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Sphingomonas frigidaeris sp. nov., isolated from an air conditioning system

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Abstract

A strictly aerobic Gram-stain-negative bacterium, designated strain KER25-10^T, was isolated from a laboratory air conditioning system in South Korea. Cells were yellow-pigmented, non-motile rods showing catalase- and oxidase-positive reactions. The strain grew at pH 4.0–9.0 (optimum, pH 6.0–7.0) and 10–40 °C (optimum, 30 °C) and in the presence of 0–3 % (w/v) NaCl (optimum, 0 %). The G+C content of the genomic DNA was 65.1 mol%. Strain KER25-10^T contained ubiquinone-10 (Q-10) as the predominant isoprenoid quinone and $C_{16:0}$, $C_{17:1}\omega6c$, summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$) and summed feature 8 (comprising $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) as the major fatty acids. The major polar lipids were sphingoglycolipid, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine. Only spermidine was detected as the polyamine. Phylogenetic analysis based on 16S rRNA sequences indicated that strain KER25-10^T formed a distinct phylogenetic lineage within the genus *Sphingomonas* of the family *Sphingomonadaceae* and the strain was most closely related to *Sphingomonas kyeonggiense* THG-DT81^T with a 96.8 % 16S rRNA gene sequence similarity. On the basis of phenotypic, chemotaxonomic and molecular features, strain KER25-10^T clearly represents a novel species of the genus *Sphingomonas*, for which the name *Sphingomonas frigidaeris* sp. nov. is proposed. The type strain is KER25-10^T (=KACC 19285^T=JCM 32053^T).

The genus *Sphingomonas*, belonging to the family *Sphingomonadaceae* of the class *Alphaproteobacteria*, was first proposed by Yabuuchi *et al.* [1] with *Sphingomonas paucimobilis* as the type species. The genus *Sphingomonas* has now been divided into four genera, *Sphingomonas*, *Sphingobium*, *Novosphingobium* and *Sphingopyxis* [2, 3]. At the time of writing, the genus *Sphingomonas* comprises 108 species with validly published names (www.bacterio.net/sphingomonas.html), which have been isolated from a variety of environmental habitats such as air, soil, aquatic habitats and plants [4–9]. Members of the genus *Sphingomonas* are Gram-stain-negative, yellow-pigmented, non-spore-forming, rod-shaped, non-motile or motile bacteria and contain sphingolipids as the characteristic polar lipids, 2-hydroxymyristic acid ($C_{14:0}$ 2-OH) as the major 2-hydroxy fatty acid and ubiquinone-10 (Q-10) as the major ubiquinone [1, 2, 9]. In this study, we isolated a presumably novel species belonging to the genus *Sphingomonas*, designated strain KER25-10^T, from an air conditioning system, and characterized it further taxonomically by using a polyphasic approach.

Strain KER25-10^T was isolated from an air conditioning system that operated in a laboratory in Seoul, South Korea. For initial cultivation, a filter of the air conditioning system was resuspended in phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.2). Aliquots of each serial dilution were spread on R2A agar (BD, USA) and incubated aerobically at 25 °C for 3 days. Colonies grown on R2A agar were randomly selected, and their 16S rRNA genes were PCR-amplified by using universal primers F1 and R13. Then, the PCR amplicons were analysed based on the fragment patterns generated by *Hae*III and *Hha*I digestion, as described previously [10]. The PCR products with different restriction fragment patterns were partially sequenced using the 340F primer (5′-CCT ACG GGA GGC AGC AG-3′). The resulting 16S rRNA gene sequences were compared with those of all reported type strains by using the Nucleotide Similarity Search program in the EzTaxon-e server (http://www.ezbiocloud.net/) [11]. From the analysis, a putative novel strain belonging to the genus *Sphingomonas*, designated KER25-10^T, was selected for further phenotypic and phylogenetic analyses. Strain KER25-10^T was routinely cultured aerobically on R2A agar at 30 °C for 3 days, except where indicated, and stored at −80 °C in R2A broth (BD) supplemented with 15 % (v/v) glycerol for a long-term

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Abbreviations: ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; Q-10, ubiquinone-10; RDP, ribosomal database project.
The GenBank accession number for the 16S rRNA gene sequence of strain KER25-10^T is KY873312.
One supplementary table and five supplementary figures are available with the online Supplementary Material.

preservation. The type strains of *Sphingomonas paucimobilis* (KACC 10198^T) and *Sphingomonas kyeonggiense* (KACC 17173^T) were obtained from a culture collection centre (Korean Agricultural Culture Collection, Korea) as reference strains for the comparison of phenotypic properties and fatty acid compositions.

The 16S rRNA gene amplicon of strain KER25-10^T that was PCR-amplified using the F1 and R13 primers was further sequenced using the universal primers 518R (5′-ATT ACC GCG GCT GCT GG-3′) and 805F (5′-GAT TAG ATA CCC TGG TAG TC-3′) at Macrogen (Republic of Korea) to obtain an almost-complete 16S rRNA gene sequence (1409 nucleotides). The 16S rRNA gene sequence of strain KER25-10^T and closely related type strains were aligned using the fast secondary structure aware Infernal aligner in the Ribosomal Database Project (RDP) (http://pyro.cme.msu.edu/spring/align.spr) [12]. Phylogenetic relationships between strain KER25-10^T and closely related type strains were inferred using the DNADIST and DNAPARS programs based on the neighbour-joining (NJ) algorithm with the Kimura two-parameter model and the maximum-parsimony (MP) algorithm through a heuristic search, respectively, in the PHYLIP software (version 3.695) [13] and their tree topologies were evaluated through bootstrap analyses based on a 1000 resamplings. Maximum-likelihood (ML) analysis with bootstrap values was performed using RAxML-HPC BlackBox (version 8.2.9) available in the Cyber-Infrastructure for Phylogenetic Research project (CIPRES, www.phylo.org) [14].

Comparative analysis based on the 16S rRNA gene sequences revealed that strain KER25-10^T was most closely related to *S. kyeonggiense* THG-DT81^T with a 96.8 % sequence similarity. The 16S rRNA gene sequence similarities of strain KER25-10^T with other validly reported type strains were less than 96.5 %. The phylogenetic analysis using the NJ algorithm indicated that strain KER25-10^T formed a distinct phylogenic lineage within the genus *Sphingomonas* of the family *Sphingomonadaceae* with a low bootstrap value (Fig. 1), which was also supported by the MP and ML algorithms (Fig. S1, available in the online Supplementary Material). Recently, it was been suggested that 98.65–98.70 % similarity of 16S rRNA gene sequences can be used as a new alternative threshold for DNA–DNA hybridization (DDH) in bacterial classification [15–17]. The similarities of 16S rRNA gene sequence between strain KER25-10^T and closely related type strains were much lower than the new threshold value, which suggests that strain KER25-10^T represents a novel species of the genus *Sphingomonas* without performing DDH experiments.

Growth of strain KER25-10^T was assessed at 30 °C for 3 days on several bacteriological media: R2A agar, Luria–Bertani agar (LB; MP Biomedicals), nutrient agar (NA; BD) and trypticase soy agar (TSA; BD). Growth of strain KER25-10^T was tested on R2A agar at different temperatures (5–50 °C at 5 °C intervals) for 3 days. Both pH and NaCl tolerances were determined in R2A broth with different pH values (1.0–10.0 at 1.0 pH unit intervals) and NaCl concentrations (0–5 % at 1 % intervals) for 3 days, respectively. The pH values below 8.0 and pH 8.0–10.0 were prepared by using Na_2HPO_4-NaH_2PO_4 and Tris-HCl buffers, respectively, and were adjusted again if necessary after sterilization (121 °C for 15 min). NaCl concentrations (0–15 % at 1 % intervals) were prepared in the laboratory according to the BD formula. Gram staining was conducted using the Gram stain kit (bioMérieux) according to the manufacturer's instructions. Anaerobic growth was assessed on R2A at 30 °C for 3 weeks under the anaerobic condition (with 4–10 % CO_2) using the GasPak Plus system (BBL). Cell morphology and motility of strain KER25-10^T were observed using transmission electron microscopy (JEM-1010, JEOL) and phase-contrast microscopy with cells grown on R2A agar at 30 °C for 2 days. Catalase and oxidase activities of strain KER25-10^T were tested by the production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution and the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck), respectively [18]. Yellow pigments were extracted from lyophilized cells by addition of methanol for 60 min and analysed by using a UV-Visible spectrometer (BioTek). The following properties of strain KER25-10^T and two reference strains were investigated in parallel under the same conditions in this study. Hydrolysis of Tween 20, Tween 80, casein, starch, tyrosine and aesculin was checked on R2A agar by following the protocols of Lányí [19] and Smibert and Krieg [18]. Additional enzymatic activities, biochemical features and oxidations of carbon compounds were tested using the API ZYM and API 20NE kits (bioMérieux) and the GN2 MicroPlate system (Biolog), respectively, according to the manufacturers' instructions; cells of strain KER25-10^T and reference strains resuspended in 0.85 % (w/v) saline were used as inocula for the tests.

Strain KER25-10^T grew well on all the tested media: R2A agar, LB agar, NA and TSA (optimum, R2A agar). Cells were Gram-stain-negative non-motile rods of approximately 0.5–0.8 μm in width and 0.8–2.0 μm in length, as measured under the transmission electron microscope (Fig. S2). Colonies were yellow-pigmented, circular, smooth and convex with 0.2–0.4 mm in diameter on R2A agar after 3 days of incubation. The yellow pigment of strain KER25-10^T had two major peaks at 449 and 475 nm, similar to those of members within the genus *Sphingomonas* (Fig. S3) [20]. Anaerobic growth was not observed after incubation on R2A agar at 30 °C for 3 weeks. In the Biolog GN2 MicroPlate, strain KER25-10^T oxidized Tween 40, L-arabinose, D-galactose, α-D-glucose, lactose, maltose, trehalose, methyl pyruvate, β-hydroxy butyric acid, succinic acid, L-alaninamide, L-glutamic acid, glycyl-L-glutamic acid, L-fucose, D-sorbitol, D-galactonic acid lactone, D-galacturonic acid, glycogen, α-keto glutaric acid, D,L-lactic acid, succinamic acid, 2-aminoethanol, glycerol and L-rhamnose, but did not oxidize *N*-acetyl-D-galactosamine, adonitol, D-arabitol, *i*-erythritol, *m*-inositol, D-mannitol, D-psicose, xylitol, acetic acid, citric acid, formic acid, D-glucosaminic acid, D-

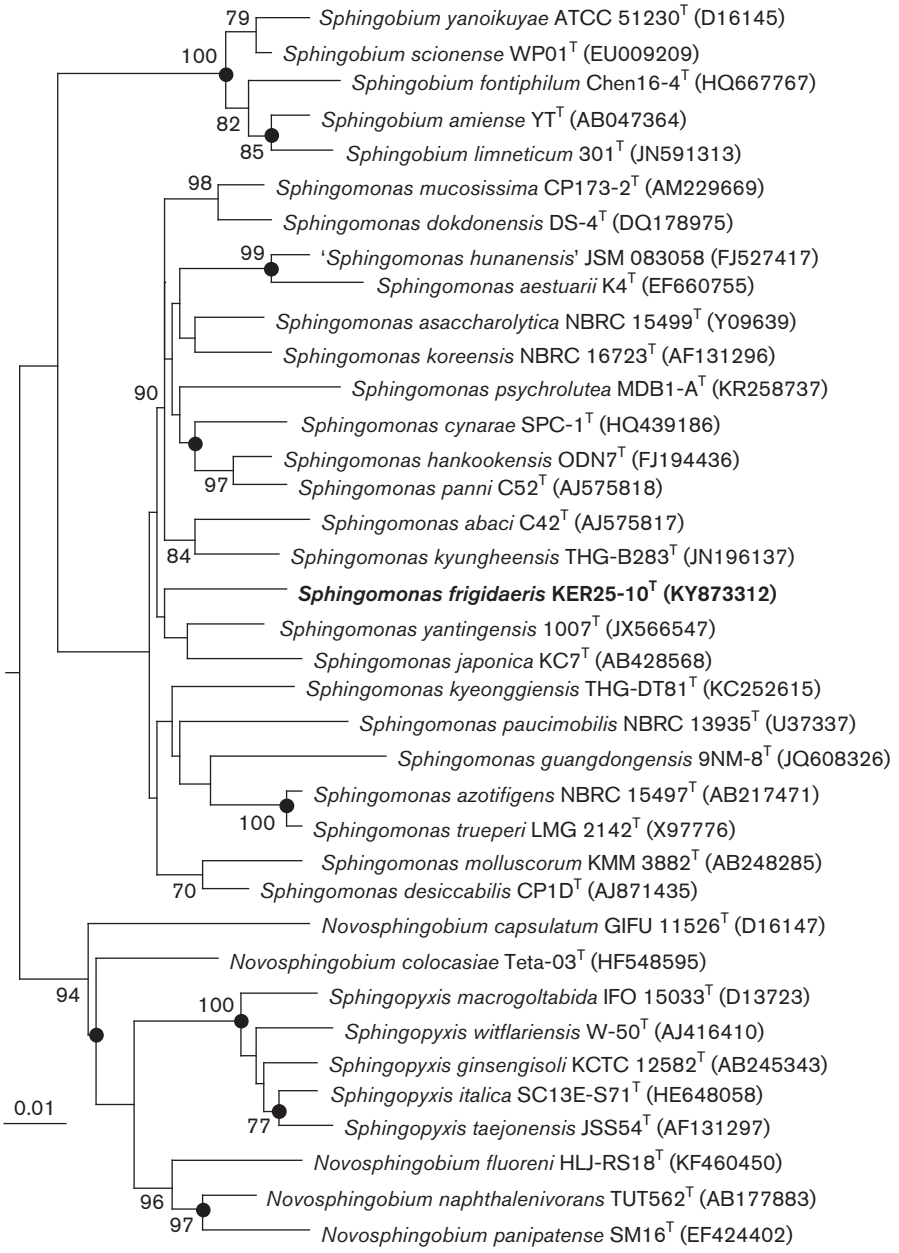

Fig. 1. A neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain KER25-10^T and related taxa. Bootstrap values with more than 70 % are shown on the nodes as percentages of 1000 replicates. Filled circles (●) indicate that the corresponding nodes were also recovered in the trees constructed by the ML and MP algorithms. *Rhodanobacter lindanoclasticus* RP5557^T (AF039167) was used as an outgroup (not shown). Bar, 0.01 changes per nucleotide position.

glucuronic acid, γ-hydroxy butyric acid, *p*-hydroxy phenylacetic acid, itaconic acid, malonic acid, quinic acid, D-fructose, lactulose, raffinose, mono-methyl-succinate, α-hydroxy butyric acid, α-keto butyric acid, α-keto valeric acid, bromo succinic acid, L-alanine, L-alanyl-glycine, L-ornitine, L-pyroglutamic acid, L-serine, putrescine, 2,3-butanediol, D-gluconic acid, propionic acid, sebacic acid, D-saccharic acid, glucuronamide, D-alanine, L-asparagine, L-asparagine, L-histidine, L-leucine, L-phenylalanine, L-threonine, D,L-carnitine, γ-amino butyric acid, urocanic acid, inosine uridine, thymidine, phenyethylamine, D,L-α-glycerol phosphate, D-serine, glycyl-L-aspartic acid, α-cyclodextrin, dextrin, Tween 80, *N*-acetyl-D-glucosamine, cellobiose, centiobiose, D-mannose, melibiose, *m*ethyl β-D-glucoside, sucrose, turanose, *cis*-aconitic acid, L-aspartic acid, hydroxy-L-proline, glucose-1-phosphate, glucose-6-phosphate and L-proline. Almost allcharacteristics of strain KER25-10^T such as yellow-pigmention, Gram-stain-

negative, strictly aerobic growth, rod-shape, non-motile, and oxidase and catalase activities were in agreement with those considered to be characteristic of the genus *Sphingomonas*, whereas others such as NaCl and pH tolerances allowed the differentiation of strain KER25-10^T from other closely related species of the genus [3, 8]. Phenotypic characteristics of strain KER25-10^T are presented in the species description and compared with those of the closely related type strains in Tables 1 and S1.

The isoprenoid quinone of strain KER25-10^T was analysed by using a high performance liquid chromatography (HPLC; model LC-20A, Shimadzu) system equipped with a reversed-phase column (250 Í 4.6 mm, Kromasil, Akzo Nobel) and a diode array detector (SPD-M20A, Shimadzu) using methanol-isopropanol (2 : 1, v/v) as an eluent (1 ml min^{-1}), as described by Komagata and Suzuki [21]. Polyamines of strain KER25-10^T were extracted and analysed using an HPLC system, as described by Busse *et al.* [22]. For the analysis of cellular fatty acids, strain KER25-10^T and two reference strains were cultivated in R2A broth at 30 °C and the cells were harvested at the same growth phase (exponential phase, OD=0.8 at 600 nm). The fatty acids were saponified, methylated, extracted and washed according to the standard MIDI protocol and the fatty acid methyl esters were analysed by using a gas chromatography system (model 6890; Hewlett Packard) based on the TSBA6 database in the Microbial Identification System (Sherlock version 6.2B) [23]. The DNA G+C content of strain KER25-10^T was determined by a fluorometric method using SYBR Green I and a real-time PCR thermocycler (Bio-Rad) [24]. The polar lipids of strain KER25-10^T were analysed by thin-layer chromatography using cells harvested at the exponential growth phase according to the method described previously [25]. The following reagents were used to detect different polar lipids: 10 % ethanolic molybdatophosphoric

Table 1. Phenotypic comparisons of strain KER25-10^T and the type strains of closely related *Sphingomonas* species

Taxa: 1, strain KER25-10^T; 2, *S. kyeonggiense* KACC 17173^T [8]; 3, *S. paucimobilis* KACC 10198^T [3]. All strains are positive for the following characteristics: strictly aerobic growth, activity* of oxidase, catalase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phospatase, naphtol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase and 4-nitrophenyl-β-D-galactopyranoside, assimilation* of D-glucose, L-arabinose, maltose and malic acid, and hydrolysis* of aesculin. All strains are negative for the following characteristics: Gram reaction, nitrate reduction*, indole production*, activity* of arginine dihydrolase, urease, gelatin hydrolysis, α-mannosidase and α-fucosidase, assimilation* of phenylacetic acid, and hydrolysis* of casein and starch. +, Positive; –, negative; W, weakly positive.

Characteristics	1	2	3
Isolation source	Air conditioning system	Soil	Hospital respirator
Growth at:			
Temperature (optimum, °C)	10–40 (30)	15–30 (25–28)	4–40 (20–37)
pH (optimum)	4.0–9.0 (6.0–7.0)	6.0–9.5 (7.0)	5.0–8.0 (7.0)
NaCl (optimum, %)	0–3 (0)	0–0.5 (0)	0–4.0 (1.0–2.0)
Glucose fermentation*	–	+	–
Hydrolysis* of:			
Tyrosine	+	–	+
Tween 20	–	+	+
Tween 80	–	–	+
Assimilation (API 20NE)* of:			
D-Mannose	–	+	+
D-Mannitol	–	–	W
N-Acetyl-glucosamine	–	+	+
Potassium gluconate	–	W	W
Adipic acid	–	–	W
Trisodium citrate	–	–	+
Enzyme activity (API ZYM)* of:			
Lipase (C14)	–	W	+
Cystine arylamidase	+	–	–
Trypsin	–	+	+
α-Chymotrypsin	+	–	+
α-Galactosidase	W	–	+
β-Glucuronidase	–	+	–
N-Acetyl-β-glucosaminidase	–	+	+
DNA G+C content (mol%)	65.1	66.8	65

*These analyses were conducted under the same conditions in this study.

acid (for total polar lipids), ninhydrin (for aminolipids), Dittmer–Lester reagent (for phospholipids) and α-naphthol (for glycolipids).

The predominant respiratory quinone of strain KER25-10^T was ubiquinone-10 (Q-10, 90.3 %), in line with all other members of the family *Sphingomonadaceae*. Another, ubiquinone-9 (Q-9, 9.7 %), was also detected as a minor quinone. Only spermidine as the polyamine was detected from strain KER25-10^T (Fig. S4). This was different from most of the other *Sphingomonas* species, including *S. kyeonggiense* the most closely related species, which contain *sym*-homospermidine as the major polyamine [4, 6, 8]. However, spermidine as the major polyamine in strain KER25-10^T was in accordance with those in many other type species of the genus *Sphingomonas* including *Sphingomonas piscinae* and *Sphingomonas lacus* [9, 26, 27]. Major fatty acids (>5 %) were summed feature 8 (comprising $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$, 48.3 %), summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$, 5.2 %) and $C_{17:1}\omega6c$ (17.0 %) and $C_{16:0}$ (9.5 %) (Table 2). $C_{14:0}$ 2-OH (4.5 %) was a major hydroxy fatty acid and 3-hydroxy fatty acid was absent. The overall fatty acid profile of strain KER25-10^T was similar to those of the genus *Sphingomonas*, but there were some differences in the respective compositions of some fatty acid components, as shown in Table 2. In particular, the presence of $C_{17:1}\omega6c$ as a major fatty acid in strain KER25-10^T differentiated strain KER25-10^T from closely related *Sphingomonas* species (Table 2). The DNA G+C content of strain KER25-10^T was 65.1 mol%, which was similar to that of *S. kyeonggiense* THG-DT81^T (66.8 mol%) [8, 26, 27]. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and sphingoglycolipid. An unknown phospholipid and three unknown lipids were also detected as minor polar lipids (Fig. S5). The general polar lipid profile of strain KER25-10^T was similar to those of the reference strains of the genus *Sphingomonas* [8]. In conclusion, the phenotypic and chemotaxonomic features of strain KER25-10^T and the phylogenetic inference support its assignment to a novel species of the genus *Sphingomonas,* for which the name *Sphingomonas frigidaeris* sp. nov. is proposed.

Table 2. Cellular fatty acid compositions (%) of strain KER25-10^T and the type strains of closely related *Sphingomonas* species

Strains: 1, strain KER25-10^T; 2, *S. kyeonggiense* KACC 17173^T; 3, *S. paucimobilis* KACC 10198^T. All data were from this study. The data are expressed as percentages of the total fatty acids. Major fatty acid components (>5.0 %) are highlighted in bold. –, Not detected; TR, trace amount (<0.5 %).

	1	2	3
Saturated fatty acid:			
$C_{16:0}$	**9.5**	**20.7**	**9.8**
$C_{17:0}$	1.7	TR	TR
$C_{17:0}$ cyclo	–	–	1.9
$C_{18:0}$	TR	TR	0.5
Unsaturated fatty acid:			
$C_{16:1}\omega5c$	2.9	0.9	1.0
$C_{17:1}\omega6c$	**17.0**	1.1	–
$C_{17:1}\omega8c$	1.0	–	–
$C_{18:1}\omega5c$	1.1	1.0	1.7
$C_{19:0}$ cyclo $\omega8c$	–	–	1.5
11-methyl $C_{18:1}\omega7c$	4.2	**7.2**	–
Hydroxyl fatty acid:			
$C_{14:0}$ 2-OH	4.5	3.8	4.9
$C_{15:0}$ 2-OH	1.7	TR	–
Summed feature*:			
3	**5.2**	1.5	3.9
8	**48.3**	**61.5**	**72.9**

*Summed features represent groups of two or three fatty acids that cannot be separated by gas–liquid chromatography with the MIDI system. Summed feature 3, $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; Summed feature 8, $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

DESCRIPTION OF *SPHINGOMONAS FRIGIDAERIS* SP. NOV.

Sphingomonas frigidaeris (fri.gid.a′e.ris. L. adj. *frigidus* cold; L. n. *aer* air; N.L. gen. n. *frigidaeris* of cold air, as the strain was isolated from an air conditioning system).

Cells are Gram-stain-negative, strictly aerobic, non-motile rods, oxidase- and catalase-positive, and do not form spores. Cells are 0.5–0.8 µm in width and 0.8–2.0 µm in length. Colonies are yellow-pigmented, circular, smooth and convex. Growth occurs at 10–40 °C (optimum, 30 °C), at pH 4.0–9.0 (optimum, pH 6.0–7.0) and in the presence of 0–3.0 % (w/v) NaCl (optimum, 0 %). Tyrosine and aesculin are hydrolysed, but casein, Tween 20 and Tween 80, starch and gelatin are not. Does not reduce nitrate. Indole is not produced, and glucose is not fermented. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, α-chymotrypsin, acid phospatase, naphtol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and 4-nitrophenyl-β-D-galactopyranoside activities are positive, but lipase (C14), trypsin, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase, urease and gelatin hydrolysis activities are negative. Tests for assimilation of D-glucose, L-arabinose, maltose and malic acid are positive, but those for assimilation of D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid are negative. The polar lipids consist of phosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, sphingoglycolipid, an unknown phospholipid and three unknown lipids. The major fatty acids are summed feature 8 (comprising $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$), summed feature 3 (comprising $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$), $C_{17:1}\omega6c$ and $C_{16:0}$. Ubiquinone-10 is the major respiratory quinone. Only spermidine is detected as the polyamine.

The type strain is KER25-10^T (=KACC 19285^T=JCM 32053^T), isolated from an air conditioning system that

operated in a laboratory in South Korea. The DNA G+C content is 65.1 mol%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. Yabuuchi E, Yano I, Oyaizu H, Hashimoto Y, Ezaki T *et al.* 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* 1990;34:99–119.
2. Takeuchi M, Hamana K, Hiraishi A. 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* 2001;51:1405–1417.
3. Yabuuchi E, Kosako Y, Fujiwara N, Naka T, Matsunaga I *et al.* 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* 2002;52:1485–1496.
4. Kim SJ, Moon JY, Lim JM, Ahn JH, Weon HY *et al.* *Sphingomonas aerophila* sp. nov. and *Sphingomonas naasensis* sp. nov., isolated from air and soil, respectively. *Int J Syst Evol Microbiol* 2014;64:926–932.
5. Yoon JH, Kang SJ, Lee SY, Oh TK. *Sphingomonas insulae* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* 2008;58:231–236.
6. Sheu SY, Chen YL, Chen WM. *Sphingomonas fonticola* sp. nov., isolated from spring water. *Int J Syst Evol Microbiol* 2015;65:4495–4502.
7. Zhu L, Si M, Li C, Xin K, Chen C *et al.* *Sphingomonas gei* sp. nov., isolated from roots of *Geum aleppicum*. *Int J Syst Evol Microbiol* 2015;65:1160–1166.
8. Son HM, Kook M, Tran HT, Kim KY, Park SY *et al.* *Sphingomonas kyeonggiense* sp. nov., isolated from soil of a ginseng field. *Antonie van Leeuwenhoek* 2014;105:791–797.
9. Chen H, Jogler M, Rohde M, Klenk HP, Busse HJ *et al.* Reclassification and emended description of *Caulobacter leidyi* as *Sphingomonas leidyi* comb. nov., and emendation of the genus *Sphingomonas*. *Int J Syst Evol Microbiol* 2012;62:2835–2843.
10. Lee HJ, Jeong SE, Cho MS, Kim S, Lee SS *et al.* *Flavihumibacter solisilvae* sp. nov., isolated from forest soil. *Int J Syst Evol Microbiol* 2014;64:2897–2901.
11. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y *et al.* Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
12. Nawrocki EP, Eddy SR. Query-dependent banding (QDB) for faster RNA similarity searches. *PLoS Comput Biol* 2007;3:e56.
13. Felsenstein J. *PHYLIP (Phylogeny Inference Package), Version 3.6a*. Seattle: Department of genetics, University of Washington, Seattle, WA, USA; 2002.
14. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 2014;30:1312–1313.
15. Kim M, Oh HS, Park SC, Chun J. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. *Int J Syst Evol Microbiol* 2014;64:346–351.
16. Rosselló-Móra R, Amann R. Past and future species definitions for *Bacteria* and *Archaea*. *Syst Appl Microbiol* 2015;38:209–216.
17. Stackebrandt E, Ebers J. Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today* 2006;33:152–155.
18. Smibert RM, Krieg NR. Phenotypic characterization. In: Gerhardt P (editor). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology; 1994. pp. 607–654.
19. Lányí B. Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* 1987;19:1–67.
20. Chen X, Wang H, Xu J, Song D, Sun G *et al.* *Sphingobium hydrophobicum* sp. nov., a hydrophobic bacterium isolated from electronic-waste-contaminated sediment. *Int J Syst Evol Microbiol* 2016;66:3912–3916.
21. Komagata K, Suzuki K. Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* 1987;19:161–208.
22. Busse H-J, Bunka S, Hensel A, Lubitz W. Discrimination of members of the family *Pasteurellaceae* based on polyamine patterns. *Int J Syst Bacteriol* 1997;47:698–708.
23. Sasser M. *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.; 1990.
24. Gonzalez JM, Saiz-Jimenez C. A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ Microbiol* 2002;4:770–773.
25. Minnikin DE, Patel PV, Alshamaony L, Goodfellow M. Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* 1977;27:104–117.
26. Chen WM, Li YS, Sheu SY. *Sphingomonas piscinae* sp. nov., isolated from a fish pond. *Int J Syst Evol Microbiol* 2016;66:5301–5308.
27. Kim JH, Kim SH, Kim KH, Lee PC. *Sphingomonas lacus* sp. nov., an astaxanthin-dideoxyglycoside-producing species isolated from soil near a pond. *Int J Syst Evol Microbiol* 2015;65:2824–2830.