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Chemotaxonomic characterisation of *Sphingomonas*

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Based on published results and investigations done for this study, chemotaxonomic characteristics of all validly described species of the genus *Sphingomonas*, as well as unnamed strains of this genus and related genera such as *Rhizomonas* and *Blastomonas*, are presented. All representatives of this group, here designated as sphingomonads, contain ubiquinone (Q-10). The two different polyamine patterns characterized either by the predominant polyamine *sym*-homospermidine or spermidine separate the sphingomonads into two major groups. Complex polar lipid profiles were found in sphingomonads in addition to the characteristic compound sphingoglycolipid. Identical profiles were found only in a few phylogenetically highly related species. Common to all sphingomonads is a fatty acid composition with 2-hydroxy fatty acids (14:0 2OH in all currently recognized species) and the lack of 3-hydroxy acids, which distinguishes them from taxa outside this group. Qualitative and quantitative differences in the fatty acid compositions, in several cases, were also suitable for identification at the species level. Thus, the differences in the chemotaxonomic characteristics demonstrate that the analyses of these low molecular weight cell compounds are suitable for classification of sphingomonads.

Keywords: *Sphingomonas*; chemotaxonomy; ubiquinone system; polyamine patterns; polar lipid profiles; fatty acid composition

Introduction

Members of the genus *Sphingomonas* [40] are Gram-negative, aerobic, yellow-pigmented, nonspore-forming, nonfermenting motile rods (by means of a single polar flagellum) or non-motile rods. The type species of the genus is *S. paucimobilis*. The genus encompasses species that were originally described as members of the genera *Flavobacterium*, *Pseudomonas* and *Arthrobacter*, eg *S. paucimobilis* (formerly *F. devorans* and *P. paucimobilis*), *S. capsulata* (formerly *F. capsulatum*) [40], *S. chlorophenolica* (certain strains of this species were formerly described as members of the genera *Arthrobacter*, *Flavobacterium* and *Pseudomonas*) [24], *S. subarctica* (strains of this species were formerly described as *P. saccharophilia*) [23], *S. trueperi* (formerly *P. azotocolligans*) [14] and *S. echinoides* (formerly *P. echinoides*) [5], as well as numerous newly described species from different sources [1,33,34,42].

Phylogenetically, the genus *Sphingomonas* clusters within the α-4 subclass of the *Proteobacteria* together with the genera *Blastomonas*, *Rhizomonas*, *Zymomonas*, and the aerobic bacteriochlorophyll-*a* containing genera *Porphyrobacter*, *Erythrobacter*, *Erythromicrobium*, *Erythromonas* and *Sandaracinobacter* [1,35,41]. On the basis of 16S rRNA sequence comparisons, the species of the genus *Sphingomonas* are divided into at least four major clusters. Cluster I, which is assumed to represent the genus *Sphingomonas* sensu stricto [43] contains the species *S. paucimobilis*, *S. parapaucimobilis*, *S. sanguinis*, *S. adhaesiva*, *S. trueperi*, *S. echinoides*, *S. pruni*, *S. mali* and *S. assacharolytica*. Cluster II contains *S. yanoikuyae*, *S. herbicidovorans*, *S. chlorophenolica* and *S. xenophaga*. Cluster III consists of the species *S. capsulata*, *S. rosa*, *S. subarctica*, *S. aromaticivorans*, *S. stygia* and *S. subterranea* and cluster IV is constituted by *S. macrogoltabidus* and *S. terrae*. A more detailed discussion of the phylogeny of sphingomonads is given in this issue by Crawford. Also several well-known degraders of xenobiotic compounds such as RW1, HH69–3, the misnamed strains *Sphingomonas 'paucimobilis'* EPA505 and '*Alcaligenes*' sp A175, were shown to cluster with the genus *Sphingomonas* (Figure 1) [1,16,20] and each of these strains can be considered to represent a distinct species. Phylogenetically, *S. 'paucimobilis'* EPA505 groups with cluster II. In contrast, the allocation of strains HH69–3, RW1 and '*Alcaligenes*' sp A175 is not very clear. In some phylogenetic trees, they represent the deepest branching within cluster I [1,16,20] whereas in others they constitute a separate cluster (Figure 1) [23]. These differences in the tree topology might be explained by different methods used for calculation of the phylogenetic trees.

The high catabolic and physiological versatility of sphingomonads [7,8,10,16–19,22–24,26,27,32,38,42], which are subjects of numerous contributions in this issue, encourages studies with strains of this group. Due to the pathogenic potential of certain species such as *S. paucimobilis* and *S. parapaucimobilis* [11,21,25], care is needed when studies with new isolates of this genus are initiated. At least a detailed characterisation and classification of the isolate should be done to assure that it does not belong to any of the opportunistic pathogens, *S. paucimobilis* and *S. parapaucimobilis*. Here we present selected chemotaxonomic features of the species of the genus *Sphingomonas* and relatives (which are here designated as sphingomonads) to

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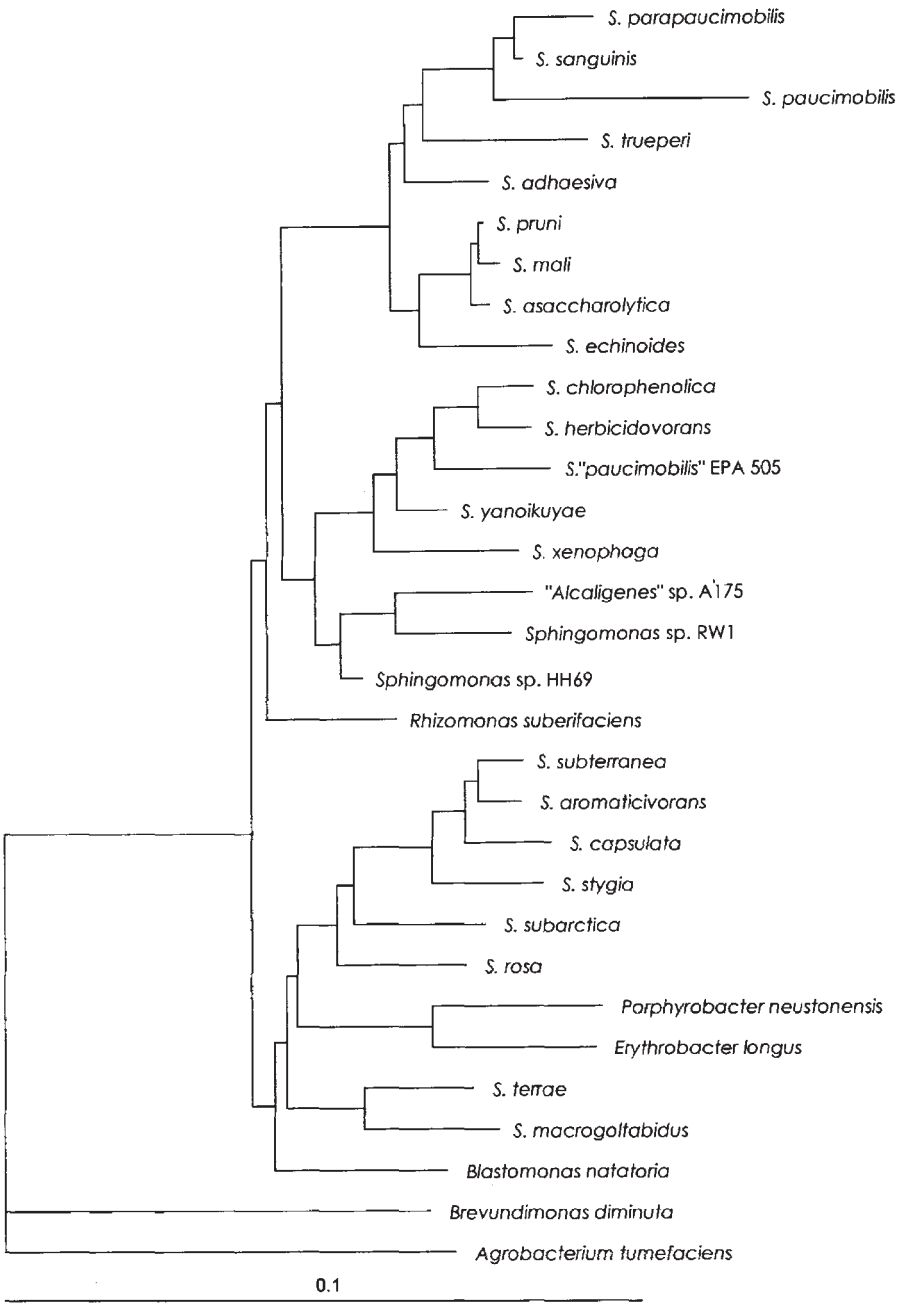

Figure 1 Phylogenetic tree of *Sphingomonas* species, strains considered as members of the genus *Sphingomonas* and selected α-*Proteobacteria*, based on nearly complete 16S rRNA gene sequences. The PHYLIP program [6] was used to calculate evolutionary distances by the method of Jukes and Cantor [13] after which the FITCH algorithm was used to estimate phylogenies from distances matrix data. *Agrobacterium tumefaciens* was used as the outgroup. Sequences were obtained from the EMBL Nucleotide Sequence Database. Sequences extracted from the EMBL database are as follows, including species names, strain designations and accession numbers: *Agrobacterium tumefaciens* DSM 30150, M11223; '*Alcaligenes*' sp A175, X94099; *Blastomonas natatoria* ACM 2057^T, X73043; *Brevundimonas diminuta* ATCC 11568^T, M59064; *Erythrobacter longus* JCM 6170^T, D12699; *Porphyrobacter neustonensis*, ACM 2844^T, L01785; *Rhizomonas suberifaciens* IFO 15211^T, D13737; *Sphingomonas adhaesiva* JCM 7370^T, X72720; *Sphingomonas aromaticivorans* SMCC F199^T, U20756; *Sphingomonas asaccharolytica* IFO 15499^T, Y09639; *Sphingomonas capsulata* ATCC 14666^T, D16147; *Sphingomonas chlorophenolica* ATCC 33790^T, X87161; *Sphingomonas echinoides* DSM 1805^T, AJ012461; *Sphingomonas herbicidovorans* DSM 11019^T, AB022428; *Sphingomonas* sp strain HH69–3 (DSM 7135), X87166; *Sphingomonas macrogoltabidus* IFO 15033^T, D13723; *Sphingomonas mali* IFO 15500^T, Y09638; *Sphingomonas parapaucimobilis* JCM 7510^T, X72721; *Sphingomonas pruni* IFO 15498^T, OY09637; *Sphingomonas paucimobilis* DSM 1098^T, X72722; *Sphingomonas 'paucimobilis'* EPA 505 (DSM 7526), X94100; *Sphingomonas* sp strain RW1 (DSM 6014), X72723; *Sphingomonas rosa* IFO 15208^T, D13945; *Sphingomonas sanguinis*, IFO 13937^T, D13726; *Sphingomonas subarctica* HAMBI 2110^T, X94102; *Sphingomonas subterranea* SMCC B0478^T, U20773; *Sphingomonas stygia* SMCC B0712^T, U20775; *Sphingomonas terrae* IFO 15098^T, D13727; *Sphingomonas trueperi* LMG 2142^T, X97776; *Sphingomonas xenophaga* DSM 6383^T, X94098; *Sphingomonas yanoikuyae* JCM 7371^T, X72725. The bar represents the scale of estimated evolutionary distance (ie an average of five substitutions at any nucleotide position, per 100 nucleotide positions) which has occurred from the point of divergence of the 16S rRNA gene sequences.

Table 1 Polyamine profiles detected in sphingomonads. An overview of the results for the same strain obtained from different groups is given. If not indicated by a footnote, the amounts of polyamines are presented as micromoles per gram of dry weight biomass (μmol g^{-1} dry weight)

Organism	μmol g^{-1} dry weight									
	DAP	PUT	CAD	TYR	NSPD	SPD	HSPD	SPM	AGM	Reference
S. paucimobilis DSM 1098^{T}						0.6	53.8	0.6		[3]
S. paucimobilis IFO 13935^{T,a}							2.55			[34]
S. paucimobilis LMG 1227^{T,a}							4.8	t		[28]
S. paucimobilis DSM 30198		0.4				2.9	56.4	0.5		[3]
S. parapaucimobilis IFO 15100^{T,a}							2.35			[34]
S. parapaucimobilis LMG 10923^{T,a}							5.0	t		[28]
S. adhaesiva IFO 15099^{T,a}						1.90				[34]
S. adhaesiva LMG 10922^{T,a}							1.8			[28]
S. sanguinis IFO 13937^{T,a}							1.97			[34]
S. sanguinis LMG 10925^{T,a}							4.8			[28]
S. pruni IFO 15498^{T,a}							1.42			[34]
S. mali IFO 15500^{T,a}							0.75			[34]
S. asaccharolytica IFO 15499^{T,a}						3.6	56.3	1.2		[14]
S. asaccharolytica IFO 15499^{T,a}						0.61			0.2	[34]
S. trueperi ATCC 7225^{T}		1.4				4.3	34.9	0.9		[3]
S. echinoides DSM 1805^{T}	0.5	8.0				7.0	37.4	0.2		[5]
'*Alcaligenes*' sp A175	1.0	7.1				6.6	50.1	1.9		This study
Sphingomonas sp RWI (DSM 6014)		0.3				2.2	48.9	1.3		This study
Sphingomonas sp HH69–3 (DSM 7135)		0.2				30.8	0.1	2.5		This study
S. capsulata DSM 30196^{T}		1.0				34.8		0.5		[3]
S. capsulata LMG 2830^{T,a}		t	t		t	2.5				[28]
S. capsulata IFO 12533^{T,a}		0.10				1.80			0.10	[34]
S. stygia SMCC0712^{T}		0.3		t	t	56.7		1.3		This study
S. aromaticivorans SMCC F199^{T}		0.2	0.1		t	47.9		2.2		This study
S. subarctica HAMBI 2110^{T}	0.3	0.2	t	0.2	t	72.1		4.6		[23]
S. rosa IFO 15208^{T,a}						1.30				[34]
S. chlorophenolica ATCC 39723	0.1	0.2			t	31.7		2.5		This study
S. herbicidovorans DSM 11019^{T,a}	0.1	0.2				24.3		5.3		This study
Sphingomonas sp SS3 (DSM 6432)	0.1	0.1			t	40.1		t		This study
S. yanoikuyae IFO 15102^{T}						28.2		1.2		This study
S. yanoikuyae IFO 15102^{T,a}						1.57			0.03	[34]
S. yanoikuyae[a,b]		(t)			(t)	2.9–4.0		(t)		[28]
S. yanoikuyae NCTC 10591		t				32.3		0.4		[3]
S. xenophaga DSM 6383^{T}						31.7		3.5		[31]
S. 'paucimobilis' EPA 505 (DSM 7626)		0.2			0.1	31.2		2.3		This study
S. macrogoltabidus IFO 15033^{T,a}		0.02				2.10				[34]
S. terrae IFO 15098^{T,a}		0.02				2.24			t	[34]
S. terrae LMG 10924^{T,a}						t	3.2			[28]
Blastomonas natatorius DSM 3183^{T}		0.7				42.4		5.3		This study
Rhz. suberifaciens IFO 15211^{T,a}		0.01				0.58				[34]
Rhizomonas sp W14 LMG 11032		0.1			t	25.4		2.9		This study

[a]The data are reported in micromoles per gram of wet weight of cells.
[b]Four strains of *S. yanoikuyae* were analyzed and the range of values is given. Some of these strains contained traces of certain polyamines given in brackets. Abbreviations: DAP, 1,3-diaminopropane; PUT, putrescine; CAD, cadaverine; TYR, tyramine; NSPD, *sym*-norspermidine; SPD, spermidine; HSPD, *sym*-homospermidine; SPM, spermine; AGM, agmatine; t, traces. ATCC, American Type Culture Collection, Rockville, MD, USA; DSM, DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; HAMBI, Culture Collection of the Department of Applied Chemistry and Microbiology, Helsinki, Finland; IFO, Institute for Fermentation, Osaka, Japan; LMG, Laboratorium voor Microbiologie, Universiteit Gent, Gent, Belgium; NCIB, National Collection of Industrial Bacteria, Aberdeen, UK; NCPPB, National Collection of Plant Pathogenic Bacteria, Hertfordshire, UK; SMCC, Subsurface Microbial Culture Collection, Florida State University, Tallahassee, USA.

demonstrate the suitability of the analysis of small molecular weight cell components for classification within this group.

Materials and methods

Source of strains

S. herbicidovorans (DSM 11019^{T}) was obtained from C Zipper (Dübendorf, Switzerland), *S. subterranea* (SMCC B 0478^{T}), *S. stygia* (SMCC B0712^{T}), and *S. aromaticivorans* (SMCC F199^{T}) from DL Balkwill (Tallahassee, FL, USA), and *S. subarctica* (HAMBI 2110^{T}), *S. 'paucimobilis'* EPA505 (DSM 7526), *B. natatoria* (DSM 3183^{T}) and the strains '*Alcaligenes*' sp A175, *Sphingomonas* sp RW1 (DSM 6014) and HH-69–3 (DSM 7135) from M Salkinoja-Salonen (Helsinki, Finland). *Rhizomonas* sp WI4 (LMG 11032) was supplied by the Culture Collection of the Laboratorium voor Microbiologie, Gent, Belgium.

Chemotaxonomic analyses

Polyamines were extracted as described by Busse and Auling [3] and analyzed according to Busse *et al* [4]. Polar lipids were prepared according to the method of Tindall [36]. Fatty acid methyl esters were prepared by the standard protocol of the Microbial Identification System (MIDI; Microbial ID, Newark, DE, USA and analyzed as described previously [15].

Results and discussion

Pigment

Colonies of the majority of species of the genera *Sphingomonas*, *Rhizomonas* and *B. natatoria* are intensively yellow pigmented [1,5,14,33,34]. This pigment can be easily extracted with acetone and the visible absorption spectrum is characterized by maxima at 452 and 480 nm [2,39]. The yellow pigment of *S. paucimobilis* was identified as nostoxanthin [12]. In contrast, strains of *S. yanoikuyae* are known to be less pigmented and the strains RW1 and '*Alcaligenes*' sp A175 are nonpigmented. Recently, some airborne orange pigmented sphingomonads have been isolated (described elsewhere in this issue). Thus, yellow pigmentation can not be considered as a characteristic feature of the genus *Sphingomonas*.

Quinone system

The species of the genus *Sphingomonas* which have been analzyed for their quinone system as well as *Rhizomonas suberifaciens* contain ubiquinone with ten isoprenoic units in the side chain (Q-10) [1,4,14,23,24,31,34]. This characteristic is not restricted to sphingomonads and is found in the majority of taxa belonging to the α-subclass of *Proteobacteria*. From the homogeneity of sphingomonads with respect to their quinone system, it is most likely that all sphingomonads contain ubiquinone Q–10 and it would be

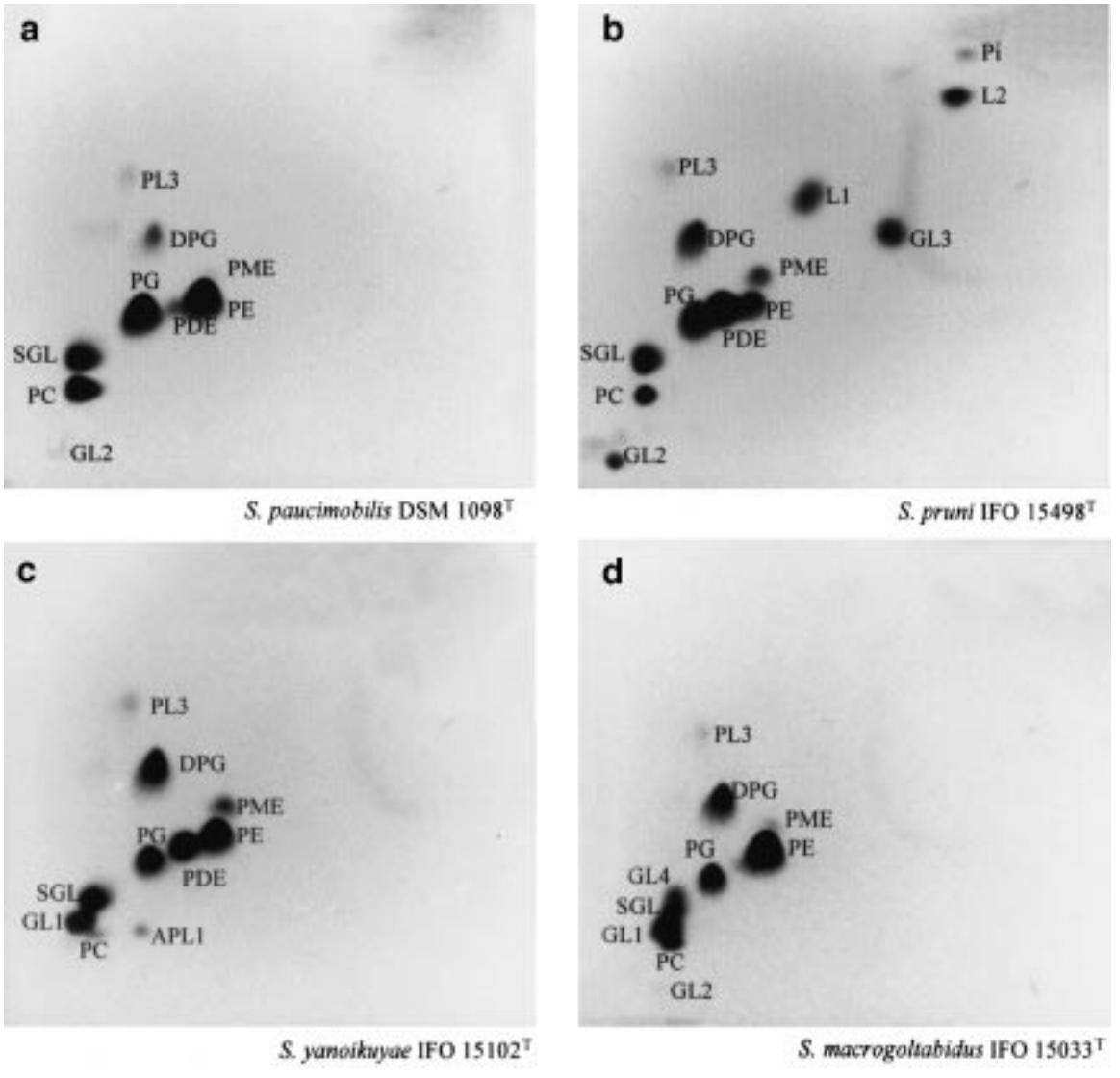

Figure 2 Polar lipid distribution in four representatives of the genus *Sphingomonas*: (a) *S. paucimobilis* DSM 1098^T, (b) *S. pruni* IFO 15498^T, (c) *S. yanoikuyae* IFO 15102^T and (d) *S. macrogoltabidus* IFO 15033^T after two-dimensional thin layer chromatography. Abbreviations as indicated in Table 2.

Table 2 Polar lipid distribution in sphingomonads. The results shown for *S. herbicidovorans* (DSM 11019^T), *S. subarctica* (HAMBI 2110^T), *S. subterranea* (SMCC B 0478^T), *S. stygia* (SMCC B0712^T), *S. aromaticivorans* (SMCC F199^T), *S. 'paucimobilis'* EPA505 (DSM 7526), *B. natatoria* (DSM 3183^T) and the strains '*Alcaligenes*' sp A175, *Sphingomonas* spp RW1 (DSM 6014) and HH69–3 (DSM 7135) were generated during the course of this study. Other polar lipid profiles have been published previously [5,14,31]

Organism	Polar lipids[a]																	
	PME	PE	PG	DPG	PDE	PC	SGL	APL1	PL1	PL2	PL3	PL4	GL1	GL2	GL3	GL4	L1	L2
S. paucimobilis DSM 1098^T	□	■	■	□	□	■	■	–	–	–	□	–	–	□	–	–	–	–
S. parapaucimobilis DSM 7463^T	□	■	■	■	□	■	□	–	–	□	□	–	■	□	–	–	–	–
S. sanguinis IFO 13937^T	–	■	■	■	□	■	□	–	–	–	□	–	□	□	–	–	–	–
S. adhaesiva DSM 7418^T	□	■	■	■	□	■	■	□	–	■	□	□	–	□	–	–	–	–
S. trueperi DSM 7225^T	□	■	■	■	■	□	■	–	□	–	□	–	–	□	–	–	■	□
S. mali DSM 10565^T	□	□	■	■	■	□	■	–	□	–	□	–	–	□	□	–	■	□
S. pruni DSM 10566^T	□	□	■	■	■	□	■	–	–	–	□	–	–	□	□	–	■	□
S. asaccharolytica DSM 10564^T	□	□	■	■	■	□	■	–	□	–	□	–	–	□	□	–	■	□
S. echinoides DSM 1805^T	–	■	■	□	–	–	■	■	□	–	–	–	–	–	□	–	■	–
Sphingomonas sp RW1 (DSM 6014)	■	■	■	■	■	□	■	–	–	–	□	–	□	–	–	□	–	–
'*Alcaligenes*' sp A175	–	■	■	■	–	□	■	–	–	–	□	–	□	–	–	□	–	–
Sphingomonas sp HH69-3 (DSM 7135)	–	■	■	■	■	□	■	–	–	–	□	–	–	□	–	–	–	–
S. yanoikuyae IFO 15102^T	□	■	■	■	■	□	■	□	–	–	□	–	■	–	–	–	–	–
S. chlorophenolica ATTC 33790^T	□	■	■	■	■	□	■	–	–	–	□	□	–	–	–	–	–	–
S. xenophaga DSM 6383^T	□	■	■	■	■	□	■	–	–	–	□	■	–	–	–	–	■	–
S. herbicidovorans DSM 11019^T	□	■	■	■	■	■	■	–	–	–	□	–	–	–	–	–	–	–
S. 'paucimobilis' EPA505 (DSM 7526)	□	■	■	■	■	■	■	–	–	–	□	–	–	–	–	–	–	–
S. capsulata DSM 31096^T	□	■	■	■	■	□	■	□	–	□	□	–	–	□	–	–	–	–
S. rosa DSM 7285^T	□	■	■	■	■	□	■	□	–	□	□	□	–	□	–	–	–	–
S. subarctica HAMBI 2110^T	□	□	■	□	■	□	■	–	–	–	□	–	–			–	–	–
S. subterranea SMCC B0478^T	□	■	■	□	■	□	■	–	–	–	□	–	–	□	–	–	–	–
S. stygia SMCC B0712^T	□	■	■	□	■	□	■	–	□	–	□	–	–	□	–	□	–	–
S. aromaticivorans SMCC F199^T	□	■	■	■	■	□	■	–	–	–	□	–	–	□	–	–	–	–
S. macrogoltabidus DSM 8826^T	□	■	■	■	–	□	□	–	–	–	□	–	□	□	–	□	–	–
S. terrae DSM 8831^T	□	■	■	■	□	■	■	–	–	–	□	–	□	–	–	–	–	–
B. natatoria DSM 3183^T	□	■	■	□	■	□	■	–	–	–	□	–	–	–	–	□	–	–

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

expected that new isolates of the genus will also share this characteristic.

Polyamine patterns

Within the sphingomonads, two major polyamine patterns have been observed (Table 1). One pattern is characterized by the predominant triamine *sym*-homospermidine and varying minor amounts of putrescine, spermidine and spermine (Table 1). The major polyamine in the second pattern is the triamine spermidine and minor amounts of putrescine and spermine. The former pattern with *sym*-homospermidine is found only in representatives of cluster I considered to represent the genus *Sphingomonas* sensu stricto [43] and in the strains *Sphingomonas* sp RW1 and '*Alcaligenes*' sp A175 whose phylogenetic position is not clear. The pattern with spermine as the predominant polyamine is found in the remaining species of the genus as well as in *B. natatoria*, *R. suberifaciens* and *Rhizomonas* sp WI4. However, controversal results were shown for *S. adhaesiva*, *S. asaccharolytica* and *S. terrae*. Hamana and Matsuzaki [9] and Segers *et al* [28] demonstrated that the type strain of *S. adhaesiva* contains *sym*-homospermidine as the predominant compound in the polyamine pattern, which is in agreement with the polyamine patterns (Table 1) of the phylogenetic relatives (Figure 1). In contrast, Takeuchi *et al* [34] reported that the type strain of *S. adhaesiva* contains spermidine as the predominant amine. This observation might indicate that two totally different strains, both desigated *S. adhaesiva*, were analyzed for their polyamine patterns. This assumption is also supported by the fact that two clearly different 16S rRNA gene sequences of the type strain of *S. adhaesiva* have been deposited in DNA data banks (accession numbers X72720 and D13722). As a result of these two different sequences, *S. adhaesiva* (accession No. X72720) groups with the species of the genus *Sphingomonas* sensu stricto (cluster I) [20] and with cluster IV (accession No. D13722) [33]. The 150 nucleotide 16S rRNA gene fragment sequence from the type strain of *S. adhaesiva*, published by Yabuuchi *et al* [40], and the corresponding sequence within a larger 16S rRNA gene sequence (accession No. X72720) differ in one nucleotide. In contrast, the corresponding sequence within a second nearly complete 16S rRNA gene (accession No. D13722), shares only 92% identity with the previous two fragment sequences. Thus, it is most likely that the 16S rRNA gene sequence (accession No. D13722) was not derived from the type strain of *S. adhaesiva* but from another sphingomonad closely related to *S. macrogoltabidus* and *S. terrae*. In a

Table 3 Fatty acid profiles of sphingomonads as generated by our group ([5,14,31] this study). Cells were grown on Trypticase soy broth agar and *S. pruni* and *S. mali* were grown on Czapek Dox agar

Strain	14:0	15:0	Summed feature 4[a]	16:0	Summed feature 7[a]	16:1 ω5c	17:1 ω6c	17:1 ω8c	17:0	18:1 ω5c	12:0 2OH	13:0 2OH	14:0 2OH	15:0 2OH	16:0 2OH	18:0	19:0 cyclo	16:0 iso 2OH
S. paucimobilis DSM 1098^T	1.4		2.7	8.7	74.6		3.0			3.2			6.4					
S. parapaucimobilis DSM 7463^T	1.0		8.6	9.6	65.0	1.9	3.8			3.5			6.7					
S. adhaesiva DSM 7418^T			6.2	6.5	47.3		21.2						13.8					
S. sanguinis IFO 13937^T	1.0	1.1	6.7	13.6	64.6	1.0	2.4		0.5	3.5			5.0					
S. trueperi DSM 7225^T				9.8	64.2		13.6		1.6	4.0			6.7			0.6		
S. mali DSM 10565^T				11.8	70.5		9.3						8.4					
S. pruni DSM 10566^T				16.0	73.1								10.9					
S. asaccharolytica DSM 10564^T				13.3	67.8								18.8					
S. echinoides DSM 1805^T	0.9		2.45	10.0	73.1	0.8	2.9			2.7			7.2					
Sphingomonas sp RW1 (DSM 6014)	1.7		9.3	12.8	55.6		0.7		6.0	1.4			7.4				5.0	
'*Alcaligenes*' sp A175	0.7		17.9	7.0	62.6	2.4							7.4		2.2			
Sphingomonas sp HH69-3 (DSM 7135)			21.8	6.7	52.6	2.4	4.6	0.7		2.1			6.3	0.5	0.4			1.8
S. macrogoltabidus DSM 8826^T			31.8	12.8	41.2	2.9	4.5			0.9			3.0		2.9			
S. terrae DSM 8831^T		5.9	8.0	4.5	13.4	2.1	48.1	7.9	3.8	0.6			0.8	4.9				
S. yanoikuyae IFO 15102^T	0.5		16.3	11.2	56.0	2.7	3.2			2.1			7.1		0.9			
S. chlorophenolica ATCC 33790^T			9.6	9.5	60.1	2.4	6.4			2.5			9.4					
S. xenophaga DSM 6386^T			23.9	8.0	55.2	2.0	2.5			1.6			6.7					
S. xenophaga DSM 8566			27.9	9.3	47.2	2.0	3.0			1.5			10.0		1.0			
S. herbicidovorans DSM 11019^T			13.4	7.4	68.3	1.6	1.7			2.0			5.5					
S. 'paucimobilis' EPA505 (DSM 7526)			8.6	5.5	73.7	1.8	1.8			2.0			6.6					
S. capsulata DSM 31096^T			6.7	8.1	64.8	1.7							14.7					
S. rosa DSM 7285^T	1.5		22.8	10.9	52.0								12.8					
S. stygia SMCC B0712^T			23.1	2.5	49.3	0.6	4.1	0.8		0.8	0.6	0.6	14.9	2.5				
S. subterranea	0.9		20.1	4.7	48.2		6.4	1.0					13.0	3.3		3.2		
S. aromaticivorans SMCC F199^T	0.5	2.6	20.0	2.4	37.7		10.2	4.5	0.7		1.0	2.6	16.8		0.9			
S. subarctica HAMBI 2110^T			12.6	10.2	61.1	2.9	3.3						7.6		2.1			

[a]Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 4 contained one or more of the following fatty acids: 16:1 ω7c and 15:0 iso 2OH. Summed feature 7 contained one or more of the following isomers: 18:1 ω7c, 18:1 ω9t, and/or 18:1 ω12t (*cis* and *trans* isomers are indicated by c and t, respectively).

Table 4 Fatty acid profiles of sphingomonads as generated by Nohynek *et al* [23,24] and van Bruggen *et al* [37]

Strain	14:0	15:0	16:0	16:1	17:0	17:1	18:1	14:0 2OH	Other
S. paucimobilis ATCC 29837^{T,a}			9	4		4	74	7	2
S. parapaucimobilis DSM 7463^{T,a}			10	10		2	67	7	4
S. adhaesiva DSM 7418^{T,a}			6	5		23	37	9	20
S. sanguinis IFO 13937^{T,a}			16	8		1	66	5	4
Sphingomonas sp RW1 (DSM 6014)[a]			15	10			60	7	8
'*Alcaligenes*' sp A175[a]			10	13		4	61	7	5
Sphingomonas sp HH69-3 (DSM 7135)[a]			6	29			53	7	5
S. yanoikuyae DSM 7462^{T,a}			10	19		tr	58	8	5
S. chlorophenolica ATCC 39723^{T,a}			14	14			57	11	4
S. xenophaga BN6 (DSM 6383)^{T,a}			11	25			53	6	5
S. 'paucimobilis' EPA 505 (DSM 7526)[a]			7	8		3	68	7	7
S. macrogoltabidus IFO 15033^{T,a}			13	35		tr	44	4	4
S. terrae IFO 15098^{T,a}			8	4		tr	80	1	7
S. capsulata DSM 30196^{T,a}			8	7		4	63	15	3
S. subarctica KF1^{T,a} (HAMBI 2110^T)			10	14		1	61	8	6
R. suberifaciens ATCC 49355^{T,b}	0.6	0.4	13.2	10.8	0.4	3.5	56.5	6.1	6.1
Rhizomonas sp W14[b]	2.1		18.0	7.1		0.9	65.9	6.0	tr

tr, Trace amounts.
[a]Cells subjected to fatty acid analysis were grown on trypticase soy agar. The 18:1 fatty acid contains one or more of the isomers 18:1 *cis*-9, 18:1 *trans*-6, 18:1 *cis*-11.
[b]Cells subjected to fatty acid analysis were grown on S-medium (5.0 g L^{-1} enzymatic casein hydrolysate, 2.5 g L^{-1} glucose, 1.3 g L^{-1} $H_2HPO_4 \cdot 3H_2O$, 0.5 g L^{-1} KNO_3, 0.5 g L^{-1} $MgSO_4 \cdot 7H_2O$, 60 mg L^{-1} $Ca(NO_3)_2 \cdot 4H_2O$ and 11 g L^{-1} Noble agar, pH 7.2).

Table 5 Fatty acid profiles of sphingomonads as generated by Takeuchi *et al* [33,34] and Sittig and Hirsch [29]. The percentages of the total non-polar acids and the total 2-hydroxyacids are shown separately

Strain	14:0	14:1	15:0	16:0	16:1	17:0	17:1	18:1	18:0	14:0 2OH	14:1 2OH	15:0 2OH	16:0 2OH	16:1 2OH	17:1 2OH	Other
S. paucimobilis IFO 13935^T				13				87		100						
S. parapaucimobilis IFO 15100^T	9			11	7			73		100						
S. sanguinis IFO 12533^T	18			17				65		100						
S. adhaesiva IFO 15099^T				18	7		12	63		82		18				
S. assacharolytica IFO 15499^T			4	8	tr	9	29	51	tr	34		66				
S. pruni IFO 15498^T			4	10	tr	4	17	68	tr	47		47	8			
S. mali IFO 15500^T		tr		11	1		4	82	2	82		13	5			
S. yanoikuyae IFO 15102^T				13	17			70		100						
S. capsulata IFO 12533^T				13	7			80		100						
S. rosa IFO 15208^T		5		15	26		2	50		100		tr				
S. macrogoltabidus IFO 15033^T				11	41			47		85			15			
S. terrae IFO 15098^T			5	6	13	6	45	26		35		63	3			
B. natatoria ATCC 35951^{T,a}	0.9		0.3	17.2	14.6	0.3	3.0	61.5	1.2	52.2	0.6	3.9	25.7	17.4	0.4	0.7

If not indicated otherwise, cells subjected to fatty acid analyses were grown on PY medium (1% peptone, 0.2% yeast extract, 0.2% NaCl, 0.2% glucose, pH 7.0).
tr, Trace amounts.
[a]Cells subjected to fatty acid analyses were grown on PYGV medium [30]. The 16:1 fatty acid contains the isomers n16:1d9 and/or n16:1d11, the fatty acid 17:1 contains the isomers n17:1d9 and/or n17:1d11 and the 16:1 2OH fatty acid contains the isomers n16:1d9 2OH and/or n16:1d11 2OH.

strain closely related to the latter two species a polyamine pattern with spermidine as the predominant compound would be expected (Table 1). Therefore, the type strain of *S. adhaesiva* can be considered to be characterized by a polyamine pattern with *sym*-homospermidine as the predominant compound whereas the strain displaying spermidine as the predominant polyamine does not represent the species *S. adhaesiva* sensu stricto. Also controversal are the polyamine patterns reported for *S. asaccharolytica*. This species was described to contain spermidine as the predominant polyamine [34], whereas a reinvestigation showed that this species contains *sym*-homospermidine [14]. The phylogenetic analysis of the *sym*-homospermidine-containing strain clearly demonstrated that *S. asaccharolytica* (DSM 10564^T) belongs to cluster I (Figure 1) [14] where a polyamine pattern with *sym*-homospermidine would be expected. Although growth conditions do not significantly affect the polyamine patterns [3], it can not be excluded here that this difference is related to different compositions of the growth media and/or time of harvesting the cells used for polyamine analysis. A similar situation is found with *S. terrae*. Segers *et al* [28] reported that *S. terrae* LMG 10924^T contains *sym*-homospermidine as the predominant polyamine, whereas Takeuchi *et al* [34] showed for *S. terrae* IFO15098^T that it contains spermidine as the major compound. Since both strain numbers indicate that they represent the type strain of *S. terrae*, the two strains should be identical and should also display highly similar polyamine patterns. If no strain confusion has occurred here, variation in growth conditions might be responsible for the dramatic differences in the polyamine patterns. Due to the result that the close phylogenetic neighbour *S. macrogoltabidus* contains spermidine as the predominant polyamine (Table 1), it can be assumed that *S. terrae* is also characterized by the presence of this polyamine. Since it appears to be difficult to study the history of the strains representing the type species of *S. adhaesiva*, *S. asaccharolytica* or *S. terrae*, it can not be clarified whether strain confusion has occurred for any of these species. However, extensive studies are needed to demonstrate whether differences in growth conditions such as media composition, temperature or pH can cause the dramatic changes in the polyamine patterns reported for the type strains of *S. adhaesiva*, *S. asaccharolytica* and *S. terrae*.

A polyamine pattern with spermidine was found in the strain *S. 'paucimobilis'* EPA505 which belongs to cluster II (Figure 1). Thus the polyamine pattern is in agreement with the phylogenetic position of *S. 'paucimobilis'* EPA505 and confirms that it is not a strain of *S. paucimobilis*. Interestingly, the strains *Sphingomonas* sp RW1 and '*Alcaligenes*' sp A175 are characterized by a polyamine pattern with the predominant compound being *sym*-homospermidine. Since this polyamine pattern is characteristic for cluster I this observation might indicate that these two strains are members of the genus *Sphingomonas* sensu stricto. A unique polyamine pattern was detected in the strain HH69–3. This strain displayed a polyamine pattern with spermidine as the predominant polyamine and trace amounts of *sym*-homospermidine. Since none of the other strains with spermidine as the major polyamine was found to additionally contain *sym*-homospermidine, this observation might indicate a separate position of strain HH69–3 within the sphingomonads or some relatedness with cluster I.

Polar lipid profiles

The polar lipid profiles are helpful for classification of sphingomonads [4,8,31]. In Figure 2 the polar lipid profiles of four species, *S. paucimobilis* DSM 1098^T, *S. yanoikuyae* IFO 15102^T, *S. macrogoltabidus* IFO 150333^T, and *S. pruni* IFO 15498^T are shown. As shown in Table 2, the sphingomonad species differ qualitatively and quantitatively in their polar lipid composition. Identical polar lipid profiles

were detected only in the close relatives, *S. asaccharolytica* and *S. mali*, and *S. herbicidovorans* and *S. 'paucimobilis'* EPA505 (Figure 1). All sphingomonads contained in their polar lipid profiles phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG) and sphingoglycolipid (SGL). The majority of strains also contained phosphatidylmonomethylethanolamine (PME), phosphatidyldimethylethanolamine (PDE), the unidentified phospholipid PL1 and the unidentified glycolipid GL2. Other unidentified polar lipids such as the aminolipid APL1, the phospholipids PL1, PL2 and PL3, the glycolipids GL1, GL3 and GL4, and the lipids L1 and L2 were detected only sporadically. The presence of these unidentified lipids contributed significantly to the unique polar lipid profiles observed for the majority of sphingomonads. The different polar lipid profiles of sphingomonads do not appear to show characteristics which are in agreement with the phylogenetic grouping such as cluster I–cluster IV, but the high variability in the profiles appears to be useful for characterization and preliminary identification of sphingomonads. This is confirmed by the analysis of two additional strains of *S. yanoikuyae* which qualitatively and quantitatively displayed the same polar lipid profile as the type strain of this species (results not shown).

Fatty acid profiles

With respect to fatty acid profiles, sphingomonads share with the majority of *α-Proteobacteria* the predominance of 18:1 acid and relatively high levels of 16:0 acid (Tables 3–5). A characteristic feature of the fatty acid profile of sphingomonads is the presence of 2-hydroxy myristic acid (14:0 2OH), sporadically other 2-hydroxy fatty acids and the lack of 3-hydroxy fatty acids (Table 3–5) which distinguishes them from other *α-Proteobacteria*. Similar to other taxa, fatty acid profiles of sphingomonads are highly suitable for identification of most sphingomonads at the species level as shown for different strains of *S. xenophaga* (Table 3), *S. chlorophenolica* [24], *S. subarctica* [23], *S. yanoikuyae* [17], *S. macrogoltabidus*, *S. terrae* [33] and *S. aromaticivorans* [1]. A problem concerning species identification of sphingomonads is caused by the fact that many species descriptions are based on only one strain. Thus, the variability of the fatty acid profiles in certain species can not be evaluated so far. On the other hand, fatty acid profiles of sphingomonads appear not to be useful for displaying relationships as demonstrated by phylogenetic analyses. As shown in dendrograms generated from Euclidean distance calculations based on fatty acid compositions [23,24], members of the different phylogenetic clusters of sphingomonads are not grouped in comparable fatty acid clusters. It has to be emphasized that the standardization of growth conditions for cultivation of cells which have to be analyzed for their fatty acid profiles is essential. As shown in Tables 3 and 4, growth of the same strain on different media can result in qualitative and quantitative changes in the fatty acid profiles and comparison of these different profiles do not necessarily result in an identification at the species level. To help overcome these problems with the interpretation of the fatty acid profiles of new sphingomonad strains, reference strains should be included in the fatty acid analyses. Usually, fatty acid profiles generated in the same laboratory are quite reproducible.

Dihydrosphingosine composition

Dihydrosphingosines often designated as long-chain bases have been considered for characterization of members of the genus *Sphingomonas* [23,33,40]. The type strains of *S. paucimobilis*, *S. parapaucimobilis*, *S. adhaesiva* and *S. capsulata* contain the major long-chain base 18:0 and significant amounts of 20:1, 21:1, 22:1 and/or 23:1 [40]. Strains of *S. subarctica* contain the major bases 18:0, 18:1, 19:1, 20:1 and 21:1 [23]. Strains of the species *S. macrogoltabidus* and *S. terrae* contain the major long-chain bases 18:0, 19:1 and 20:1 and *S. sanguinis* contains 18:0 as the major base [33]. *S. yanoikuyae* was reported to contain the long-chain acid 20:1 as the most abundant, and high proportions of 18:0 and 21:1 [40], *S. aromaticivorans* to contain as the predominant bases cy21:0 and 18:0 and *S. stygia* and *S. subterranea* to contain as the major bases cy20:0 and 18:0, [1]. The importance of analysing long-chain bases for classification of sphingomonads is not clearly demonstrated. This characteristic can be highly variable even when strains of the same species are compared, as demonstrated for *S. parapaucimobilis* and *S. aromaticivorans* [1,40]. Thus, this approach needs to be evaluated in more detail to elucidate the validity of using this method for classification of sphingomonads.

Conclusions

The employment of chemotaxonomic methods is suitable for the classification and identification of sphingomonads. The analyses of polyamine patterns and the quinone system is useful only for a preliminary characterization of sphingomonad strains. In contrast, the analyses of the polar lipid and the fatty acid profiles are an excellent approach for identification at the family level based on the detection of the sphingoglycolipid, 2-hydroxy fatty acids and the lack of 3-hydroxy fatty acids. Usually an identification at the species level seems to be possible when these two approaches are applied in combination. Both the polar lipids and fatty acid profiles are composed of numerous components by which the majority of species can be distinguished from each other due to qualitative and/or quantitative variations of components in these profiles. Identical or highly similar profiles in both approaches give good evidence for the assignment of a new strain to an established species or at least to demonstrate a high degree of relatedness. The degree of relatedness can then be investigated by other methods such as genetic fingerprinting, polyacrylamide gel electrophoresis of whole-cell proteins or DNA-DNA hybridizations.

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