Sphingomonas pseudosanguinis sp. nov., isolated from the water reservoir of an air humidifier

Peter Kämpfer,¹ Uwe Meurer,² Michael Esser,³ Thomas Hirsch³ and Hans-Jürgen Busse⁴

Correspondence

Peter Kämpfer
peter.kaempfer@agrar.
uni-giessen.de

¹Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität, D-35390 Giessen, Germany

²Technical Department, MEDISANA AG, D-40724 Hilden, Germany

³Stadtwerke Solingen GmbH, T 3.2 Labor, D-42699 Solingen, Germany

⁴Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität Wien, A-1210 Wien, Austria

A yellow-pigmented bacterial isolate, strain G1-2^T, obtained from the surface of an air humidifier, was characterized taxonomically. 16S rRNA gene sequence analysis, physiological characterization and estimation of the ubiquinone and polar lipid patterns and fatty acid composition revealed that strain G1-2^T was similar to *Sphingomonas yabuuchiae* and *Sphingomonas sanguinis*, but also showed pronounced differences. On the basis of these results, a novel species of the genus *Sphingomonas* is described, for which the name *Sphingomonas pseudosanguinis* sp. nov. is proposed. The type strain is G1-2^T (= CCUG 54232^T = CIP 109431^T).

The genus *Sphingomonas*, proposed by Yabuuchi *et al.* (1990), contains Gram-negative, non-fermentative rods that are characterized chemotaxonomically by the presence of ubiquinone Q-10, *sym*-homospermidine as the key polyamine, a lipid pattern consisting of phosphatidylethanolamine (with the exception of *Sphingomonas echinoides* DSM 1805^T; Denner *et al.*, 1999), phosphatidylglycerol, diphosphatidylglycerol, sphingoglycolipid and phosphatidylcholine as the major lipids and by the presence of 2-hydroxymyristic acid (2-OH 14:0) and the absence of 3-hydroxy fatty acids in their fatty acid profiles (Busse *et al.*, 1999; Takeuchi *et al.*, 2001; Zhang *et al.*, 2005; Yoon *et al.*, 2006). Members of the genus *Sphingomonas* have been isolated from clinical samples, for example *Sphingomonas sanguinis* (Yabuuchi *et al.*, 1990; Takeuchi *et al.*, 1993), and also from some very unusual environments such as the Russian space laboratory Mir, from where *Sphingomonas yabuuchiae* was isolated (Li *et al.*, 2004). In this study, we describe a novel species of the genus *Sphingomonas* that shows an intermediate position between *S. sanguinis* and *S. yabuuchiae* on the basis of a polyphasic characterization.

Strain G1-2^T was isolated from the water reservoir of an air humidifier (Medibreeze 60002; Medisana) on DEV nutrient agar at 36 °C. The novel strain was isolated after several unsuccessful attempts to disinfect the air humidifier.

The novel strain was maintained on nutrient agar (Oxoid) at 30 °C and showed a yellow pigmentation when grown on this medium. Cells of strain G1-2^T stained Gram-negative with the modified Hucker method (Gerhardt *et al.*, 1994). Cell morphology was observed under a Zeiss light microscope at ×1000 using cells grown for 24 h at 30 °C on Medium 65 (DSMZ). The 16S rRNA gene sequence was analysed as described by Kämpfer *et al.* (2003). Phylogenetic analysis was performed using the MEGA version 3.0 software package (Kumar *et al.*, 2004) after multiple alignment of the data by CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura-2 model) and clustering with the neighbour-joining and maximum-parsimony methods were performed by using bootstrap values based on 1000 replications (Fig. 1). The 16S rRNA gene sequence of strain G1-2^T was a continuous stretch of 1371 bp. Sequence similarity calculations performed after a neighbour-joining analysis indicated that the closest relatives of strain G1-2^T were *S. sanguinis* IFO 13937^T (GenBank accession no. D13726) with 99.2 % sequence similarity, *S. yabuuchiae* A1-18^T (AB071955), 99.2 % similarity, and *Sphingomonas parapaucimobilis* JCM 7510^T (D84525), 99.1 % similarity. Lower sequence similarities (<98 %) were found with all other recognized species of the genus *Sphingomonas*.

Chemotaxonomic analyses were performed as follows. Respiratory quinones and polar lipids were determined according to Tindall (1990a, b) and Altenburger *et al.* (1996), respectively. Polyamines were analysed as described by Busse & Auling (1988) and Stolz *et al.* (2007).

Abbreviations: pNA, para-nitroanilide; pNP, para-nitrophenol.

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain G1-2^T is AM412238.

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession nos are given in parentheses) constructed after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). Distance (distance options according to the Kimura-2 model) and clustering with the neighbour-joining method were performed by using the MEGA version 3.0 software package (Kumar *et al.*, 2004). Bootstrap values based on 1000 replications are given as percentages at the branching points. Bar, 0.02 nucleotide substitutions per nucleotide position.

The detection of a quinone system consisting of ubiquinone Q-10 (96%) and Q-9 (4%) for strain G1-2^T was in accordance with the characteristics of species of the genus *Sphingomonas sensu stricto* and of the family *Sphingomonadaceae* (Busse *et al.*, 1999; Kosako *et al.*, 2000). The polyamine pattern showed a predominance of the sym-homospermidine [48.3 µmol (g dry weight)⁻¹], the key characteristic of *Sphingomonas sensu stricto* (Busse *et al.*, 1999; Takeuchi *et al.*, 2001), and minor amounts of spermidine [1.0 µmol (g dry weight)⁻¹] and putrescine [0.1 µmol (g dry weight)⁻¹]. The polar lipid profiles of strain G1-2^T contained the major compounds phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, sphingoglycolipid, phosphatidylcholine, a highly hydrophilic unknown glycolipid GL1 and an unknown phospholipid. Another highly hydrophobic unknown glycolipid GL2, an unknown aminophosphoglycolipid and an unknown polar lipid were also detected in moderate amounts. Minor to trace amounts of phosphatidyl-dimethylethanolamine and three unknown polar lipids were also detected (Fig. 2). This profile is in excellent agreement with the characteristics found for species of the genus *Sphingomonas sensu stricto* (Busse *et al.*, 1999). The absence of phosphatidylmonomethylethanolamine, as well as presence of only minor amounts of phosphatidyl-dimethylethanolamine, are in agreement with the characteristics of the close phylogenetic neighbour *S. sanguinis* and are useful characteristics for distinguishing the novel strain G1-2^T from the majority of other species of the genus *Sphingomonas* (Busse *et al.*, 1999, 2005). However, the presence of an unknown aminophosphoglycolipid and the presence of only a single sphingoglycolipid in strain G1-2^T enables it to be differentiated unambiguously from *S. sanguinis* which shows a profile containing an unknown glycolipid (GL1) assumed to represent another sphingoglycolipid (Busse *et al.*, 1999).

GC was used to analyse the fatty acid profiles of the strain, as described previously (Kämpfer & Kroppenstedt, 1996; Kämpfer *et al.*, 1997). Strain G1-2^T showed a fatty acid profile typical for members of this genus with 2-OH C_{14:0} as the key hydroxylated fatty acid (Table 1).

Physiological and biochemical characterizations were performed according to previously described procedures (Kämpfer *et al.*, 1991, 1997).

Strain G1-2^T is capable of assimilating several carbon sources and the utilization profile is similar to that of *S. sanguinis* and *S. yabuuchiae*. However, strain G1-2^T could be differentiated from *S. sanguinis* and *S. yabuuchiae* on the basis of several carbon substrate utilization tests (Table 2). Due to the high similarity of strain G1-2^T to *S. sanguinis* and *S. yabuuchiae*, DNA–DNA hybridization experiments were

Fig. 2. Polar lipid profile of strain G1-2^T after two-dimensional TLC and detection with molybdatophosphoric acid. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PDE, phosphatidyl-dimethylethanolamine; PC, phosphatidylcholine; SGL, sphingoglycolipid; PL, unknown phospholipid; GL1–2, unknown glycolipids; L1–4, unknown polar lipids; Pigm, yellow pigment.

Table 1. Fatty acid content of selected species of the genus *Sphingomonas*

Taxa: 1, strain G1-2^T; 2, *S. sanguinis* IFO 13937^T; 3, *S. yabuuchiae* A1-18^T; 4, *S. paucimobilis* ATCC 29837^T; 5, *S. parapaucimobilis* JCM 7510^T; 6, *S. trueperi* ATCC 12417^T. Values are percentages of total fatty acids. tr, Trace (<1.0%); –, not detected. Data are taken from the present study and Denner *et al.* (2001). Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system (Microbial ID). Summed feature 4 contains one or more of the following fatty acids: 16:1 ω 7*t*, 15:0 iso 2-OH and 16:1 ω 7*c*. Summed feature 7 contains one or more of the following fatty acids: 18:1 ω 7*c*, 18:1 ω 9*t* and/or 18:1 ω 12*t*.

Fatty acid	1	2	3	4	5	6
14:0	1.0	1.0	1.3	1.4	1.0	–
14:0 2-OH	5.4	6.7	8.7	6.4	5.0	6.7
15:0	–	–	–	–	1.1	–
16:0	12.9	9.6	9.9	8.7	13.6	9.8
16:1 ω 5 <i>c</i>	–	1.9	tr	–	1.0	–
Summed feature 4	4.8	8.6	5.9	2.7	6.7	–
17:0	–	–	1.2	–	0.5	1.6
17:1 ω 6 <i>c</i>	0.8	3.8	5.1	3.0	2.4	13.6
18:0	1.6	–	tr	–	0.6	–
Summed feature 7	72.0	65	62.5	74.6	64.6	64.2
18:1 ω 5 <i>c</i>	1.4	3.5	2.3	3.2	3.5	4.0

performed with the type strains of these two species using the method described by Ziemke *et al.* (1998), except that for nick translation 2 μ g of DNA was labelled during a 3 h incubation at 15 °C. Strain G1-2^T showed relatively low DNA–DNA relatedness values to *S. sanguinis* IFO 13937^T (46%; reciprocal 39.6%) and *S. yabuuchiae* DSM 14562^T (51.9%; reciprocal 43.2%).

On the basis of the 16S rRNA gene sequence analysis, DNA–DNA hybridization values and phenotypic data, we propose that strain G1-2^T represents a novel species, *Sphingomonas pseudosanguinis* sp. nov.

Description of *Sphingomonas pseudosanguinis* sp. nov.

Sphingomonas pseudosanguinis (pseu.do.san'gui.nis. Gr. adj. *pseudos* false; L. gen. n. *sanguinis* of the blood; N.L. gen. n. *pseudosanguinis* indicating that the strains are similar to *Sphingomonas sanguinis*).

Cells are rod-shaped, 0.8–1.5 μ m in length and 0.4–0.6 μ m in diameter. Colonies are yellow-coloured. The following are used as substrates for growth: L-arabinose, p-arbutin, D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, D-maltose, sucrose, D-trehalose, D-xylose, citrate, fumarate, DL-3-hydroxybutyrate, L-malate, pyruvate, L-alanine, L-leucine and L-proline. *N*-acetyl-D-glucosamine, gluconate, α -D-melibiose, L-rhamnose, ribose, salicin, D-adonitol, maltitol, D-mannitol, D-sorbitol, acetate, propionate,

Table 2. Differentiating characteristics of strain G1-2^T and related *sym*-homospermidine-containing *Sphingomonas* species

Taxa: 1, strain G1-2^T; 2, *S. yabuuchiae* DSM 24562^T; 3, *S. sanguinis* IFO 13937^T; 4, *Sphingomonas pituitosa* EDIV^T; 5, *Sphingomonas trueperi* ATCC 12417^T; 6, *S. paucimobilis* ATCC 29837^T; 7, *S. parapaucimobilis* JCM 7510^T; 8, *Sphingomonas roseiflava* IAM 14832^T. All data were obtained using the same method (Kämpfer *et al.*, 1991). +, Positive; (+), weakly positive; –, negative; pNA, para-nitroanilide; pNP, para-nitrophenol. Data for strain G1-2^T, *S. yabuuchiae* and *S. roseiflava* are from this study. All other data are from Kämpfer *et al.* (1997) and Denner *et al.* (2001). All taxa were negative in tests for acid production from D-mannitol and trehalose.

Characteristic	1	2	3	4	5	6	7	8
Acid produced from:								
Cellobiose	–	–	–	(+)	–	(+)	(+)	–
Glucose	–	(+)	–	(+)	–	–	–	–
Maltose	–	–	–	(+)	–	(+)	(+)	–
D-Mannose	–	–	–	–	–	(+)	(+)	–
Melibiose	–	–	–	–	–	(+)	(+)	–
Methyl D-glucoside	–	–	–	–	–	+	–	–
Rhamnose	–	–	–	–	–	–	(+)	–
Salicin	–	–	–	–	–	+	–	–
Sorbitol	–	–	–	–	+	–	–	–
D-Xylose	–	–	–	(+)	–	+	(+)	–
Hydrolysis of:								
Aesculin	+	+	–	+	–	(+)	(+)	+
L-Alanine pNA	+	+	+	+	–	+	+	+
pNP β -D-glucuronide	–	(+)	+	+	+	–	+	–
pNP phosphorylcholine	+	–	+	+	+	–	+	–
L-Proline pNA	+	+	–	–	–	–	+	+
Assimilation of:								
Acetate	–	+	+	+	+	–	+	–
<i>cis</i> -Aconitate	–	+	–	–	–	+	+	+
<i>trans</i> -Aconitate	–	+	–	–	–	+	–	+
L-Alanine	+	+	+	+	+	–	+	(+)
L-Aspartate	–	–	+	–	+	–	–	–
Citrate	+	+	–	–	–	–	+	–
D-Fructose	+	+	+	–	+	–	–	+
Fumarate	+	+	+	+	+	+	–	+
Gluconate	–	+	–	–	–	–	+	+
Glutarate	+	+	–	–	+	–	+	–
4-Hydroxybenzoate	–	–	–	–	+	–	–	–
DL-3-Hydroxybutyrate	+	+	+	+	+	–	+	+
DL-Lactate	–	+	+	–	+	–	+	–
L-Leucine	+	–	+	–	+	–	+	(+)
Maltitol	–	+	+	–	+	–	–	–
α -D-Melibiose	–	+	+	+	+	–	+	–
L-Ornithine	–	–	+	–	–	–	–	–
Oxoglutarate	+	+	+	–	–	+	+	+
L-Proline	+	+	+	+	–	–	–	+
Propionate	–	–	+	–	+	–	+	–
L-Rhamnose	–	–	–	–	–	–	(+)	–
Salicin	–	+	+	+	+	–	+	–
L-Serine	–	–	–	–	+	–	–	–

azelate, *cis*-aconitate, adipate, 4-aminobutyrate, DL-lactate, L-aspartate, L-histidine, L-ornithine, L-phenylalanine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate are not used as substrates. Positive in tests for the hydrolysis of aesculin, pNP α -D-glucopyranoside, pNP β -D-glucopyranoside, pNP β -D-galactopyranoside, bis-pNP phosphate, pNP phenyl phosphonate, 2-deoxythymidine-5'-pNP phosphate, pNP phosphorylcholine, L-alanine pNA, L-glutamate- γ -3-carboxy-pNA and L-proline pNA. pNP β -D-glucuronide is not hydrolysed. The main polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, sphingoglycolipid, phosphatidylcholine, a highly hydrophilic unknown glycolipid GL1 and an unknown phospholipid. The species is characterized by the major fatty acids 18:1 (Summed feature 7) and 16:0. Minor fatty acids are 14:0, 18:0, 17:1 ω 6c and 18:1 ω 5c. The major hydroxy-fatty acid is 2-hydroxy-14:0. The major polyamine is *sym*-homospermidine with the following minor polyamines: diaminopropane, putrescine, cadaverine, spermidine and spermine. Produces predominantly ubiquinone Q-10.

The type strain, G1-2^T (=CCUG 54232^T=CIP 109431^T), was isolated from the surface of an air humidifier.

References

- Altenburger, P., Kämpfer, P., Makristathis, A., Lubitz, W. & Busse, H.-J. (1996). Classification of bacteria isolated from a medieval wall painting. *J Biotechnol* **47**, 39–52.
- Busse, H. J. & Auling, G. (1988). Polyamine pattern as a chemotaxonomic marker within the *Proteobacteria*. *Syst Appl Microbiol* **11**, 1–8.
- Busse, H.-J., Kämpfer, P. & Denner, E. B. M. (1999). Chemotaxonomic characterisation of *Sphingomonas*. *J Ind Microbiol Biotechnol* **23**, 242–251.
- Busse, H.-J., Hauser, E. & Kämpfer, P. (2005). Description of two novel species, *Sphingomonas abaci* sp. nov. and *Sphingomonas panni* sp. nov. *Int J Syst Evol Microbiol* **55**, 2565–2569.
- Denner, E. B. M., Kämpfer, P., Busse, H.-J. & Moore, E. R. B. (1999). Reclassification of *Pseudomonas echinoides* Heumann 1962, 343^{AL}, in the genus *Sphingomonas* as *Sphingomonas echinoides* comb. nov. *Int J Syst Bacteriol* **49**, 1103–1109.
- Denner, E. B. M., Paukner, S., Kämpfer, P., Moore, E. R. B., Abraham, W. R., Busse, H.-J., Wanner, G. & Lubitz, W. (2001). *Sphingomonas pituitosa* sp. nov., an exopolysaccharide-producing bacterium that secretes an unusual type of sphingane. *Int J Syst Evol Microbiol* **51**, 827–841.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kämpfer, P., Steiof, M. & Dott, W. (1991). Microbiological characterisation of a fuel-oil contaminated site including numerical identification of heterotrophic water and soil bacteria. *Microb Ecol* **21**, 227–251.
- Kämpfer, P., Denner, E. B. M., Meyer, S., Moore, E. R. B. & Busse, H.-J. (1997). Classification of “*Pseudomonas azotocolligans*” Anderson 1955, 132, in the genus *Sphingomonas* as *Sphingomonas trueperi* sp. nov. *Int J Syst Bacteriol* **47**, 577–583.
- Kämpfer, P., Dreyer, U., Neef, A., Dott, W. & Busse, H.-J. (2003). *Chryseobacterium defluvii* sp. nov., isolated from wastewater. *Int J Syst Evol Microbiol* **53**, 93–97.
- Kosako, Y., Yabuuchi, E., Naka, T., Fujiwara, N. & Kobayashi, K. (2000). Proposal of *Sphingomonadaceae* fam. nov., consisting of *Sphingomonas* Yabuuchi *et al.* 1990, *Erythrobacter* Shiba and Shimidu 1982, *Erythromicrobium* Yurkov *et al.* 1994, *Porphyrobacter* Fuerst *et al.* 1993, *Zymomonas* Kluyver and van Niel 1936, and *Sandara-cinobacter* Yurkov *et al.* 1997, with the type genus *Sphingomonas* Yabuuchi *et al.* 1990. *Microbiol Immunol* **44**, 563–575.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- Li, Y., Kawamura, Y., Fujiwara, N., Naka, T., Liu, H., Huang, X., Kobayashi, K. & Ezaki, T. (2004). *Sphingomonas yabuuchiae* sp. nov. and *Brevundimonas nasdae* sp. nov., isolated from the Russian space laboratory Mir. *Int J Syst Evol Microbiol* **54**, 819–825.
- 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: amended description of the genus *Sphingomonas* and new description of *Sphingomonas macroglutabidus* 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.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Tindall, B. J. (1990a). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Tindall, B. J. (1990b). A comparative study of the lipid composition of *Halobacterium saccharovororum* from various sources. *Syst Appl Microbiol* **13**, 128–130.
- 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.
- Yoon, J.-H., Lee, M.-H., Kang, S.-J., Lee, S.-Y. & Oh, T. K. (2006). *Sphingomonas dokdonensis* sp. nov., isolated from soil. *Int J Syst Evol Microbiol* **56**, 2165–2169.
- Zhang, Y.-Q., Chen, Y.-G., Li, W.-J., Tian, X.-P., Xu, L.-H. & Jiang, C.-L. (2005). *Sphingomonas yunnanensis* sp. nov., a novel Gram-negative bacterium from a contaminated plate. *Int J Syst Evol Microbiol* **55**, 2361–2364.
- Ziemke, F., Höfle, M. G., Lalucat, J. & Rosselló-Mora, R. (1998). Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *Shewanella baltica* sp. nov. *Int J Syst Bacteriol* **48**, 179–186.