Pedobacter composti sp. nov., isolated from compost

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A Gram-negative, aerobic, rod-shaped, non-motile, non-spore-forming bacterial strain, designated TR6-06^T, was isolated from a compost sample in South Korea and characterized taxonomically by using a polyphasic approach. The organism grew optimally at 30 °C and pH 6.5–7.0. The isolate was positive for catalase and oxidase tests, but negative for gelatinase and urease and for indole and H₂S production. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain TR6-06^T was most closely affiliated with members of the genus *Pedobacter* of the family *Sphingobacteriaceae*. Strain TR6-06^T exhibited 16S rRNA gene sequence similarity values of 89.9–93.5 % to the type strains of species of the genus *Pedobacter*. The G+C content of the genomic DNA of strain TR6-06^T was 41.9 mol%. The predominant respiratory quinone was MK-7. The major fatty acids were iso-C_{15:0}, iso-C_{17:0} 3-OH, C_{16:1}ω7c and anteiso-C_{15:0}. These chemotaxonomic data support the affiliation of strain TR6-06^T to the genus *Pedobacter*. However, on the basis of its phenotypic properties and phylogenetic distinctiveness, strain TR6-06^T (=KCTC 12638^T=LMG 23490^T) should be classified as the type strain of a novel species, for which the name *Pedobacter composti* sp. nov. is proposed.

The genus *Pedobacter* was proposed by Steyn *et al.* (1998) to accommodate species characterized by Gram-negative rods that produce heparinase, are obligately aerobic, with or without gliding motility, are negative for urease, lipase, gelatinase, arginine dihydrolase, indole production and nitrate reduction and contain iso-C_{15:0}, iso-C_{15:0} 2-OH, iso-C_{15:0} 3-OH, C_{16:0}, C_{16:1}ω5c, C_{16:1}ω7c, C_{16:0} 3-OH, iso-C_{17:0} 3-OH and iso-C_{17:1}ω9c fatty acids. At the time of writing, the genus comprised 16 recognized species, including the recently described species *Pedobacter lentus* and *Pedobacter terricola* (Yoon *et al.*, 2007). In this study, taxonomic characterization of a *Pedobacter*-like bacterial strain, TR6-06^T, is reported.

Strain TR6-06^T was originally isolated from a compost sample composed of cow dung and rice straw near Daejeon city in South Korea. This compost sample was suspended in 50 mM phosphate buffer (pH 7.0) and the suspension was spread on R2A agar plates (Difco) after serial dilution with 50 mM phosphate buffer (pH 7.0). Plates were incubated at 30 °C for 2 weeks. Single colonies on these plates were purified by transferring them onto new plates and subjecting them to an additional incubation for 3 days

at 30 °C. Purified colonies were tentatively identified using partial 16S rRNA gene sequences. Strain TR6-06^T was cultured routinely on R2A agar at 30 °C 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. Motility was tested using the hanging drop technique. 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 20E, API 20NE, API 32GN, API 50CH and API ZYM test kits (bioMérieux). Anaerobic growth was tested in serum bottles containing R2A broth supplemented with sodium thioglycollate (1 g l^{–1}) with the upper air layer substituted by nitrogen gas. Growth at different temperatures (4, 15, 20, 25, 30, 37 and 42 °C) and pH (pH 5.0–10.0 at intervals of 0.5 pH units) was assessed after 5 days incubation. Salt tolerance was determined on R2A agar supplemented with 1–10 % (w/v) NaCl after 5 days incubation. Growth on nutrient agar, trypticase soy agar (TSA) and MacConkey agar was also evaluated at 30 °C.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain TR6-06^T is AB267720.

Cells of strain TR6-06^T were strictly aerobic, Gram-negative, non-motile, non-spore-forming and rod-shaped. Colonies grown on R2A agar plates for 4 days were 0.8–1.5 mm in diameter. The isolate grew well on nutrient agar and TSA, but did not grow on MacConkey agar. Other physiological characteristics of strain TR6-06^T are sum-

marized in the species description. Phenotypic characteristics that differentiate the novel isolate from its closest phylogenetic relatives are listed in Table 1.

For measurement of the chromosomal DNA G+C content, genomic DNA of the novel strain was extracted

Table 1. Comparison of the phenotypic characteristics of strain TR6-06^T and related type strains of species of the genus *Pedobacter*

Strains: 1, TR6-06^T; 2, *P. africanus* DSM 12126^T; 3, *P. cryoconitis* DSM 14825^T; 4, *P. himalayensis* HHS 22^T; 5, *P. heparinus* DSM 2366^T; 6, *P. piscium* DSM 11725^T. All strains are positive for aerobic growth, catalase, oxidase, phosphatase, β -galactosidase, aesculin hydrolysis, and utilization of *N*-acetylglucosamine, maltose and salicin as sole carbon sources. All strains are negative for Gram staining, indole and H₂S production, urease, nitrate reduction to nitrite, utilization of inositol and L-histidine as sole carbon sources, and acid production from adonitol, D-lactose, melibiose and D-rhamnose. +, Positive; –, negative; $\pm$, variable; NA, data not available. Data for taxa 2–6 are from Shivaji *et al.* (2005).

Characteristic	1	2	3	4	5	6
Habitat	Compost	Soil, sludge	Glacier cryoconite	Glacier water	Soil	Fish
Colony diameter (mm)	0.8–1.5	2–4	1.6–2.0	1.6–2	1–4	2–4
Colony shape	Round, convex, smooth with entire margins	Round, convex, smooth with entire scalloped margins	Round, convex, mucoid with entire margins	Round, convex, mucoid with entire margins	Round, convex or slightly umbonate with entire margins	Round, convex, opaque, smooth with entire margins
Colony colour	Pale orange	Translucent yellow	Creamish white	Pale white	Translucent yellow	Yellow or creamish white
Growth range (°C)	25–37	NA	1–25	4–25	5–30	5–30
Maximum growth temperature (°C)	37	37	25	25	37	30
Motility	Non-motile	Non-motile	Motile/gliding	Non-motile	Gliding	Non-motile
Cell shape	Short rods	NA	Long rods	Long rods	Short rods	Long rods
Gelatinase	–	$\pm$	+	+	–	–
Lysine decarboxylase	–	+	–	–	+	+
Tryptophan deaminase	–	–	–	+	–	NA
Arginine dihydrolase	–	–	–	+	–	NA
β -Glucosidase	+	$\pm$	+	+	–	+
Acid production from:						
D-Arabinose	–	$\pm$	–	–	$\pm$	$\pm$
D-Glucose	–	+	–	–	+	+
D-Mannose	–	–	–	+	–	NA
Mannitol	–	–	+	+	–	$\pm$
Inositol	–	–	–	+	–	–
Sorbitol	–	–	–	+	–	–
Utilization of carbon sources:						
D-Arabinose	+	$\pm$	–	+	–	–
Ribose	–	$\pm$	–	+	–	–
D-Glucose	+	–	+	+	–	–
L-Fucose	–	$\pm$	+	+	+	–
L-Rhamnose	–	+	–	+	+	$\pm$
Melibiose	–	+	+	+	+	+
Sucrose	–	+	+	+	+	+
Glycogen	–	–	+	–	–	–
Mannitol	–	–	–	+	+	–
D-Sorbitol	–	–	–	+	+	–
L-Alanine	–	$\pm$	–	+	–	–
L-Serine	–	+	–	+	$\pm$	$\pm$
L-Proline	–	$\pm$	–	+	–	+
DNA G + C content (mol%)	42	43–45	43	41	42–43	40–43

and purified as described by Moore & Dowhan (1995) and degraded enzymically into nucleosides; G + C content was determined as described by Mesbah *et al.* (1989) using reverse-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 then analysed by HPLC, as described previously (Hiraishi *et al.*, 1996). Cellular fatty acid profiles were determined for strains grown on TSA (Difco) for 3 days. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids analysed by GC (Hewlett Packard 6890) were identified by the Microbial Identification software package (Sasser, 1990).

The G + C content of genomic DNA of strain TR6-06^T was 41.9 mol%. The predominant menaquinone was MK-7. The fatty acid profile of strain TR6-06^T was composed of branched-chain, saturated and unsaturated fatty acids; the major fatty acids were iso-C_{15:0} (31.7%), anteiso-C_{15:0} (8.9%), iso-C_{17:0} 3-OH (12.5%) and C_{16:1}ω7c (11.8%). Fatty acid data for strain TR6-06^T and related type strains of *Pedobacter* species are given in Table 2. The fatty acid profile of strain TR6-06^T, including iso-C_{15:0}, iso-C_{17:0}

3-OH and C_{16:1}ω7c as the major fatty acids, is similar to those of species of the genus *Pedobacter*, although there are differences in the proportions of some fatty acids, perhaps because of differences in extraction and cultivation conditions (Shivaji *et al.*, 2005; Vanparys *et al.*, 2005; Ten *et al.*, 2006).

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* (2005). Full sequences of the 16S rRNA gene were compiled using SEQMAN software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed with the program CLUSTAL_X (Thompson *et al.*, 1997). Gaps were edited in the program BIOEDIT (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were constructed by using the neighbour-joining method (Saitou & Nei, 1987) with the MEGA3 program (Kumar *et al.*, 2004) with bootstrap values based on 1000 replications (Felsenstein, 1985).

Nearly complete 16S rRNA gene sequences (1430 bp) of strain TR6-06^T were determined and subjected to phylogenetic analysis; results indicated that strain TR6-06^T belonged to the genus *Pedobacter* (Fig. 1). Strain TR6-06^T showed the highest level of 16S rRNA gene sequence similarity to *Pedobacter africanus* DSM 12126^T (93.3% similarity), followed by *Pedobacter cryoconitis* DSM 14825^T (93.1%), *Pedobacter himalayensis* HHS 22^T (93.0%), *Pedobacter heparinus* DSM 2366^T (92.6%) and *Pedobacter piscium* DSM 11725^T (92.5%). The values (<97%) were low enough, according to Stackebrandt & Goebel (1994), to classify strain TR6-06^T as a representative of a novel species in the genus *Pedobacter*.

On the basis of the data described above, strain TR6-06^T should be assigned to the genus *Pedobacter* as the type strain of a novel species, for which the name *Pedobacter composti* sp. nov. is proposed.

Description of *Pedobacter composti* sp. nov.

Pedobacter composti (com.pos'ti. N.L. gen. n. *composti* of compost).

Cells are Gram-negative, strictly aerobic, non-motile and 0.2–0.3 × 1.2–1.6 μm after cultivation for 4 days on R2A agar. Colonies grown on R2A agar (Difco) for 3 days are smooth, circular, convex and pale orange in colour. Good growth occurs at 30 °C and pH 7.0. Growth is observed at 15–37 °C, at pH 5.0–8.0 and in 0–1% (w/v) NaCl. Unable to reduce nitrate. Anaerobic growth does not occur. Substrate utilization, enzyme production and other physiological characteristics are indicated in Table 1. In addition, utilizes L-arabinose, glucose, maltose, mannose, N-acetylglucosamine and salicin as sole carbon sources. Does not utilize acetate, adipate, L-alanine, caprate, citrate, L-fucose, gluconate, glycogen, L-histidine, 3- or 4-hydroxybenzoate, 3-hydroxybutyrate, inositol, itaconate, 2- or

Table 2. Fatty acid composition (%) of strain TR6-06^T and related type strains of species of the genus *Pedobacter*

Strains: 1, TR6-06^T; 2, *P. africanus* DSM 12126^T; 3, *P. cryoconitis* DSM 14825^T; 4, *P. himalayensis* HHS 22^T; 5, *P. heparinus* DSM 2366^T; 6, *P. piscium* DSM 11725^T. Strain TR6-06^T was grown in TSA medium at 30 °C for the determination of fatty acid composition in the present study. Data for taxa 2–6 are from Shivaji *et al.* (2005). tr, Trace (<1%); –, not detected; ECL, equivalent chain-length.

Fatty acid	1	2	3	4	5	6
ECL 13.566	1.3	2.6	—	—	5.0	tr
C _{14:0}	tr	1.5	1.0	1.0	1.1	1.3
C _{15:0}	6.0	tr	2.0	2.0	1.1	2.0
C _{15:1} ω6c	3.8	tr	2.0	2.0	2.0	2.0
iso-C _{15:0}	31.7	26.6	15.0	33.0	28.2	26.2
anteiso-C _{15:0}	8.9	tr	2.0	2.0	tr	2.6
iso-C _{15:0} 2-OH	—	1.0	2.0	2.0	1.0	1.1
iso-C _{15:0} 3-OH	5.2	2.1	1.9	1.5	2.5	2.5
C _{16:0}	2.8	3.8	9.1	7.3	3.0	3.3
C _{16:1} ω5c	tr	2.1	2.0	2.0	1.4	3.5
C _{16:1} ω7c	11.8	23.7	27	20.0	20.2	31.4
C _{16:0} 3-OH	1.1	3.1	2.0	2.0	1.5	4.5
iso-C _{16:0} 3-OH	3.6	tr	tr	tr	tr	tr
iso-C _{16:1} OH	1.9	—	—	—	—	—
C _{16:0} 10-methyl	2.1	—	—	—	—	—
ECL 16.580	tr	1.0	2.0	2.0	1.0	tr
iso-C _{17:1} ω9c	—	4.4	8.6	4.0	6.3	1.6
anteiso-C _{17:1} ω9c	1.5	2.0	2.0	2.0	2.0	1.2
iso-C _{17:0} 3-OH	12.5	14.7	4.2	6.0	15.2	9.2

Fig. 1. Phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationship between *Pedobacter composti* TR6-06^T and related species. The tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) with a two-parameter distance matrix (Kimura, 1983) and pairwise deletion. Bootstrap values (expressed as percentages of 1000 replications) greater than 60% are shown at the branch points. Bar, 0.02 substitutions per nucleotide position.

5-ketogluconate, lactate, malate, malonate, mannitol, melibiose, phenylacetate, L-proline, propionate, rhamnose, ribose, L-serine, sorbitol, suberate, sucrose or valerate as sole carbon sources. In API 50CH tests, acid is produced only from aesculin; acid is not produced from *N*-acetylglucosamine, adonitol, amygdalin, D- or L-arabinose, D- or L-arabitol, arbutin, cellobiose, dulcitol, erythritol, D- or L-fucose, fructose, galactose, gentiobiose, glucose, gluconate, glycerol, glycogen, inositol, inulin, 2- or 5-ketogluconate, lactose, D-lyxose, maltose, mannitol, mannose, melibiose, melezitose, methyl α -D-glucoside, methyl α -D-mannoside, methyl β -D-xyloside, raffinose, rhamnose, ribose, salicin, sorbitol, sorbose, starch, sucrose, D-tagatose, trehalose, turanose or D- or L-xylose. According to the API ZYM gallery, positive for acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, *N*-acetyl- β -glucosaminidase, valine arylamidase, β -galactosidase and β -glucosidase, weakly positive for α -glucosidase, and tests negative for lipase (C14), naphthol-AS-BI-phosphohydrolase, trypsin, α -chymotrypsin, α -fucosidase, α -galactosidase, α -mannosidase and β -glucuronidase. The predominant menaquinone is MK-7. The major fatty acids are iso-C_{15:0}, iso-C_{17:0} 3-OH, C_{16:1} ω 7c and anteiso-C_{15:0}. Other phenotypic characteristics are given in Table 1.

The type strain, TR6-06^T (=KCTC 12638^T=LMG 23490^T), was isolated from compost near Daejeon city, South Korea. The genomic DNA G+C content of the type strain is 41.9 mol%.

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