oxidase activity was evaluated via the oxidation of 1 % *p*-aminodimethylaniline oxalate. Catalase activity was determined by measurements of bubble production after the application of 3 % (v/v) H₂O₂ solution. Acid production from carbohydrates was assessed by the procedures outlined by Cappuccino & Sherman (2002). Growth at 4, 15, 25, 30, 37 and 42 °C was assessed on R2A agar and growth at various pH values was assessed in R2A broth. Growth was also evaluated at 30 °C on nutrient agar (NA), trypticase soy agar (TSA) and MacConkey agar. The API 20NE and API ID32 GN microtest systems were employed according to the recommendations of the manufacturer (bioMérieux).

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), purified via TLC and subsequently analysed by HPLC as described previously (Collins & Jones, 1981; Shin *et al.*, 1996). In order to perform fatty acid methyl ester analysis, the strain was allowed to grow on TSA for 48 h at 30 °C and then two loops of well-grown cells were harvested. Fatty acid methyl esters were prepared, separated and identified with the Sherlock Microbial Identification System (MIS) (Sasser, 1990). Polyamines were extracted and analysed according to Busse & Auling (1988) and Schenkel *et al.* (1995), respectively. Polar lipids were extracted and examined by using two-dimensional TLC (Minnikin *et al.*, 1984).

The genomic DNA of strain Jip14^T was extracted and purified with the Qiagen Genomic-tip system 100/G and was then enzymically degraded into nucleosides as described previously (Tamaoka & Komagata, 1984; Mesbah *et al.*, 1989).

Genomic DNA was extracted and purified with the Genomic DNA isolation kit (Core Bio System). The 16S rRNA gene was amplified from chromosomal DNA of strain Jip14^T using the universal bacterial primers 9F and 1512R (Weisburg *et al.*, 1991) and the purified PCR products were sequenced by Genotec (Daejeon, Korea) (Kim *et al.*, 2005). The full sequence of the 16S rRNA gene was compiled with SeqMan software and the 16S rRNA gene sequence of the test strain was edited using the BioEdit program (Hall, 1999). The 16S rRNA gene sequences of related taxa were obtained from GenBank and multiple alignments were performed with the CLUSTAL X program (Thompson *et al.*, 1997). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). The phylogenetic tree was constructed via the neighbour-joining method (Saitou & Nei, 1987) in the MEGA2 program (Kumar *et al.*, 2001). Bootstrap analysis with 1000 replicates was also conducted in order to obtain confidence levels for the branches (Felsenstein, 1985). All species of the genera *Pedobacter* and *Sphingobacterium* were included in the phylogenetic tree.

Strain Jip14^T was cultured on R2A agar (Difco) at 30 °C, yielding white, circular colonies. Strain Jip14^T was found to be an aerobic, Gram-negative, non-motile and rod-shaped bacterium. Strain Jip14^T was also determined to be able to grow at 25–42 °C, but did not grow at 4 °C. Growth at 37 °C was not observed on MacConkey agar. Physiological characteristics of strain Jip14^T are summarized in the species description and a comparison of differential characteristics with related type strains is shown in Table 1.

Strain Jip14^T was similar to members of the family *Sphingobacteriaceae* in that it was a Gram-negative, non-motile, aerobic, non-spore-producing, oxidase- and catalase-positive, rod-shaped bacterium. Strain Jip14^T has properties similar to those of species of the genus *Pedobacter* in that it has no urease or α -mannosidase activity. In contrast to species in the genera *Pedobacter* and *Sphingobacterium*, strain Jip14^T utilizes inositol as a sole carbon source and does not have esterase activity.

The cellular fatty acid profiles of strain Jip14^T and related *Pedobacter* and *Sphingobacterium* type strains are shown in Table 2. The major cellular fatty acids in strain Jip14^T included C_{15:0} iso (33.6 %), C_{16:0} (6.0 %), C_{16:0} 10-methyl (7.1 %), C_{17:0} iso 3-OH (14.4 %) and summed feature 3 (C_{15:0} iso 2-OH/C_{16:1} ω 7c; 27.8 %). Minor amounts of C_{14:0} (1.1 %), C_{16:0} anteiso (0.5 %), C_{15:1} ω 6c (1.0 %), C_{15:0} 3-OH (1.7 %), C_{15:0} iso 3-OH (2.3 %), C_{16:0} 2-OH (1.1 %) and C_{16:0} 3-OH (1.9 %) were also present. The fatty acid profile of strain Jip14^T is different from those of members of related genera. There are a number of qualitative differences; for instance, strain Jip14^T has no C_{16:1} ω 5c or C_{17:1} iso ω 9c. These fatty acids are common to *Pedobacter* species but absent in *Sphingobacterium*.

Strain Jip14^T contained a menaquinone with seven isoprene units (MK-7) as the predominant isoprenoid quinone. Menaquinone MK-7 is commonly found in species in the family *Sphingobacteriaceae* (Steyn *et al.*, 1998). The major polyamine of strain Jip14^T was homospermidine, as is the case for other species in the family *Sphingobacteriaceae*. Sphingolipid, which is a characteristic feature of the family *Sphingobacteriaceae*, was also detected.

The 16S rRNA gene sequence of strain Jip14^T was found to be a continuous stretch of 1481 nucleotides. Strain Jip14^T was determined to belong to the class *Sphingobacteria*, order *Sphingobacteriales* and family *Sphingobacteriaceae*. The highest degrees of sequence similarity were found with *Pedobacter saltans* DSM 12145^T (88.5 %), *Pedobacter africanus* DSM 12126^T (87.6 %), *Pedobacter heparinus* DSM 2366^T (87.1 %) and *Pedobacter caeni* LMG 22862^T (86.9 %). In the phylogenetic tree (Fig. 1), strain Jip14^T clearly belonged to the family *Sphingobacteriaceae* lineage, as evidenced by the high bootstrap value. Based on 16S rRNA gene sequencing, the phylogenetic position of strain Jip14^T among members of the family *Sphingobacteriaceae* was unique and distinct.

Table 1. Differential phenotypic characteristics between strain Jip14^T, *Pedobacter* species and other related species

Strains: 1, strain Jip14^T; 2, *Pedobacter africanus* LMG 10353^T; 3, *Pedobacter caeni* LMG 22862^T; 4, *Pedobacter cryoconitis* DSM 14825^T; 5, *Pedobacter heparinus* LMG 10339^T; 6, *Pedobacter himalayensis* HHS 22^T; 7, *Pedobacter piscium* LMG 140^T; 8, *Pedobacter saltans* LMG 10337^T; 9, *S. faecium* DSM 14022^T; 10, *S. mizutaii* ATCC 33299^T; 11, *S. multivorum* LMG 8342^T; 12, *S. spiritivorum* LMG 8347^T; 13, *S. thalpophilum* LMG 11520^T. +, Positive; –, negative; V, variable; ND, no data available. Data for reference *Pedobacter* strains, with the exception of *Pedobacter caeni* LMG 22862^T (data from Vanparys *et al.*, 2005), were taken from Shivaji *et al.* (2005); data for reference *Sphingobacterium* strains were taken from Steyn *et al.* (1998).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Gliding motility	–	–	+	+	+	–	–	–	–	–	–	–	–
Optimum growth temperature (°C)	37	37	37	25	37	25	25–30	25–30	ND	ND	ND	ND	ND
Growth at 37 °C	+	V	+	ND	V	ND	–	V	+	+	+	+	+
Nitrate reduction	–	–	–	–	–	–	–	–	–	–	–	–	+
Enzyme activities													
N-Acetyl-β-glucosaminidase	+	+	–	+	+	+	+	+	+	+	+	+	+
Arginine dihydrolase	–	–	–	–	–	+	ND	–	ND	ND	ND	ND	ND
Esterase (C8)	–	+	+	+	+	+	+	+	+	+	+	+	+
α-Glucosidase	+	+	–	+	+	+	+	+	+	+	+	+	+
β-Glucosidase	+	V	+	+	–	+	+	+	+	+	+	+	+
β-Glucuronidase	–	ND	–	ND	ND	ND	ND	ND	V	–	V	+	+
Lipase	–	+	+	–	–	–	V	+	V	+	V	+	–
α-Mannosidase	–	–	–	ND	ND	ND	ND	ND	+	+	+	+	+
Protease	–	V	–	+	–	+	–	–	–	–	V	–	–
Urease	–	–	–	–	–	–	–	–	+	+	+	+	+
Valine arylamidase	+	ND	–	ND	ND	ND	ND	ND	+	+	+	+	+
Growth on MacConkey agar	–	–	ND	–	–	+	–	–	ND	ND	ND	ND	ND
Glucose fermentation	+	+	–	–	+	–	+	V	+	+	+	+	+
Assimilation tests													
Acetate	–	–	ND	–	–	–	–	–	–	–	–	–	–
Caprate	–	ND	–	ND	ND	ND	ND	ND	–	–	–	–	–
Citrate	–	–	–	–	–	–	–	–	–	V	–	–	–
L-Arabinose	–	V	+	ND	+	ND	+	+	+	V	+	–	+
L-Fucose	+	V	–	+	+	+	–	–	–	–	–	–	–
D-Glucose	+	–	+	+	–	+	–	+	+	+	+	+	+
Maltose	+	V	+	+	+	+	+	+	+	+	+	+	+
Melibiose	–	+	+	+	+	+	+	V	+	+	+	+	+
L-Rhamnose	–	+	–	–	+	+	V	+	ND	ND	ND	ND	ND
Ribose	+	V	–	–	–	+	–	–	–	–	–	–	–
Sucrose	+	+	+	+	+	+	+	V	+	+	+	+	+
Inositol	+	–	–	–	–	–	–	–	–	–	–	–	–
Mannitol	+	–	–	–	+	+	–	–	–	–	–	+	–
Sorbitol	+	–	–	–	+	+	–	–	–	–	–	–	–
L-Alanine	–	V	ND	–	–	+	–	–	ND	ND	ND	ND	ND
L-Histidine	–	V	ND	–	–	–	–	–	–	+	V	–	–
L-Proline	–	V	ND	–	–	+	+	–	–	+	–	–	–
L-Serine	+	+	ND	–	V	+	V	–	–	V	–	–	–
N-Acetyl-D-glucosamine	+	+	+	+	+	+	+	+	ND	ND	ND	ND	ND
Salicin	+	V	+	+	+	+	+	+	+	+	+	+	+
Glycogen	–	–	–	+	–	–	–	–	–	–	V	–	–
DNA G + C content (mol%)	45.6	43–45	42.7	43	42–43	41	40–43	36–38	37.3	39.3–30	39.9–40.5	39.8	44–44.2

The G + C content of the genomic DNA of the strain Jip14^T was 45.6 mol%, which is slightly higher than values reported for other species in the family *Sphingobacteriaceae*. Yabuuchi

et al. (1983) reported that members of the genus *Sphingobacterium*, the type genus of the family, showed DNA G + C contents of 39–42 mol%.

Table 2. Cellular fatty acid profiles of strain Jip14^T, *Pedobacter* species and other related species

Strains: 1, strain Jip14^T; 2, *Pedobacter africanus* LMG 10353^T; 3, *Pedobacter caeni* LMG 22862^T; 4, *Pedobacter cryoconitis* DSM 14825^T; 5, *Pedobacter heparinus* LMG 10339^T; 6, *Pedobacter himalayensis* HHS 22^T; 7, *Pedobacter piscium* LMG 140^T; 8, *Pedobacter saltans* LMG 10337^T; 9, *S. faecium* DSM 14022^T; 10, *S. mizutaii* ATCC 33299^T; 11, *S. multivorum* LMG 8342^T; 12, *S. spiritivorum* LMG 8347^T; 13, *S. thalpophilum* LMG 11520^T. Data for reference strains were taken from Steyn *et al.* (1998), with the exception of *Pedobacter caeni* LMG 22862^T (data from Vanparys *et al.*, 2005) and *Pedobacter himalayensis* HHS 22^T (Shivaji *et al.*, 2005). For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain; *cis* isomers are indicated by the suffix *c*. Values are percentages of total fatty acids; tr, trace amount; ECL, equivalent chain length.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12	13
Saturated													
C _{14:0}	1.1	1.5	2.5	1.0	1.1	1.0	1.3	tr	tr	tr	2.7	1.0	3.2
C _{15:0}		tr			1.1			1.4					
C _{15:0} anteiso		tr	tr		tr		2.6	2.9	tr	tr		tr	
C _{15:0} iso	33.6	26.6	21.3	15.0	28.2	33.0	26.2	31.4	24.6	30.0	22.2	30.1	17.7
C _{16:0}	6.0	3.8	3.0	9.1	3.0	7.3	3.3	4.0	4.5	tr	7.8	3.5	6.0
C _{16:0} anteiso	0.5												
C _{16:0} 10-methyl	7.1								1.4				
Unsaturated													
C _{15:1} ω 6 <i>c</i>	1.1	tr						1.4					
C _{16:1} ω 5 <i>c</i>		2.1	2.5		1.4		3.5	tr	1.5	tr		tr	
C _{16:1} ω 7 <i>c</i>	—*	23.7	—	27.0	20.2	20.0	31.4	19.6	32.2	9.5	31.6	21.1	23.2
C _{17:1} iso ω 9 <i>c</i>		4.4	2.2	8.6	6.3	4.0	1.6	6.6					
C _{17:1} anteiso ω 9 <i>c</i>			tr				1.2						
Hydroxy													
C _{15:0} 2-OH			tr										
C _{15:0} iso 2-OH	—	10.4	—	2.0	10.4	2.0	10.7	8.9	15.9	25.6	17.4	21.5	24.6
C _{15:0} 3-OH	1.7												
C _{15:0} iso 3-OH	2.3	2.1	3.3	1.9	2.5	1.5	2.5	2.8	3.7	3.0	3.2	2.2	4.3
C _{16:0} 2-OH	1.1	tr									tr		3.2
C _{16:0} 3-OH	1.9	3.1	2.8		1.5		4.5	tr					
C _{17:0} 2-OH			1.3										
C _{17:0} iso 3-OH	14.4	14.7	12.1	4.2	15.2	6.0	9.2	12.7	10.0	22.1	7.1	12.5	10.0
Summed feature 3*	27.8	(34.1)	39.9	(29.0)	(30.6)	(22.0)	(42.1)	(28.5)	(48.1)	(35.1)	(49.0)	(42.6)	(47.8)
Unknown fatty acids													
ECL 13.566	0.8	2.6			5.0		tr	1.1	tr	1.3		tr	1.4
ECL 16.580		1.0			1.0		1.0	tr	tr	tr	tr	tr	
ECL 16.582	0.7												

*Summed feature 3 contains C_{15:0} iso 2-OH and/or C_{16:1} ω 7*c*, which could not be separated reliably by GLC with the Microbial Identification System. Steyn *et al.* (1998) and Shivaji *et al.* (2005) used a more polar chromatographic system to separate these two components; their combined contents are given in parentheses.

On the basis of the phenotypic, chemotaxonomic and phylogenetic data, we conclude that strain Jip14^T is a member of a novel genus and species of the family *Sphingobacteriaceae*, for which the name *Parapedobacter koreensis* gen. nov., sp. nov. is proposed.

Description of *Parapedobacter* gen. nov.

Parapedobacter (Pa.ra.pe'do.bac.ter. Gr. pref. *para-* like, beside; N.L. masc. n. *Pedobacter* a bacterial genus; N.L. masc. n. *Parapedobacter* like *Pedobacter*, referring to the close relationship to the genus).

Cells are Gram-negative. Oxidase- and catalase-positive. MK-7 is present in the respiratory chain. The major fatty acids are C_{15:0} iso, C_{16:0}, C_{16:0} 10-methyl, C_{17:0} iso 3-OH and summed feature 3 (C_{15:0} iso 2-OH/C_{16:1} ω 7*c*). The major polyamine is homospermidine and sphingolipid is also detected. The type species is *Parapedobacter koreensis*.

Description of *Parapedobacter koreensis* sp. nov.

Parapedobacter koreensis (ko.re.en'sis. N.L. masc. adj. *koreensis* of Korea, from where the type strain was isolated).

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences, showing the phylogenetic relationships between strain Jip14^T and *Pedobacter* and *Sphingobacterium* species. The neighbour-joining method was used. Bar, 0.02 substitutions per nucleotide position.

Displays the following properties in addition to those given in the genus description. Cells are aerobic, non-motile rods after growth on R2A agar (Difco) at 30 °C for 5 days. Colonies grown on R2A agar for 5 days are white and circular. Optimal growth temperature is 37 °C. Grows in a salt concentration of 3 %, but it cannot grow above 4 %. Produces *N*-acetyl- β -glucosaminidase, acid and alkaline phosphatases, α -chymotrypsin, trypsin, α - and β -glucosidases, α - and β -galactosidases and cystine, leucine and valine arylamidases but does not produce arginine dihydrolase, esterase, α -fucosidase, β -glucuronidase, lipase, α -mannosidase, naphthol-AS-BI-phosphohydrolase, protease or urease. Assimilates gluconate, L-fucose, D-glucose, D-maltose, D-mannose, D-ribose, sucrose, inositol, D-mannitol, D-sorbitol, L-serine, *N*-acetyl-D-glucosamine and salicin. Does not assimilate 2-ketogluconate, 3-hydroxybenzoate, 3-hydroxybutyrate, 4-hydroxybenzoate, 5-ketogluconate, acetate, adipate, caprate, citrate, itaconate, lactate, L-malate, malonate, phenylacetate, propionate, suberate, n-valerate, L-arabinose, D-melibiose, L-rhamnose, L-alanine, L-histidine, L-proline or glycogen. The DNA G+C content of the type strain is 45.6 mol% as determined by HPLC. The major cellular fatty acids are C_{15:0} iso (33.57 %), C_{16:0} (6.02 %), C_{16:0} 10-methyl (7.08 %), C_{17:0} iso 3-OH (14.39 %) and summed feature 3 (27.8 %). Minor components C_{14:0} (1.12 %), C_{16:0} anteiso (0.54 %), C_{15:1} ω 6c (1.05 %), C_{15:0} 3-OH (1.7 %), C_{15:0} iso 3-OH (2.34 %), C_{16:0} 2-OH (1.06 %) and C_{16:0} 3-OH (1.9 %) are also present. Does not reduce nitrate to nitrite or nitrogen gas.

The type strain, Jip14^T (=KCTC 12643^T=LMG 23493^T), was isolated from dried rice straw in South Korea.

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