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

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Strain BZ30^T 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 BZ30^T were summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) (37.4 %), C_{18:1}ω7c (19.6 %), C_{16:0} (8.2 %), C_{14:0} 2-OH (8.0 %) and C_{16:0} 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 BZ30^T belonged to the family *Sphingomonadaceae* of the α-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. taejonensis* (97.4 %) and *S. ginsengisoli* (97.2 %). On the basis of the phenotypic characteristics, phylogenetic analysis and DNA–DNA relatedness data, strain BZ30^T represents a novel species of the genus *Sphingopyxis*, for which the name *Sphingopyxis bauzanensis* is proposed. The type strain is BZ30^T (=DSM 22271^T =CGMCC 1.8959^T =CIP 110136^T).

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 macrogoltabida* (Takeuchi *et al.*, 1993, 2001), *Sphingopyxis*

terrae (Takeuchi *et al.*, 1993, 2001), *Sphingopyxis taejonensis* (Lee *et al.*, 2001; Pal *et al.*, 2006), *Sphingopyxis witflariensis* (Kämpfer *et al.*, 2002), *Sphingopyxis alaskensis* (Vancanneyt *et al.*, 2001; Godoy *et al.*, 2003), *Sphingopyxis chilensis* (Godoy *et al.*, 2003), ‘*Sphingopyxis granulifera*’ (Kim *et al.*, 2005), *Sphingopyxis flavimaris* (Yoon & Oh, 2005), *Sphingopyxis baekryungensis* (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 from an industrial site contaminated with hydrocarbons.

Strain BZ30^T 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 BZ30^T.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BZ30^T is GQ131578.

Cellular fatty acid compositions and a two-dimensional thin-layer chromatogram of polar lipids of strain BZ30^T are available with the online version of this paper.

Sphingopyxis witflariensis W-50^T, *Sphingopyxis ginsengisoli* LMG 23390^T, *Sphingopyxis taejonensis* DSM 15583^T and *Sphingopyxis chilensis* DSM 14889^T shared the highest sequence similarities with strain BZ30^T 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 BZ30^T grouped with members of the genus *Sphingopyxis* (Fig. 1) and formed a cluster with *Sphingopyxis witflariensis* W-50^T, *Sphingopyxis chilensis* LMG 20986^T and *Sphingopyxis taejonensis* KCTC 2884^T. 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 rRNA of strain BZ30^T 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 BZ30^T 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,N',N'-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 BZ30^T and of the four reference strains *Sphingopyxis witflariensis* W-50^T, *Sphingopyxis ginsengisoli* LMG 23390^T, *Sphingopyxis taejonensis* DSM 15583^T and *Sphingopyxis chilensis* DSM 14889^T. Activities of β-galactosidase, amylase and protease were additionally tested by using R₂A agar plates supplemented with appropriate substrates (Margesin

Fig. 1. Phylogenetic relationship of strain BZ30^T 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 11170^T was used as the outgroup. GenBank accession numbers of 16S rRNA sequences are given in parentheses. Bar, 2 % sequence divergence.

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 BZ30^T are given in the species description (see below) and are shown in Table 1. The features that serve to differentiate strain BZ30^T 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 BZ30^T 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 BZ30^T 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). Spermidine was the major polyamine component (17.1 $\mu\text{mol g}^{-1}$ dry mass), whereas 1,3-diaminopropane and putrescine were detected in only small amounts (0.3 and

Table 1. Phenotypic characteristics that differentiate strain BZ30^T from closely related species of the genus *Sphingopyxis*

Strains: 1, *Sphingopyxis bauzanensis* sp. nov. BZ30^T; 2, *Sphingopyxis chilensis* DSM 14889^T; 3, *Sphingopyxis ginsengisoli* LMG 23390^T; 4, *Sphingopyxis taejonensis* DSM 15583^T; 5, *Sphingopyxis witflariensis* DSM 14551^T. 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 phenylacetic acid, and for fermentative metabolism. +, Positive; w, weakly positive; –, negative.

Characteristic	1	2	3	4	5
Isolation source	Contaminated soil (Italy)	Contaminated river sediment (Chile)	Soil from ginseng field (South Korea)	Natural mineral water (South Korea)	Activated sludge from wastewater plant (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 $\mu\text{mol g}^{-1}$ dry mass, respectively). Cadaverine and spermine were detected in trace amounts ($<0.1 \mu\text{mol g}^{-1}$ 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 BZ30^T and two reference strains (*Sphingopyxis witflariensis* W-50^T and *Sphingopyxis chilensis* DSM 14889^T) 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 BZ30^T were summed feature 3 ($\text{C}_{16:1\omega7c}$ and/or iso- $\text{C}_{15:0}$ 2-OH) (37.4 %), $\text{C}_{18:1\omega7c}$ (19.6 %), $\text{C}_{16:0}$ (8.2 %), $\text{C}_{14:0}$ 2-OH (8.0 %) and $\text{C}_{16:0}$ 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 BZ30^T 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 BZ30^T was 64.4 mol%. The DNA–DNA relatedness between strain BZ30^T and *Sphingopyxis chilensis* DSM 14889^T, *Sphingopyxis witflariensis* W-50^T, *Sphingopyxis ginsengisoli* LMG 23390^T and *Sphingopyxis taejonensis* DSM 15583^T 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 BZ30^T 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 BZ30^T 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 BZ30^T 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 ($0.4\text{--}0.6 \times 0.8\text{--}1.1 \mu\text{m}$) 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 aesculin and for activities of alkaline phosphatase, acid phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and α -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, glycogen, inositol, sorbitol, rhamnose, melibiose, amygdalin and L-arabinose. Sensitive to ampicillin (50 $\mu\text{g ml}^{-1}$), penicillin G, tetracycline, chloramphenicol, rifampicin and trimethoprim (each 100 $\mu\text{g ml}^{-1}$), but resistant to streptomycin (100 $\mu\text{g ml}^{-1}$) and cyclosporin A (100 $\mu\text{g ml}^{-1}$). Growth in presence of kanamycin (100 $\mu\text{g ml}^{-1}$) is delayed. Q-10 is the major ubiquinone. The predominant cellular fatty acids are summed feature 3 ($\text{C}_{16:1\omega7c}$ and/or iso- $\text{C}_{15:0}$ 2-OH), $\text{C}_{18:1\omega7c}$, $\text{C}_{16:0}$, $\text{C}_{14:0}$ 2-OH and $\text{C}_{16:0}$ 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 BZ30^T (=DSM 22271^T =CGMCC 1.8959^T =CIP 110136^T) and was isolated from hydrocarbon-contaminated soil in Bozen, South Tyrol, Italy.

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References

- Brosius, J., Palmer, M. L., Kennedy, P. J. & Noller, H. F. (1978). Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc Natl Acad Sci U S A* **75**, 4801–4805.

- Busse, H.-J. & Auling, G. (1988). Polyamine pattern as a chemotaxonomic marker within the *Proteobacteria*. *Syst Appl Microbiol* **11**, 1–8.
- Busse, H.-J., Bunka, S., Hensel, A. & Lubitz, W. (1997). Discrimination of members of the family *Pasteurellaceae* based on polyamine patterns. *Int J Syst Bacteriol* **47**, 698–708.
- Busse, H.-J., Kämpfer, P. & Denner, E. B. M. (1999). Chemotaxonomic characterisation of *Sphingomonas*. *J Ind Microbiol Biotechnol* **23**, 242–251.
- Busse, H.-J., Denner, E. B. M., Buczolits, S., Salkinoja-Salonen, M., Bennisar, A. & Kämpfer, P. (2003). *Sphingomonas aurantiaca* sp. nov., *Sphingomonas aerolata* sp. nov. and *Sphingomonas faeni* sp. nov., air- and dustborne and Antarctic, orange-pigmented, psychrotolerant bacteria, and emended description of the genus *Sphingomonas*. *Int J Syst Evol Microbiol* **53**, 1253–1260.
- Collins, M. D. (1985). Isoprenoid quinone analysis in classification and identification. In *Chemical Methods in Bacterial Systematics*, pp. 267–287. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Godoy, F., Vancanneyt, M., Martínez, M., Steinbüchel, A., Swings, J. & Rehm, B. H. A. (2003). *Sphingopyxis chilensis* sp. nov., a chlorophenol-degrading bacterium that accumulates polyhydroxyalkanoate, and transfer of *Sphingomonas alaskensis* to *Sphingopyxis alaskensis* comb. nov. *Int J Syst Evol Microbiol* **53**, 473–477.
- HuB, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.
- Kämpfer, P., Witzemberger, R., Denner, E. B. M., Busse, H.-J. & Neef, A. (2002). *Sphingopyxis witfariensis* sp. nov., isolated from activated sludge. *Int J Syst Evol Microbiol* **52**, 2029–2034.
- Kates, M. (1986). *Techniques of Lipidology*, 2nd edn. Amsterdam: Elsevier.
- Kim, M. K., Im, W.-T., Ohta, H., Lee, M. & Lee, S.-T. (2005). *Sphingopyxis granuli* sp. nov., a β -glucosidase-producing bacterium in the family *Sphingomonadaceae* in α -4 subclass of the *Proteobacteria*. *J Microbiol* **43**, 152–157.
- Kim, B.-S., Lim, Y. W. & Chun, J. (2008). *Sphingopyxis marina* sp. nov. and *Sphingopyxis litoris* sp. nov., isolated from seawater. *Int J Syst Evol Microbiol* **58**, 2415–2419.
- Lee, J.-S., Shin, Y. K., Yoon, J.-H., Takeuchi, M., Pyun, Y.-R. & Park, Y.-H. (2001). *Sphingomonas aquatilis* sp. nov., *Sphingomonas koreensis* sp. nov. and *Sphingomonas taejonensis* sp. nov., yellow-pigmented bacteria isolated from natural mineral water. *Int J Syst Evol Microbiol* **51**, 1491–1498.
- Lee, M., Ten, L. N., Lee, H.-W., Oh, H. W., Im, W.-T. & Lee, S.-T. (2008). *Sphingopyxis ginsengisoli* sp. nov., isolated from soil of a ginseng field in South Korea. *Int J Syst Evol Microbiol* **58**, 2342–2347.
- Margesin, R., Gander, S., Zacke, G., Gounot, A. M. & Schinner, F. (2003). Hydrocarbon degradation and enzyme activities of cold-adapted bacteria and yeasts. *Extremophiles* **7**, 451–458.
- Pal, R., Bhasin, V. K. & Lal, R. (2006). Proposal to reclassify [*Sphingomonas*] *xenophaga* Stolz et al. 2000 and [*Sphingomonas*] *taejonensis* Lee et al. 2001 as *Sphingobium xenophagum* comb. nov. and *Sphingopyxis taejonensis* comb. nov., respectively. *Int J Syst Evol Microbiol* **56**, 667–670.
- Sambrook, J., Frisch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Stolz, A., Busse, H.-J. & Kämpfer, P. (2007). *Pseudomonas knackmussii* sp. nov. *Int J Syst Evol Microbiol* **57**, 572–576.
- Takeuchi, M., Kawai, F., Shimada, Y. & Yokota, A. (1993). Taxonomic study of polyethylene glycol-utilizing bacteria: emended description of the genus *Sphingomonas* and new descriptions of *Sphingomonas macrogoltabidus* sp. nov., *Sphingomonas sanguis* sp. nov. and *Sphingomonas terrae* sp. nov. *Syst Appl Microbiol* **16**, 227–238.
- Takeuchi, M., Hamana, K. & Hiraishi, A. (2001). Proposal of the genus *Sphingomonas sensu stricto* and three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, on the basis of phylogenetic and chemotaxonomic analyses. *Int J Syst Evol Microbiol* **51**, 1405–1417.
- Vancanneyt, M., Schut, F., Snauwaert, C., Goris, J., Swings, J. & Gottschal, J. C. (2001). *Sphingomonas alaskensis* sp. nov., a dominant bacterium from a marine oligotrophic environment. *Int J Syst Evol Microbiol* **51**, 73–79.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Wu, C., Lu, X., Qin, M., Wang, Y. & Ruan, J. (1989). Analysis of menaquinone compound in microbial cells by HPLC. *Microbiology [English translation of Microbiology (Beijing)]* **16**, 176–178.
- Yabuuchi, E., Yano, I., Oyaizu, H., Hashimoto, Y., Ezaki, T. & Yamamoto, H. (1990). Proposals of *Sphingomonas paucimobilis* gen. nov. and comb. nov., *Sphingomonas parapaucimobilis* sp. nov., *Sphingomonas yanoikuyae* sp. nov., *Sphingomonas adhaesiva* sp. nov., *Sphingomonas capsulata* comb. nov., and two genospecies of the genus *Sphingomonas*. *Microbiol Immunol* **34**, 99–119.
- Yabuuchi, E., Kosako, Y., Fujiwara, N., Naka, T., Matsunaga, I., Ogura, H. & Kobayashi, K. (2002). Emendation of the genus *Sphingomonas* Yabuuchi et al. 1990 and junior objective synonymy of the species of three genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, in conjunction with *Blastomonas ursincola*. *Int J Syst Evol Microbiol* **52**, 1485–1496.
- Yoon, J.-H. & Oh, T.-K. (2005). *Sphingopyxis flavimaris* sp. nov., isolated from sea water of the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **55**, 369–373.
- Yoon, J.-H., Lee, C.-H., Yeo, S.-H. & Oh, T.-K. (2005). *Sphingopyxis baekryungensis* sp. nov., an orange-pigmented bacterium isolated from sea water of the Yellow Sea in Korea. *Int J Syst Evol Microbiol* **55**, 1223–1227.
- Zhang, D.-C., Wang, H.-X., Liu, H.-C., Dong, X.-Z. & Zhou, P.-J. (2006). *Flavobacterium glaciei* sp. nov., a novel psychrophilic bacterium isolated from the China No.1 glacier. *Int J Syst Evol Microbiol* **56**, 2921–2925.