Pedobacter suwonensis sp. nov., isolated from the rhizosphere of Chinese cabbage (*Brassica campestris*)

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A mesophilic bacterium, strain 15-52^T, was isolated from the rhizosphere of Chinese cabbage (*Brassica campestris*). On the basis of phenotypic and genotypic characteristics, the bacterium was identified as representing a novel species belonging to the genus *Pedobacter*. The strain is non-flagellated, non-spore-forming and grows at temperatures in the range 1–37 °C. Physiological tests of the strain showed the presence of oxidase, catalase, protease (gelatin and casein hydrolysis), β -glucosidase and β -galactosidase activities. The highest levels of 16S rRNA gene sequence similarity were found with respect to *Pedobacter roseus* CL-GP80^T (97.3 %) and *Pedobacter sandarakinus* DS-27^T (97.2 %). A phylogenetic analysis based on 16S rRNA gene sequence data indicated that strain 15-52^T is a member of the genus *Pedobacter*. DNA–DNA hybridization analysis revealed low levels of relatedness (<42.3 %) between the isolate and two phylogenetically related type strains, *P. roseus* KCCM 42272^T and *P. sandarakinus* KCTC 12559^T. The DNA G + C content is 44.2 mol% and the predominant fatty acids are iso-C_{15:0} (35.4 %), iso-C_{15:0} 2-OH and/or C_{16:1} ω 7c (27.8 %) and iso-C_{17:0} 3-OH (15.8 %). On the basis of these data, strain 15-52^T represents a novel species of the genus *Pedobacter*, for which the name *Pedobacter suwonensis* sp. nov. is proposed. The type strain is 15-52^T (=KACC 11317^T = DSM 18130^T).

Pedobacter strains have been isolated from soils (Steyn *et al.*, 1998; Yoon *et al.*, 2006), water (Vanparys *et al.*, 2005; Gallego *et al.*, 2006; Hwang *et al.*, 2006) and glaciers (Margesin *et al.*, 2003; Shivaji *et al.*, 2005). One distinctive characteristic of members of the genus is their ability to grow in temperatures in the range 1–37 °C. Their colonies exhibit various colours, from dirty yellow to creamy white or pink (Gallego *et al.*, 2006), but the pigment does not produce the typical flexirubin reaction. Some species can degrade heparin: these include *Pedobacter heparinus*, *Pedobacter africanus*, *Pedobacter saltans* and *Pedobacter himalayensis*.

During the characterization of bacteria isolated from the rhizosphere of Chinese cabbage (*Brassica campestris* L.), we isolated a mesophilic strain, 15-52^T, belonging to the genus *Pedobacter*. Strain 15-52^T was cultured on trypticase soy agar (TSA; Difco) at 30 °C and maintained on TSA medium. The

cellular morphology was determined by using phase-contrast microscopy with 2-day-old cells. Gliding motility was observed by direct microscopic examination of the edge of colonies grown on 1/10-strength trypticase soy broth (TSB) supplemented with 1 % agar. Gram staining, catalase and oxidase activities, indole production and the hydrolysis of gelatin and aesculin were determined according to the methods of Smibert & Krieg (1994). Heparinase production was determined by using the method of Joubert *et al.* (1984). Flexirubin pigments were tested for by suspending cells in 20 % KOH (Fautz & Reichenbach, 1980). In addition, strain 15-52^T was characterized by using the complete test spectrum provided by the API 20NE and API ZYM kits, according to the instructions of the manufacturer (bioMérieux). Carbon assimilation was tested by using the API 50 CH gallery (bioMérieux), according to a standardized procedure (Kerstens *et al.*, 1984). The temperature ranges for the growth of strain 15-52^T were determined by using a temperature-gradient incubator (TN-2612; Advantec) and TSB at 7–45 °C (i.e. at 7, 14, 17, 21, 23, 26, 28, 31, 34, 37, 40 and 45 °C); additional temperatures

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 15-52^T is DQ097274.

(1–5 °C) were tested with TSA slants by using a bench-top incubator (e-Cooling Bucket; Taitec).

Fatty acid methyl esters were extracted and prepared by using the standard protocol of the Microbial Identification System (MIDI; Microbial ID), with cells grown on TSA for 48 h at 28 °C. Isoprenoid quinones were analysed by HPLC as described previously (Groth *et al.*, 1996). The DNA G + C content (mol%) was determined by HPLC analysis of the deoxyribonucleosides, as described by Mesbah *et al.* (1989), using a reverse-phase column (Supelcosil LC-18-S; Supelco).

The 16S rRNA gene sequence of strain 15-52^T was analysed as described by Kwon *et al.* (2003). The sequences were aligned together with those of representative members of selected genera by using the CLUSTAL W program (Thompson *et al.*, 1994). The evolutionary tree for the datasets was inferred from the neighbour-joining method of Saitou & Nei (1987) by using MEGA version 3.1 (Kumar *et al.*, 2004) (Fig. 1). The stability of relationships was assessed by performing bootstrap analyses of the neighbour-joining data, on the basis of 1000 resamplings. DNA–DNA hybridization was carried out using the filter hybridization method described by Seldin & Dubnau (1985). Probe labelling was conducted by using the non-radioactive DIG-High Prime system (Roche), and hybridized DNA was visualized using the DIG luminescent detection kit (Roche). DNA–DNA relatedness was quantified by using a densitometer (Bio-Rad).

The phenotypic features that distinguish strain 15-52^T from related *Pedobacter* species are shown in Table 1. The strain grew well on R2A, TSA and nutrient agar (Difco), but did not grow on MacConkey agar (Difco). The minimum doubling time of strain 15-52^T at 34 °C was about 2.6 h. In API ZYM tests, strain 15-52^T gave positive responses for 13 enzyme tests. In the API 50 CH tests, the strain assimilated a total of 26 carbohydrates, including D-glycerol.

The predominant cellular fatty acids of strain 15-52^T were iso-C_{15:0} (35.4 %), iso-C_{17:0} 3-OH (15.8 %) and iso-C_{15:0} 2-OH and/or C_{16:1}ω7c (27.8 %; the two fatty acids cannot be separated by GLC with the MIDI system) (Table 2).

Smaller amounts of iso-C_{17:1}ω9c (7.4 %), iso-C_{15:0} 3-OH (2.4 %), C_{16:0} (1.6 %), C_{16:1}ω5c (1.4 %) and C_{16:0} 3-OH (1.2 %) were also present. The DNA G + C content was 44.2 mol%, and the major respiratory quinones were MK-7 (90.5 %), MK-9 (5.6 %) and MK-8 (3.9 %).

Comparison of the 16S rRNA gene sequence of the strain with publicly available sequences of some *Pedobacter* strains showed that strain 15-52^T clustered in a monophyletic group together with nine *Pedobacter* species (not including *P. saltans*) at a bootstrap value of 100 %. The strain showed the highest levels of 16S rRNA gene sequence similarity with respect to *P. roseus* CL-GP80^T (97.3 %) and *P. sandarakinus* DS-27^T (97.2 %). The levels of similarity between strain 15-52^T and the type strains of other *Pedobacter* species ranged from 90.6 % (*P. saltans*) to 95.9 % (*P. aquatilis*). Strain 15-52^T exhibited DNA–DNA reassociation values of 42.3 and 28.9 %, respectively, with respect to *P. roseus* KCCM 42272^T and *P. sandarakinus* KCTC 12559^T, indicating that it is not related to them at the species level (Wayne *et al.*, 1987).

On the basis of this polyphasic taxonomic approach, strain 15-52^T represents a novel species of the genus *Pedobacter*, for which the name *Pedobacter suwonensis* sp. nov. is proposed.

Description of *Pedobacter suwonensis* sp. nov.

Pedobacter suwonensis (su.won.en'sis. N.L. masc. adj. *suwonensis* referring to Suwon, the city in which the type strain was isolated).

Cells are Gram-negative and rod-shaped (0.65 × 1.4–3.2 µm). After 2 days on TSA, colonies are circular, 2–4 mm in diameter, smooth, convex with entire margins and pinkish yellow. Grows well on R2A, TSA and nutrient agar, but does not grow on MacConkey agar. Cells are aerobic, non-motile, non-spore-forming and mesophilic. Flexirubin-type pigments are absent. Growth occurs at pH 5.0–8.0 and 0–3 % NaCl, with optimal growth at pH 6.5–7.0 and 0–0.5 % NaCl. Grows at temperatures in the range 1–37 °C, with optimum growth at 34 °C. Positive

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain 15-52^T with respect to *Pedobacter* species. *Hymenobacter roseosalivarius* AA-688 was used as the outgroup. Numbers at nodes indicate percentages of bootstrap support, based on 1000 resampled datasets; values below 50 % are not shown. Bar, 2 substitutions per 100 nucleotides.

Table 1. Phenotypic features that distinguish strain 15-52^T from *Pedobacter* species

Taxa: 1, strain 15-52^T; 2, *P. himalayensis*; 3, *P. cryoconitis*; 4, *P. heparinus*; 5, *P. africanus*; 6, *P. piscium*; 7, *P. caeni*; 8, *P. sandarakinus*; 9, *P. aquatilis*; 10, *P. roseus*. Data for reference species are from Steyn *et al.* (1998), Shivaji *et al.* (2005), Vanparys *et al.* (2005), Yoon *et al.* (2006), Gallego *et al.* (2006) and Hwang *et al.* (2006). +, Positive; –, negative; (+), weak; v, variable; ND, no data available. All taxa are positive for aerobic growth, the presence of catalase, oxidase, acid and alkaline phosphatases and β -galactosidase, aesculin hydrolysis and assimilation of D-cellobiose, D-glucose, D-mannose, lactose and N-acetylglucosamine. All taxa are negative for the Gram stain and for sporulation, indole production, nitrate reduction, urease and assimilation of erythritol, L-sorbose, dulcitol and inositol.

Characteristic	1	2	3	4	5	6	7	8	9	10
Colony colour	Pinkish yellow	Pale white	Creamy white	Translucent yellow	Translucent yellow	Yellow or creamy white	Yellow	Orange	Pink	Pink
Temperature for growth (°C)										
Range	1–37	4–25	1–25	5–30	ND	5–30	?–37	4–33	4–30	5–33
Maximum	37	25	25	37	37	25–30	37	33	30	33
Motility	Non-motile	Non-motile	Motile/ gliding	Gliding	Non-motile	Gliding	Non-motile	Non-motile	Non-motile	Non-motile
Growth on MacConkey agar	–	+	–	–	–	–	ND	–	–	+
Gelatinase	+	+	+	–	v	–	–	–	–	+
Heparinase	–	+	–	+	+	–	ND	ND	ND	–
Lipase	–	–	–	–	+	+	–	–	–	–
Arginine dihydrolase	–	+	–	–	–	–	–	–	–	–
Assimilation of carbon sources										
D-Glycerol	–	+	–	–	–	–	–	–	–	+
D-Arabinose	–	+	–	–	v	–	–	–	–	ND
L-Arabinose	+	+	+	+	v	+	+	–	+	+
D-Ribose	–	+	–	–	v	–	–	–	–	–
L-Xylose	–	+	–	–	–	–	+	–	–	+
D-Adonitol	–	+	–	+	–	–	–	–	–	ND
D-Galactose	+	+	+	+	+	v	–	+	+	+
D-Fructose	(+)	+	+	+	v	+	–	–	+	+
L-Rhamnose	+	+	–	+	+	v	–	–	+	+
D-Mannitol	–	+	–	+	–	–	–	–	–	–
D-Sorbitol	–	+	–	+	–	–	–	–	–	–
Methyl α -D-mannoside	+	+	–	+	+	–	+	(+)	+	ND
Inulin	–	+	(+)	–	–	–	–	–	–	+
D-Melezitose	+	–	–	–	–	–	–	–	+	ND
D-Raffinose	+	+	+	–	–	+	+	–	+	+
Starch	+	+	+	–	v	+	+	+	+	ND
Glycogen	+	–	+	–	–	–	–	+	–	+
Xylitol	–	–	–	–	–	–	+	–	–	ND
L-Fucose	–	+	–	+	v	–	–	–	–	ND
DNA G+C content (mol%)	44.2	41.0	43.0	42–43	43–45	40–43	42.7	39.7	38	41.3

for catalase, oxidase, protease (hydrolysis of gelatin and casein) and aesculin hydrolysis. Negative for lipase, urease, arginine dihydrolase, indole production and nitrate reduction. Utilizes L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, methyl α -D-mannoside, methyl α -D-glucoside, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, D-cellobiose, D-maltose, D-lactose, D-melibiose, sucrose, D-trehalose, D-melezitose,

D-raffinose, starch, glycogen, β -gentiobiose and D-turanose as sole carbon sources. Enzymic activity is observed for alkaline and acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, β -galactosidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase and α -fucosidase (API ZYM). The DNA G+C content is 44.2 mol%. The major menaquinone is MK-7 and the

Table 2. Fatty acid compositions (%) of strain 15-52^T and related *Pedobacter* species

Taxa: 1, strain 15-52^T; 2, *P. himalayensis*; 3, *P. cryoconitis*; 4, *P. heparinus*; 5, *P. africanus*; 6, *P. piscium*; 7, *P. caeni*; 8, *P. sandarakinus*; 9, *P. aquatilis*; 10, *P. roseus*. Data for reference strains are from Steyn *et al.* (1998), Shivaji *et al.* (2005), Vanparys *et al.* (2005), Yoon *et al.* (2006), Gallego *et al.* (2006) and Hwang *et al.* (2006). tr, Trace (<1.0%); –, not detected/not reported.

Fatty acid	1	2	3	4	5	6	7	8	9	10
Straight-chain saturated										
C _{14:0}	tr	1.0	1.0	1.1	1.5	1.3	2.5	–	1.0	tr
C _{15:0}	–	–	–	1.1	tr	–	–	4.2	1.6	tr
C _{16:0}	1.6	7.3	9.1	3.0	3.8	3.3	3.0	–	1.9	tr
C _{15:0} 2-OH	–	–	–	tr	tr	–	tr	1.2	–	tr
C _{16:0} 3-OH	tr	–	–	1.5	3.1	4.5	2.8	–	3.5	1.0
C _{17:0} 2-OH	–	–	–	2.7	tr	–	1.3	–	tr	tr
Branched saturated										
iso-C _{15:0}	35.4	33.0	15.0	28.2	26.6	26.2	21.3	35.8	28.9	37.0
iso-C _{17:0}	–	–	–	–	–	–	–	1.8	–	–
iso-C _{15:0} 3-OH	2.4	1.5	1.9	2.5	2.1	2.5	3.3	3.0	2.7	2.7
iso-C _{17:0} 3-OH	15.8	6.0	4.2	15.2	14.7	9.2	12.1	17.7	13.6	11.3
anteiso-C _{15:0}	tr	–	–	tr	tr	2.6	tr	–	tr	1.9
Summed feature 3*	27.2	22.0	29.0	30.6	34.1	42.1	39.9	21.7	30.7	24.5
Monounsaturated										
C _{15:1} ω6c	–	–	–	–	tr	–	–	1.6	2.1	1.6
C _{16:1} ω5c	1.4	–	–	1.4	2.1	3.5	2.5	–	3.2	1.3
C _{17:1} ω9c	–	–	–	–	–	–	–	1.1	–	–
iso-C _{17:1} ω9c	7.4	4.0	8.6	6.3	4.4	1.6	2.2	3.8	2.6	7.6
anteiso-C _{17:1} ω9c	–	–	–	–	–	1.2	tr	–	–	–
Unknown†										
ECL 13.56	1.3	–	–	5.0	2.6	tr	–	–	–	1.6
ECL 16.58	1.5	–	–	1.0	1.0	tr	–	–	–	1.5

*Summed feature 3 contains iso-C_{15:0} 2-OH and/or C_{16:1}ω7c (the two fatty acids cannot be separated by GLC with the MIDI system).

†ECL, Equivalent chain length.

predominant cellular fatty acids are iso-C_{15:0}, iso-C_{17:0} 3-OH and iso-C_{15:0} 2-OH and/or C_{16:1}ω7c.

The type strain, 15-52^T (= KACC 11317^T = DSM 18130^T), was isolated from the rhizosphere of Chinese cabbage (*Brassica campestris* L.).

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