Pedobacter bauzanensis sp. nov., isolated from soil

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A Gram-negative, aerobic, rod-shaped, non-motile bacterium, designated BZ42^T, was isolated from the soil of an industrial site. Strain BZ42^T was able to grow at 5–25 °C. The major fatty acids were iso-C_{15:0} (46.2%), C_{16:1ω7c} and/or iso-C_{15:0} 2-OH (23.2%) and iso-C_{17:0} 3-OH (9.1%). The predominant menaquinone was MK-7. The genomic DNA G + C content was 36.5 mol% (HPLC). 16S rRNA gene sequence phylogenetic analysis revealed that strain BZ42^T was a member of the genus *Pedobacter*, family *Sphingobacteriaceae*, and 16S rRNA gene sequence similarities between strain BZ42^T and the type strains of species of the genus *Pedobacter* with validly published names were 90.4–93.2%. On the basis of phenotypic, chemotaxonomic and phylogenetic distinctiveness, strain BZ42^T was considered to represent a novel species of the genus *Pedobacter*, for which the name *Pedobacter bauzanensis* sp. nov. is proposed. The type strain is BZ42^T (=DSM 22554^T =CGMCC 1.10187^T =CIP 110134^T).

The genus *Pedobacter* was first 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*. The genus accommodates bacteria that are Gram-negative, obligately aerobic rods with MK-7 as the major menaquinone and a DNA G + C content of 36–45 mol%. Other characteristics of the genus are the presence of catalase, oxidase and alkaline phosphatase and absence of nitrate reduction, glucose fermentation and production of urease, indole and H₂S from thiosulfate. Since the genus was first described, the number of *Pedobacter* species has increased considerably and at the time of writing the genus comprised 30 recognized species. Members of the genus *Pedobacter* have been found worldwide, in climatic conditions ranging from cold to temperate, and most have been isolated from environmental terrestrial and aquatic habitats. In this study, we report on the characterization of a novel bacterium of the genus *Pedobacter* isolated from soil contaminated with hydrocarbons.

Soil containing large amounts of heavy oil and heavy metals was collected under sterile conditions from an industrial site in Bozen, South Tyrol, Italy, in spring 2008. A sample of the soil (10 g) was shaken with 90 ml sterile 1% sodium pyrophosphate at 150 r.p.m. for 20 min and appropriate dilutions were prepared with sterile saline solution (0.9% NaCl). Aliquots (0.1 ml) were spread on R2A agar (0.05% yeast extract, 0.05% peptone, 0.05% Casamino acids, 0.05% glucose, 0.05% starch, 0.03% sodium pyruvate, 0.03% K₂HPO₄, 0.005% MgSO₄, 1.5% agar; pH 7) and incubated at 20 °C. Strain BZ42^T was isolated and purified by subcultivation. Strain BZ42^T was

routinely cultured in R2A medium at 20 °C for 10 days and maintained as a suspension in skimmed milk (10%, w/v) at –80 °C.

DNA was extracted and purified as described by Sambrook *et al.* (1989). The gene encoding the 16S rRNA was amplified by PCR with two universal primers (Zhang *et al.*, 2006). The amplification products were cloned using the pGEM-T Easy Vector system (Promega), according to the manufacturer's instructions. Sequencing reactions were carried out using an ABI BigDye 3.1 sequencing kit and an automated DNA sequencer (ABI 3730; Applied BioSystems). The nearly complete 16S rRNA gene sequence (1514 bp) of strain BZ42^T was submitted to GenBank and EMBL to search for similar sequences using the BLAST algorithm. A neighbour-joining phylogenetic tree was constructed using Kimura's two-parameter model and pairwise-deletion analysis using MEGA version 3.0 (Kumar *et al.*, 2004). The phylogenetic analysis showed that strain BZ42^T clustered with members of the genus *Pedobacter* (Fig. 1). A similar topology was found in the tree generated with the maximum-parsimony algorithm (not shown). Thus, it was clear that the isolate belonged to the genus *Pedobacter*. Low levels of 16S rRNA gene sequence similarity (<93.2%) between the isolate and the type strains of previously described species of the genus *Pedobacter* indicated that strain BZ42^T represented a novel species of this genus (Stackebrandt & Goebel, 1994).

The morphology of cells was examined by phase-contrast microscopy (×1000 magnification). The Gram reaction was tested by classic Gram staining and was confirmed by the KOH lysis test. Catalase activity was determined by bubble production in 3% (v/v) H₂O₂ and oxidase activity was determined using 1% (w/v) N,N,N',N'-tetramethyl-*p*-phenylenediamine. Cell motility was evaluated on motility agar by light microscopy (×1000). Physiological and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BZ42^T is GQ161990.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic positions of strain BZ42^T, recognized members of the genus *Pedobacter* and representatives of some other related taxa. Bootstrap values (>50%) based on 1000 replicates are shown at branch nodes. *Cytophaga hutchinsonii* ATCC 33406^T was used as an outgroup. Bar, 2% sequence divergence.

biochemical characteristics and enzyme activities were determined at 20 °C using the API 20 E, API 20 NE and API ZYM systems (bioMérieux), according to the manufacturer's instructions. The assimilation of a number of carbon sources (0.2%, w/v) was tested in 10 ml liquid culture using a pH-neutral phosphate-buffered mineral medium supplemented with a vitamin solution (Schlegel, 1992) and/or a trace element solution. The assimilation of glucose was tested at 0.025–0.2% (w/v). β -Galactosidase, amylase and protease activities were tested on R2A agar supplemented with appropriate substrates (Margesin *et al.*, 2003). Due to the extremely low biomass production in liquid culture, growth at 1–30 °C, at pH 5–9 and with 0–5% (w/v) NaCl was assessed on R2A agar. Antibiotic susceptibility was determined on R2A agar supplemented with various antibiotics and incubated at 20 °C.

The morphological, physiological and biochemical characteristics of strain BZ42^T are given in the species description and in Table 1. Strain BZ42^T could be distinguished from its four closest phylogenetic neighbours by its lower maximum temperature for growth and its inability to utilize glucose as a sole carbon source, even in the presence of vitamins and trace elements. Strain BZ42^T exhibited slow growth, a characteristic that is also observed for *Pedobacter lentus* (Table 1).

Respiratory quinones were extracted and purified according to Collins (1985) and were analysed by HPLC (Wu *et al.*,

1989). For fatty acid analysis, cell mass was harvested from TSA plates after incubation at 25 °C for 2 days and the fatty acid methyl esters were extracted, prepared and analysed according to the standard protocol of the Sherlock Microbial Identification System (MIDI; Sasser, 1990). The G+C content of the DNA was determined from cells grown on R2A agar using standard HPLC analysis (Mesbah *et al.*, 1989). Strain BZ42^T contained MK-7 as the major menaquinone. The predominant cellular fatty acids of strain BZ42^T (>1% of the total fatty acids) were iso-C_{15:0} (46.2%), C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH (23.2%), iso-C_{17:0} 3-OH (9.1%), C_{17:0} cyclo (3.8%), iso-C_{15:0} 3-OH (3.8%), iso-C_{17:1} ω 9c (2.3%), C_{16:0} (1.7%), C_{16:1} ω 5c (1.6%) and anteiso-C_{15:0} (1.2%). This fatty acid profile was similar to those of other *Pedobacter* species (Steyn *et al.*, 1998; Yoon *et al.*, 2006, 2007; An *et al.*, 2009), although there were differences in the proportions of some fatty acids, perhaps due to different cultivation and extraction conditions. The DNA G+C content of strain BZ42^T was 36.5 mol%.

On the basis of phenotypic, chemotaxonomic and phylogenetic evidence, strain BZ42^T was identified as a representative of a novel species of the genus *Pedobacter*, for which the name *Pedobacter bauzanensis* sp. nov. is proposed.

Description of *Pedobacter bauzanensis* sp. nov.

Pedobacter bauzanensis (bau.zan.en'sis. M.L. masc. adj. *bauzanensis* referring to Bauzanum, the medieval Latin

Table 1. Phenotypic characteristics that differentiate strain BZ42^T from its closest phylogenetic neighbours and the type strain of the type species of the genus *Pedobacter*

Strains: 1, *Pedobacter bauzanensis* sp. nov. BZ42^T; 2, *P. daechungensis* Dae 13^T (data from An *et al.*, 2009); 3, *P. lentus* DS-40^T (Yoon *et al.*, 2007); 4, *P. terricola* DS-45^T (Yoon *et al.*, 2007); 5, *P. glucosidilyticus* KCTC 22438^T (Luo *et al.*, 2010); 6, *P. heparinus* DSM 2366^T (data from this study and Steyn *et al.*, 1998; Takeuchi & Yokota, 1992; Yoon *et al.*, 2007). All strains were positive for aerobic growth, aesculin hydrolysis, activities of catalase, oxidase, alkaline phosphatase and esterase lipase (C8) and assimilation of D-mannose and maltose. All strains were negative for the Gram reaction, sporulation, nitrate reduction, indole and H₂S production, activities of urease, β -glucuronidase and trypsin and (no data for *P. glucosidilyticus* KCTC 22438^T) assimilation of citrate, malate, capric acid, adipic acid and phenylacetic acid. +, Positive; v, variable; w, weakly positive; –, negative.

Characteristic	1	2	3	4	5	6
Isolation source	Contaminated soil	Lake sediment	Soil	Soil	Soil	Soil
Temperature range (°C)						
Minimum	5	15	4	4	15	5
Maximum	25	30	31	34	37	30–37 (v)
Enzyme activities						
Acid phosphatase	+	–	+	+	+	+
Naphthol-AS-BI-phosphohydrolase	+	–	+	+	+	+
α -Fucosidase	–	–	–	–	+	–
β -Glucosidase	+	+	–	–	+	v
β -Galactosidase	–	–	+	+	w	+
α -Glucosidase	+	–	+	+	+	+
N-Acetyl- β -glucosaminidase	+	–	+	+	+	+
α -Chymotrypsin	–	–	+	+	–	v
Assimilation of:						
D-Glucose	–	+	+	+	+	+
Potassium gluconate	–	+	–	–	–	–
<i>myo</i> -Inositol	–	+	–	–	–	–
D-Mannitol	–	+	–	–	–	+
Sorbitol	–	–	–	–	–	+
Sucrose	+	+	–	–	+	+
DNA G + C content (mol%)	36.5	33.8	36.0	36.8	37.2–37.6	42.3–43.0

name for Bozen/Bolzano, a city in South Tyrol, Italy, where the species was first found).

Cells are Gram-negative, aerobic and non-motile rods, 0.5–0.6 $\times$ 1.9–2.2 μ m. After 10 days on R2A agar at 20 °C, colonies are white, round, glossy and convex with entire margins, about 1 mm in diameter. Growth occurs on R2A agar, nutrient agar and tryptic soy agar; growth in liquid media is very poor. On R2A agar, growth occurs at 5–25 °C but not at 30 °C (fastest growth at 20–25 °C), at pH 7–8 and with 0–3 % (w/v) NaCl. Positive for catalase, cytochrome oxidase, aesculin hydrolysis and alkaline phosphatase, acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, α - and β -glucosidases and N-acetyl- β -glucosaminidase. Weakly positive for lipase (C14), valine arylamidase and cystine arylamidase. Negative for nitrate reduction, indole and H₂S production, citrate utilization, hydrolysis of urea, starch, protein, gelatin and Tween 80 and activities of arginine dihydrolase, lysine dihydrolase, ornithine dihydrolase, α - and β -galactosidase, β -glucuronidase, trypsin, α -chymotrypsin, α -fucosidase and α -mannosidase. Assimilates D-mannose, maltose, N-acetyl- β -glucosamine, sucrose and salicin (enhanced

growth in the presence of vitamins), but not D-glucose, D-mannitol, potassium gluconate, *myo*-inositol, D-sorbitol, citrate, malic acid, capric acid, adipic acid or phenylacetic acid. Does not ferment glucose, ribose, xylose, mannitol, maltose, lactose, sucrose, glycogen, inositol, sorbitol, rhamnose, melibiose, amygdalin or L-arabinose. Sensitive to ampicillin (50 μ g ml^{–1}), penicillin G, streptomycin, tetracycline, chloramphenicol, rifampicin and trimethoprim (each 100 μ g ml^{–1}), but resistant to kanamycin and cyclosporin A (each 100 μ g ml^{–1}). MK-7 is the major menaquinone. The predominant cellular fatty acids are iso-C_{15:0}, C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH and iso-C_{17:0} 3-OH. The DNA G+C content of the type strain is 36.5 mol%.

The type strain is BZ42^T (=DSM 22554^T =CGMCC 1.10187^T =CIP 110134^T), isolated from soil of an industrial site in Bozen, South Tyrol, Italy.

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