**Sphingopyxis bauzanensis** sp. nov., a psychrophilic bacterium isolated from soil

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Strain BZ30ᵀ was isolated from hydrocarbon-contaminated soil. The Gram-negative, aerobic bacterium was psychrophilic and able to grow at temperatures ranging from 1 to 30 °C. The predominant cellular fatty acids of strain BZ30ᵀ were summed feature 3 (C₁₆:₁ω7c and/or iso-C₁₅:₀ 2-OH) (37.4 %), C₁₈:₁ω7c (19.6 %), C₁₆:₀ (8.2 %), C₁₄:₀ 2-OH (8.0 %) and C₁₆:₀ 2-OH (5.0 %). The predominant ubiquinone was Q-10. Major polar lipids were sphingoglycolipids, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine. Spermidine was the major polyamine. The genomic DNA G+C content was 64.4 mol%.

Phylogenetic analysis based on 16S rRNA gene sequence similarity showed that strain BZ30ᵀ belonged to the family Sphingomonadaceae of the β-4 group of the phylum Proteobacteria, and was related to the members of the genus Sphingopyxis, sharing the highest sequence similarities with the type strains of Sphingopyxis chilensis (98.3 %), S. witflariensis (98.2 %), S. taenonisens (97.4 %) and S. ginsengisoli (97.2 %). On the basis of the phenotypic characteristics, phylogenetic analysis and DNA–DNA relatedness data, strain BZ30ᵀ represents a novel species of the genus Sphingopyxis, for which the name *Sphingopyxis bauzanensis* is proposed. The type strain is BZ30ᵀ (= DSM 22271ᵀ = CGMCC 1.8959ᵀ = CIP 110136ᵀ).

The genus *Sphingomonas* was first proposed by Yabuuchi et al. (1990), with five species and two genomospecies. Based on phylogenetic, chemotaxonomic and physiological analyses, the genus *Sphingomonas* has been divided into four genera: *Sphingomonas*, *Sphingobium*, *Novosphingobium* and *Sphingopyxis* (Takeuchi et al., 2001). These four genera differ in their polyamine patterns, nucleotide signatures and some phenotypic characteristics (e.g. ability or inability for nitrate reduction) and were separated into four clusters (I–IV) based on distance matrix analysis of 16S rRNA gene sequences (Takeuchi et al., 2001). However, Yabuuchi et al. (2002) emphasized that the three proposed genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, should be considered as later homotypic synonyms of species of the genus *Sphingomonas* because there is no phenotypic and phylogenetic evidence. So far the nomenclature of Takeuchi et al. (2001) is generally used (Busse et al., 2003; Yoon & Oh, 2005). At the time of writing, the genus *Sphingopyxis* comprises 12 species: *Sphingopyxis macrogolitabida* (Takeuchi et al., 1993, 2001), *Sphingopyxis bauzanensis* sp. nov., *Sphingopyxis chilensis* (Lee et al., 2005), *Sphingopyxis flavimaris* (Vancanneyt et al., 2001; Godoy et al., 2003), *Sphingopyxis chilensis* (Godoy et al., 2003), *Sphingopyxis granuli* (Kim et al., 2005), *Sphingopyxis flavimaris* (Yoon & Oh, 2005), *Sphingopyxis baikyungensis* (Yoon et al., 2005), *Sphingopyxis ginsengisoli* (Lee et al., 2008), *Sphingopyxis litoris* and *Sphingopyxis marina* (Kim et al., 2008). The type strains of these species have been isolated from various environmental sources, such as soil, sludge, sediment, seawater and mineral water. In this study, we report on the characterization of a novel, psychrophilic bacterium of the genus *Sphingopyxis* isolated from soil containing high amounts of heavy oil and located in Bozen, South Tyrol, Italy. The soil was collected under sterile conditions in spring 2008. A 10 g sample was shaken with 90 ml of sterile 1 % sodium pyrophosphate for 20 min at 150 r.p.m. Appropriate dilutions, prepared with sterile saline solution (0.9 % NaCl), were plated on R₂A agar incubated at 20 °C. One of the pure cultures was yellow-pigmented and was designated BZ30ᵀ.

Strain BZ30ᵀ was isolated from soil from an industrial site containing high amounts of heavy oil and located in Bozen, South Tyrol, Italy. The soil was collected under sterile conditions in spring 2008. A 10 g sample was shaken with 90 ml of sterile 1 % sodium pyrophosphate for 20 min at 150 r.p.m. Appropriate dilutions, prepared with sterile saline solution (0.9 % NaCl), were plated on R₂A agar incubated at 20 °C. One of the pure cultures was yellow-pigmented and was designated BZ30ᵀ.
Sphingopyxis witflariensis W-50T, Sphingopyxis ginsengisoli LMG 23390T, Sphingopyxis taejonensis DSM 15583T and Sphingopyxis chilensis DSM 14889T shared the highest sequence similarities with strain BZ30T and were thus used as reference strains for DNA–DNA hybridization and for analysis of phenotypic parameters and fatty acid composition. They were routinely grown in nutrient broth (0.3 % yeast extract, 0.5 % peptone; Merck) at 25 °C.

DNA was extracted and purified as described by Sambrook et al. (1989). The gene encoding 16S rRNA was amplified by PCR with two universal primers (Zhang et al., 2006). PCR products were cloned in pGEM-T vectors using the pGEM-T easy vector system (Promega) according to the manufacturer’s instructions. Sequencing reactions and phylogenetic analysis were performed as described by Zhang et al. (2006). Phylogenetic analysis based on a consensus 1314 bp length of 16S rRNA gene sequences showed that strain BZ30T grouped with members of the genus Sphingopyxis (Fig. 1) and formed a cluster with Sphingopyxis witflariensis W-50T, Sphingopyxis chilensis LMG 20986T and Sphingopyxis taejonensis KCTC 2884T. The highest sequence similarities were shown with the type strains of Sphingopyxis chilensis (98.3 %), Sphingopyxis witflariensis (98.2 %), Sphingopyxis taejonensis (97.4 %) and Sphingopyxis ginsengisoli (97.2 %). Similar tree topologies were found in the trees generated with the maximum-parsimony and maximum-likelihood algorithms (data not shown). Nucleotide signatures specific to the 16S RNA of strain BZ30T were the same as those of the genus Sphingopyxis (Cluster IV) reported by Takeuchi et al. (2001), i.e. C–G at position 52 : 359, G at position 134, U at position 593, G–C at position 987 : 1218 and U–G at position 990 : 1215 (Escherichia coli numbering; Brosius et al., 1978).

Strain BZ30T was routinely cultured in the low-strength medium R2A at 20 °C and maintained as a suspension in skim milk (10 %, w/v) at −80 °C. The cell morphology was examined by phase-contrast microscopy (×1000) of cells grown on R2A agar plates at 20 °C. The colonial morphology was also observed on R2A agar plates. The API M system (bioMérieux) was used to evaluate cell motility. Gram-reaction was tested by classical Gram-staining and was confirmed by the KOH-lysis test. Catalase activity was determined by bubble production in 3 % (v/v) H2O2; oxidase activity was determined using 1 % (w/v) N,N,N9,N9-tetramethyl-p-phenylenediamine. API strips (API 20 E, API 20 NE, API ZYM; bioMérieux) incubated at 25 °C were used according to the manufacturer’s instructions to determine physiological and biochemical characteristics as well as enzyme activities of strain BZ30T and of the four reference strains Sphingopyxis witflariensis W-50T, Sphingopyxis ginsengisoli LMG 23390T, Sphingopyxis taejonensis DSM 15583T and Sphingopyxis chilensis DSM 14889T. Activities of β-galactosidase, amylase and protease were additionally tested by using R2A agar plates supplemented with appropriate substrates (Margesin et al., 2010).

Fig. 1. Phylogenetic relationship of strain BZ30T and related genera based on 16S rRNA gene sequence data (neighbour-joining method). Bootstrap values (expressed as percentages of 1000 replicates) greater than 50 % are shown at branch points. Rhodospirillum rubrum ATCC 11170T was used as the outgroup. GenBank accession numbers of 16S rRNA sequences are given in parentheses. Bar, 2 % sequence divergence.

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et al., 2003). Gelatinase activity was tested on Nutrient Gelatin (0.3 % beef extract, 0.5 % peptone, 12 % gelatin; Difco). Growth at different temperatures (1–37 °C) was assessed on R2A agar plates and in R2A liquid medium at 150 r.p.m. Growth at various pH (5–9) and salt tolerance [0–10 % (w/v) NaCl] were determined on R2A agar plates. Antibiotic susceptibility was determined on R2A agar plates supplemented with various antibiotics and incubated at 20 °C. Morphological, physiological and biochemical characteristics of strain BZ30T are given in the species description (see below) and are shown in Table 1. The features that serve to differentiate strain BZ30T from the phylogenetically most closely related members of the genus Sphingopyxis 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 cellular polar lipids were extracted and analysed on silica gel plates (Kieselgel 60 F; Merck) by TLC (Kates, 1986). Glycolipid spots were detected by spraying the plate with 0.5 % (w/v) α-naphthol in 50 % (v/v) methanol, followed by spraying with 50 % sulfuric acid/50 % ethanol (v/v) and heating at 120 °C for 5 min. Phospholipids were visualized with the Zinzadze reagent (Kates, 1986). Total lipids were stained with 5 % (w/v) ethanolic molybdatophosphoric acid spray. For polyamine analysis, strain BZ30T was grown in modified R2A medium (without starch) since it was not able to grow in PYE medium (0.3 % yeast extract, 0.3 % peptone, pH 7) or in diluted (1:3 or 1:6) PYE medium, which is commonly used to determine polyamine patterns. Polyamines were extracted and analysed as described by Busse & Auling (1988), Busse et al. (1997) and Stolz et al. (2007).

Strain BZ30T contained Q-10 as the major ubiquinone. Major polar lipids were sphingoglycolipids, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine. Small amounts of phosphatidylmonomethylethanolamine and an unidentified glycolipid are also present (see two-dimensional thin-layer chromatogram available as Supplementary Fig. S1 in IJSEM Online).

Table 1. Phenotypic characteristics that differentiate strain BZ30T from closely related species of the genus Sphingopyxis

| Strains: 1, Sphingopyxis bauzanensis sp. nov. BZ30T; 2, Sphingopyxis chilensis DSM 14889T; 3, Sphingopyxis ginsengisoli LMG 23390T; 4, Sphingopyxis taejonensis DSM 15583T; 5, Sphingopyxis witflariensis DSM 14551T. All data from this study. All species are Gram-negative, rod-shaped, non-spore-forming, motile, positive for activities of catalase, oxidase, acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase, and assimilate glucose. All species are negative for nitrate reduction, indole production, hydrolysis of urea and gelatin, activities of lipase (C14), α-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase, assimilation of citrate, N-acetylglucosamine and phenylactic acid, and for fermentative metabolism. +, Positive; W, weakly positive; –, negative. |
|---|---|---|---|---|---|
| Characteristic | 1 | 2 | 3 | 4 | 5 |
| Isolation source | Contaminated soil | Contaminated river sediment | Soil from ginseng field | Natural mineral water | Activated sludge from wastewater plant |
| | (Italy) | (Chile) | (South Korea) | (South Korea) | (Germany) |
| Growth temperature range (°C)* | 1–30 | 1–37 | 10–37 | 10–37 (weak at 5) | 1–30 |
| Growth in presence of 7.5% (w/v) NaCl * | – | + | – | – | – |
| Enzyme activities (25 °C) | | | | | |
| Hydrolysis of aesculin | + | + | + | – | – |
| Arginine dihydrolase | – | – | + | – | – |
| Cystine arylamidase | – | + | + | + | + |
| Trypsin | – | – | + | – | – |
| α-Chymotrypsin | – | – | + | – | – |
| α-Glucosidase | + | + | – | + | – |
| Assimilation of (25 °C): | | | | | |
| l-Arabinose | – | + | – | – | – |
| D-Mannitol | – | + | – | – | – |
| Maltose | + | + | – | – | + |
| D-Mannose | – | W | – | – | – |
| Potassium gluconate | – | + | – | – | – |
| Capric acid | – | – | – | w | + |
| Malic acid | + | – | + | – | – |

*Data obtained on R2A agar plates.
0.2 μmol g⁻¹ dry mass, respectively). Cadaverine and spermine were detected in trace amounts (<0.1 μmol g⁻¹ dry mass). This polyamine pattern is in agreement with the characteristics of members of the genus *Sphingopyxis* (Busse et al., 1999; Takeuchi et al., 2001).

For fatty acid methyl ester analysis, strain BZ30ᵀ and two reference strains (*Sphingopyxis witflariensis* W-50ᵀ and *Sphingopyxis chilensis* DSM 14889ᵀ) were grown on tryptic soy agar plates (TSA) at 25 °C for 3 days. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI (Microbial Identification) system (Sasser, 1990). The predominant cellular fatty acids of strain BZ30ᵀ were summed feature 3 (C₁₆:₁₀7c and/or iso-C₁₅:₀2-OH (37.4 %), C₁₈:₁₀7c (19.6 %), C₁₆:₀ (8.2 %), C₁₄:₀ 2-OH (8.0 %) and C₁₆:₀ 2-OH (5.0 %); this fatty acid profile was in agreement with those of other species of the genus *Sphingopyxis* (Kämpfer et al., 2002; Godoy et al., 2003; Lee et al., 2008). The fatty acid profiles of strain BZ30ᵀ and other species of the genus *Sphingopyxis* are available as Supplementary Table S1.

The DNA G+C content was determined by the thermal denaturation method with *Escherichia coli* K-12 as the reference, 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 BZ30ᵀ was 64.4 mol%. The DNA–DNA relatedness between strain BZ30ᵀ and *Sphingopyxis chilensis* DSM 14889ᵀ, *Sphingopyxis witflariensis* W-50ᵀ, *Sphingopyxis ginsengisoli* LMG 23390ᵀ and *Sphingopyxis taejonensis* DSM 15583ᵀ was 21.2, 20.3, 19.9 and 18.4 %, respectively. These values are below the threshold value (approx. 70 %) for possible relatedness at the species level (Wayne et al., 1987).

Bacteria belonging to the genus *Sphingopyxis* are Gram-negative, non-fermentative, aerobic, non-spore-forming, yellow-pigmented or whitish-brown, catalase- and oxidase-positive, contain the ubiquinone Q-10 and sphingoglycolipids, and have a DNA G+C content of 63–69 mol% (Godoy et al., 2003; Lee et al., 2008). Strain BZ30ᵀ displayed all of these properties. In addition, nucleotide signatures and some important phenotypic characteristics, such as the polyamine pattern and the inability to reduce nitrate, further confirmed the taxonomic position of strain BZ30ᵀ as determined by phylogenetic analysis. The strain is a psychrophilic (cold-adapted) representative of the genus *Sphingopyxis*, showing good growth at 1–25 °C. Based on phylogenetic, phenotypic and genomic evidence, strain BZ30ᵀ was identified as a representative of a novel species of the genus *Sphingopyxis*, for which the name *Sphingopyxis bauzanensis* sp. nov. is proposed.

**Description of *Sphingopyxis bauzanensis* sp. nov.**

*Sphingopyxis bauzanensis* (bau.zan.en’sis. M.L. fem. adj. bauzanensis referring to Bauzanum, the medieval Latin name for Bozen/Bolzano, a city in South Tyrol, Italy, where the type strain was first found).

Cells are Gram-negative, aerobic, rod-shaped (0.4–0.6 × 0.8–1.1 μm) and motile. Colonies on R₂A agar are yellow, round, convex and with entire margin; colony diameter is approximately 1 mm after 6 days at 20 °C on R₂A agar. Growth occurs in liquid R₂A medium and on agar plates at 1–30 °C; growth rate is fastest at 25 °C and absent at 37 °C; good growth occurs at 1–5 °C. On R₂A agar plates, grows at pH 6–8 and in the presence of 0–3 % (w/v) NaCl. Produces catalase and cytochrome oxidase. Positive for hydrolysis of asesculin and for activities of alkaline phosphatase, acid phosphatase, esterase (C₄), esterase lipase (C₈), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and x-glucosidase. Negative for nitrate reduction, production of indole (tryptophan deaminase activity) and H₂S, citrate utilization, and hydrolysis of urea, starch, gelatin and TWEEN 80. Negative for activities of arginine dihydrolase, lysine dihydrolase, ornithine dihydrolase, lipase (C₁₄), cystine arylamidase, β-glucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, trypsin, α-chymotrypsin, α-fucosidase and α-mannosidase. Assimilates D-glucose, maltose and malic acid, but does not assimilate L-arabinose, citrate, D-mannose, D-mannitol, N-acetylglucosamine, potassium gluconate, capric acid or phenylacetic acid as sole carbon sources. Negative for fermentation of glucose, ribose, xylose, mannitol, maltose, lactose, sucrose, glycerogen, inositol, sorbitol, rhamsone, melibiose, amygdalin and L-arabinose. Sensitive to ampicillin (50 μg ml⁻¹), penicillin G, tetracycline, chloramphenicol, rifampicin and trimethoprim (each 100 μg ml⁻¹), but resistant to streptomycin (100 μg ml⁻¹) and cyclosporin A (100 μg ml⁻¹). Growth in presence of kanamycin (100 μg ml⁻¹) is delayed. Q-10 is the major ubiquinone. The predominant cellular fatty acids are summed feature 3 (C₁₆:₁₀7c and/or iso-C₁₅:₀ 2-OH), C₁₈:₁₀7c, C₁₆:₀, C₁₄:₀ 2-OH and C₁₆:₀ 2-OH. Major polar lipids are sphingoglycolipids, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine. Spermidine is the major polyamine. The G+C content of the genomic DNA is 64.4 mol%.

The type strain is BZ30ᵀ (=DSM 22271ᵀ =CGMCC 1.8959ᵀ =CIP 110136ᵀ) and was isolated from hydrocarbon-contaminated soil in Bozen, South Tyrol, Italy.

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**References**


