Sphingopyxis italica sp. nov., isolated from Roman catacombs

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A Gram-stain-negative, aerobic, motile, rod-shaped bacterium, strain SC13E-S71T, was isolated from tuff, volcanic rock, where the Roman catacombs of Saint Callixtus in Rome, Italy, was excavated. Analysis of 16S rRNA gene sequences revealed that strain SC13E-S71T belongs to the genus Sphingopyxis, and that it shows the greatest sequence similarity with Sphingopyxis chilensis DSM 14889T (98.72 %), Sphingopyxis taejonensis DSM 15583T (98.65 %), Sphingopyxis golsengioli LMG 23390T (98.16 %), Sphingopyxis panaciterrae KCTC 12580T (98.09 %), Sphingopyxis alaskensis DSM 13593T (98.09 %), Sphingopyxis witkleriensis DSM 14551T (98.09 %), Sphingopyxis bauzanensis DSM 22271T (98.02 %), Sphingopyxis granuli KCTC 12209T (97.73 %), Sphingopyxis macrogotabida KACC 10927T (97.49 %), Sphingopyxis ummariensis DSM 24316T (97.37 %) and Sphingopyxis panaciterrae KCTC 22112T (97.09 %). The predominant fatty acids were C18 : 1w7c, summed feature 3 (iso-C15 : 0 2-OH and/or C16 : 1w6c), C14 : 0 2-OH and C16 : 0. The predominant menaquinone was MK-10. The major polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phospha-tidylcholine and sphingoglycolipid. These chemotaxonomic data are common to members of the genus Sphingopyxis. However, a polyphasic approach using physiological tests, DNA base ratios, DNA–DNA hybridization and 16S rRNA gene sequence comparisons showed that the isolate SC13E-S71T belongs to a novel species within the genus Sphingopyxis, for which the name Sphingopyxis italica sp. nov. is proposed. The type strain is SC13E-S71T (=DSM 25229T = CECT 8016T).

The genus Sphingopyxis was proposed by Takeuchi et al. (2001) to include the type species Sphingopyxis macrogotabida and Sphingopyxis terrae, which Takeuchi et al. (1993) had previously described as Sphingomonas macrogotabida and Sphingomonas terrae. At the time of writing, the genus Sphingopyxis comprises 16 species: Sphingopyxis macrogotabida (Takeuchi et al., 1993, 2001), Sphingopyxis terrae (Takeuchi et al., 1993, 2001), Sphingopyxis alaskensis (Vancanneyt et al., 2001; Godoy et al., 2003), Sphingopyxis taejonensis (Lee et al., 2001; Pal et al., 2006), Sphingopyxis witkleriensis (Kämpfer et al., 2002), Sphingopyxis chilensis (Godoy et al., 2003), Sphingopyxis baekryuensis (Yoon et al., 2005), Sphingopyxis granuli (Kim et al., 2005), Sphingopyxis panaciterrae (Lee et al., 2008a), Sphingopyxis golsengioli (Lee et al., 2008b), Sphingopyxis ummariensis (Sharma et al., 2010), Sphingopyxis panaciterrae (Srinivasan et al., 2010), Sphingopyxis bauzanensis (Zhang et al., 2010), Sphingopyxis soli (Choi et al., 2010), Sphingopyxis rigui (Baik et al., 2013) and Sphingopyxis woomoenensis (Baik et al., 2013). Members of this genus are Gram-negative, non-spore-forming, aerobic, chemo-organotrophic, catalase-positive, yellow or whitish-brown-pigmented bacteria with a DNA G + C content of 58.0–69.2 mol%. The type strains of these species have been isolated from sediment, soil, sludge and water; however, novel species of Sphingopyxis have not yet been described either in subterranean environments or volcanic rock. In this study, we describe strain SC13E-S71T retrieved from tuff, a volcanic rock from the Roman catacombs of Saint Callixtus in Rome, Italy. A polyphasic approach showed that this isolate belongs to a novel species within the genus Sphingopyxis.

Strain SC13E-S71T was isolated on tryptose soy agar (TSA; Oxoid) after 2 weeks at 28 °C. The methods used in this study have been described previously (Jurado et al., 2005a, b), unless indicated otherwise. Morphological, physiological and chemotaxonomic studies were carried out in triplicate on cultures on R2A agar (Difco) at 28 °C. Cell morphology, dimensions and motility were examined by phase-contrast microscopy. Furthermore, motility was also checked on R2A broth containing 0.3 % agar (Tambalo et al., 2010). Oxidase activity was determined by monitoring the oxidation of
over the range 4–45°C. Tolerance to NaCl was studied on R2A supplemented with 0–15% (w/v) NaCl. Growth at different pH values (4.0–11.0 at intervals of 0.5 pH unit) was assessed with R2A broth and R2A agar. Different media were tested for spore production: oatmeal agar (Difco), nutrient agar (Difco) and Bennett’s agar (Jones, 1949). Cellular fatty acid compositions were analysed in triplicate after 3 days on TSA at 28°C according to the standard methodology described by Jurado et al. (2009). Polymyamines were extracted and analysed by thin layer chromatography (TLC) according to Pedrol & Tiburcio (2001). Polar lipid profile, respiratory quinones and G+C content of genomic DNA were determined by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). Genomic DNA extraction and amplification of 16S rRNA genes were performed as described by Laiz et al. (2009). The identification of phylogenetic neighbours was carried out by applying BLAST (Altschul et al., 1990) to the GenBank sequence database and the EzTaxon database (Kim et al., 2012). Pairwise 16S rRNA gene sequence similarities among the most closely related strains were determined using the global alignment algorithm on the EzTaxon server (http://eztaxon-e.ezbiocloud.net/) (Kim et al., 2012). For phylogenetic analyses, the nearly complete 16S rRNA gene sequence of strain SC13E-S71T was aligned and compared with corresponding sequences of members of the genus Sphingopyxis and other representatives of taxa of the family Sphingomonadaceae using the multiple sequence alignment program CLUSTAL X (Thompson et al., 1997). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 5 (Tamura et al., 2011) and PHYLO_WIN (Galtier et al., 1996) with three treeing algorithms: the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) methods. Tree robustness was assessed by bootstrap resampling (1000 replicates each). The degree of genomic relatedness among strain SC13E-S71T and the most closely related species on the basis of 16S rRNA gene sequence similarity was determined by DNA–DNA hybridization as described by Urdiaín et al. (2008).

Cells of strain SC13E-S71T were aerobic, Gram-negative, non-sporulating rods, catalase- and oxidase-positive. Strain SC13E-S71T showed weak growth at concentrations of 2% (w/v) NaCl, although optimal growth occurred in the absence of NaCl. Growth of strain SC13E-S71T occurred in the temperature range of 10–30°C, with an optimum at 25–30°C. Table 1 shows other physiological characteristics of strain SC13E-S71T, as well as numerous phenotypic differences from the phylogenetically closest species of the Sphingopyxis genus. Obvious differences were related with the presence or absence of enzymic activities, assimilation of N-acetylg glucosamine, adipic acid, arabinose, malate and mannose. Other differences included the production of acid from N-acetylg glucosamine and aesculin. Further dissimilarities were noticed in fatty acid composition. The fatty acid composition of the strain SC13E-S71T was similar to those of other type strains, but contained different amounts of fatty acids (Table S1, available in IJSEM Online). The presence of C18:1ω7c (30.0%), summed feature 4 (consisting of iso-C15:0 2-OH and/or C16:1ω7c; 27.3%), C14:0-2-OH (19.1%) and C16:0 (7.2%) as the major fatty acids are a characteristic feature of the genus Sphingopyxis (Takeuchi et al., 2001). The characteristic difference between strain SC13E-S71T and the other type strains of the genus Sphingopyxis was the absence of the fatty acid C17:0ω6c, which is generally found in members of this genus. Other differences were the presence of C17:1ω6c (4.7%), which is absent in other type strains, and the high value of the fatty acid C14:0 2-OH. Strain SC13E-S71T contained ubiquinone Q-10 as the major respiratory quinone. Polar lipid analysis showed that strain SC13E-S71T possessed diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, sphingoglycolipid and glycolipid (Fig. S1). Strain SC13E-S71T contained spermidine and spermine.

The 16S rRNA gene sequence of strain SC13E-S71T indicated a phylogenetic relationship to the genus Sphingopyxis, as shown in the 16S rRNA gene sequence phylogenetic tree (Fig. 1), where strain SC13E-S71T formed a separate line of descent in the phylogenetic cluster of the genus Sphingopyxis. These results were supported by a high bootstrap value (96%). Most of the species included in this cluster shared 97% similarity in their 16S rRNA sequences. The closest taxa to strain SC13E-S71T based on EzTaxon similarity searches were S. chilensis DSM 14889T (98.72% 16S rDNA gene sequence similarity), S. taeniosens DSM 15583T (98.65%), S. ginsengisoli LMG 23390T (98.16%), S. panaciterrae KCTC 12580T (98.09%), S. alaskensis DSM 13593T (98.09%), S. witflariensis DSM 14551T (98.09%), S. bauzanensis DSM 22271T (98.02%), S. granuli KCTC 12209T (97.73%), S. macrogolubida KACC 10927T (97.49%), S. ummariensis DSM 24316T (97.37%) and S. panaciterrae KCTC 22112T (97.09%). Strain SC13E-S71T showed DNA–DNA relatedness of 35.0% with S. chilensis DSM 14889T (reciprocal, 55.7%), 30.2% with S. taeniosens DSM 15583T (reciprocal, 39.2%), 48.9% with S. ginsengisoli LMG 23390T (reciprocal, 59.0%), 42.9% with S. panaciterrae KCTC 12580T (reciprocal, 56.3%) and 53.4% with S. bauzanensis DSM 22271T. These results indicate that strain SC13E-S71T shows sufficient genomic coherence and hybridization similarities with S. chilensis.
Table 1. Phenotypic characteristics of strain SC13E-S71^T^ and its closest neighbours

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Data in columns 1–10 are from this study using cells grown under the same conditions and data in columns 11 and 12 are from Srinivasan et al. (2010). +, Positive; ( + ), weakly positive; −, negative.

The phenotypic and genotypic characteristics described above and in the species description below, together with the differences observed between strain SC13E-S71^T^ and previously described species of the genus *Sphingopyxis* reveal that strain SC13E-S71^T^ is a novel species within the genus *Sphingopyxis*. The name *Sphingopyxis italica* sp. nov. is proposed.

**Description of Sphingopyxis italica** sp. nov.

*Sphingopyxis italica* (i.ta.li.ca. L. fem. adj. italica from Italy, the origin of the type strain).

Cells are aerobic, motile, Gram-negative, non-sporulating and rod-shaped (0.5–0.9 × 1.0–2.0 μm). Colonies are pale yellow, smooth, circular and 0.5 mm in diameter after 3 days at 28 °C on R2A agar. Catalase- and oxidase-positive. Does not reduce nitrate to nitrite. Growth occurs between 10 and 30 °C, optimum at 25–30 °C. Cells grow optimally in the absence of NaCl, with poor growth at 2% NaCl. The pH range for growth is between 4.5 and 8.5, with an optimum between pH 7.0 and 8.0. Does not produce indole. Produces acid from L-arabinose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-2-d-mannopyranoside, methyl-2-d-glucopyranoside, N-acetylgalcosamine, amygdalin, arbutin, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycerogen, xylitol, turanose, D-tagatose, DL-arabitol, potassium glutonate, potassium 2-ketogluconate or L-fucose; variable acid production from glycerol, D-arabinose, D-ribose, DL-xylene, D-adonitol, methyl β-D-xylpyranoside, salicin, cellobiose, maltose, lactose, melibiose, gentiobiose, D-lyxose, D-fucose and potassium 5-ketogluconate. Produces β-glucosidase, β-galactosidase, alkaline phosphatase, esterase (C1), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, β-glucuronidase, α,β-glucosidase, N-acetyl-β-glucosaminidase, valine arylamidase and trypsin, but not arginine dihydrolase, urease, gelatinase, lipase (C14), cystine arylamidase, x-chymotrypsin, x-galactosidase, x-mannosidase or x-fucosidase. Assimilates glucose, arabinose, mannose, N-acetylgalcosamine, malate and maltose, but not mannitol, potassium glutonate, capric acid, adipic acid, trisodium citrate or phenylactic acid. The predominant fatty acids are C18:1ω7c, summed feature 3 (C16:1ω7c or iso-C15:0 2-OH), C14:0 2-OH and C16:0. The predominant respiratory lipoquinone is Q-10. The major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine.
and sphingoglycolipid. The polyamines are spermidine and spermine.

The type strain, SC13E-S71T (=DSM 25229T=CECT 8016T), was isolated from the tuff walls of the Roman catacombs of Saint Callixtus, Rome, Italy. The DNA G+C content of the type strain is 65.7 mol%.

Acknowledgements

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References


