Proposal of the genus *Sphingomonas sensu stricto* and three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, on the basis of phylogenetic and chemotaxonomic analyses

Mariko Takeuchi,¹ Koei Hamana² and Akira Hiraishi³

¹ Institute for Fermentation
Osaka, 17-85, Jusohonmachi 2-chome,
Yodogawa-ku, Osaka
532-8686, Japan

² Department of Laboratory
Sciences, Gunma University
School of Health Sciences,
39-15 Showa-machi 3-
chome, Maebashi, Gunma
371-8514, Japan

³ Department of Ecological
Engineering, Toyohashi
University of Technology,
Tenpaku-cho, Toyohashi
441-8580, Japan

Author for correspondence: Mariko Takeuchi. Tel: +81 6 6300 6555. Fax: +81 6 6300 6814.
e-mail: takeuchi-mariko@ifo.or.jp

Phylogenetic analyses of 16S rRNA gene sequences by distance matrix and parsimony methods indicated that the currently known species of the genus *Sphingomonas* can be divided into four clusters. Some chemotaxonomic and phenotypic differences were noted among these clusters. Three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, are proposed in addition to the genus *Sphingomonas sensu stricto*. The genus *Sphingobium* is proposed to accommodate *Sphingomonas chlorophenolica*, *Sphingomonas herbicidovorans* and *Sphingomonas yanoikuyae*. The genus *Novosphingobium* is proposed for *Sphingomonas aromaticivorans*, *Sphingomonas capsulata*, *Sphingomonas rosa*, *Sphingomonas stygia*, *Sphingomonas subarctica* and *Sphingomonas subterranea*. *Sphingomonas macrogoltabidus* and *Sphingomonas terrae* are reclassified in the genus *Sphingopyxis*. The type species of *Sphingobium*, *Novosphingobium* and *Sphingopyxis* are *Sphingobium yanoikuyae*, *Novosphingobium capsulatum* and *Sphingopyxis macrogoltabida*, respectively.

Keywords: *Sphingomonas sensu stricto*, *Sphingobium*, *Novosphingobium*, *Sphingopyxis*

INTRODUCTION

The genus *Sphingomonas* was created by Yabuuchi *et al.* (1990) to accommodate strictly aerobic, chemoheterotrophic, yellow-pigmented, Gram-negative, rod-shaped bacteria that contain glycosphingolipids (GSLs) as cell envelope components. The species of the genus described were *Sphingomonas adhaesiva*, *Sphingomonas capsulata*, *Sphingomonas parapaucimobilis*, *Sphingomonas paucimobilis* (type species) and *Sphingomonas yanoikuyae*, most of which consisted of strains of clinical origin. Since then, large numbers of phenotypically and phylogenetically similar strains have been isolated from various environments and

described as novel species of this genus. These include *Sphingomonas macrogoltabidus*, *Sphingomonas sanguinis*, *Sphingomonas terrae* (Takeuchi *et al.*, 1993), *Sphingomonas asaccharolytica*, *Sphingomonas mali*, *Sphingomonas pruni*, *Sphingomonas rosa* (Takeuchi *et al.*, 1995), *Sphingomonas chlorophenolica* (Nohynek *et al.*, 1995), *Sphingomonas herbicidovorans* (Zipper *et al.*, 1996), *Sphingomonas subarctica* (Nohynek *et al.*, 1996), *Sphingomonas aromaticivorans*, *Sphingomonas stygia*, *Sphingomonas subterranea* (Balkwill *et al.*, 1997), *Sphingomonas trueperi* (Kämpfer *et al.*, 1997), ‘*Sphingomonas agrestis*’ strain HV3 (Yrjälä *et al.*, 1998) and *Sphingomonas echinoides* (Denner *et al.*, 1999). Yabuuchi *et al.* (1999) transferred *Blastomonas natatoria* (Sly & Cahill, 1997), *Erythromonas ursincola* (Yurkov *et al.*, 1997) and *Rhizomonas suberifaciens* (Van Bruggen *et al.*, 1990, 1993) to the genus *Sphingomonas* as *Sphingomonas natatoria*, *Sphingomonas ursincola* and *Sphingomonas suberifaciens*, respectively. Thus, the increase in the number of species in the genus *Sphingomonas* has complicated *Sphingo-*

Abbreviations: BChl, bacteriochlorophyll; GSLs, glycosphingolipids.

The DDBJ accession numbers for the 16S rDNA sequences of *Sphingomonas* spp. IFO 15914, IFO 15496, IFO 15497, IFO 15495, IFO 15915 and IFO 15917 and *Sphingomonas herbicidovorans* IFO 16415^T are AB033945–AB033950 and AB042233, respectively.

monas classification. The proposal of Yabuuchi *et al.* (1999) would place a broader range of species, including photosynthetic and phytopathogenic species, in the genus *Sphingomonas*.

In contrast, Hiraishi *et al.* (2000) emended the description of *Blastomonas* as a genus of aerobic photosynthetic bacteria and reclassified *Erythromonas* (*Sphingomonas*) *ursincola* as a species of the genus *Blastomonas*. Segers *et al.* (1994) suggested that *S. yanoikuyae* and *S. capsulata* should belong to a genus separate from the genus *Sphingomonas*; this suggestion was supported by polyamine profiling (Nohynek *et al.*, 1996; Denner *et al.*, 1999). In addition, Kämpfer *et al.* (1997) indicated that the *Sphingomonas* species containing *sym*-homospermidine as the major polyamine, i.e. *S. paucimobilis*, *S. mali*, *S. pruni* and *S. trueperi*, formed a relatively homogeneous subgroup within the genus.

The genus now contains genetically relatively diverse species that can be classified into at least four clusters within the α -4 subclass of the *Proteobacteria* based on 16S rDNA sequences (Stackebrandt *et al.*, 1988; Takeuchi *et al.*, 1994; Yurkov *et al.*, 1997). The chemotaxonomic and phylogenetic heterogeneity existing in the genus *Sphingomonas* calls for further study regarding this genus and related sphingomonads.

In this study, almost-complete 16S rRNA gene sequences of several strains of the genus *Sphingomonas* and related genera were examined and sequences were compared with published sequences of species belonging to the α -4 subclass of the *Proteobacteria*. Fatty acid profiles, polyamine patterns and some other traits of the sphingomonads were also examined. Based on the results of these phylogenetic, chemotaxonomic and phenotypic approaches, the transfer of several species from the genus *Sphingomonas* to three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, is proposed. The description of the genus *Sphingomonas* is also emended.

METHODS

Bacterial strains and cultivation. The strains used in this study are listed in Table 1. All of the test strains were cultured aerobically at 30 °C in PY medium, which contained (l⁻¹ distilled water): 10 g peptone, 2 g yeast extract and 1 g MgSO₄·7H₂O (pH 7.0). For fatty acid analysis of sphingolipids, all strains were also cultured in R (Rich) medium. R medium contained (l⁻¹ distilled water): 10 g peptone, 5 g yeast extract, 5 g malt extract, 5 g Bacto-Casamino acids, 2 g beef extract, 2 g glycerol, 50 mg Tween 80 and 1 g MgSO₄·7H₂O (pH 7.0). For the analysis of polyamines, organisms other than *Sphingomonas rosa* IFO 15208^T were cultured aerobically at 30 °C with shaking in PY medium. *S. rosa* IFO 15208^T was cultured at 30 °C in PTYG medium containing (l⁻¹ distilled water): 0.25 g peptone, 0.25 g tryptone, 0.5 g yeast extract, 0.5 g glucose, 0.6 g MgSO₄·7H₂O and 70 mg CaCl₂·2H₂O (pH 7.0).

Cellular fatty acid analysis. Cells were harvested from

cultures at the mid-exponential phase of growth and freeze-dried. Cellular fatty acids were extracted from dried cells (50 mg) by acid methanolysis, purified (Yokota *et al.*, 1993) and analysed by GLC-MS with a Shimadzu GCMS-QP5000 spectrometer combined with a CLASS-5000 MS Workstation computer system. GLC was performed with a Shimadzu GC-17A GC equipped with a BPX70 capillary column (SGE) containing 70% cyanopropyl equivalent modified siloxane (50 m × 0.25 mm). Components were separated with helium as carrier gas at a flow rate of 1.4 ml min⁻¹ under the following thermal conditions: 80 °C for 2 min, 80–150 °C at 15 °C min⁻¹, 150–250 °C at 8 °C min⁻¹ and 250 °C for 5 min.

Glycosphingolipid (GSL) and quinone analyses. GSLs were extracted from dried cells (50 mg) with chloroform/methanol (1:3, v/v) and treated with 0.1 M NaOH at 100 °C for 1 h. The lipids were separated by TLC with Merck Kieselgel 60F₂₅₄ plates using chloroform/methanol/acetic acid/water (100:20:12:5 by vol.) as the developing solvent (Kawahara *et al.*, 1991). The major spot, corresponding to the monosaccharide-type GSL, was scraped from the plate and subjected to fatty acid analysis as noted above.

Analysis of isoprenoid quinones was performed as described previously (Takeuchi *et al.*, 1993).

Polyamine analysis (Hamana & Takeuchi, 1998). Cells were homogenized in 0.5 M perchloric acid (HClO₄) and centrifuged. Polyamines in the resultant supernatant were analysed by HPLC on an L-6000 high-speed liquid chromatograph (Hitachi) as described previously (Hamana *et al.*, 1994).

Biochemical tests and photosynthetic properties. Biochemical tests were performed using API 20NE systems according to the manufacturer's instructions (API bioMérieux).

Bacteriochlorophyll (BChl *a*) was extracted with acetone/methanol (7:2, v/v) from fresh wet cells and analysed by reverse-phase HPLC as described previously (Hiraishi *et al.*, 1998). The *puf* genes, encoding the photosynthetic reaction centre and core light-harvesting complex of purple bacteria, were detected by PCR as described by Hiraishi *et al.* (1998).

16S rDNA sequence determination and phylogenetic analysis. 16S rDNA fragments corresponding to positions 8–1540 in the *Escherichia coli* numbering system (Brosius *et al.*, 1978) were amplified by PCR, sequenced directly with a Thermo Sequenase cycle sequencing kit with 7-deaza-dGTP (Amersham Life Science) and analysed with a LI-COR 4200 (two-dye system) DNA sequencer according to the manufacturer's instructions.

The 16S rDNA sequences were aligned with published sequences available from DDBJ, EMBL and GenBank. Some positions, including gaps and alignment uncertainties, were omitted from the analysis and the final analysis included 1245 bases. Nucleotide substitution rates (K_{nuc} values) (Kimura, 1980) were calculated, a phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) and the topology of the tree was evaluated by the bootstrap resampling method (Felsenstein, 1985) with 1000 replicates with the CLUSTAL W program (Thompson *et al.*, 1994). Maximum-parsimony analysis was performed with PAUP* (version 4.0b4a) (Swofford, 2000). Parsimonious trees were found by 100 replicates of random-sequence addition heuristic searches with tree bisection–reconstruction branch swapping.

Table 1. Bacterial strains used in this study and 16S rDNA sequence accession numbers

Only accession numbers submitted from this study are given. Abbreviations: ATCC, American Type Culture Collection, Manassas, VA, USA; CIP, Collection of the Institut Pasteur, Paris, France; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; GIFU, Department of Microbiology, Gifu University School of Medicine, Gifu, Japan; IAM, Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, Japan; IFO, Institute for Fermentation, Osaka, Japan; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research (RIKEN), Hirosawa, Wako, Japan; LMG, Laboratorium voor Microbiologie, Universiteit Gent, Gent, Belgium; NCIB, National Collection of Industrial Bacteria, NCIMB, Aberdeen, UK; NCPPB, National Collection of Plant-pathogenic Bacteria, Harpenden, UK; NCTC, National Collection of Type Cultures, Central Public Health Laboratory Service, London, UK; SMCC, Subsurface Microbial Culture Collections, Florida State University, FL, USA.

Species	IFO no.	Other designation(s)	Source	Accession no.
<i>Sphingomonas adhaesiva</i>	15099 ^T	ATCC 51229 ^T , DSM 7418 ^T , GIFU 11458 ^T	Sterile water	
<i>Sphingomonas aromaticivorans</i>	15084 ^T	CIP 105152 ^T , SMCC F199 ^T	Coastal plain sediments	
<i>Sphingomonas asaccharolytica</i>	15499 ^T	ATCC 51839 ^T	Apple tree roots	
<i>Sphingomonas capsulata</i>	12533 ^T	ATCC 14666 ^T , DSM 30196 ^T , GIFU 11526 ^T	Stocked distilled water	
<i>Sphingomonas chlorophenolica</i>	16172 ^T	ATCC 33790 ^T , JCM 10275 ^T	Soil	
<i>Sphingomonas echinoides</i>	15742 ^T	ATCC 14820 ^T , DSM 1805 ^T , NCIB 9420 ^T	Plate contaminant	
<i>Sphingomonas herbicidovorans</i>	16415 ^T	DSM 11019 ^T	Soil	AB042233
<i>Sphingomonas macrogoltabidus</i>	15033 ^T	ATCC 51380 ^T , DSM 8826 ^T	Soil	
<i>Sphingomonas mali</i>	15500 ^T	ATCC 51840 ^T , DSM 10565 ^T , JCM 10193 ^T	Apple tree roots	
<i>Sphingomonas parapaucimobilis</i>	15100 ^T	ATCC 51231 ^T , DSM 7463 ^T , GIFU 11387 ^T	Urine	
<i>Sphingomonas paucimobilis</i>	13935 ^T	ATCC 29837 ^T , DSM 1098 ^T , NCTC 11030 ^T	Hospital respirator	
<i>Sphingomonas pruni</i>	15498 ^T	JCM 10277 ^T	<i>Prunus persica</i> roots	
<i>Sphingomonas rosa</i>	15208 ^T	NCPPB 2661 ^T , IAM 14222 ^T	<i>Rosa</i> sp. roots	
<i>Sphingomonas sanguinis</i>	13937 ^T	JCM 7514 ^T	Blood	
<i>Sphingomonas stygia</i>	16085 ^T	ATCC 70280 ^T , CIP 10514 ^T , SMCC B0712 ^T	Coastal plain sediments	
<i>Sphingomonas subarctica</i>	16058 ^T	DSM 10700 ^T , JCM 10398 ^T	Fluidized-bed reactor	
<i>Sphingomonas subterranea</i>	16086 ^T	ATCC 70279 ^T , CIP 105153 ^T , SMCC B0478 ^T	Coastal plain sediments	
<i>Sphingomonas terrae</i>	15098 ^T	ATCC 51381 ^T , CIP 10419 ^T , JCM 10195 ^T	Activated sludge	
<i>Sphingomonas trueperi</i>	16157 ^T	ATCC 12417 ^T , DSM 7225 ^T , LMG 2142 ^T	Soil	
<i>Sphingomonas yanoikuyae</i>	15102 ^T	ATCC 51230 ^T , DSM 7462 ^T , GIFU 9882 ^T	Clinical specimen	
<i>Sphingomonas</i> sp. OGS-47*	15495	—	Paddy soil	AB033948
<i>Sphingomonas</i> sp. Y-22*	15496	—	Paddy soil	AB033946
<i>Sphingomonas</i> sp. Y-39*	15497	—	Paddy soil	AB033947
<i>Sphingomonas</i> sp. Sus-A†	15914	—	Corrosion of stainless steel welds	AB033945
<i>Sphingomonas</i> sp. Cu-B†	15915	—	Underground water	AB033949
<i>Sphingomonas</i> sp. Cu-D†	15917	—	Underground water	AB033950
<i>Blastomonas natatoria</i>	15649 ^T	ATCC 35951 ^T , DSM 3183 ^T , JCM 10396 ^T	Freshwater swimming pool	
<i>Erythrobacter longus</i>	14126 ^T	ATCC 33941 ^T , DSM 6997 ^T , JCM 6170 ^T	Seaweed (<i>Enteromorpha linza</i>)	
<i>Rhizomonas suberifaciens</i>	15211 ^T	ATCC 49355 ^T , JCM 8521 ^T	Corked lettuce root	

* Oyaizu-Masuchi & Komagata (1988).

† Kikuchi *et al.* (1998).

Fig. 1. Phylogenetic tree of *Sphingomonas* species and their relatives, based on a distance matrix analysis of the 16S rDNA sequences (accession numbers given in parentheses). Numbers at the nodes indicate the percentages of bootstrap samplings, derived from 1000 samples, supporting the internal branches (Felsenstein, 1985). Bar, 0.01 K_{nuc} unit.

RESULTS

Phylogenetic analysis

Almost complete 16S rDNA sequences of the *Sphingomonas* strains (1395–1468 nt) were determined and compared with the sequences of 30 established and two invalidated species of the genus *Sphingomonas* and related genera available from the databases. A phylogenetic tree depicting the results of the distance matrix of the 16S rDNA sequences shows that the currently known species of *Sphingomonas* were clearly separated into four clusters, designated here clusters I–IV (Fig. 1). Each of the four clusters could be regarded as a monophyletic and distinct phylogenetic group, as supported by high levels of bootstrap confidence

(> 86%). The six test strains of unidentified *Sphingomonas* species were positioned in clusters I and IV. '*Sphingomonas agrestis*' strain HV3 (Yrjälä *et al.*, 1998) was positioned in cluster II. The levels of sequence similarity among the *Sphingomonas* species within each cluster were more than 95.8%, but those between the species of different clusters were lower, ranging from 92.6 to 96.5% (Table 2). These inter-relationships among the four *Sphingomonas* clusters suggest that each of the clusters corresponds to a single genus from a phylogenetic viewpoint.

Two types of phylogenetic trees were obtained by applying the maximum-parsimony method and both trees also showed that species of the genus *Sphingomonas* were clearly separated into four clusters (data

Table 2. Levels of 16S rDNA sequence similarity among species in the four clusters of *Sphingomonas* and related micro-organisms

Species: 1, *S. paucimobilis*; 2, *S. yanoikuyae*; 3, *S. capsulata*; 4, *S. macrogoltabidus*.

Species and strain	Similarity (%) to species:			
	1	2	3	4
Cluster I (genus <i>Sphingomonas</i>)	95.8–98.7	94.7–95.7	93.2–94.1	93.4–95.3
Cluster II (genus <i>Sphingobium</i>)	93.4–94.9	96.9–97.4	92.6–94.2	93.6–94.3
Cluster III (genus <i>Novosphingobium</i>)	93.2–94.3	94.2–95.1	96.7–97.8	94.8–96.5
Cluster IV (genus <i>Sphingopyxis</i>)	94.1–94.9	94.7–95.7	94.1–94.8	97.6–98.0
<i>Blastomonas natatoria</i> DSM 3183 ^T	94.2	95.3	95.8	96.2
<i>Blastomonas ursincola</i> DSM 9006 ^T	94.2	95.3	95.8	96.2
<i>Erythrobacter litoralis</i> DSM 8509 ^T	93.7	93.8	94.4	94.1
<i>Erythrobacter longus</i> ATCC 33941 ^{T*}	92.9	93.9	94.2	93.7
<i>Erythromicrobium ramosum</i> DSM 8510 ^T	93.6	93.7	94.8	94.9
<i>Porphyrobacter neustonensis</i> DSM 9434 ^T	93.2	93.6	94.7	94.2
<i>Porphyrobacter tepidarius</i> DSM 10594 ^T	93.3	93.4	94.5	94.2
<i>Rhizomonas suberifaciens</i> IFO 15211 ^T	94.5	96.4	94.7	95.6

* Yurkov *et al.* (1994).

Table 3. Nucleotide signatures of 16S rRNA useful for differentiation of the four clusters of *Sphingomonas* and other sphingolipid-containing genera in the α -4 subclass of the *Proteobacteria*

Phylogenetic cluster and genus	No. of species*	Signature at position:				
		52:359	134	593	987:1218	990:1215
Cluster I (genus <i>Sphingomonas</i>)	9 (4)	C:G	G	G	G:C	U:G
Cluster II (genus <i>Sphingobium</i>)	4†	U:A	G	U	A:U	U:G
Cluster III (genus <i>Novosphingobium</i>)	6	C:G	G	U	G:C	U:A
Cluster IV (genus <i>Sphingopyxis</i>)	2 (2)	C:G	G	U	G:C	U:G
Genus <i>Rhizomonas</i>	1	C:G	A	A	G:C	U:G
Genus <i>Blastomonas</i>	2	C:G	G	U	G:C	U:A
Genus <i>Erythrobacter</i>	2	C:G	G	U/C	G:C	U:A
Genus <i>Porphyrobacter</i>	2	C:G	G	U	G:C	U:A

* Figures in parentheses indicate the number of test strains of unidentified *Sphingomonas* species.

† Includes '*Sphingomonas agrestis*' strain HV3 (Yrjälä *et al.*, 1998), which has not been validly described.

Table 4. Cellular fatty acid composition of species in the four clusters of *Sphingomonas* and related micro-organisms

Values are percentages of total fatty acid content. —, Not detected.

Taxon	Non-polar														2-OH			
	14:0	14:1	14:2	15:0	15:1	16:0	16:1	16:2	17:0	17cy	17:1	18:0	18:1	18:2	14:0	15:0	16:0	16:1
Cluster I (genus <i>Sphingomonas</i>)																		
<i>S. adhaesiva</i> IFO 15099 ^T	—	—	—	—	—	13	7	—	—	—	6	—	47	—	28	—	—	—
<i>S. asaccharolytica</i> IFO 15499 ^T	—	—	—	—	—	6	—	—	4	—	16	12	22	—	15	24	—	—
<i>S. echinoides</i> IFO 15742 ^T	—	—	—	4	—	11	2	—	2	—	15	4	33	—	23	6	—	—
<i>S. mali</i> IFO 15500 ^T	—	—	—	—	—	10	—	—	—	—	10	16	36	—	20	8	—	—
<i>S. parapaucimobilis</i> IFO 15100 ^T	—	—	—	—	—	7	6	—	—	—	—	—	56	—	32	—	—	—
<i>S. paucimobilis</i> IFO 13935 ^T	—	—	—	—	—	10	2	—	—	—	—	—	50	—	38	—	—	—
<i>S. pruni</i> IFO 15498 ^T	—	—	—	—	—	10	2	—	—	—	—	—	50	—	27	—	—	—
<i>S. sanguinis</i> IFO 13937 ^T	1	—	—	—	—	11	6	1	—	—	—	—	50	—	31	—	—	—
<i>S. trueperi</i> IFO 16157 ^T	—	—	8	—	—	14	—	2	—	—	2	—	53	—	25	—	—	—
<i>Sphingomonas</i> sp. IFO 15495	—	—	8	8	—	15	—	—	3	—	8	6	31	—	21	4	—	—
<i>Sphingomonas</i> sp. IFO 15496	—	—	7	3	—	17	—	—	2	—	7	7	32	—	22	4	—	—
<i>Sphingomonas</i> sp. IFO 15497	—	—	5	—	—	24	—	—	1	—	2	9	33	—	25	1	—	—
<i>Sphingomonas</i> sp. IFO 15914	—	—	—	—	—	—	—	—	—	8	47	—	12	4	5	20	—	—
Cluster II (genus <i>Sphingobium</i>)																		
<i>S. chlorophenolica</i> IFO 16172 ^T	—	—	—	—	—	7	9	3	—	—	3	2	46	—	26	1	2	3
<i>S. herbicidovorans</i> IFO 16415 ^T	—	—	—	—	—	5	7	—	—	—	—	5	49	—	28	—	3	3
<i>S. yanoikuyae</i> IFO 15102 ^T	—	—	8	—	—	10	8	2	—	—	—	4	32	—	34	—	3	—
Cluster III (genus <i>Novosphingobium</i>)																		
<i>S. aromaticivorans</i> IFO 16084 ^T	—	—	—	—	—	23	—	—	—	—	3	—	42	—	23	4	—	—
<i>S. capsulata</i> IFO 12533 ^T	—	—	—	—	—	5	3	—	—	—	3	—	52	—	35	2	—	—
<i>S. rosa</i> IFO 15208 ^T	—	—	—	—	—	13	7	—	—	—	—	—	50	—	33	—	—	—
<i>S. stygia</i> IFO 16085 ^T	—	—	—	—	—	25	—	—	—	—	11	—	36	—	24	3	—	—
<i>S. subarctica</i> IFO 16058 ^T	—	—	—	—	—	14	10	—	—	—	—	—	52	—	20	—	—	—
<i>S. subterranea</i> IFO 16086 ^T	—	—	—	—	—	6	11	—	—	—	16	—	38	—	21	9	—	—
Cluster IV (genus <i>Sphingopyxis</i>)																		
<i>S. macrogoltabidus</i> IFO 15033 ^T	2	2	—	13	3	10	21	6	—	—	—	2	23	—	10	—	5	—
<i>S. terrae</i> IFO 15098 ^T	—	—	—	4	—	5	11	—	2	5	32	—	18	—	7	18	—	—
<i>Sphingomonas</i> sp. IFO 15915	—	—	—	—	—	15	20	—	—	—	11	3	18	—	10	8	11	—
<i>Sphingomonas</i> sp. IFO 15917	—	—	—	3	—	4	4	—	4	6	34	2	16	—	4	24	—	—
<i>Blastomonas natatoria</i> IFO 15649 ^T	—	—	—	—	—	13	8	—	—	—	16	—	35	10	6	4	—	—
<i>Erythrobacter longus</i> IFO 14126 ^T	—	—	—	3	—	6	8	—	5	6	31	2	18	4	18	2	—	—
<i>Rhizomonas suberifaciens</i> IFO 15211 ^T	—	—	—	3	—	5	5	—	4	6	31	2	16	4	21	2	—	—

Table 5. 2-Hydroxy fatty acid composition in sphingolipids of species of the genus *Sphingomonas* and related genera grown in different media

Percentages of total 2-hydroxy fatty acid content in the medium listed are shown.

Taxon	PY medium				R medium		
	14:0	15:0	16:0	16:1	14:0	15:0	16:0
Cluster I (genus <i>Sphingomonas</i>)							
<i>S. adhaesiva</i> IFO 15099 ^T	100	—	—	—	100	—	—
<i>S. asaccharolytica</i> IFO 15499 ^T	39	61	—	—	18	82	—
<i>S. echinoides</i> IFO 15742 ^T	79	21	—	—	100	—	—
<i>S. mali</i> IFO 15500 ^T	71	29	—	—	75	25	—
<i>S. parapaucimobilis</i> IFO 15100 ^T	100	—	—	—	100	—	—
<i>S. paucimobilis</i> IFO 13935 ^T	100	—	—	—	100	—	—
<i>S. pruni</i> IFO 15498 ^T	86	14	—	—	100	—	—
<i>S. sanguinis</i> IFO 13937 ^T	100	—	—	—	100	—	—
<i>S. trueperi</i> IFO 16157 ^T	98	2	—	—	100	—	—
<i>Sphingomonas</i> sp. IFO 15495	81	19	—	—	100	—	—
<i>Sphingomonas</i> sp. IFO 15496	84	16	—	—	100	—	—
<i>Sphingomonas</i> sp. IFO 15497	96	4	—	—	100	—	—
<i>Sphingomonas</i> sp. IFO 15914	18	82	—	—	25	75	—
Cluster II (genus <i>Sphingobium</i>)							
<i>S. chlorophenolica</i> IFO 16172 ^T	84	3	5	8	100	—	—
<i>S. herbicidovorans</i> IFO 16415 ^T	83	—	8	9	100	—	—
<i>S. yanoikuyae</i> IFO 15102 ^T	92	—	8	—	100	—	—
Cluster III (genus <i>Novosphingobium</i>)							
<i>S. aromaticivorans</i> IFO 16084 ^T	87	13	—	—	100	—	—
<i>S. capsulata</i> IFO 12533 ^T	96	4	—	—	100	—	—
<i>S. rosa</i> IFO 15208 ^T	100	—	—	—	100	—	—
<i>S. stygia</i> IFO 16085 ^T	89	11	—	—	100	—	—
<i>S. subarctica</i> IFO 16058 ^T	87	—	13	—	100	—	—
<i>S. subterranea</i> IFO 16086 ^T	70	30	—	—	100	—	—
Cluster IV (genus <i>Sphingopyxis</i>)							
<i>S. macrogoltabidus</i> IFO 15033 ^T	67	—	33	—	62	—	38
<i>S. terrae</i> IFO 15098 ^T	32	51	12	5	39	52	9
<i>Sphingomonas</i> sp. IFO 15915	34	27	28	1	ND	—	—
<i>Sphingomonas</i> sp. IFO 15917	13	75	7	5	ND	—	—
<i>Blastomonas natatoria</i> IFO 15649 ^T	35	23	15	27	16	79	5
<i>Blastomonas ursincola</i> DSM 9006 ^T	60	26	14	—	59	22	19
<i>Erythrobacter longus</i> IFO 14126 ^T	13	75	6	6	16	74	10
<i>Porphyrobacter neustonensis</i> DSM 9434 ^T	54	24	22	—	ND	—	—
<i>Rhizomonas suberifaciens</i> IFO 15211 ^T	90	10	—	—	84	16	—

ND, Not determined; —, not detected.

not shown). Each of the phylogenetic trees derived from the neighbour-joining and maximum-parsimony methods showed that the phytopathogenic genus *Rhizomonas* (Van Bruggen *et al.*, 1990) and the photosynthetic genera *Blastomonas* (Sly & Cahill, 1997), *Erythrobacter* (Shiba & Simidu, 1982) and *Porphyrobacter* (Fuerst *et al.*, 1993) were nested in the whole cluster of *Sphingomonas* species, but were clearly separated from the above-noted four clusters.

Attempts to find nucleotide signatures specific to the 16S rRNA of the four *Sphingomonas* clusters gave some positive results (Table 3). Cluster I was charac-

terized by the presence of G at position 593, cluster II by a U:A pair at position 52:359, cluster III by a U:A pair at position 990:1215 and cluster IV by a combination of signatures at these positions. This set of nucleotide signatures may be useful for the circumscription of the four clusters and for their placement in different genera.

Cellular fatty acid profiles

As shown in Table 4, most of the *Sphingomonas* strains tested contained 18:1 as the primary component and 16:0 as the second most common type of whole-cell

Table 6. Cellular concentrations of polyamines in *Sphingomonas* species and related micro-organisms

Values are μmol polyamine (g wet cells)⁻¹. Abbreviations: Put, putrescine; Spd, spermidine; HSpd, homospermidine; Agm, agmatine. Data for *Blastomonas natatoria* and *Erythrobacter longus* were taken from Hamana & Takeuchi (1998). —, Not detected. All strains were grown on PY medium with the exception of *S. rosa* IFO 15208^T, which was grown on PTYG medium.

Taxon	Put	Spd	HSpd	Agm
Cluster I (genus <i>Sphingomonas</i>)				
<i>S. adhaesiva</i> IFO 15099 ^T	—	—	1.90	—
<i>S. asaccharolytica</i> IFO 15499 ^T	—	—	1.30	—
<i>S. echinoides</i> IFO 15742 ^T	—	—	1.76	—
<i>S. mali</i> IFO 15500 ^T	—	—	0.75	—
<i>S. parapaucimobilis</i> IFO 15100 ^T	—	—	2.35	—
<i>S. paucimobilis</i> IFO 13953 ^T	—	—	2.55	—
<i>S. pruni</i> IFO 15498 ^T	—	—	1.42	—
<i>S. sanguinis</i> IFO 13937 ^T	—	—	1.97	—
<i>S. trueperi</i> IFO 16157 ^T	—	—	1.10	—
<i>Sphingomonas</i> sp. IFO 15495	—	—	0.98	—
<i>Sphingomonas</i> sp. IFO 15496	—	—	1.46	—
<i>Sphingomonas</i> sp. IFO 15497	—	—	1.20	—
<i>Sphingomonas</i> sp. IFO 15914	—	—	2.10	—
Cluster II (genus <i>Sphingobium</i>)				
<i>S. chlorophenolica</i> IFO 16172 ^T	—	0.95	—	—
<i>S. herbicidovorans</i> IFO 16415 ^T	—	1.25	—	—
<i>S. yanoikuyae</i> IFO 15102 ^T	—	1.57	—	0.03
Cluster III (genus <i>Novosphingobium</i>)				
<i>S. aromaticivorans</i> IFO 15084 ^T	—	1.05	—	—
<i>S. capsulata</i> IFO 12533 ^T	0.10	1.80	—	0.10
<i>S. rosa</i> IFO 15208 ^T	—	1.30	—	—
<i>S. stygia</i> IFO 16085 ^T	—	0.95	—	—
<i>S. subarctica</i> IFO 16058 ^T	—	1.00	—	—
<i>S. subterranea</i> IFO 16086 ^T	—	1.05	—	—
Cluster IV (genus <i>Sphingopyxis</i>)				
<i>S. macrogoltabidus</i> IFO 15033 ^T	0.02	2.10	—	—
<i>S. terrae</i> IFO 15098 ^T	0.02	2.24	—	—
<i>Sphingomonas</i> sp. IFO 15915	—	0.85	—	0.04
<i>Blastomonas natatoria</i> IFO 15649 ^T	—	0.89	—	0.01
<i>Erythrobacter longus</i> IFO 14126 ^T	—	0.43	—	—
<i>Rhizomonas suberifaciens</i> IFO 15211 ^T	0.01	0.58	—	—

fatty acid. Considerable variations were noted in other non-polar fatty acid components among these strains. To determine the usefulness of cellular fatty acid composition, fatty acid profiles of the organisms grown in different media and at different temperatures were examined. However, noticeable differences in cellular fatty acid composition were not found among the test organisms grown under any culture conditions.

GSLs and 2-hydroxy fatty acids

In TLC experiments, GSLs were detected in all test strains of the genera *Sphingomonas*, *Rhizomonas*, *Blastomonas*, *Erythrobacter* and *Porphyrobacter*. The majority of the *Sphingomonas* species contained not only a monosaccharide-type GSL but also one or more

oligosaccharide types of GSL, which were distinguishable from each other in their TLC mobility (data not shown). On the other hand, all test strains of *Rhizomonas*, *Blastomonas*, *Erythrobacter* and *Porphyrobacter* contained monosaccharide-type GSLs only.

GSL fatty acid profiles of the test organisms changed to some extent depending upon the growth medium (Table 5). The fatty acid patterns of cells grown in R medium appeared to be simpler and more useful for taxonomic purposes than those obtained with PY medium. Namely, when grown in R medium, strains of *Sphingomonas* cluster I contained 2-OH 14:0 or 2-OH 15:0 as the major components, those of clusters II and III contained 2-OH 14:0 only and those of cluster IV contained 2-OH 14:0, 2-OH 15:0 and 2-OH 16:0.

Table 7. Some phenotypic characteristics of *Sphingomonas* species and related genera

Characteristics are scored as: +, positive; (+), weakly positive; –, negative; ND, not determined.

Taxon	Nitrate reduction	Aesculin	β -Galactosidase	BChl <i>a</i> production
Cluster I (genus <i>Sphingomonas</i>)				
<i>S. adhaesiva</i> IFO 15099 ^T	–	+	+	–
<i>S. asaccharolytica</i> IFO 15499 ^T	+	+	+	–
<i>S. echinoides</i> IFO 15742 ^T	+	(+)	–	–
<i>S. mali</i> IFO 15500 ^T	+	+	+	–
<i>S. parapaucimobilis</i> IFO 15100 ^T	(+)	+	+	–
<i>S. paucimobilis</i> IFO 13953 ^T	+	+	+	–
<i>S. pruni</i> IFO 15498 ^T	+	+	+	–
<i>S. sanguinis</i> IFO 13937 ^T	+	+	+	–
<i>S. trueperi</i> IFO 16157 ^T	+	+	+	–
<i>Sphingomonas</i> sp. IFO 15495	+	+	+	ND
<i>Sphingomonas</i> sp. IFO 15496	(+)	+	+	ND
<i>Sphingomonas</i> sp. IFO 15497	+	+	+	ND
<i>Sphingomonas</i> sp. IFO 15914	–	+	–	ND
Cluster II (genus <i>Sphingobium</i>)				
<i>S. chlorophenolica</i> IFO 16172 ^T	–	+	+	–
<i>S. herbicidovorans</i> IFO 16415 ^T	–	+	+	–
<i>S. yanoikuyae</i> IFO 15102 ^T	–	+	+	–
Cluster III (genus <i>Novosphingobium</i>)				
<i>S. aromaticivorans</i> IFO 15084 ^T	+	+	+	–
<i>S. capsulata</i> IFO 12533 ^T	+	(+)	+	–
<i>S. rosa</i> IFO 15208 ^T	+	+	+	–
<i>S. stygia</i> IFO 16085 ^T	+	+	(+)	–
<i>S. subarctica</i> IFO 16058 ^T	+	+	+	–
<i>S. subterranea</i> IFO 16086 ^T	+	+	+	–
Cluster IV (genus <i>Sphingopyxis</i>)				
<i>S. macrogoltabidus</i> IFO 15033 ^T	–	+	+	–
<i>S. terrae</i> IFO 15098 ^T	–	–	–	–
<i>Sphingomonas</i> sp. IFO 15915	–	+	–	ND
<i>Sphingomonas</i> sp. IFO 15917	–	+	(+)	ND
<i>Blastomonas natatoria</i> IFO 15649 ^T	–	+	–	+
<i>Blastomonas ursincola</i> DSM 9006 ^T	–	–	–	+
<i>Erythrobacter longus</i> IFO 14126 ^T	–	+	–	+
<i>Rhizomonas suberifaciens</i> IFO 15211 ^T	–	+	–	–

Rhizomonas suberifaciens contained 2-OH 14:0 and 2-OH 15:0 and the *Blastomonas*, *Erythromonas* and *Porphyrobacter* species contained 2-OH 14:0, 2-OH 15:0 and 2-OH 16:0. These results indicate that, when the culture conditions are standardized, fatty acid analysis provides more reliable information for determining the taxonomy of sphingomonads.

Polyamine patterns

As shown in Table 6, homospermidine was detected as the major polyamine only in species belonging to cluster I. All other strains examined contained spermidine as the major polyamine. In a previous study, Takeuchi *et al.* (1995) reported that *S. asaccharolytica* had spermidine as the predominant component, but in this study, the major component

detected was homospermidine [1.30 μ mol (g wet cell)^{–1}], as described by Kämpfer *et al.* (1997).

Other characteristics

In experiments with the API 20NE system, all test strains exhibited negative reactions for indole production, glucose fermentation, arginine dihydrolase, urease and gelatin hydrolysis. Representative strains of the four *Sphingomonas* clusters tested assimilated arabinose, D-mannose, maltose, D-mannitol, *N*-acetylglucosamine, adipate, *n*-caproate, citrate, gluconate and DL-malic acid. Some phenotypic differences were observed among the test strains (nitrate reduction, aesculin hydrolysis and β -galactosidase activity) (Table 7). In particular, it is worth noting that most test strains of *Sphingomonas* cluster I and all strains of cluster III were positive for nitrate reduction, whereas

none of the test strains of clusters II and IV reduced nitrate to nitrite. BChl *a* was detected from the test strains of *Blastomonas* and *Erythrobacter*, but was not detected in appreciable amounts from those of *Sphingomonas* or *Rhizomonas*.

PCR detection of *puf* genes from *Sphingomonas* strains was unsuccessful. In all test strains of *Sphingomonas*, *Rhizomonas*, *Blastomonas* and *Erythrobacter*, ubiquinone-10 was the major respiratory quinone, accounting for more than 93% of the total quinone content.

DISCUSSION

Since the proposal of the genus *Sphingomonas* by Yabuuchi *et al.* (1990), large numbers of novel species from various environments have been added to this genus. As a result, the genus *Sphingomonas* has come to encompass a relatively broad range of species with respect to physiology, genetics and ecology. One of the most characteristic features of the genus *Sphingomonas* is the presence of GSLs. Recently, Yabuuchi *et al.* (1999) proposed that *Blastomonas natatoria*, *Erythromonas ursincola* and *Rhizomonas suberifaciens* should be reclassified as members of the genus *Sphingomonas* in view of the fact that GSLs are present in all these species. However, GSLs are found in species of other genera of the α -4 subclass of the *Proteobacteria*, such as *Erythrobacter* and *Porphyrobacter*, as reported here and elsewhere (Fuerst *et al.*, 1993). Although the presence of GSLs is an important chemotaxonomic criterion, the assignment of species to the genus *Sphingomonas* should not be based on this criterion alone. *Blastomonas natatoria*, *Erythromonas ursincola* and *Rhizomonas suberifaciens* not only form a line of descent distinct from the four clusters of *Sphingomonas* species (Fig. 1), but also differ from the latter clusters in having photosynthetic or phytopathogenic traits. Hiraishi *et al.* (2000) have recently proposed to emend the description of *Blastomonas* as a genus of aerobic photosynthetic bacteria and to reclassify *Erythromonas ursincola* as a species of the genus *Blastomonas*. Thus, it is concluded that the genera *Blastomonas* and *Rhizomonas* should be retained as they stand, because of the taxonomic importance of photosynthetic and phytopathogenic properties and the phylogenetic evidence for their taxonomic positions. *Blastomonas* and *Rhizomonas* can be distinguished from all *Sphingomonas* species at the generic level by a combined use of chemotaxonomic and phenotypic markers, as discussed below.

As reported in this study and previously (Yurkov *et al.*, 1997), 16S rDNA-based phylogenetic analysis indicated that members of the genus *Sphingomonas* can be divided into four phylogenetic groups, here designated clusters I–IV. All these clusters were considered to be monophyletic, based on high levels (86%) of bootstrap confidence. While the levels of sequence similarity among species within each cluster were more than 95.8%, the similarity values between species of

different clusters were lower, ranging from 92.6 to 96.5%. The levels of similarity among the four clusters appear to be low enough to justify different generic assignments. A comparative analysis of the primary and secondary 16S rRNA structures has also revealed that the four clusters can be identified on the basis of a set of characteristic nucleotide signatures (Table 3).

Some chemotaxonomic and phenotypic features support the phylogenetic evidence that the four clusters of *Sphingomonas* represent different genera. Firstly, 2-hydroxy fatty acid profiles of GSLs provide valuable information for the differentiation of the four clusters. Members of cluster I contained 2-OH 14:0 and/or 2-OH 15:0 as the major components, whereas those of cluster IV were characterized by the presence of 2-OH 15:0 and/or 2-OH 16:0 in addition to 2-OH 14:0. Members of clusters II and III contained 2-OH 14:0 only as the major component, as shown in Table 5, although minor components were detected when grown in PY medium. Secondly, polyamine patterns are quite useful in distinguishing cluster I from all other clusters: the major polyamine was homospermidine in cluster I and spermidine in all other clusters. Thirdly, the activity of nitrate reduction was a useful character to distinguish the four clusters from each other. Therefore, the four clusters of the genus *Sphingomonas* can be differentiated clearly by a combination of these chemotaxonomic and phenotypic markers. The other sphingolipid-containing genera in the α -4 subclass, *Blastomonas*, *Erythrobacter* and *Porphyrobacter*, are distinguishable from any of the *Sphingomonas* clusters by a combination of sphingolipid fatty acid composition, polyamine pattern, BChl *a* production, nitrate reduction and β -galactosidase activity.

In conclusion, the creation of three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, is proposed to accommodate the species of clusters II, III and IV, respectively. According to Principle and Rule 39a of the Bacteriological Code (Lapage *et al.*, 1992), the genus *Sphingomonas*, type species *S. paucimobilis*, is retained and emended.

Emended description of the genus *Sphingomonas* (Yabuuchi *et al.* 1990) emend. Yabuuchi *et al.* 1999

Cells are Gram-negative, non-sporulating rods measuring 0.3–0.8 $\times$ 1.0–1.9 μ m. Motile or non-motile. Colonies are yellow or off-white. Strictly aerobic and chemo-organotrophic. Catalase-positive. Respiratory quinone is predominantly Q-10. Major fatty acids are 18:1, saturated 16:0 and/or 17:1. Major 2-hydroxy fatty acids are 2-OH 14:0 or 2-OH 15:0. GSLs are present. Homospermidine is the major polyamine component. The DNA G + C content is 62–68 mol%. Phylogenetic position is in the α -4 subclass of *Proteobacteria*. Characteristic 16S rRNA signatures are found at positions 52:359 (C:G), 134 (G), 593 (G), 987:1218 (G:C) and 990:1215 (U:G). Habitat: soil and clinical specimens. Nine species are assigned to

the emended genus *Sphingomonas*: *Sphingomonas adhaesiva*, *Sphingomonas asaccharolytica*, *Sphingomonas echinoides*, *Sphingomonas mali*, *Sphingomonas parapaucimobilis*, *Sphingomonas paucimobilis*, *Sphingomonas pruni*, *Sphingomonas sanguinis* and *Sphingomonas trueperi*. Type species: *Sphingomonas paucimobilis* Yabuuchi *et al.* 1990.

Description of *Sphingobium* gen. nov.

Sphingobium (Sphin.go'bi.um. Gr. adj. *Sphingos* of sphinx; Gr. n. *bios* life, living; N.L. fem. n. *Sphingobium* sphingosine-containing life).

Cells are Gram-negative, non-sporulating rods, measuring $0.3\text{--}0.7 \times 1.0\text{--}3.5\text{ }\mu\text{m}$. Motile or non-motile. Colonies are yellow or whitish-brown. Strictly aerobic and chemo-organotrophic. Catalase-positive. Nitrate is not reduced. Ubiquinone is predominantly Q-10. The dominant fatty acid is 18:1 and 16:0 is a minor component. Major 2-hydroxy fatty acid is 2-OH 14:0. GSLs are present. Spermidine is the major polyamine component. The DNA G+C content is 62–67 mol %. Characteristic 16S rRNA signatures are present at positions 52:359 (U:A), 134 (G), 593 (U), 987:1218 (A:U) and 990:1215 (U:G). Habitat: soil, pentachlorophenol-contaminated soil and clinical specimens. The type species is *Sphingobium yanoikuyae* (Yabuuchi *et al.* 1990) Takeuchi *et al.* 2001.

Description of *Sphingobium chlorophenolicum* comb. nov.

With the addition of the details listed in the genus description above, the description is the same as that given by Nohynek *et al.* (1995).

Description of *Sphingobium herbicidovorans* comb. nov.

With the addition of the details listed in the genus description above, the description is the same as that given by Zipper *et al.* (1996).

Description of *Sphingobium yanoikuyae* comb. nov.

With the addition of the details listed in the genus description above, the description is the same as that given by Yabuuchi *et al.* (1990).

Description of *Novosphingobium* gen. nov.

Novosphingobium (No.vo.sphin.go'bi.um. L. adj. *novus* new; Gr. adj. *sphingos* of sphinx; Gr. n. *bios* life, living; N.L. fem. n. *Novosphingobium* new sphingosine-containing life).

Cells are Gram-negative, non-sporulating rods measuring $0.3\text{--}0.5 \times 1.0\text{--}3.0\text{ }\mu\text{m}$. Motile or non-motile. Colonies are yellow or whitish-brown. Strictly aerobic and chemo-organotrophic. Catalase-positive. Nitrate is reduced. Ubiquinone is predominantly Q-10. Satu-

rated 18:1 is the dominant fatty acid and 16:0 is present as a minor component. Major 2-hydroxy fatty acid is 2-OH 14:0. GSLs are present. Spermidine is the major polyamine component. The DNA G+C content is 62–67 mol %. Characteristic 16S rRNA signatures are present at positions 52:359 (U:A), 134 (G), 593 (U), 987:1218 (A:U) and 990:1215 (U:G). Habitat: soil, roots of rose tree, clinical specimens, stocked distilled water, coastal plain sediments and fluidized-bed reactor. The type species is *Novosphingobium capsulatum* (Yabuuchi *et al.* 1990) Takeuchi *et al.* 2001.

Description of *Novosphingobium aromaticivorum* comb. nov.

With the addition of the details listed in the genus description above, the description is the same as that given by Balkwill *et al.* (1997).

Description of *Novosphingobium capsulatum* comb. nov.

With the addition of the details listed in the genus description above, the description is the same as that given by Yabuuchi *et al.* (1990).

Description of *Novosphingobium roseae* comb. nov.

With the addition of the details listed in the genus description above, the description is the same as that given by Takeuchi *et al.* (1995).

Description of *Novosphingobium stygiae* comb. nov.

With the addition of the details listed in the genus description above, the description is the same as that given by Balkwill *et al.* (1997).

Description of *Novosphingobium subarcticum* comb. nov.

With the addition of the details listed in the genus description above, the description is the same as that given by Nohynek *et al.* (1996).

Description of *Novosphingobium subterraneae* comb. nov.

With the addition of the details listed in the genus description above, the description is the same as that given by Balkwill *et al.* (1997).

Description of *Sphingopyxis* gen. nov.

Sphingopyxis (Sphin.go.pyx'is. Gr. adj. *sphingos* of sphinx; Gr. n. *pyxis* jewel box; N.L. fem. n. *Sphingopyxis* jewel box of sphingolipid-containing life).

Cells are Gram-positive, non-sporulating rods measuring $0.3\text{--}0.5 \times 0.9\text{ }\mu\text{m}$. Motile or non-motile.

Colonies are yellow or whitish-brown. Strictly aerobic and chemo-organotrophic. Catalase-positive. Nitrate is not reduced. Ubiquinone is predominantly Q-10. Major fatty acids are 18:1, saturated 16:0 and 16:1. Major 2-hydroxy fatty acids are 2-OH 14:0, 2-OH 15:0 and/or 2-OH 16:0. GSLs are present. Spermidine is the major polyamine component. The DNA G+C content is 63.0–65.0 mol%. Characteristic 16S rRNA signatures are present at positions 52:359 (C:G), 134 (G), 593 (U), 987:1218 (G:C) and 990:1215 (U:G). Habitat: soil and underground water. The type species is *Sphingopyxis macrogoltabida* (Takeuchi *et al.* 1993) Takeuchi *et al.* 2001.

Description of *Sphingopyxis macrogoltabida* comb. nov.

With the addition of the details listed in the genus description above, the description is the same as that given by Takeuchi *et al.* (1993).

Description of *Sphingopyxis terrae* comb. nov.

With the addition of the details listed in the genus description above, the description is the same as that given by Takeuchi *et al.* (1993).

ACKNOWLEDGEMENTS

We are grateful to Dr Kazunori Hatano, Institute for Fermentation, Osaka, for valuable suggestions and discussions. We thank Ms Yayoi Yamaguchi for her help with the fatty acid analysis. A part of this study (by A.H.) was supported by a Grand-in-Aid from the Ministry of Health and Welfare, Japan (Research in Environmental Health, H11 Seikatsu-015).

REFERENCES

Balkwill, D. L., Drake, G. R., Reeves, R. H. & 7 other authors (1997). Taxonomic study of aromatic-degrading bacteria from deep-terrestrial-subsurface sediments and description of *Sphingomonas aromaticivorans* sp. nov., *Sphingomonas subterranea* sp. nov., and *Sphingomonas stygia* sp. nov. *Int J Syst Bacteriol* **47**, 191–201.

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

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.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Fuerst, J. A., Hawkins, J. A., Holmes, A., Sly, L. I., Moore, C. J. & Stackebrandt, E. (1993). *Porphyrobacter neustonensis* gen. nov., sp. nov., an aerobic bacteriochlorophyll-synthesizing budding bacterium from fresh water. *Int J Syst Bacteriol* **43**, 125–134.

Hamana, K. & Takeuchi, M. (1998). Polyamine profiles as chemotaxonomic marker within alpha, beta, gamma, delta, and epsilon subclasses of class *Proteobacteria*: distribution of 2-

hydroxyputrescine and homospermidine. *Microbiol Cult Coll* **14**, 1–14.

Hamana, K., Sakane, T. & Yokota, A. (1994). A polyamine analysis of the genera *Aquaspirillum*, *Magnetospirillum*, *Oceanospirillum* and *Spirillum*. *J Gen Appl Microbiol* **40**, 75–82.

Hiraishi, A., Nagashima, K. V. P., Matsuura, K., Shimada, K., Takaichi, S., Wakao, N. & Katayama, Y. (1998). Phylogeny and photosynthetic features of *Thiobacillus acidophilus* and related acidophilic bacteria: its transfer to the genus *Acidiphilium* as *Acidiphilium acidophilum* comb. nov. *Int J Syst Bacteriol* **48**, 1389–1398.

Hiraishi, A., Kuraishi, H. & Kawahara, K. (2000). Emendation of the description of *Blastomonas natatoria* (Sly 1985) Sly and Cahill 1997 as an aerobic photosynthetic bacterium and reclassification of *Erythromonas ursincola* Yurkov *et al.* 1997 as *Blastomonas ursincola* comb. nov. *Int J Syst Evol Microbiol* **50**, 1113–1118.

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.

Kawahara, K., Seydel, U., Matsuura, M., Danbara, H., Rietschel, E. T. & Zähringer, U. (1991). Chemical structure of glyco-sphingolipids isolated from *Sphingomonas paucimobilis*. *FEBS Lett* **292**, 107–110.

Kikuchi, Y., Ozawa, M., Tohmoto, K., Kanamaru, T. & Sakane, T. (1998). Corrosion behavior of copper and its welds by bacteria in underground water. *Trans Joining Welding Res Inst Osaka Univ* **27**, 53–62.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Lapage, S. P., Sneath, P. H. A., Lessel, E. F., Skerman, V. B. D., Seeliger, H. P. R. & Clark, W. A. (editors). (1992). *International Code of Nomenclature of Bacteria (1990 Revision)*. Washington, DC: American Society for Microbiology.

Nohynek, L. J., Suhonen, E. L., Nurmiäho-Lassila, E.-L., Hantula, J. & Salkinoja-Salonen, M. (1995). Description of four pentachlorophenol-degrading bacterial strains as *Sphingomonas chlorophenolica* sp. nov. *Syst Appl Microbiol* **18**, 527–538.

Nohynek, L. J., Nurmiäho-Lassila, E.-L., Suhonen, E. L., Busse, H.-J., Mohammadi, M., Hantula, J., Rainey, F. & Salkinoja-Salonen, M. S. (1996). Description of chlorophenol-degrading *Pseudomonas* sp. strains KF1^T, KF3, and NK1 as a new species of the genus *Sphingomonas*, *Sphingomonas subarctica* sp. nov. *Int J Syst Bacteriol* **46**, 1042–1055.

Oyaizu-Masuchi, Y. & Komagata, K. (1988). Isolation of free-living nitrogen-fixing bacteria from the rhizosphere of rice. *J Gen Appl Microbiol* **34**, 127–164.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Segers, P., Vancanneyt, M., Pot, B., Torck, U., Hoste, B., Dewettinck, D., Falsen, E., Kersters, K. & De Vos, P. (1994). Classification of *Pseudomonas diminuta* Leifson and Hugh 1954 and *Pseudomonas vesicularis* Büsing, Döll, and Freytag 1953 in *Brevundimonas* gen. nov. as *Brevundimonas diminuta* comb. nov. and *Brevundimonas vesicularis* comb. nov., respectively. *Int J Syst Bacteriol* **44**, 499–510.

Shiba, T. & Simidu, U. (1982). *Erythrobacter longus* gen. nov., sp. nov., an aerobic bacterium which contains bacteriochlorophyll a. *Int J Syst Bacteriol* **32**, 211–217.

- Sly, L. I. & Cahill, M. M. (1997). Transfer of *Blastobacter natatorius* (Sly 1985) to the genus *Blastomonas* gen. nov. as *Blastomonas natatoria* comb. nov. *Int J Syst Bacteriol* **47**, 566–568.
- Stackebrandt, E., Murray, R. G. E. & Trüper, H. G. (1988). *Proteobacteria* classis nov., a name for the phylogenetic taxon that includes the “purple bacteria and their relatives”. *Int J Syst Bacteriol* **38**, 321–325.
- Swofford, D. L. (2000). PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4.0b4a. Sunderland, MA: Sinauer Associates.
- Takeuchi, M., Kawai, F., Shimada, Y. & Yokota, A. (1993). Taxonomic study of polyethylene glycol-utilizing bacteria: emended description of the genus *Sphingomonas* and new descriptions of *Sphingomonas macrogoltabidus* sp. nov., *Sphingomonas sanguis* sp. nov., and *Sphingomonas terrae* sp. nov. *Syst Appl Microbiol* **16**, 227–238.
- Takeuchi, M., Sawada, H., Oyaizu, H. & Yokota, A. (1994). Phylogenetic evidence for *Sphingomonas* and *Rhizomonas* as nonphotosynthetic members of the alpha-4 subclass of the *Proteobacteria*. *Int J Syst Bacteriol* **44**, 308–314.
- Takeuchi, M., Sakane, T., Yanagi, M., Yamasato, K., Hamana, K. & Yokota, A. (1995). Taxonomic study of bacteria isolated from plants: proposal of *Sphingomonas rosa* sp. nov., *Sphingomonas pruni* sp. nov., *Sphingomonas asaccharolytica* sp. nov., and *Sphingomonas mali* sp. nov. *Int J Syst Bacteriol* **45**, 334–341.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Van Bruggen, A. H. C., Jochimsen, K. N. & Brown, P. R. (1990). *Rhizomonas suberifaciens* gen. nov., sp. nov., the causal agent of corky root of lettuce. *Int J Syst Bacteriol* **40**, 175–188.
- Van Bruggen, A. H. C., Jochimsen, K. N., Steinberger, E. M., Segers, P. & Gillis, M. (1993). Classification of *Rhizomonas suberifaciens*, an unnamed *Rhizomonas* species, and *Sphingomonas* spp. in rRNA superfamily IV. *Int J Syst Bacteriol* **43**, 1–7.
- Yabuuchi, E., Yano, I., Oyaizu, H., Hashimoto, Y., Ezaki, T. & Yamamoto, H. (1990). Proposals of *Sphingomonas paucimobilis* gen. nov. and comb. nov., *Sphingomonas parapaucimobilis* sp. nov., *Sphingomonas yanoikuyae* sp. nov., *Sphingomonas adhaesiva* sp. nov., *Sphingomonas capsulata* comb. nov., and two genospecies of the genus *Sphingomonas*. *Microbiol Immunol* **34**, 99–119.
- Yabuuchi, E., Kosako, Y., Naka, T., Suzuki, S. & Yano, I. (1999). Proposal of *Sphingomonas suberifaciens* (van Bruggen, Jochimsen and Brown 1990) comb. nov., *Sphingomonas natatoria* (Sly 1985) comb. nov., *Sphingomonas ursincola* (Yurkov *et al.* 1997) comb. nov., and emendation of the genus *Sphingomonas*. *Microbiol Immunol* **43**, 339–349.
- Yokota, A., Takeuchi, M. & Weiss, N. (1993). Proposal of two new species in the genus *Microbacterium*: *Microbacterium dextranolyticum* sp. nov. and *Microbacterium aurum* sp. nov. *Int J Syst Bacteriol* **43**, 549–554.
- Yrjölä, K., Suomalainen, S., Suhonen, E. L., Kilpi, S., Paulin, L. & Romantschuk, M. (1998). Characterization and reclassification of an aromatic- and chloroaromatic-degrading *Pseudomonas* sp., strain HV3, as *Sphingomonas* sp. HV3. *Int J Syst Bacteriol* **48**, 1057–1062.
- Yurkov, V., Stackebrandt, E., Holmes, A. & 7 other authors (1994). Phylogenetic positions of novel aerobic, bacteriochlorophyll *a*-containing bacteria and description of *Roseococcus thiosulfatophilus* gen. nov., sp. nov., *Erythromicrobium ramosum* gen. nov., sp. nov., and *Erythrobacter litoralis* sp. nov. *Int J Syst Bacteriol* **44**, 427–434.
- Yurkov, V., Stackebrandt, E., Buss, O., Vermeglio, A., Gorlenko, V. & Beatty, J. T. (1997). Reorganization of the genus *Erythromicrobium*: description of “*Erythromicrobium sibiricum*” as *Sandaracinobacter sibiricus* gen. nov., sp. nov., and of “*Erythromicrobium ursincola*” as *Erythromonas ursincola* gen. nov., sp. nov. *Int J Syst Bacteriol* **47**, 1172–1178.
- Zipper, C., Nickel, K., Angst, W. & Kohler, H. (1996). Complete microbial degradation of both enantiomers of the chiral herbicide mecoprop [(*RS*)-2-(4-chloro-2-methylphenoxy) propionic acid] in an enantioselective manner by *Sphingomonas herbicidovorans* sp. nov. *Appl Environ Microbiol* **62**, 4318–4322.