Classification of “*Pseudomonas azotocolligans*” Anderson 1955, 132, in the Genus *Sphingomonas* as *Sphingomonas trueperi* sp. nov.

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“*Pseudomonas azotocolligans*” ATCC 12417^T (T = type strain), which was described as a diazotrophic bacterium, was reinvestigated to clarify its taxonomic position. 16S ribosomal DNA sequence comparisons demonstrated that this strain clusters phylogenetically with species of the genus *Sphingomonas* and represents a new species. The results of investigations of the fatty acid patterns, polar lipid profiles, and quinone system supported this placement. The substrate utilization profile and biochemical characteristics displayed no obvious similarity to the characteristics of any previously described species of the genus *Sphingomonas*. The new name *Sphingomonas trueperi* is proposed on the basis of these results and previously published data for the G+C content of the genomic DNA and the polyamine pattern.

In a study of nitrogen-fixing soil bacteria, Anderson (1a) described a diazotrophic, polarly flagellated, rod-shaped organism and proposed the name “*Pseudomonas azotocolligans*” for it. Only one strain was placed in “*P. azotocolligans*,” and this strain was deposited in the National Collection of Industrial Bacteria as strain NCIB 9391^T (T = type strain). However, the name “*P. azotocolligans*” has not been validated. The G+C content of the DNA of this organism was determined to be 65.6 mol% (5), and, based on the detection of peritrichous flagella, De Ley (5) proposed that “*P. azotocolligans*” should be removed from the genus *Pseudomonas*. Subsequently, it was shown that this organism could not fix nitrogen (8). In the American Type Culture Collection catalog (1), strain NCIB 9391^T is listed as a patent strain for the production of coenzyme Q-10. The major component in the polyamine pattern has been demonstrated to be sym-homospermidine (4). However, neither ubiquinone 10 nor sym-homospermidine is found in the species belonging to the genus *Pseudomonas* sensu stricto (2, 4, 17), as defined phylogenetically (6, 24). The results of DNA-rRNA hybridization studies distinguished “*P. azotocolligans*” from the authentic *Pseudomonas* species and placed it close to the misnamed organisms *Pseudomonas paucimobilis* and *Flavobacterium capsulatum* phylogenetically (3, 7). Recently, the latter two species have been reclassified as species of the genus *Sphingomonas* (25), which now includes the following validly described species: *Sphingomonas paucimobilis* (type species of the genus), *Sphingomonas adhaesiva*, *Sphingomonas asaccharolytica*, *Sphingomonas capsulata*, *Sphingomonas chlorophenolica*, *Sphingomonas macrogoltabidus*, *Sphingomonas mali*, *Sphingomonas parapaucimobilis*, *Sphingomonas pruni*, *Sphingomonas rosa*, *Sphingomonas sanguis*, *Sphingomonas terrae*, and *Sphingomonas yanoikuyae* (13, 15, 16, 21, 22, 25). Phylogenetically, the species of the genus *Sphingomonas* form a rather homogeneous cluster that is also supported by several common chemotaxonomic features. Sphingomonads are characterized by the presence of ubiquinone 10, the presence of a unique sphingoglycolipid, the presence of 2-hydroxy fatty acids, the

absence of 3-hydroxy fatty acids, and the presence of spermidine or sym-homospermidine as the major polyamine (4, 16, 17, 22, 25).

Only one strain of “*P. azotocolligans*” is available in culture collections. We reinvestigated the taxonomic position of “*P. azotocolligans*” ATCC 12417^T (= NCIB 9391^T = LMG 2142^T = DSM 7225^T), which was obtained from G. Auling (Institute of Microbiology, University of Hannover, Hannover, Germany). “*P. azotocolligans*” was characterized by analyzing the quinone system and the polar lipids by thin-layer chromatography (14, 23), by performing a gas-liquid chromatographic analysis of the fatty acids (10), by determining physiological and biochemical characteristics (9, 18), and by determining the 16S rRNA gene sequence (15) and was compared to the previously established species of the genus *Sphingomonas*.

“*P. azotocolligans*” contained a ubiquinone with 10 isoprenoid units in the side chain (ubiquinone Q-10) and a sphingoglycolipid. The overall polar lipid pattern of this strain was similar to the patterns determined for the other *Sphingomonas* species in that it includes phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol as the major compounds (Fig. 1). Other lipids detected in “*P. azotocolligans*” were high levels of phosphatidylmethylethanolamine and an unidentified lipid and minor levels of phosphatidylcholine, phosphatidylmonomethylamine, an unidentified glycolipid (GL2), an unidentified lipid, and two unidentified phospholipids (PL1 and PL3). This polar lipid profile clearly distinguished “*P. azotocolligans*” from the other *Sphingomonas* species (Table 1).

Fatty acid methyl esters were extracted and prepared by the standard protocol of the Microbial Identification System (MIDI; Microbial ID, Inc., Newark, Del.). Extracts were analyzed by using a Hewlett-Packard model HP6890A gas chromatograph equipped with a flame ionization detector, an automatic sampler, an integrator, and a computer, as described previously (11). All *Sphingomonas* strains contained the fatty acids 16:0, summed feature 7 (18:1 ω 7c, 18:1 ω 9t, and/or 18:1 ω 12t), and 14:0 2OH, and all but four strains contained summed feature 4 (16:1 ω 7c and/or 15:0 iso 2OH) (Table 2). These fatty acids have also been detected in previous studies (21, 22, 25) and are characteristic of all validly described species of the genus *Sphingomonas*. A more precise comparison of the results of this study with the results described by Yabuuchi et al. (25) and Takeuchi et al. (21, 22) was not possible due to the different

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FIG. 1. Polar lipids of *S. trueperi* DSM 7225^T after separation by two-dimensional thin-layer chromatography. Abbreviations: PE, phosphatidylethanolamine; PME, phosphatidylmonomethylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PDE, phosphatidylmethylethanolamine; PC, phosphatidylcholine; SGL, sphingoglycolipid; PL1 and PL3, unidentified phospholipids; GL2, unidentified glycolipid; L, unidentified polar lipids; P, pigment.

methods of fatty acid extraction and analysis used. Table 2 shows that the type strains of *Sphingomonas* species exhibit considerable heterogeneity in their fatty acid profiles, which correlates with the heterogeneity found in the polar lipid profiles. The presence of 2-hydroxymyristic acid (14:0 2OH) as the dominant hydroxylated fatty acid (0.8 to 18.8%) and the absence of 3-hydroxylated fatty acids are consistent with and confirm the results of other studies. However, the relatively high percentage of 2-hydroxymyristic acid described in previous reports (21, 22) could not be confirmed. *S. terrae* IFO 15098^T was observed to contain 15:0 2OH as a second hydroxylated fatty acid, which could not be detected in the type strain

of any other species. This finding has been described previously by Takeuchi et al. (21). In contrast to the results of Takeuchi et al. (22), no 15:0 2OH was detected in the type strains of *S. pruni*, *S. asaccharolytica*, and *S. mali*, whereas 16:0 2OH was detected in *S. macrogoltabidus* (in agreement with the results of Takeuchi et al. [21]) and *S. yanoikuyae* (in agreement with the results of Takeuchi et al. [22]) but not in *S. mali* and *S. pruni* (in contrast to the findings of Takeuchi et al. [22]).

Furthermore, all strains were characterized on the basis of 87 biochemical and physiological characteristics as described previously (12), and the results are shown in Table 3. Where possible, a detailed comparison was made with all previously published data (21, 22, 25). Table 3 shows that nearly all test results which could be compared directly with data from previously published papers were confirmed. However, it should be noted that acid formation from various sugars often resulted in very weak positive results; these test results should be interpreted with care. Table 3 shows that the species described in this paper, *Sphingomonas trueperi*, can be differentiated from all other *Sphingomonas* species on the basis of the results of several tests. Additional biochemical characteristics of *S. trueperi* are given below. A numerical analysis of the data was performed by using the simple matching coefficient and the Jaccard coefficient (S_j) (20), and phenograms were generated by using the unweighted pair group with mathematical average (UPGMA) algorithm. Similar phenograms were generated with the two coefficients. The results of the UPGMA- S_j analysis are shown in Fig. 2.

A nearly complete 16S rRNA gene sequence (nucleotide positions 28 to 1524; *Escherichia coli* numbering) of "*P. azotocolligans*," comprising 1,442 nucleotide positions, was determined by PCR amplification and direct sequencing of the PCR-amplified 16S ribosomal DNA (rDNA). A comparative analysis and estimation of phylogenetic relationships demonstrated that "*P. azotocolligans*" clusters within the genus *Sphingomonas* and is most closely related to *S. paucimobilis*, *S. parapaucimobilis*, *S. sanguis*, and *S. adhaesiva* (Fig. 3). However, a comparison of levels of 16S rDNA sequence similarity (Table 4) revealed that "*P. azotocolligans*" represents a distinct species of the genus *Sphingomonas*. The levels of sequence similarity between the 16S rRNA genes of "*P. azotocolligans*" and other *Sphingomonas* species ranged from 95.8% (with the sequence of *S. paucimobilis* and with the nearly complete sequences of *S.*

TABLE 1. Polar lipid distribution in *Sphingomonas* species

Organism	Distribution of the following polar lipid ^a															
	PME	PE	PG	DPG	PDE	PC	SGL	APL1	PL1	PL2	PL3	GL1	GL2	GL3	GL4	L1
<i>S. trueperi</i> DSM 7225 ^T	□	■	■	■	■	□	■	—	□	—	□	—	□	—	—	■
<i>S. mali</i> IFO 15500 ^T	□	□	■	■	■	□	■	—	□	—	□	—	□	□	—	■
<i>S. pruni</i> IFO 15498 ^T	□	□	■	■	■	□	■	—	□	—	□	—	□	□	—	■
<i>S. asaccharolytica</i> IFO 15499 ^T	□	□	■	■	■	□	■	—	□	—	□	—	□	□	—	■
<i>S. paucimobilis</i> DSM 1098 ^T	—	■	■	□	—	■	■	—	—	—	□	—	□	—	—	—
<i>S. parapaucimobilis</i> IFO 15100 ^T	□	■	■	■	□	■	□	—	—	□	□	■	□	—	—	—
<i>S. adhaesiva</i> IFO 15099 ^T	□	■	■	■	□	■	■	□	—	■	□	—	□	—	—	—
<i>S. sanguis</i> IFO 13937 ^T	—	■	■	■	□	■	□	—	—	—	□	□	□	—	—	—
<i>S. yanoikuyae</i> IFO 15102 ^T	□	■	■	■	■	□	■	□	□	—	—	■	—	—	—	—
<i>S. chlorophenolica</i> ATCC 33790 ^T	■	■	■	■	■	□	■	—	—	□	□	—	—	—	—	—
<i>S. macrogoltabidus</i> IFO 15033 ^T	□	■	■	■	—	□	□	—	—	—	□	□	□	—	□	—
<i>S. terrae</i> IFO 15090 ^T	□	■	■	■	□	■	■	—	—	—	□	□	—	—	—	—
<i>S. rosa</i> IFO 15208 ^T	□	■	■	■	■	□	■	□	—	□	□	—	□	—	—	—
<i>S. capsulata</i> DSM 30196 ^T	□	■	■	■	■	□	■	□	—	□	□	—	□	—	—	—

^a PME, phosphatidylmonomethylethanolamine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PDE, phosphatidylmethylethanolamine; PC, phosphatidylcholine; SGL, sphingoglycolipid; APL1, unidentified aminophospholipid; PL1, PL2, and PL3, unidentified phospholipids; GL1, GL2, GL3, and GL4, unidentified glycolipids; L1 and L2, unidentified lipids. ■, present in major amounts; □, present in minor amounts; —, not detected.

TABLE 2. Major fatty acid compositions of the type strains of 14 *Sphingomonas* species^a

Compound ^b	% in:													
	<i>S. macroglabridus</i>	<i>S. terrae</i>	<i>S. chlorophenolica</i>	<i>S. yanoikuyae</i>	<i>S. asaccharolytica</i>	<i>S. adhaesiva</i>	<i>S. paucimobilis</i>	<i>S. parapaucimobilis</i>	<i>S. trueperi</i>	<i>S. sanguis</i>	<i>S. rosa</i>	<i>S. capsulata</i>	<i>S. mali</i>	<i>S. pruni</i>
Saturated fatty acids														
14:0				0.5			1.4	1.0		1.0	1.5			
15:0		5.9								1.1				
16:0	12.8	4.5	9.5	11.2	13.3	6.5	8.7	9.6	9.8	13.6	10.9	8.1	11.8	16.0
17:0		3.8							1.6	0.5				
18:0										0.6				
Unsaturated fatty acids														
16:1 ω 5c	2.9	2.1	2.4	2.7				1.9		1.0		1.7		
17:1 ω 6c	4.5	48.1	6.4	3.2		21.2	3.0	3.8	13.6	2.4		4.0	9.3	
17:1 ω 8c		7.9												
18:1 ω 5c	0.9	0.6	2.5	2.1			3.2	3.5	4.0	3.5				
Hydroxy fatty acids														
14:0 2OH	3.0	0.8	9.4	7.1	18.8	13.8	6.4	6.7	6.7	5.0	12.8	14.7	8.4	10.9
15:0 2OH		4.9												
16:0 2OH	2.9			0.9										
Summed features														
Summed feature 4	31.8	8.0	9.6	16.3		6.2	2.7	8.6		6.7	22.8	6.7		
Summed feature 7	41.2	13.4	60.1	56.0	67.8	47.3	74.6	65.0	64.2	64.6	52.0	64.8	70.5	73.1

^a All strains except *S. pruni* IFO 15498^T and *S. mali* IFO 15500^T were grown on Trypticase soy broth agar at 28°C for 48 h prior to fatty acid analysis. As *S. pruni* IFO 15498^T and *S. mali* IFO 15500^T did not grow on Trypticase soy broth agar, they were cultured on Czapek Dox agar for 48 h at 28°C.

^b For unsaturated fatty acids the position of the double bond is located by counting from the methyl (ω) end of the carbon chain. *cis* is indicated by the suffix c. Summed features are groups of two or three fatty acids that cannot be separated by gas-liquid chromatography with the MIDI system. Summed feature 4 contained one or more of the following fatty acids: 16:1 ω 7c and 15:0 iso 2OH. Summed feature 7 contained one or more of the following isomers: 18:1 ω 7c, 18:1 ω 9t, and 18:1 ω 12t (*trans* isomers are indicated by the suffix t).

mali, *S. pruni*, and *S. asaccharolytica*, which were determined in this study) to 90.8% (with the sequence of *S. capsulata*). These data demonstrate that "*P. azotocolligans*," as well as *S. mali*, *S. pruni*, and *S. asaccharolytica*, are located on the *S. paucimobilis* phylogenetic branch (Fig. 3). "*P. azotocolligans*" and other species belonging to the *S. paucimobilis* branch (Fig. 3), including *S. pruni* and *S. mali*, have been shown to contain *sym*-homospermidine as their major polyamine (4, 18, 22). In contrast, *S. asaccharolytica* has been described as containing spermidine as the predominant compound (22). A reinvestigation of the polyamine pattern of *S. asaccharolytica*, performed under standardized conditions as described previously (4), revealed that this species also contained the major compound *sym*-homospermidine (56.2 μ mol/g [dry weight]), whereas spermidine (3.6 μ mol/g [dry weight]) and spermine (1.2 μ mol/g [dry weight]) were present only in minor amounts. As other branches in the genus, represented by *S. capsulata*, *S. terrae*, and *S. yanoikuyae* (Fig. 3), were characterized by having spermidine as the major polyamine in an amended description of the genus, the presence of *sym*-homospermidine may be a characteristic of the genus *Sphingomonas* sensu stricto (that is, the species that cluster with *S. paucimobilis*, the type species of the genus).

The substrate utilization profiles and the results of the biochemical investigation demonstrated the separate position of "*P. azotocolligans*." These results, combined with previously published data, justify a proposal that "*P. azotocolligans*" should be described as a new species of the genus *Sphingomonas*. However, the name "*P. azotocolligans*" did not appear on the Ap-

proved Lists of Bacterial Names (19), nor has it been validated. As it has been demonstrated that "*P. azotocolligans*" lacks the ability to fix molecular nitrogen (8), it would be misleading to retain the species epithet. Therefore, we propose the new name *Sphingomonas trueperi*. The new *Sphingomonas* species is described below.

Description of *Sphingomonas trueperi* sp. nov. *Sphingomonas trueperi* (true'per.i. N. L. gen. n. *trueperi*, of Trüper, in honor of Hans G. Trüper, a German microbiologist, in recognition of his numerous contributions to the taxonomy of the *Proteobacteria*) is a gram-negative, rod-shaped, non-spore-forming, motile, peritrichously flagellated organism (5). Colonies are yellow, circular, entire, low convex, and smooth. Growth occurs in the presence of 10% sodium chloride and 0.02% sodium azide. Oxidase positive. Catalase positive. Indole, urease, and arginine dihydrolase are not produced. β -Galactosidase positive. Esculin and gelatin are hydrolyzed. The following compounds are hydrolyzed: *p*-nitrophenyl-*N*-acetyl- β -D-glucosaminide, *p*-nitrophenyl- α -L-arabinopyranoside, *p*-nitrophenyl- β -D-galacturonide, *p*-nitrophenyl- α -D-glucopyranoside, *p*-nitrophenyl- β -D-glucopyranoside, *p*-nitrophenyl- β -D-glucuronide, *p*-nitrophenyl- β -D-melibiose, *p*-nitrophenyl- β -D-xyloside, bis-*p*-nitrophenyl-phosphate, *p*-nitrophenyl-phenyl-phosphonate, *p*-nitrophenyl-phosphoryl-choline, 2-deoxythymidine-5'-*p*-nitrophenyl-phosphate, D-alanine-*p*-nitroanilide, L-alanine-*p*-nitroanilide, L-arginine-*p*-nitroanilide, γ -L-glutamate-*p*-nitroanilide, glutamate-1-*p*-nitroanilide, glycine-*p*-nitroanilide, L-lysine-*p*-nitroanilide, L-phenylalanine-*p*-nitroanilide, and L-valine-*p*-nitroanilide.

p-Nitrophenyl-*N*-acetyl- α -D-glucosamine, *p*-nitrophenyl- α -

TABLE 3. Physiological characteristics of the type strains of 14 *Sphingomonas* species^d

Characteristic ^e	<i>S. macrogolabidus</i>	<i>S. terrae</i>	<i>S. chlorophenolica</i>	<i>S. yanokuyae</i>	<i>S. asaccharolytica</i>	<i>S. adhaesiva</i>	<i>S. paucimobilis</i>	<i>S. parupaucimobilis</i>	<i>S. trueperi</i>	<i>S. sanguis</i>	<i>S. rosa</i>	<i>S. capsulata</i>	<i>S. mali</i>	<i>S. pruni</i>
Acid produced from ^e :														
Glucose	— ^f	—	—	— ^a	(+) ^c	— ^a	— ^a	— ^a	—	—	(+) ^c	— ^a	(+) ^c	(+) ^c
Lactose	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sucrose	+	—	—	—	—	—	—	—	—	—	—	—	—	—
D-Mannitol	—	—	—	— ^a	+	— ^a	— ^a	— ^a	—	—	—	— ^a	—	—
Dulcitol	+	—	—	— ^a	—	— ^a	— ^a	— ^a	—	—	—	— ^a	—	—
Salicin	—	—	—	—	(+) ^c	+	+	—	—	—	(+) ^c	—	— ^c	— ^c
Adonitol	—	—	—	— ^a	—	— ^a	— ^a	— ^a	—	—	—	— ^a	—	—
Inositol	—	+	—	— ^a	+	— ^a	— ^a	— ^a	—	—	—	— ^a	—	—
Sorbitol	—	—	—	— ^a	—	— ^a	— ^a	— ^a	+	—	—	— ^a	—	—
L-Arabinose	+	—	—	—	—	—	—	—	—	—	—	—	—	—
Raffinose	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rhamnose	—	—	—	(+) ^a	(+) ^c	(+) ^a	— ^a	(+) ^a	—	—	(+) ^c	—	(+) ^a	— ^c
Maltose	—	—	—	(+) ^a	—	(+) ^a	(+) ^a	(+) ^a	—	—	—	(+) ^a	—	—
D-Xylose	—	—	+	(+) ^a	—	(+) ^a	+	(+) ^a	—	—	—	(+) ^a	—	—
Trehalose	—	—	—	+	+	—	—	—	—	—	—	—	—	—
Cellobiose	—	—	—	(+) ^a	—	(+) ^a	(+) ^a	(+) ^a	—	—	—	(+) ^a	—	—
Methyl-D-glucoside	—	+	+	—	—	+	+	—	+	—	—	—	—	—
Erythritol	—	—	—	—	—	+	—	—	—	—	—	—	—	—
Melibiose	—	—	—	(+) ^a	—	(+) ^a	(+) ^a	(+) ^a	—	—	—	(+) ^a	—	—
D-Arabitol	—	+	—	—	—	—	—	—	—	—	—	—	—	—
D-Mannose	—	—	—	(+) ^a	—	(+) ^a	(+) ^a	(+) ^a	—	—	—	(+) ^a	—	—
Hydrolysis of:														
Esculin	+ ^b	—	—	+ ^a	—	—	(+)	(+)	+	—	(+)	—	(+) ^c	(+) ^c
pNP-β-D-galactopyranoside	+	—	—	(+) ^a	(+) ^c	(+) ^a	+ ^a	+ ^a	+	+	+ ^c	+	+ ^c	(+) ^c
pNP-β-D-glucuronide	—	—	—	+	+	+	—	+	+	+	+	+	+	+
pNP-α-D-glucopyranoside	—	+	—	+ ^a	+	+ ^a	+ ^a	+ ^a	+	+	+	+ ^a	+	+
pNP-β-D-glucopyranoside	+	—	—	+ ^a	+	+ ^a	+ ^a	+ ^a	+	+	+	+ ^a	+	+
bis-pNP-phosphate	+	+	+	+	+	+	+	+	+	+	+	+	+	+
pNP-phenyl-phosphonate	+	+	+	+	+	+	+	+	+	+	+	+	+	+
pNP-phosphoryl-choline	+	+	—	+	+	+	—	+	+	+	+	+	+	+
2-Deoxythymidine-5'-pNP-phosphate	+	+	+	+	+	+	+	+	+	+	+	+	+	+
pNP-phosphate	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Alanine-pNA	+	+	+	+	+	+	+	+	+	+	+	+	+	+
L-Glutamate-γ-3-carboxy-pNA	+	+	+	+	+	+	+	+	+	+	—	+	+	+
L-Proline-pNA	+	+	+	—	+	—	—	+	—	—	—	—	+	+
Assimilation of:														
N-Acetyl-D-glucosamine	—	—	—	+ ^a	+ ^c	+ ^a	+ ^a	+ ^a	+	+	(+) ^c	+ ^a	+	+ ^c
L-Arabinose	—	—	—	+ ^a	+ ^c	— ^a	+ ^a	+ ^a	+	+ ^b	+ ^c	(+) ^a	+ ^c	+ ^c
p-Arbutin	—	—	—	+ ^c	—	+	+	+	+	+	+	+	+	+
D-Cellobiose	— ^b	— ^b	+	+ ^a	(+) ^c	+ ^a	+ ^a	+ ^a	+	+ ^b	+ ^c	+ ^a	+ ^c	+ ^c
D-Fructose	— ^b	— ^b	—	—	—	+	—	—	+	+ ^b	+ ^c	—	—	—
D-Galactose	— ^b	—	—	+ ^a	+	+	+	+	+	+ ^b	—	+	+ ^c	+ ^c
Gluconate	— ^b	— ^b	—	+ ^c	— ^c	— ^c	— ^c	+ ^c	—	— ^b	+ ^c	+	+ ^c	— ^c
D-Glucose	+	—	+	+ ^c	+ ^c	+	+	+	+	+	+ ^c	+	+ ^c	+ ^c
D-Mannose	— ^b	— ^b	—	— ^a	+ ^c	+ ^a	+ ^a	+ ^a	+	+ ^b	+ ^c	(+) ^a	+ ^c	+ ^c
D-Maltose	—	—	—	+ ^a	+ ^c	+ ^a	+ ^a	+ ^a	+	+ ^b	+ ^c	+ ^a	+ ^c	+ ^c
α-D-Melibiose	—	—	—	+ ^c	+ ^c	+	—	+	+	+	+ ^c	—	+ ^c	+ ^c
L-Rhamnose	—	—	—	+ ^c	+ ^c	—	— ^c	(+) ^c	—	—	+ ^c	+	+ ^c	+ ^c
D-Ribose	— ^b	— ^b	+	— ^a	—	— ^a	— ^a	— ^a	—	— ^b	—	— ^a	—	—
Sucrose	—	—	—	+ ^c	(+) ^c	+	+	+	+	+	+ ^c	+	+ ^c	+ ^c
Salicin	—	—	—	+ ^c	(+) ^c	+	—	+	+	+ ^b	+ ^c	—	+ ^c	(+) ^c
D-Trehalose	+ ^b	— ^b	—	+ ^c	(+) ^c	+	+	+	+	+ ^b	— ^c	+	+ ^c	+ ^c
D-Xylose	— ^b	— ^b	—	+ ^c	(+) ^c	— ^c	+ ^c	+ ^c	+	+	+ ^c	+	+ ^c	+ ^c
Adonitol	— ^b	— ^b	—	— ^a	— ^c	— ^a	— ^a	— ^a	—	— ^b	— ^c	— ^a	— ^c	— ^c
i-Inositol	—	—	—	— ^c	— ^c	—	—	—	—	—	— ^c	—	— ^c	— ^c
Maltitol	—	—	—	+	+	—	—	—	+	+	—	—	—	—
Putrescine	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Acetate	+	—	+	+ ^c	— ^c	—	—	+	+	+	—	—	+	—
Propionate	+	—	+	+	—	—	—	+	+	+	—	—	—	—
cis-Aconitate	—	—	—	—	—	—	+	+	—	—	—	—	—	—

Continued on following page

TABLE 3—Continued

Characteristic ^a	<i>S. macrogoltabidus</i>	<i>S. terrae</i>	<i>S. chlorophenolica</i>	<i>S. yanoikuyae</i>	<i>S. asaccharolytica</i>	<i>S. adhaesiva</i>	<i>S. paucimobilis</i>	<i>S. parapaucimobilis</i>	<i>S. trueperi</i>	<i>S. sanguis</i>	<i>S. rosa</i>	<i>S. capsulata</i>	<i>S. mali</i>	<i>S. pruni</i>
<i>trans</i> -Aconitate	—	—	—	—	—	—	+	—	—	—	—	—	—	—
Adipate	— ^c	(+) ^c	— ^c	— ^a	— ^c	— ^a	— ^a	— ^a	—	—	— ^c	— ^a	(+) ^c	— ^c
4-Aminobutyrate	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Azelate	+	—	—	—	—	—	—	—	—	—	—	—	—	—
Citrate	—	—	—	+ ^c	— ^c	— ^c	— ^c	+ ^c	— ^c	— ^c	— ^c	— ^c	— ^c	— ^c
Fumarate	+ ^b	+	+	+ ^c	(+) ^c	+	+	—	+	+	+	+	+ ^c	+ ^c
Glutarate	—	—	—	—	—	—	—	+	+	—	—	—	—	—
DL-3-Hydroxybutyrate	+	+	+	+	+	—	—	+	+	+	—	—	+	+
Itaconate	—	—	—	—	—	—	—	—	—	—	—	—	—	—
DL-Lactate	—	—	—	+ ^c	— ^c	—	—	+	+	+	— ^c	—	— ^c	— ^c
L-Malate	— ^b	+	+	+ ^c	— ^c	+	+	+	+	+	+	+	— ^c	— ^c
Mesaconate	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Oxoglutarate	—	—	—	—	—	+	+	+	—	+	—	—	—	—
Pyruvate	—	—	+	+	—	—	+	+	+	+	—	—	+	—
Suberate	+	—	+	—	—	+	—	—	—	—	+	—	+	—
L-Alanine	+	—	—	—	+	+	—	+	+	+	—	—	—	—
β-Alanine	—	—	—	—	—	—	—	—	—	—	—	—	—	—
L-Aspartate	+	—	—	+	+	+	—	—	+	+	—	+	+	+
L-Histidine	+	—	—	+	—	—	—	—	—	—	+	+	+	+
L-Leucine	+	—	+	+	—	+	—	+	+	+	—	+	—	—
L-Ornithine	—	—	—	—	—	—	—	—	—	+	—	—	—	—
L-Phenylalanine	+	+	—	+	—	—	—	—	—	—	—	—	—	—
L-Proline	+	—	—	+	+	+	—	—	—	+	—	—	—	—
L-Serine	—	—	—	—	—	—	—	—	+	—	—	—	—	—
L-Tryptophan	+	—	—	—	—	—	—	—	—	—	—	—	—	—
3-Hydroxybenzoate	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4-Hydroxybenzoate	—	—	—	+	—	—	—	—	+	—	—	—	—	—
Phenylacetate	— ^b	—	—	— ^a	—	— ^a	— ^a	— ^a	—	— ^b	—	— ^a	—	—

^a Data are in agreement with the data of Yabuuchi et al. (22).
^b Data are in agreement with the data of Takeuchi et al. (18).
^c Data are in agreement with the data of Takeuchi et al. (19).
^d Test results were determined after 72 h of incubation at 30°C.
^e Acid formation from carbohydrates in most cases was very weak (even after prolonged incubation); these tests cannot be recommended for differentiation.
^f —, negative; +, positive; (+), weakly positive.
^g pNP, *para*-nitrophenyl; pNA, *para*-nitroanilide.

L-fucopyranoside, *p*-nitrophenyl-β-D-galactosaminide, *p*-nitrophenyl-β-D-galactopyranoside, *p*-nitrophenyl-β-D-cellobioside, *p*-nitrophenyl-β-D-fucopyranoside, *p*-nitrophenyl-β-D-lactoside, *p*-nitrophenyl-α-D-maltoside, *p*-nitrophenyl-β-D-maltoside, *p*-nitrophenyl-α-D-mannopyranoside, *p*-nitrophenyl-α-L-rhamnopyranoside, *p*-nitrophenyl-sulfate, acetyl-L-alanine-*p*-nitro-anilide, L-glutamate-γ-3-carboxyl-*p*-nitroanilide, succinyl-L-phenylalanine-*p*-nitroanilide, acetyl-L-leucine-*p*-nitroanilide, benzoyl-DL-arginine-*p*-nitroanilide, and L-proline-*p*-nitroanilide are not hydrolyzed.

The following compounds are assimilated: D-glucose, L-arabinose, D-mannose, D-xylose, D-fructose, α-methyl-D-mannoside, α-methyl-D-glucoside, esculin, salicin, lactose, sucrose, trehalose, D-raffinose, amidon, β-gentiobiose, D-turanose, L-fucose, L-malate, DL-malate, succinate, fumarate, DL-lactate, DL-3-hydroxybutyrate, pyruvate, *N*-acetyl-D-glucosamine, *p*-arbutin, D-cellobiose, D-galactose, D-glucosamine, D-glucuronate, lactulose, D-maltose, D-mannoheptulose, α-D-melezitose, α-D-melibiose, α-methyl-D-mannoside, α-methyl-D-glucopyranoside, starch, D-sucrose, D-trehalose, acetamidocaprinate, acetyl-L-glutamine, acetyl-L-proline, L-alanine, DL-2-aminoadipate, L-asparagine, L-aspartate, DL-aspartate, D-glutamate, L-glutamate, L-glutamine, L-isoleucine, L-leucine, phenylglycine, L-proline,

FIG. 2. Phenogram of *Sphingomonas* species generated by using the UPGMA algorithm and *S_j* (20).

FIG. 3. Unrooted dendrogram showing the estimated phylogenetic relationships between *S. trueperi* and other species of the genus *Sphingomonas* as derived by evolutionary distance calculations from pairwise comparisons of nearly complete 16S rRNA gene sequences, including the Jukes-Cantor correction factor. Nucleotide insertions-deletions were weighted 0.5 times the value of nucleotide substitutions. The dendrogram was generated by using the least-squares distance algorithm of the FITCH program in PHYLIP (Phylogeny Inference Package, version 3.5c). The EMBL accession numbers for sequences obtained from the EMBL Nucleotide Sequence Database are as follows: *S. paucimobilis* DSM 1098^T, X72722; *S. adhaesiva* JCM 7370^T, X72720; *S. asaccharolytica* IFO 15499^T, Y09639; *S. capsulata* IFO 12533^T, D16147; *S. chlorophenolica* ATCC 33790^T, X87161; *S. macrogoltabidus* IFO 15033^T, D13723; *S. mali* IFO 15500^T, Y09638; *S. parapaucimobilis* JCM 7510^T, X72721; *S. pruni* IFO 15498^T, Y09637; *S. rosa* IFO 15208^T, D13945; *S. sanguis* IFO 13937^T, D13726; *S. terrae* IFO 15098^T, D13727; *S. yanoikuyae* JCM 7371^T, X72725; *Rhizomonas suberifaciens* IFO 15211^T, D13737; *Agrobacterium tumefaciens* DSM 30150, M11223; *Brevundimonas diminuta* ATCC 11568^T, M59064; *Erythrobacter longus* JCM 6170^T, D12699; and *Porphyrobacter neustonensis* ACM 2844^T, L01785. Bar = 5 substitutions per 100 nucleotide positions (estimated evolutionary distance from the point of divergence of the 16S rRNA gene sequences).

L-valine, casein, malitol, tributyrin, DL-2-hydroxyisobutyrate, 2-hydroxyvalerate, oxalacetate, 2-oxoglutarate, 2-oxoisocaproate, 1-butanol, L-leucineamide, *p*-hydroxybenzoate, and protocatechuate.

Acid is produced from *N*-acetyl-D-glucosamine, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucosamine, D-glucose, lactose, lactulose, D-mannose, salicin, D-trehalose, D-turanose, and D-xylose.

The following compounds are not assimilated: glycerol, erythritol, D-arabinose, ribose, L-xylose, adonitol, β-methyl-xyloside, L-sorbose, rhamnose, dulcitol, sorbitol, inulin, glycogen, L-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, 5-ketogluconate, *N*-acetylgalactosamine, amygdalin, α-D-galacturonate, D-glucarate, D-glucosaminic acid, glycogen, glycyrrhizinate, L-mannose, β-methyl-D-xyloside, mucate, 5-oxogluconate, L-rhamnose, D-ribose, *m*-erythritol, i-inositol, D-mannitol, D-xylitol, 1-hexanol, acetyl-L-glutamate, acetyl-glycine, acetyl-DL-methionine, D-alanine, 4-aminobutyrate, L-arginine, betaine, L-citrulline, dimethylglycine, L-histidine, L-hydroxyproline, DL-isoleucine, D-leucine, L-lysine, D-norvaline, L-norvaline, L-ornithine, L-phenylalanine, D-proline, sarcosine, L-serine, L-threonine, carnitine, creatine, putrescine, spermidine, tyramine, allantoin, cytosine, glycineamide, acetate, propionate, butyrate, isobutyrate, *n*-valerate, isovalerate, *N*-caproate, heptanoate, caprylate, pelargonate, caprate, oxalate, malonate, maleate, glutarate, adipate, pimelate, suberate, azelate, sebacate, *cis*-aconitate, *trans*-aconitate, glycerophosphate, glyoxylate, 4-hydroxybutyrate, DL-2-hydroxyisovalerate, D-lactate, L-lactate, mesoxalate, 2-butanol, ethanol, hippurate, thiamine, ureidosuccinate, L-valinamide, anthranilate, 3-aminobenzoate, 4-aminobenzoate, 3-hydroxyphenylacetate, 4-hydroxyphenylacetate, quinate, salicylate, tropic acid, vanillate, glycolate, DL-glycerate, D-malate, D-tartrate, L-

tartrate, *m*-tartrate, levulinate, 2-ketogluconate, citraconate, itaconate, mesaconate, aconitate, citrate, phenylacetate, benzoate, *o*-hydroxybenzoate, *m*-hydroxybenzoate, D-mandelate, L-mandelate, phthalate, isophthalate, terephthalate, arabonic acid, dextran, sorbate, L-2-hydroxyisocaproate, DL-isocitrate, tartronate, 1,8-octandiol, β-alanine, DL-aminobutyrate, DL-3-aminobutyrate, DL-2-aminoisobutyrate, aminoxycetate, D-arginine, D-asparagine, L-cysteinate, DL-2,4-diaminobutyrate, diaminopimelate, 2,3-diaminopropionate, L-glycine, glycineanhydride, D-histidine, L-homoserine, DL-8-hydroxylysine, D-lysine, D-methioninesulfone, L-norleucine, D-phenylalanine, serine, D-tryptophan, L-tryptophan, butylamine, cadaverine, ethanolamine, ethylamine, urea, hexylamine, spermine, taurine, trigonelline, tryptamine, acetamide, indole-3-acetate, oxamide acid, salicylamide, uracil, furan-2-carbonic acid, 2-hydroxyphenylacetate, phenoxyacetate, phenyllactate, phenylpropionate, and phenylpyruvate. Acetate, butyrate, isobutyrate, isovalerate, malonate, glutarate, glycerate, glycolate, and propionate are weakly assimilated after 7 days of incubation.

No acid is produced from D-arabinose, arabonic acid, dextran, α-D-galacturonate, gluconate, glucuronate, inulin, lactobionic acid, L-lyxose, D-maltose, glycogen, α-D-melezitose, α-D-melibiose, L-rhamnose, D-sucrose, α-methyl-D-mannoside, methyl-α-D-glucopyranoside, β-methyl-D-xyloside, mucate, α-D-raffinose, D-ribose, L-sorbose, starch, D-tagatose, L-xylose, dulcitol, *m*-erythritol, glycerol, i-inositol, maltitol, D-xylitol, and tributyrin.

The G+C content of the DNA is 65.6 mol% (5). The major isoprenoid quinone is ubiquinone Q-10. The main component in the polyamine pattern is *sym*-homospermidine (4). The major nonpolar fatty acids are 18:1 and 16:0, and the major 2-hydroxy fatty acid is 14:0 2OH.

Sphingoglycolipid, phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylmethylethanolamine, and an unknown lipid are the predominant polar lipids. Phosphatidylmonomethylethanolamine and four unknown lipids are present in minor concentrations.

Isolated from soil.

TABLE 4. Levels of sequence similarity between the 16S rRNA gene of *S. trueperi* and the 16S rRNA genes of other *Sphingomonas* species and species belonging to the α subclass of the *Proteobacteria*

Species	Strain ^a	% Sequence similarity to <i>S. trueperi</i>
<i>Sphingomonas paucimobilis</i>	DSM 1098 ^T	95.8
<i>Sphingomonas parapaucimobilis</i>	JCM 7510 ^T	95.4
<i>Sphingomonas adhaesiva</i>	JCM 7370 ^T	95.5
<i>Sphingomonas sanguis</i>	IFO 13937 ^T	95.6
<i>Sphingomonas mali</i>	IFO 15500 ^T	95.8
<i>Sphingomonas pruni</i>	IFO 15498 ^T	95.8
<i>Sphingomonas asaccharolytica</i>	IFO 15499 ^T	95.8
<i>Sphingomonas yanoikuyae</i>	JCM 7370 ^T	93.1
<i>Sphingomonas chlorophenolica</i>	ATCC 33790 ^T	92.4
<i>Sphingomonas macrogoltabidus</i>	IFO 15033 ^T	91.1
<i>Sphingomonas terrae</i>	IFO 15098 ^T	90.9
<i>Sphingomonas capsulata</i>	ATCC 14666 ^T	90.8
<i>Sphingomonas rosa</i>	IFO 15208 ^T	92.1
<i>Rhizomonas suberifaciens</i>	IFO 15211 ^T	92.4
<i>Erythrobacter longus</i>	JCM 6170 ^T	90.9
<i>Brevundimonas diminuta</i>	ATCC 11568 ^T	87.2
<i>Agrobacterium tumefaciens</i>	DSM 30150	86.4

^a DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; JCM, Japanese Collection of Microorganisms, Wako, Saitama, Japan; IFO, Institute of Fermentation, Yodogawa-ku, Osaka, Japan; ATCC, American Type Culture Collection, Rockville, Md.

The type strain is strain NCIMB 9391 (= ATCC 12417 = LMG 2142 = DSM 7225).

The 16S rDNA sequence has been deposited in the EMBL data bank under accession number X97776.

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