NOTE

Reclassification of *Pseudomonas echinoides* Heumann 1962, 343^{AL}, in the genus *Sphingomonas* as *Sphingomonas echinoides* comb. nov.

Ewald B. M. Denner,¹ Peter Kämpfer,² Hans-Jürgen Busse^{1,3} and Edward R. B. Moore⁴

Author for correspondence: Ewald B. M. Denner. Tel: +43 1 4277 54632. Fax: +43 1 4277 12876. e-mail: denner@gem.univie.ac.at

¹ Institut für Mikrobiologie und Genetik, Universität Wien, A-1030 Wien, Austria

² Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, D-35390 Giessen, Germany

³ Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität, Veterinärplatz 1, A-1210 Wien, Austria

⁴ Bereich Mikrobiologie, Gesellschaft für Biotechnologische Forschung, D-38124 Braunschweig, Germany

[*Pseudomonas*] *echinoides* DSM 1805^T (= ATCC 14820^T, DSM 50409^T, ICBP 2835^T, NCIB 9420^T) has been reinvestigated to clarify its taxonomic position. 16S rDNA sequence comparisons demonstrated that this species clusters phylogenetically with species of the genus *Sphingomonas*. Investigation of fatty acid patterns, polar lipid profiles, polyamine patterns and quinone systems supported this delineation. Substrate utilization profiles and biochemical characteristics displayed no distinct overall similarity to any validly described species of the genus *Sphingomonas*. Therefore, the reclassification of [*Pseudomonas*] *echinoides* as *Sphingomonas echinoides* comb. nov. is proposed, based upon the estimated phylogenetic position derived from 16S rRNA gene sequence data, chemotaxonomic data and previously published genomic DNA G+C content data.

Keywords: *Sphingomonas echinoides* comb. nov., phylogenetic position, chemotaxonomy, phenotypical characteristics, reclassification

[*Pseudomonas*] *echinoides* was initially proposed as the name for a yellow-pigmented bacterial strain, designated B13 (Heumann, 1962a), which had been isolated as a plate contaminant (Heumann, 1960). The species description was based on morphological characteristics, i.e. a Gram-negative bacillus with polar flagellation, as well as several physiological traits. Further investigations concerned the genetics, ultra-structure and carotenoid composition of this species (Heumann, 1962b, 1972; Marx & Heumann, 1962; Heumann & Marx, 1964; Czygan & Heumann, 1967; Mayer *et al.*, 1972). The species name has been registered in the Approved Lists of bacterial names (Skerman *et al.*, 1980). Previously, it was recognized that [*P.*] *echinoides* is related genetically to *Sphingomonas paucimobilis* (basonym *Pseudomonas paucimobilis*) and related organisms (Segers *et al.*, 1994).

Jenkins *et al.* (1979) identified the yellow pigment of [*P.*] *paucimobilis*, [*P.*] *azotocolligans* (recently re-

classified as *Sphingomonas trueperi*; Kämpfer *et al.*, 1997) and [*P.*] *echinoides* as the carotenoid nostoxanthin, so far not detected in other yellow-pigmented bacteria. A degree of genetic relationship between [*P.*] *echinoides*, *S. paucimobilis*, *Sphingomonas capsulata* (basonym [*Flavobacterium*] *capsulatum*) and *S. trueperi* was observed and the reclassification of these four species in a new genus has been proposed by Owen & Jackman (1982) based on data from DNA–DNA hybridization studies. DNA–rRNA hybridization studies have clearly distinguished [*P.*] *echinoides* from the genus *Pseudomonas sensu stricto* and placed it close to [*P.*] *paucimobilis* and [*F.*] *capsulatum* (De Vos *et al.*, 1989). The G+C content of the genomic DNA of [*P.*] *echinoides* was determined to be 65.8 mol% (Owen & Jackman, 1982). More recently, using DNA–rRNA hybridization, Segers *et al.* (1994) observed that [*P.*] *echinoides* clusters with species of the genus *Sphingomonas* (Yabuuchi *et al.*, 1990).

The genus *Sphingomonas*, as it is currently described, contains phylogenetically heterogeneous species and can be divided into several sub-groups (Van Bruggen

The EMBL accession number for the *Sphingomonas echinoides* 16S rDNA sequence is AJ012461.

Fig. 1. An unrooted dendrogram of the estimated phylogenetic relationships between *Sphingomonas* (*Pseudomonas*) *echinoides* DSM 1805^T and other member species of the genus *Sphingomonas*, as derived by evolutionary distance calculations of pairwise comparisons of nearly complete 16S rRNA gene sequences, including the Jukes & Cantor (1969) correction factor. Nucleotide insertions/deletions were weighted at 0.5 × the value of nucleotide substitutions. The dendrogram was generated using the least-squares distance algorithm of the FITCH program in PHYLIP (Phylogeny Inference Package, version 3.5c). Sequences were extracted from the EMBL database and their accession numbers are as follows: *S. adhaesiva* JCM 7370^T, X72720; *S. aromaticivorans* SMCC F199^T, U20765; *S. asaccharolytica* IFO 15499^T, Y09639; *S. capsulata* IFO 12533^T, D16147; *S. chlorophenolica* ATCC 33790^T, X87161; *S. mali* IFO 15500^T, Y09638; *S. macrogoltabidus* IFO 15033^T, D13723; *S. parapaucimobilis* JCM 7510^T, X72721; *S. paucimobilis* DSM 1098^T, X72722; *S. pruni* IFO 15498^T, Y09637; *S. rosa* IFO 15208^T, D13945; *S. sanguinis* IFO 13937^T, D13726; *S. stygia* SMCC B0712^T, U20775; *S. subarctica* HAMBI 2110^T, X94102; *S. subterranea* SMCC B0478^T, U20773; *S. terrae* IFO 15098^T, D13727; *S. trueperi* ATCC 12417^T, X97776; *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; *Poryphyrobacter neustonensis* ACM 2844^T, L01785. The bar represents the scale of estimated evolutionary distance (i.e. a mean of 5 substitutions per 101 nt) from the point of divergence of the 16S rRNA gene sequences.

et al., 1993; Takeuchi *et al.*, 1994; Balkwill *et al.*, 1997). Members of the genus *Sphingomonas* are characterized by the presence of the respiratory isoprenoid quinone, ubiquinone-10 (Q-10), a unique sphingoglycolipid, the presence of 2-hydroxymyristic acid (14:0 2-OH), the absence of 3-hydroxy fatty acids and spermidine or sym-homospermidine as the major polyamine (Yabuuchi *et al.*, 1990; Takeuchi *et al.*, 1993, 1995; Nohynek *et al.*, 1996; Balkwill *et al.*, 1997; Kämpfer *et al.*, 1997). Therefore, the presence of ubiquinone Q-10, 2-hydroxymyristic acid (14:0 2-OH) and the lack of 3-hydroxy fatty acids are characteristic for all species of the genus *Sphingomonas* analysed so far. Improved resolution for discrimination within the genus *Sphingomonas* can be obtained by the determination of the polyamine content. Species of the genus *Sphingomonas sensu stricto*, as suggested by Takeuchi *et al.* (1995), including *S. paucimobilis*, *Sphingomonas parapaucimobilis*, *Sphingomonas sanguinis*, *Sphingomonas asaccharolytica*, *Sphingomonas adhaesiva*, *Sphingomonas mali*, *Sphingomonas pruni* and *S. trueperi* (Kämpfer *et al.*, 1997), are located on a separate branch of the genus (Fig. 1). These species are characterized by the presence of sym-homospermidine as the predominant polyamine. Other member species of the genus *Sphingomonas*, which are located on different branches, have a polyamine pattern that contains spermidine as the

major compound (Busse & Auling, 1988; Seger *et al.*, 1994; Hamana & Matsuzaki, 1991).

To our knowledge only one strain of [*P.*] *echinoides* has been described and is available in culture collections. Considering the recent description of the genus *Sphingomonas*, we have reinvestigated the taxonomic position of [*P.*] *echinoides* DSM 1805^T (= ATCC 14820^T, DSM 50409^T, ICBP 2835^T, NCIB 9420^T) with respect to all validly described *Sphingomonas* species.

[*P.*] *echinoides* has been characterized by determination of polyamine content, using HPLC (Busse *et al.*, 1997), the respiratory isoprenoid quinone system (Kroppenstedt, 1982) and the polar lipid pattern, using TLC (Tindall, 1990), and by analysis of fatty acid composition, using GLC (Kämpfer *et al.*, 1992), physiological and biochemical characterizations (Kämpfer & Altwegg, 1992; Kämpfer *et al.*, 1991), and 16S rRNA gene sequence determination, analysis (Moore *et al.*, 1993) and comparison with related member species of the genus *Sphingomonas*. The nearly complete 16S rRNA gene sequence (positions 28–1524, *E. coli* 16S rRNA gene sequence numbering) of [*P.*] *echinoides* (1442 nt) was determined by direct sequencing of 16S rDNA after PCR amplification. Comparative analysis and estimation of phylogenetic relationships demonstrated that [*P.*] *echinoides* clusters

Table 1. Sequence similarities between the 16S rRNA gene of *Sphingomonas* (*Pseudomonas*) *echinoides* DSM 1805^T and member species of the genus *Sphingomonas* as well as species of the α -subclass of the *Proteobacteria*

Species	Strain*	Sequence similarity (%) with <i>S. echinoides</i>
<i>S. paucimobilis</i>	DSM 1098 ^T	94.3
<i>S. parapaucimobilis</i>	JCM 7510 ^T	94.2
<i>S. trueperi</i>	LMG 2142 ^T	93.8
<i>S. adhaesiva</i>	JCM 7370 ^T	94.6
<i>S. sanguinis</i>	IFO 13937 ^T	93.9
<i>S. mali</i>	IFO 15500 ^T	96.4
<i>S. pruni</i>	IFO 15498 ^T	96.2
<i>S. asaccharolytica</i>	IFO 15499 ^T	95.9
<i>S. yanoikuyae</i>	JCM 7370 ^T	91.9
<i>S. chlorophenolica</i>	ATCC 33790 ^T	91.3
<i>S. aromaticivorans</i>	SMCC F199 ^T	92.4
<i>S. subterranea</i>	SMCC B0478 ^T	92.0
<i>S. macrogoltabidus</i>	IFO 15033 ^T	92.3
<i>S. terrae</i>	IFO 15098 ^T	91.4
<i>S. capsulata</i>	ATCC 14666 ^T	92.1
<i>S. stygia</i>	SMCC B0712 ^T	92.4
<i>S. subarctica</i>	HAMBI 2110 ^T	92.1
<i>S. rosa</i>	IFO 15208 ^T	92.6
<i>Rhizomonas suberifaciens</i>	IFO 15211 ^T	92.9
<i>Erythrobacter longus</i>	JCM 6170 ^T	90.8
<i>Brevundimonas diminuta</i>	ATCC 11568 ^T	85.6
<i>Agrobacterium tumefaciens</i>	DSM 30150	86.4

* ATCC, American Type Culture Collection, Manassas, VA, USA; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; HAMBI, Culture Collection of Faculty of Agriculture and Forestry, University of Helsinki, Finland; IFO, Institute of Fermentation, Yodogawaku, Osaka, Japan; JCM, Japan Collection of Microorganisms, Wako, Saitama, Japan; LMG, Laboratorium voor Mikrobiologie, Universiteit Gent, Gent, Belgium; SMCC, Subsurface Microbial Culture Collection, Florida State University, USA.

with species of the genus *Sphingomonas* (Fig. 1), most closely with *S. mali*, *S. pruni* and *S. asaccharolytica* (95.9–96.4% sequence similarity) and more distantly to *S. paucimobilis*, *S. parapaucimobilis*, *S. sanguinis*, *S. adhaesiva* and *S. trueperi* (93.8–94.6% sequence similarity). The values of sequence similarities (Table 1) of [*P.*] *echinoides* to other *Sphingomonas* species were in the range 91.3–92.6%. These data revealed that [*P.*] *echinoides* represents a distinct species within the genus *Sphingomonas*.

[*P.*] *echinoides* was observed to possess sym-homospermidine [37.19 μmol (g dry wt)⁻¹] as the major component in the polyamine pattern and minor amounts of putrescine [8.33 μmol (g dry wt)⁻¹], spermidine [7.55 μmol (g dry wt)⁻¹], 1,3-diamino-

Fig. 2. Polar lipid pattern of *Sphingomonas* (*Pseudomonas*) *echinoides* DSM 1805^T after separation by two dimensional TLC. PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; APL1, unidentified aminophospholipid; SGL, sphingoglycolipid; PL1, unidentified phospholipid; L1, unidentified polar lipid; GL, GL3, unidentified glycolipids.

propane [0.53 μmol (g dry wt)⁻¹] and spermine [0.22 μmol (g dry wt)⁻¹]. Ubiquinone Q-10 was determined as the major respiratory quinone. Neither sym-homospermidine, as the predominant polyamine compound, nor ubiquinone Q-10, as the respiratory quinone, is present in member species of the genus *Pseudomonas sensu stricto* (Auling *et al.*, 1991; Busse & Auling, 1988; Oyaizu & Komagata, 1983) as defined phylogenetically (De Vos & De Ley, 1983; Woese, 1987). However, sym-homospermidine has been found as the major compound in the polyamine pattern in the phylogenetic neighbours of [*P.*] *echinoides*, the species *S. mali*, *S. pruni*, *S. asaccharolytica*, *S. paucimobilis*, *S. parapaucimobilis*, *S. sanguinis*, *S. adhaesiva* and *S. trueperi*, and this supports the high degree of relationship (Yabuuchi *et al.*, 1990; Takeuchi *et al.*, 1995; Kämpfer *et al.*, 1997). The remaining species of the genus have spermidine as the predominant compound (Busse & Auling, 1988; Takeuchi *et al.*, 1993; Van Bruggen *et al.*, 1993; H.-J. Busse, unpublished results), reinforcing the proposal for reclassifying some species of the genus *Sphingomonas* (Van Bruggen *et al.*, 1993; Takeuchi *et al.*, 1994).

The overall polar lipid profile of [*P.*] *echinoides* (Fig. 2) was observed to be similar to those found in other *Sphingomonas* species (Kämpfer *et al.*, 1997). It was characterized by phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and an unidentified lipid (L1) as major compounds. Minor amounts of diphosphatidylglycerol (DPG), the characteristic sphingoglycolipid (SGL) and various unidentified lipids, including an aminophospholipid (APL1), a phospholipid (PL1) and glycolipids (GL, GL3), were

Table 2. Polar lipid distribution in *Sphingomonas (Pseudomonas) echinoides* DSM 1805^T and other phylogenetically related *sym*-homospermidine-containing *Sphingomonas* species

PME, phosphatidylmonomethylethanolamine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PDE, phosphatidylmethylethanolamine; 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; ND, not detected.

Organism	PME	PE	PG	DPG	PDE	PC	SGL	APL1	PL1	PL2	PL3	GL1	GL2	GL3	L1	L2
<i>S. echinoides</i> DSM 1805 ^T	ND	++	++	+	ND	ND	++	++	+	ND	ND	ND	ND	+	++	ND
<i>S. mali</i> IFO 15500 ^T	+	+	++	++	++	+	++	ND	+	ND	+	ND	+	+	++	+
<i>S. pruni</i> IFO 15498 ^T	+	+	++	++	++	+	++	ND	+	ND	+	ND	+	+	++	+
<i>S. asaccharolytica</i> IFO 15499 ^T	+	+	++	++	++	+	++	ND	+	ND	+	ND	+	+	++	+
<i>S. adhaesiva</i> JCM 7370 ^T	+	++	++	++	+	++	++	+	ND	++	+	ND	+	ND	ND	ND
<i>S. paucimobilis</i> DSM 1098 ^T	+	++	++	+	+	++	++	ND	ND	ND	+	ND	+	ND	ND	ND
<i>S. parapaucimobilis</i> JCM 7510 ^T	+	++	++	++	+	++	+	ND	ND	+	+	++	+	ND	ND	ND
<i>S. sanguinis</i> IFO 13937 ^T	ND	++	++	++	+	++	+	ND	ND	ND	+	+	+	ND	ND	ND
<i>S. trueperi</i> DSM 7225 ^T	+	++	++	++	++	+	++	ND	+	ND	+	ND	+	ND	++	+

Table 3. Percentage major fatty acid composition in *Sphingomonas (Pseudomonas) echinoides* DSM 1805^T and phylogenetically related *sym*-homospermidine-containing *Sphingomonas* species

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.

Compound*	<i>S. echinoides</i>	<i>S. mali</i>	<i>S. pruni</i>	<i>S. asaccharolytica</i>	<i>S. adhaesiva</i>	<i>S. paucimobilis</i>	<i>S. parapaucimobilis</i>	<i>S. sanguinis</i>	<i>S. trueperi</i>
Saturated fatty acids									
14:0	0.9					1.4	1.0	1.0	
15:0								1.1	
16:0	10.0	11.8	16.0	13.3	6.5	8.7	9.6	13.6	9.8
17:0								0.5	1.6
18:0								0.6	
Unsaturated fatty acids									
16:1 ω 5c	0.8						1.9	1.0	
17:1 ω 6c	2.9	9.3			21.2	3.0	3.8	2.4	13.6
17:1 ω 8c									
18:1 ω 5c	2.7					3.2	3.5	3.5	4.0
Hydroxy fatty acids									
14:0 2-OH	7.2	8.4	10.9	18.8	13.8	6.4	6.7	5.0	6.7
Summed features									
4	2.45				6.2	2.7	8.6	6.7	
7	73.1	70.5	73.1	67.8	47.3	74.6	65.0	64.6	64.2

* Fatty acid nomenclature. Unsaturated fatty acids: the position of the double bond can be located by counting from the methyl (ω) end of the carbon chain. 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 ω 7 and 15:0 iso 2-OH. Summed feature 7 contained one or more of the following isomers 18:1 ω 7c, 18:1 ω 9t and/or 18:1 ω 12t (*cis* and *trans* isomers are indicated by the suffixes *c* and *t*, respectively).

detected as well. Despite some similarities of the polar lipid profile of [*P.*] *echinoides* to those found in other *Sphingomonas* species, the overall profile was observed to be unique and suitable for differentiation from other species within the genus (Table 2). Fatty acid methyl-

esters were extracted and prepared by the standard protocol of the MIDI Microbial Identification System (Microbial ID). Extracts were analysed using a Hewlett Packard model HP6890A gas chromatograph equipped with a flame ionization detector, automatic

Table 4. Phenotypical characteristics of *Sphingomonas* (*Pseudomonas*) *echinoides* DMS 1805^T and phylogenetically related *sym*-homospermidine-containing *Sphingomonas* species

Test results given in the table were read after 72 h incubation at 30 °C; +, positive; – negative; (+) weak positive.

Character*	<i>S.</i> <i>echinoides</i>	<i>S.</i> <i>mali</i>	<i>S.</i> <i>pruni</i>	<i>S.</i> <i>asaccharolytica</i>	<i>S.</i> <i>adhaesiva</i>	<i>S.</i> <i>paucimobilis</i>	<i>S.</i> <i>parapaucimobilis</i>	<i>S.</i> <i>sanguinis</i>	<i>S.</i> <i>trueperi</i>
Acid produced from:†									
Glucose	–	(+)‡	(+)‡	(+)‡	–§	–§	–§	–	–
D-Mannitol	–	–	–	+	–§	–§	–§	–	–
Salicin	–	–‡	–‡	(+)‡	+	+	–	–	–
Inositol	–	–	–	+	–§	–§	–§	–	–
Sorbitol	–	–	–	–	–§	–§	–§	–	+
Rhamnose	–	–‡	–‡	(+)‡	(+)§	–§	(+)§	–	–
Maltose	–	–	–	–	(+)§	(+)§	(+)§	–	–
D-Xylose	–	–	–	–	(+)§	+	(+)§	–	–
Trehalose	–	–	–	+	–	–	–	–	–
Cellobiose	–	–	–	–	(+)§	(+)§	(+)§	–	–
Methyl D-glucoside	–	–	–	–	+	+	–	–	–
Erythritol	–	–	–	–	+	–	–	–	–
Melibiose	–	–	–	–	(+)§	(+)§	(+)§	–	–
D-Mannose	–	–	–	–	(+)§	(+)§	(+)§	–	–
Hydrolysis of:									
Aesculin	+	(+)‡	(+)‡	–	–	(+)	(+)	–	–
pNP-β-D-galactopyranoside	(+)‡	+‡	(+)‡	(+)‡	(+)‡	+§	+§	+	+
pNP-β-D-glucuronide	+	+	+	+	+	–	+	+	+
pNP-phosphorylcholine	+	+	+	+	+	–	+	+	+
L-Alanine-pNA	+	+	+	+	+	+	+	+	–
L-Glutamate-γ-(3-carboxy-pNA)	(+)	+	+	+	+	+	+	+	+
L-Proline-pNA	+	+	+	+	–	–	+	–	–
Assimilation of:									
p-Arbutin	–	+	+	–	+	+	+	+	+
Cellobiose	(+)‡	+‡	+‡	(+)‡	+§	+§	+§	+	+
D-Fructose	–	–	–	–	+	–	–	+	+
Gluconate	–	+‡	–‡	–‡	–‡	–‡	+‡	–	–
D-Glucose	(+)	+‡	+‡	+‡	+	+	+	+	+
D-Mannose	–	+‡	+‡	+‡	+§	+§	+	+	+
D-Maltose	(+)	+‡	+	+‡	+§	+§	+§	+	+
α-D-Melibiose	–	+‡	+‡	+‡	+	–	+	+	+
L-Rhamnose	–	+‡	+	+‡	–	–‡	(+)‡	–	–
Sucrose	–	+‡	+‡	(+)‡	+	+	+	+	+
Salicin	–	+‡	(+)‡	(+)‡	+	–	+	+	+
D-Trehalose	(+)	+‡	+‡	(+)‡	+	+	+	+	+
D-Xylose	–	+‡	+‡	(+)‡	–‡	+‡	+‡	+	+
Maltitol	–	–	–	+	–	–	–	+	+
Acetate	–	–	–	–‡	–	–	+	+	+
Propionate	–	–	–	–	–	–	+	+	+
cis-Aconitate	–	–	–	–	–	+	+	–	–
trans-Aconitate	–	–	–	–	–	+	–	–	–
Citrate	–	–‡	–	–‡	–‡	–‡	+	–‡	–‡
Fumarate	+	+‡	+	(+)‡	+	+	–	+	+
Glutarate	–	–	–	–	–	–	+	–	+
DL-3-Hydroxybutyrate	–	+	–	+	–	–	+	+	+
DL-Lactate	–	–‡	–	–‡	–	–	+	+	+
L-Malate	+	–‡	+	–‡	+	+	+	+	+
Oxoglutarate	–	–	–	–	+	+	+	+	–
Pyruvate	–	–	–	–	–	+	+	+	+
Suberate	–	–	–	–	+	–	–	–	–
L-Alanine	–	–	–	+	+	–	+	+	+
L-Asparagine	–	+	–	+	+	–	–	+	+
L-Histidine	–	+	–	–	–	–	–	–	–
L-Leucine	–	–	–	–	–	–	+	+	+
L-Ornithine	–	–	–	–	–	–	–	+	–
L-Proline	–	–	–	+	+	–	–	+	–
L-Serine	–	–	–	–	–	–	–	–	+
4-Hydroxybenzoate	–	–	–	–	–	–	–	–	+

* pNA, *p*-Nitroanilide, pNP, *p*-nitrophenyl.

† Acid formation from carbohydrates in most cases was very weak (even after prolonged incubation); these tests cannot be recommended for differentiation.

‡ Data in line with those published by Takeuchi *et al.* (1995).

§ Data in line with those published by Yabuuchi *et al.* (1990).

|| Data in line with those published by Takeuchi *et al.* (1993).

sampler, integrator and computer, as described previously (Kämpfer & Kroppenstedt, 1996). The fatty acid profile of [*P.*] *echinoides* contained 73.1% of summed feature 7 (18:1 ω 7c, 18:1 ω 9t and/or 18:1 ω 12t); 10.0% 16:0, as well as 7.2% 14:0 2-OH as major fatty acids (Table 3). The presence of these fatty acids is characteristic for all validly described species of the genus *Sphingomonas* (Yabuuchi *et al.*, 1990; Takeuchi *et al.*, 1993, 1995; Nohynek *et al.*, 1996; Balkwill *et al.*, 1997; Kämpfer *et al.*, 1997). Additionally, [*P.*] *echinoides* contained the fatty acids 17:1 ω 6c, 18:1 ω 5c, summed feature 4 (16:1 ω 7c and/or 15:0 iso 2-OH), 14:0 and 16:1 ω 5c, which have been traced in other *Sphingomonas* species (Takeuchi *et al.*, 1995; Balkwill *et al.*, 1997; Kämpfer *et al.*, 1997).

[*P.*] *echinoides* was also characterized on the basis of 88 biochemical and physiological characters, as described previously (Kämpfer *et al.*, 1991). It can be seen in Table 4 that [*P.*] *echinoides* is differentiated from other related *sym*-homospermidine-containing *Sphingomonas* species on the basis of several tests. Additional phenotypical characteristics for [*P.*] *echinoides* are given within the text. The substrate utilization profile and biochemical investigations confirmed the distinct position of [*P.*] *echinoides* within the genus *Sphingomonas*. Based on the data presented here, and combined with published data, we propose the transfer of [*P.*] *echinoides* from the genus *Pseudomonas* to the genus *Sphingomonas* as *Sphingomonas echinoides* comb. nov. The following description of *S. echinoides* is based on published data, as well as the data presented in this study.

Description of *Sphingomonas echinoides* comb. nov.

Sphingomonas echinoides (e.chi.noi'des. Gr. adj. *echinos* spiny appearance; Gr. n. *eidus* form, shape; M.L. adj. *echinoides* spiny shaped).

Cells are Gram-negative, slightly curved rods with sharp ends (0.8 $\times$ 1.9 μ m), non-spore-forming, motile and polar-flagellated (flagella wavelength 1.9 μ m). Yellow intracellular pigments (carotenoids) are produced. Colonies are yellow, circular, entire, low convex and smooth. In liquid culture, as well as on agar media, the organisms form cell aggregates (rosettes); in broth they can be observed as small flocs. Oxidase- and catalase-positive. Indole, urease and arginine dihydrolase are not produced. Gelatin is not liquefied. β -Galactosidase-positive. The following compounds are hydrolysed: aesculin, *p*-nitrophenyl- β -D-galactopyranoside, *p*-nitrophenyl- β -D-glucuronide, *p*-nitrophenyl- α -D-glucopyranoside, *p*-nitrophenyl- β -D-glucopyranoside, bis(*p*-nitrophenyl) phosphate, *p*-nitrophenyl-phenylphosphonate, *p*-nitrophenylphosphorylcholine, 2'-deoxythymidine 5'-*p*-nitrophenyl phosphate, L-alanine-*p*-nitroanilide, L-glutamate- γ -(3-carboxy-*p*-nitroanilide) and L-proline-*p*-nitroanilide. The following compounds are assimilated: *N*-acetyl-D-glucosamine, L-arabinose, D-cellobiose, D-galactose,

D-glucose, D-maltose, D-trehalose, fumarate and L-malate. In most cases only weak growth was obtained. The following compounds are not assimilated: *p*-arbutin, D-fructose, gluconate, D-mannose, α -D-melibiose, L-rhamnose, D-ribose, sucrose, salicin, D-xylose, adonitol, iso-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, acetate, propionate, *cis*-aconitate, *trans*-aconitate, adipate, 4-aminobutyrate, azelate, citrate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, 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 and phenylacetate. No acid is produced from glucose, lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, L-arabinose, raffinose, rhamnose, maltose, D-xylose, trehalose, cellobiose, methyl-D-glucoside, erythritol, melibiose, D-arabitol or D-mannose. The species clusters phylogenetically with validly described species of the genus *Sphingomonas* within the α -subclass of the *Proteobacteria* (Woese, 1987; Stackebrandt *et al.*, 1988). The G+C content of the genomic DNA is 65.8 mol% (Owen & Jackman, 1982). The major respiratory isoprenoid quinone is ubiquinone Q-10. The main component in the polyamine pattern is *sym*-homospermidine. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and an unknown lipid (L1). Diphosphatidylglycerol and several unknown lipids (aminophospholipid, APL1; glycolipids, GL, GL3; phospholipid, PL1) are present in minor concentrations. Sphingoglycolipid is present. The major non-polar fatty acids are 18:1 and 16:0, and the major 2-hydroxy fatty acid is 14:0 2-OH. Isolated as a laboratory contaminant on a nutrient agar plate. The type strain is DSM 1805^T (= ATCC 14820^T, DSM 50409^T, ICBP 2835^T, NCIB 9420^T).

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