Pedobacter caeni sp. nov., a novel species isolated from a nitrifying inoculum

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A Gram-negative, rod-shaped, non-spore-forming bacterium was isolated from a nitrifying inoculum. On the basis of 16S rRNA gene sequence similarity, LMG 22862^T was shown to belong to the family *Sphingobacteriaceae*, related to *Pedobacter africanus* (98.0%) and *Pedobacter heparinus* (97.6%). The results of 16S rRNA gene sequence analysis, DNA–DNA hybridization, SDS-PAGE, analysis of the fatty acid composition and physiological and biochemical tests allowed genotypic and phenotypic differentiation of LMG 22862^T from *Pedobacter* species with validly published names. LMG 22862^T therefore represents a novel species within this genus, for which the name *Pedobacter caeni* sp. nov. is proposed, with the type strain LMG 22862^T (=DSM 16990^T).

At present, the genus *Pedobacter* (family *Sphingobacteriaceae*) comprises five species: *Pedobacter heparinus*, *Pedobacter piscium*, *Pedobacter africanus*, *Pedobacter saltans* (Steyn *et al.*, 1998) and *Pedobacter cryoconitis* (Margesin *et al.*, 2003).

Amongst organisms isolated from an enriched nitrifying community, LMG 22862^T was recovered on a standard mineral medium with 1.69 mM succinate (Stanier *et al.*, 1966) incubated aerobically at 22 °C for 3 weeks. Briefly, an enrichment of the autotrophic nitrifying community from natural surface water was stimulated by adding a daily load of nitrogen [117.4 g TAN (total ammoniacal nitrogen) day⁻¹ as ammonium chloride and 58.6 g NO₂⁻-N day⁻¹ as sodium nitrite]. During this process, no organic carbon and only limited amounts of micronutrients were added. The purpose of the enriched nitrifying community is to enhance removal of ammonia and nitrite in aquaria and aquacultures; for more detailed information refer to Grommen *et al.* (2002).

The nearly complete 16S rRNA gene sequence of LMG 22862^T (AJ786798) was determined as described below. DNA was extracted according to Pitcher *et al.* (1989) as modified by Heyndrickx *et al.* (1996). PCR amplification was performed as described previously (Heyrman & Swings, 2001). PCR-amplified 16S rRNA gene products were purified using the Nucleofast 96 PCR system (Millipore). For each sequence reaction a mixture of 1 µl purified product, 1 µl of BigDye Termination RR mix version 3.1 (Perkin

Elmer), 1.5 µl BigDye buffer (5 ×) and 3 µl (20 ng µl⁻¹) of one of the six sequencing primers was used. MilliQ water was added to a total volume of 10 µl. The primers and PCR program were previously described (Heyrman & Swings, 2001). Sequence analysis was performed using an Applied Biosystems 3100 DNA Sequencer following the protocols of the manufacturer (Perkin-Elmer). The sequence was assembled with the Kodon version 2.0 software package (Applied Maths). FASTA analysis at the EMBL database (Pearson & Lipman, 1988) indicated that LMG 22862^T was most closely related to species of the genus *Pedobacter*. Phylogenetic analysis using Kodon version 2.0 showed that LMG 22862^T was most closely related to *P. africanus* (98.0%) and *P. heparinus* (97.6%) (Fig. 1).

The DNA G+C content of LMG 22862^T, determined by HPLC (Mesbah *et al.*, 1989) according to the further specifications given by Logan *et al.* (2000), was 42.7 mol%. This is within the range of 36–45 mol% given in the genus description (Steyn *et al.*, 1998). DNA–DNA hybridization experiments were performed between LMG 22862^T, *P. heparinus* LMG 4024^T and *P. africanus* LMG 10353^T using a modification of the microplate method of Ezaki *et al.* (1989) as described by Willems *et al.* (2001). A hybridization temperature of 32 °C (calculated with correction for the presence of 50% formamide) was used. LMG 22862^T showed DNA–DNA relatedness values of 12.1% to *P. heparinus* LMG 4024^T and 14.0% to *P. africanus* LMG 10353^T.

A quantitative analysis of cellular fatty acid content was performed using the GLC procedure previously described (Mergaert *et al.*, 1993), with cells grown on TSA for 48 h at 28 °C. The fatty acid profiles of LMG 22862^T, together with the profiles of the type strains of its closest phylogenetic

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of LMG 22862^T is AJ786798.

Fig. 1. Phylogenetic dendrogram obtained by neighbour-joining clustering of 16S rRNA gene sequences showing the position of LMG 22862^T among the type strains of *Pedobacter* and *Sphingobacterium* species. EMBL accession numbers are shown in parentheses. Relevant bootstrap values (expressed as percentages of 1000 replications) are shown at branch points.

neighbours *P. heparinus* and *P. africanus*, are shown in Table 1. The dominant fatty acids were iso-C_{15:0}, summed feature 3 (iso-C_{15:0} 2-OH/C_{16:1}ω7c) and iso-C_{17:0} 3-OH. Significant amounts of iso-C_{15:0} 2-OH/C_{16:1}ω7c are characteristic of all representatives of the family *Sphingobacteriaceae* (Steyn *et al.*, 1998). UPGMA clustering with the Canberra metric distance coefficient using BioNumerics version 3.5 (Applied Maths) showed 74 % similarity of LMG 22862^T to *P. heparinus* LMG 4024^T and 69 % to *P. africanus* LMG 10353^T.

For SDS-PAGE of whole-cell proteins, isolates were grown on phosphate-buffered nutrient agar (NA) (pH 6.8) and incubated aerobically at 28 °C for 48 h. The preparation of whole-cell proteins and SDS-PAGE were performed as described by Pot *et al.* (1994). Densitometric analysis, normalization and interpolation of the protein profiles were performed using the GelCompar 4.2 software package (Applied Maths). UPGMA clustering of the protein profiles

(data not shown) revealed 80 % similarity of LMG 22862^T to the type strains of *P. heparinus* and *P. africanus*. This observation further supports the conclusion that LMG 22862^T belongs to a novel species within the genus *Pedobacter*.

Cell morphology was investigated by light microscopy at $\times 1000$ of cells grown on TSA for 48 h at 28 °C. Cells were Gram stained and examined for catalase and oxidase activity. Utilization of carbon sources and enzyme production (Table 2) were tested using API 20 NE, API 50 CH and API ZYM strips (bioMérieux) according to the manufacturer's instructions. Antimicrobial susceptibilities (Table 2) were determined with the disc diffusion method using a ST6090 Disc Dispenser (Oxoid). Antibigrams were determined according to the conventional Kirby–Bauer method (Bauer *et al.*, 1966). Isolates were classified into three categories based on the quantitative interpretation criteria recommended by the NCCLS (1993).

In agreement with the genus description of *Pedobacter* (Steyn *et al.*, 1998), LMG 22862^T was positive for oxidase, catalase, acid and alkaline phosphatase, esterase lipase, leucine arylamidase and β -galactosidase. It hydrolyses aesculin and assimilates D-glucose, D-mannose, amygdalin and glucosamine. Indole production, urease, gelatinase, trypsin, α -fucosidase and the ability to assimilate D-melezitose are absent. It does not give a flexirubin reaction with 20 % KOH. In contrast to the genus description, LMG 22862^T is negative for α -glucosidase and N-acetyl- β -glucosaminidase.

Description of *Pedobacter caeni* sp. nov.

Pedobacter caeni (ca.e'ni. L. gen. neut. n. *caeni* of sludge).

After 48 h incubation at 28 °C on TSA and NA, colonies are 0.5–8 mm, smooth, convex, round, slimy, initially creamy white, turning to yellow when ageing. Cells are rod-shaped (0.5–1 µm wide and 3–10 µm long), Gram-negative, non-spore-forming. Formation of filaments is observed. No motility was observed. Cells are oxidase- and catalase-positive. Good growth is obtained after incubation for 48 h at 28 °C on TSA and NA; very slow growth occurs at 37 °C. Can be differentiated from the type strains of its closest phylogenetic neighbours, *P. heparinus* LMG 4024^T

Table 1. Cellular fatty acid composition expressed as percentages of the total fatty acid content

Strains: 1, *P. caeni* LMG 22862^T; 2, *P. africanus* LMG 10353^T; 3, *P. heparinus* LMG 4024^T. tr, Trace (<1.0%); –, not detected; Summed feature 3 contains iso-C_{15:0} 2-OH and/or C_{16:1ω7c}.

Fatty acid	1	2	3
C _{14:0}	2.5	1.2	1.1
C _{15:1} ω6 <i>c</i>	—	tr	—
iso-C _{15:0}	21.3	33.3	27.9
anteiso-C _{15:0}	tr	1.0	tr
C _{15:0} 2-OH	tr	tr	tr
iso-C _{15:0} 3-OH	3.3	2.1	3.6
C _{16:0}	3.0	2.2	2.2
C _{16:1} ω5 <i>c</i>	2.5	1.3	1.9
C _{16:0} 3-OH	2.8	1.6	2.2
iso-C _{17:1} ω9 <i>c</i>	2.2	6.5	6.4
anteiso-C _{17:1} ω9 <i>c</i>	tr	—	—
C _{17:0} 2-OH	1.3	tr	2.7
iso-C _{17:0} 3-OH	12.1	16.1	14.0
Summed feature 3	39.9	26.5	31.7

Table 2. Physiological characteristics of the type strains of *P. caeni* sp. nov., *P. africanus* and *P. heparinus*

Strains: 1, *P. caeni* LMG 22862^T; 2, *P. africanus* LMG 10353^T; 3, *P. heparinus* LMG 4024^T. +, Positive; –, negative; (+), weakly positive. All strains are positive for the following features: aesculin hydrolysis, presence of catalase, oxidase, β -galactosidase, acid and alkaline phosphatase, esterase lipase, leucine arylamidase and assimilation of the following substrates: L-arabinose, D-glucose, D-mannose, methyl α -D-mannoside, methyl α -D-glucoside, *N*-acetylglucosamine, amygdalin, aesculin, salicin, D-cellobiose, D-lactose, D-melibiose, D-sucrose, D-trehalose, D-maltose and gentiobiose. All strains are negative for the following features: reduction of nitrate, indole production, glucose fermentation, α -galactosidase, β -glucuronidase, arginine dihydrolase, urease, gelatinase, esterase, lipase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, α -fucosidase and assimilation of the following substrates: glycerol, erythritol, D-arabinose, D-ribose, methyl β -D-xyloside, L-sorbose, dulcitol, inositol, inulin, D-melezitose, glycogen, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate, 5-ketogluconate, caprate, adipate, malate, citrate and phenylacetate. All strains are resistant to the antibiotics gentamicin (10 μ g), vancomycin (30 μ g), methicillin (5 μ g), kanamycin (30 μ g), polymyxin B (300 U), ciprofloxacin (5 μ g) and ampicillin (10 μ g). R, Resistant; S, sensitive; I, intermediate. Data are from this study unless indicated.

Characteristic	1	2	3
Enzyme activities			
Naphthol-AS-BI-phosphohydrolase	–	+	+
α -Glucosidase	–	+	+
β -Glucosidase	–	+	–
<i>N</i> -Acetyl- β -glucosaminidase	–	+	+
α -Mannosidase	–	–	(+), –*
Assimilation of:			
D-Xylose	–	+	+
L-Xylose	+	–	–
D-Adonitol	–	–	+
D-Galactose	–	–, +*	+
D-Fructose	–	–	+
L-Rhamnose	–	+	+
D-Mannitol	–	–	+
D-Sorbitol	–	–	+
Arbutin	+	–	–
D-Raffinose	+	–	–
Starch	+	–	–
Xylitol	+	+, –*	–
D-Turanose	–	+	+
L-Fucose	–	–	+
Sensitivity to antibiotics			
Chloramphenicol (30 μ g)	R	S	S
Rifampicin (5 μ g)	S	I	I
Amikacin (30 μ g)	S	R	R
Tetracycline (30 μ g)	S	I	R

*Data from Steyn *et al.* (1998).

and *P. africanus* LMG 10353^T, by its ability to assimilate L-xylose, arbutin, D-raffinose and starch, by its inability to assimilate D-xylose, L-rhamnose and D-turanose and by the absence of the enzymes naphthol-AS-BI-phosphohydrolase, α -glucosidase and *N*-acetyl- β -glucosaminidase.

The type strain, LMG 22862^T (= DSM 16990^T), was isolated from a commercial nitrifying inoculum in Gent, Belgium.

Emended description of the genus *Pedobacter*

In contrast to the genus description given by Steyn *et al.* (1998), this study shows that strains belonging to *Pedobacter* are variable for the presence of α -glucosidase and *N*-acetyl- β -glucosaminidase.

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