Pseudomonas bauzanensis sp. nov., isolated from soil

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A Gram-negative, aerobic, motile rod, designated BZ93T, was isolated from soil from an industrial site. The strain grew at 5–30 °C. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain BZ93T was related to members of the genus Pseudomonas and was related most closely to Pseudomonas xiamenensis C10-2T (97.8 % 16S rRNA gene sequence similarity) and Pseudomonas pertucinogena IFO 14163T (97.4 %). The predominant cellular fatty acids of strain BZ93T were C18 : 1ω7c (54.8 %), summed feature 3 (C16 : 1ω7c and/or iso-C15 : 0 2-OH; 10.3 %), C16 : 0 (9.9 %) and C17 : 0 cyclo (7.4 %). The major quinone was ubiquinone 9. The major phospholipids were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and an unknown phospholipid. The genomic DNA G+C content was 61.8 mol%. On the basis of phenotypic characteristics, phylogenetic analysis and DNA–DNA relatedness, a novel species, Pseudomonas bauzanensis sp. nov., is proposed. The type strain is BZ93T (=DSM 22558T =CGMCC 1.9095T=LMG 26048T).

The genus Pseudomonas was originally created by Migula (1894). Bacteria of the genus Pseudomonas belong to the Gammaproteobacteria and are aerobic, chemooorganotrophic, non-spore-forming, motile, Gram-negative rods with polar flagella and a respiratory rather than a fermentative metabolism (Palleroni, 1984; Timmis, 2002). Over the years, species of the genus have been reclassified and revised several times (Sneath et al., 1981; Oyaizu & Komagata, 1983; Vancanneyt et al., 1996; Anzai et al., 2000). Members of the genus Pseudomonas are widely distributed in soil, water, air, plants and many other natural habitats (Palleroni, 1992). Some representatives of this genus colonize cold habitats (Yumoto et al., 2001; Reddy et al., 2004; Bozal et al., 2007; Margesin et al., 2008). The present study deals with the characterization of a novel strain of the genus Pseudomonas that was isolated from soil and is able to grow at low temperatures.

Strain BZ93T was isolated from soil from an industrial site (containing high amounts of heavy oil and heavy metals) in Bozen, South Tyrol, Italy, as described previously (Zhang et al., 2010). The soil was sampled and processed under sterile conditions in spring 2008. A sample (10 g) was shaken with 90 ml sterile 1 % sodium pyrophosphate for 20 min at 150 r.p.m. Appropriate dilutions were prepared with sterile 0.9 % NaCl and 0.1 ml aliquots 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 % MgSO4·7H2O; pH 7; Reasoner & Geldreich, 1985) and incubated at 20 °C. A white colony was purified and designated BZ93T. Strain BZ93T was routinely cultured on R2A medium at 20 °C and maintained as a suspension in skimmed milk (10 % w/v) at −80 °C. Pseudomonas pertucinogena LMG 1874T and Pseudomonas xiamenensis CGMCC 1.6446T were routinely grown on R2A agar at 25 °C and used as reference strains.

DNA was extracted and purified as described by Sambrook et al. (1989). The 16S rRNA gene was amplified by PCR using universal primers (27F, 5’-AGAGTTTGATCCTGGCTCAG-3’; 1541R, 5’-AAGGAGGTGATCCAGCGCA-3’). PCR products were cloned into the pGEM-T vector (Promega) according to the manufacturer’s instructions. Fasta similarity searches were done using the GenBank and EMBL databases. A pairwise comparison of the 16S rRNA gene sequences was analysed using EzTaxon (Chun et al., 2007). The 16S rRNA gene sequence of strain BZ93T showed 97.8 and 97.4 % similarity with P. xiamenensis C10-2T and P. pertucinogena IFO 14163T, respectively. Similarities between strain BZ93T and other members of the genus Pseudomonas were <96.5 %.

Phylogenetic analysis was performed using MEGA version 4.0 (Tamura et al., 2007) and PHYLIP version 3.69.
(Felsenstein, 2009) after multiple alignment of data using CLUSTAL X version 1.8 (Thompson et al., 1997). For neighbour-joining analysis (Saitou & Nei, 1987), genetic distances were calculated using Kimura's two-parameter model (Kimura, 1980). Strain BZ93\(^T\) fell within the genus *Pseudomonas* and formed a distinct cluster with *P. xiamenensis* C10-2\(^T\) and *P. pertucinogena* IFO 14163\(^T\) (Fig. 1). The relative position of strain BZ93\(^T\) was also confirmed in the maximum-likelihood tree.

Cell morphology was examined by phase-contrast microscopy (×1000) of cells grown on R\(_2\)A agar at 20 °C. Colony morphology was also observed on R\(_2\)A agar at 20 °C. Motility of cells grown at 20 °C was evaluated on motility agar (API M; bioMérieux) and under a light microscope (×1000).

All of the following tests were done with strain BZ93\(^T\) and the reference strains. The Gram reaction was tested by Gram staining and confirmed by the KOH-lysis test.

Pigment analysis was performed as described by Hildebrand et al. (1994). To test for the production of a cellular (methanol-extractable) pigment, cells were grown for 4 days at 25 °C on King’s B agar and nutrient agar. To test for the presence of a diffusible pigment, cells were grown for 48 h at 25 °C and 200 r.p.m. in King’s B broth and nutrient broth. Catalase activity was determined by bubble production in 3 % (v/v) \(\mathrm{H}_2\mathrm{O}_2\). Oxidase activity was determined using 1 % (w/v) \(\mathrm{N}_2\mathrm{N}_2\mathrm{N}_4\mathrm{N}_4\)-tetramethyl-\(\mathrm{p}\)-phenylenediamine. The API 20 E, API 20 NE and API ZYM systems (bioMérieux) and EcoPlates (Biolog) were used at 25 °C to determine physiological and biochemical characteristics and enzyme activities. \(\beta\)-Galactosidase, amylase and lipase esterase activities were additionally tested using R\(_2\)A agar supplemented with appropriate substrates (Margesin et al., 2003). Growth at 1–45 °C was assessed on R\(_2\)A agar and in R\(_2\)A liquid medium at 150 r.p.m. Growth at pH 5–9 and with 0–20 % (w/v) NaCl was determined on R\(_2\)A agar at 20 °C. Susceptibility to antibiotics was determined at 25 °C using antibiotic

\[\text{Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic position of strain BZ93}^T\text{ within the genus } Pseudomonas. \text{ Bootstrap values (>50 %) based on 1000 resamplings are shown at branch nodes. Asterisks indicate that the corresponding nodes were also recovered in the maximum-likelihood tree. Halomonas elongata ATCC 33173}^T \text{ was used as an outgroup. Bar, 0.01 nucleotide substitutions per site.}\]
discs (bioMérieux). The morphological, physiological and biochemical characteristics of strain BZ93\textsuperscript{T} are given in the species description and Table 1. The features that serve to differentiate strain BZ93\textsuperscript{T} from P. xiamenensis CGMCC 1.6446\textsuperscript{T} and P. pertucinogena LMG 1874\textsuperscript{T} are given in Table 1.

Respiratory quinones were extracted and purified according to Collins (1985) and were analysed by HPLC (Wu et al., 1989). The quinone system consisted predominantly of ubiquinone Q-9.

For fatty acid methyl ester analysis, cell mass of strain BZ93\textsuperscript{T} and the reference strains was harvested from trypticase soy agar after incubation at 25 °C for 3 days. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the Sherlock Microbiological Identification System (MIDI; Sasser, 1990). The predominant cellular fatty acids of strain BZ93\textsuperscript{T} were C\textsubscript{18:0} 3-0H (54.8 %), summed feature 3 (C\textsubscript{16:1} 7c and/or iso-C\textsubscript{15:0} 2-OH; 10.3 %), C\textsubscript{16:0} (9.9 %) and C\textsubscript{17:0} cyclo (7.4 %). The fatty acid profiles of strain BZ93\textsuperscript{T}, P. xiamenensis CGMCC1.6446\textsuperscript{T} and P. pertucinogena LMG 1874\textsuperscript{T} are shown in Supplementary Table S1 (available in IJSEM Online).

The cellular polar lipids were extracted and analysed on silica gel plates (Kieselgel 60 F; Merck) by TLC (Kates, 1986) and staining with molybdatophosphoric acid. The polar lipid profile of strain BZ93\textsuperscript{T} consisted of phosphatidyethanolamine, diphasphatidylglycerol, phosphatidylglycerol and an unknown phospholipid. An unknown aminophospholipid and 11 unknown polar lipids were also detected (Supplementary Fig. S1, available in IJSEM Online).

The G+C content was determined by the thermal denaturation method (Marmur & Doty, 1962) with Escherichia coli K-12 as the reference strain, and DNA–DNA hybridization was done by the liquid renaturation method (De Ley et al., 1970) as modified by Huß et al. (1983). Both experiments were carried out using a model Lambda 35 UV/VIS spectrometer equipped with a temperature program controller (Perkin-Elmer). The DNA G+C content of strain BZ93\textsuperscript{T} was 61.8 mol%. DNA–DNA relatedness between strain BZ93\textsuperscript{T} and P. xiamenensis CGMCC 1.6446\textsuperscript{T} and P. pertucinogena LMG 1874\textsuperscript{T} was 32.5 and 26.6 %, respectively.

The results presented above demonstrate that strain BZ93\textsuperscript{T} is a member of the genus Pseudomonas. Strain BZ93\textsuperscript{T} could be easily differentiated from its closest phylogenetic neighbours by its ability to grow well at 5 °C and with 10 % (w/v) NaCl and to utilize a number of carboxylic acids (pyruvic acid methyl ester, itaconic acid and \(\alpha\)-ketobutyric acid). In addition, strain BZ93\textsuperscript{T} could be differentiated from P. xiamenensis CGMCC 1.6446\textsuperscript{T} by its inability to reduce nitrate and to grow at pH 9 and its ability to assimilate adipic acid, as well as by having higher amounts of the cellular fatty acid C\textsubscript{18:1} 7c and smaller amounts of C\textsubscript{19:0} cyclo \(\omega\)8c. Strain BZ93\textsuperscript{T} could be differentiated from P. pertucinogena LMG 1874\textsuperscript{T} by its inability to grow at 37 °C, ferment glucose and utilize 4-hydroxybenzoic acid, by the absence of cystine arylamidase, trypsin and \(\alpha\)-chymotrypsin and its ability to assimilate trisodium citrate and grow with 7 % (w/v) NaCl and at 10 °C, as well as by having higher amounts of the cellular fatty acids C\textsubscript{18:1} 7c, C\textsubscript{17:0} cyclo and C\textsubscript{19:0} cyclo \(\omega\)8c and smaller amounts of summed feature 3.

### Table 1. Phenotypic characteristics that differentiate strain BZ93\textsuperscript{T} from its closest phylogenetic neighbours

<table>
<thead>
<tr>
<th>Characteristic</th>
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<td>Growth at with:</td>
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<tr>
<td>5 °C</td>
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<td>10 °C</td>
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<td>37 °C</td>
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<td>pH 9</td>
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<td>7 % (w/v) NaCl</td>
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<td>10 % (w/v) NaCl</td>
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<td>Nitrate reduction</td>
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<td>Enzyme activities (API ZYM)</td>
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<td>Cystine arylamidase</td>
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<td>Trypsin</td>
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<td>(\alpha)-Chymotrypsin</td>
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<td>Glucose fermentation</td>
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<td>Utilization (API 20 NE)</td>
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<tr>
<td>Adipic acid</td>
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<td>Trisodium citrate</td>
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<td>Utilization (EcoPlates)</td>
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<td>Pyruvic acid methyl ester</td>
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<td>4-Hydroxybenzoic acid</td>
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<td>Itaconic acid</td>
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<td>(\alpha)-Ketobutyric acid</td>
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<td>l-Serine</td>
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Therefore, on the basis of phenotypic, phylogenetic and genomic evidence, strain BZ93T was identified as a representative of a novel species of the genus *Pseudomonas*, for which the name *Pseudomonas bauzanensis* sp. nov. is proposed.

**Description of Pseudomonas bauzanensis sp. nov.**

*Pseudomonas bauzanensis* (bau.zan.en.sis. N.L. fem. adj. bauzanensis referring to Bauxanum medieval Latin name of Bozen/Bolzano, a city in South Tyrol, Italy, where the species was first isolated).

Cells are aerobic, Gram-negative, motile, short rods (0.7–0.9 × 1.1–1.7 μm after 4 days at 20 °C on R2A agar). Grows on R2A agar, nutrient agar, trypticase soy agar and King’s B agar. Colonies on R2A agar are white, round, convex, smooth and with entire margins (1.5–2 mm in diameter after 6 days at 20 °C). Negative for production of a cellular (methanol-extractable) or diffusible fluorescent pigment. Good growth occurs in R2A liquid medium at 5–30 °C (highest cell yields at 10–20 °C); growth at 1 and 37 °C is absent in R2A liquid culture and on R2A agar plates. On R2A agar, growth occurs at pH 7–8 and with 0–7 % (w/v) NaCl; growth is delayed with 10 % (w/v) NaCl and absent with 15 % (w/v) NaCl. Produces catalase and cytochrome oxidase. Negative for production of indole, H2S and urease, utilization of citrate, reduction of nitrate and hydrolysis of aesculin. Positive for acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), naphthol-AS-BI-phosphohydrolase and leucine arylamidase; weakly positive for valine arylamidase and lipase (C14); and negative for gelatinase, amylase, arginine dihydrolase, cysteine dihydrolase, ornithine dihydrolase, tryptophan deaminase, trypsin, N-acetyl-β-glucosaminidase, β- and γ-galactosidase, β-glucuronidase, α- and β-glucosidase, α-mannosidase and α-fucosidase. With API 20 NE, assimilates capric acid, adipic acid, malic acid and trisodium citrate, but not D-glucose, D-mannitol, maltose, L-arabinose, D-mannose, N-acetylglycosamine, glucanate or phenylacetic acid. With EcoPlates, utilizes pyruvic acid methyl ester, itaconic acid, α-ketobutyric acid, D-malic acid, Tweens 40 and 80 and weakly utilizes L-serine, but does not utilize D-glucosaminic acid, D-galacturonic acid, γ-hydroxybutyric acid, cellobiose, L-lactose, methyl β-D-glucoside, D-xylene, L-erythritol, D-mannitol, N-acetyl-D-glucosamine, glucose 1-phosphate, DL-α-glycerol phosphate, D-galacturonic acid, γ-lactone, L-arginine, L-asparagine, L-phenylalanine, L-threonine, glycyl L-glutamic acid, phenylethylamine, putrescine, 2-hydroxybenzoic acid, 4-hydroxybenzoic acid, α-cyclodextrin or glycogen. Does not ferment glucose, mannitol, sucrose, inositol, sorbitol, rhamnose, melibiose, amygdalin or L-arabinose. Resistant to (μg per disc) tetracycline (30) and erythromycin (15), and sensitive to kanamycin (30), amikacin (30), gentamicin (10), streptomycin (10), ampicillin (10), nalidixic acid (30), chloramphenicol (30) and rifampicin (30). The predominant cellular fatty acids are C16:1ω7c, summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH), C16:0 and C17:0 cyclo. The quinone system consists predominantly of ubiquinone Q-9. The polar lipid profile consists of phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol and an unknown phospholipid; an unknown aminophospholipid and 11 unknown polar lipids are also present. The DNA G+C content of the type strain is 61.8 mol%.

The type strain is BZ93T (=DSM 22558T =CGMCC 1.9903T =LMG 26048T) and was isolated from soil contaminated with hydrocarbons and heavy metals in Bozen, South Tyrol, Italy.

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**References**


