Note

Sphingopyxis chilensis sp. nov., a chlorophenol-degrading bacterium that accumulates polyhydroxyalkanoate, and transfer of *Sphingomonas alaskensis* to *Sphingopyxis alaskensis* comb. nov.

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The taxonomic position of a chlorophenol-degrading bacterium, strain S37^T, was investigated. The 16S rDNA sequence indicated that this strain belongs to the genus *Sphingopyxis*, exhibiting high sequence similarity to the 16S rDNA sequences of *Sphingomonas alaskensis* LMG 18877^T (98·8 %), *Sphingopyxis macrogoltabida* LMG 17324^T (98·2 %), *Sphingopyxis terrae* IFO 15098^T (95 %) and *Sphingomonas adhaesiva* GIFU 11458^T (92 %). These strains (except *Sphingopyxis terrae* IFO 15098^T, which was not investigated) and the novel isolate accumulated polyhydroxyalkanoates consisting of 3-hydroxybutyric acid and 3-hydroxyvaleric acid from glucose as carbon source. The G+C content of the DNA of strain S37^T was 65·5 mol%. The major cellular fatty acids of this strain were octadecenoic acid (18 : 1ω7*c*), heptadecenoic acid (17 : 1ω6*c*) and hexadecanoic acid (16 : 0). The results of DNA–DNA hybridization experiments and its physiological characteristics clearly distinguished the novel isolate from all known *Sphingopyxis* species and indicated that the strain represents a novel *Sphingopyxis* species. Therefore, the species *Sphingopyxis chilensis* sp. nov. is proposed, with strain S37^T (=LMG 20986^T =DSM 14889^T) as the type strain. The transfer of *Sphingomonas alaskensis* to the genus *Sphingopyxis* as *Sphingopyxis alaskensis* comb. nov. is also proposed.

The genus *Sphingopyxis* was created recently by Takeuchi *et al.* (2001). This genus was proposed as part of the splitting of the genus *Sphingomonas* (Yabuuchi *et al.*, 1990), because the genus *Sphingomonas* represented a broad range of heterogeneous species with respect to physiology, phylogenetics and ecology. Thus, on the basis of phylogenetic evidence and some chemotaxonomic and phenotypic features that allow differentiation between the four clusters of *Sphingomonas* species, Takeuchi *et al.* (2001) proposed to emend the genus *Sphingomonas* (*sensu stricto*) and created three new genera: *Sphingobium*, *Novosphingobium* and *Sphingopyxis*. At the time of writing, the genus *Sphingopyxis* contains the species *Sphingopyxis macrogoltabida* and *Sphingopyxis terrae*. Bacteria belonging to this genus are Gram-negative, non-fermentative, aerobic, non-spore-forming, yellow-pigmented or whitish-brown, non-motile or motile, and are characterized chemotaxonomically by the presence of ubiquinone Q-10 and 2-hydroxymyristic acid (2-OH 14 : 0). Spermidine is the major polyamine component, sphingoglycolipids are present and the DNA G+C content is 63–65 mol%.

The intracellular accumulation of polyhydroxyalkanoates (PHAs) in the genus *Sphingomonas* has not been studied in much detail. These storage compounds are polyesters of commercial interest and represent a useful taxonomic criterion for differentiating bacterial genera (Kessler & Palleroni, 2000). Strain S37^T, which degrades chlorophenols and is able to accumulate PHA, was isolated from sediments of a river polluted with chlorophenolic compounds (Godoy

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Abbreviation: PHA, polyhydroxyalkanoate.

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et al., 1999). On the basis of preliminary morphological and physiological study, strain 37^T was identified as being related to *Sphingomonas paucimobilis* (Aranda *et al.*, 1999; Yeber *et al.*, 2000).

In this study, we describe the morphological, biochemical and phylogenetic characteristics of strain S37^T, and also the analysis of reserve polymers of species belonging to the genera *Sphingopyxis* and *Sphingomonas* that are closely related to strain S37^T. On the basis of phenotypic data, DNA–DNA hybridization data and the results of the 16S rDNA sequence analysis, we propose that strain S37^T represents a novel species of the genus *Sphingopyxis*, *Sphingopyxis chilensis* sp. nov.

Bacterial strains and cultures

Strain S37^T (=LMG 20986^T=DSM 14889^T) was isolated from the subsurface of a river (Biobío River in central Chile) polluted with chlorophenolic compounds as a 2,4,6-trichlorophenol-degrading bacterium (Godoy *et al.*, 1999). *Sphingomonas adhaesiva* LMG 10922^T, *Sphingopyxis macrogoltabida* LMG 17324^T and *Sphingomonas alaskensis* LMG 18877^T were obtained from the BCCM/LMG Bacteria Collection, Gent, Belgium. All strains were grown on R2A agar (Difco) at 25 °C unless indicated otherwise.

Morphological and biochemical characterization

The strains studied were characterized biochemically using API 20 NE test strips (bioMérieux). Phenotypic characterization using API 20 NE strips and the comparison with other strains from the genus *Sphingopyxis* are summarized in Table 1. Strain S37^T is able to utilize glucose, mannose, maltose and fructose as sole carbon sources, whereas only weak growth was obtained with adipate, xylose and *N*-acetylglucosamine as sole carbon sources (Table 1). Additionally, growth on different fatty acids was examined by culturing strain S37^T on mineral salts medium (Schlegel *et al.*, 1961) in the presence of 1 % (w/v) propionate, butyrate, glutarate, gluconate, valerate, hexanoate, octanoate, fumarate or decanoate at 25 °C for 72 h. Strain S37^T showed growth with fumarate as the sole carbon source, but was not able to grow with propionate, butyrate, glutarate, gluconate, valerate, hexanoate, octanoate or decanoate (all as sodium salts). Cells of strain S37^T were Gram-negative, motile rods that grew aerobically. The strain produced small yellow colonies (2 mm) on R2A agar after 3 days incubation. The morphological, physiological and biochemical characteristics of strain S37^T are consistent with the characteristics of the genus *Sphingopyxis*, as defined by Takeuchi *et al.* (2001) (Table 1). The phenotypic characteristics of strain S37^T allow differentiation from *Sphingomonas alaskensis* and *Sphingopyxis macrogoltabida*. Unlike these other species, strain S37^T is incapable of hydrolysing lactose (β-galactosidase activity). Strain S37^T and *Sphingopyxis macrogoltabida* utilize D-mannose as a sole carbon source, whereas *Sphingomonas alaskensis* can not utilize this carbohydrate. In contrast to *Sphingopyxis macrogoltabida*, *Sphingomonas alaskensis* and strain S37^T grow with maltose as a sole carbon source.

Table 1. Physiological and biochemical characteristics of strain S37^T and type strains of the genera *Sphingomonas* and *Sphingopyxis*

Strains: 1, strain S37^T; 2, *Sphingomonas alaskensis* LMG 18877^T; 3, *Sphingopyxis macrogoltabida* LMG 17324^T; 4, *Sphingomonas adhaesiva* LMG 10922^T. +, Positive; −, negative; (+), weakly positive; ND, not determined. The sodium salts of the respective acids were used. All strains were negative for the following characteristics: nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease, hydrolysis of gelatin and assimilation of L-arabinose, D-mannitol, citrate and phenylacetate. All strains were positive for the assimilation of glucose and malate.

Characteristic	1	2	3	4
β-Galactosidase	−	+	+	(+)
Hydrolysis of aesculin	+	+	+	−
Assimilation of:				
Fructose	+	ND	ND	ND
D-Mannose	+	−	+	−
N-Acetylglucosamine	(+)	−	−	+
Maltose	+	+	−	+
D-Gluconate	−	ND	ND	−
Caproate	−	−	+	−
Adipate	(+)	(+)	−	−
Xylose	(+)	ND	ND	ND
Fumarate	+	ND	ND	ND
Propionate, butyrate, glutarate, valerate, hexanoate, octanoate, decanoate	−	ND	ND	ND

DNA analysis

DNA extraction, PCR amplification and purification of the PCR products were performed as described by Rainey *et al.* (1993). The 16S rDNA was amplified with a PCR, using a universal primer set corresponding to positions 8–27 (forward primer) and 1505–1525 (reverse primer) (Rainey *et al.*, 1993). The PCR products were sequenced using the *Taq* Dye-Deoxy Terminator cycle sequencing kit (Applied Biosystems). Sequencing reactions were electrophoresed using an 4000L DNA (LI-COR) sequencer. The 16S rDNA sequence (1452 bp) of strain S37^T was determined. Similarity values between 16S rDNA sequences were obtained using internet tools as described previously (Rehm, 2001). Initial investigations on the global 16S rDNA sequence-based position of the novel isolate used the ARB database (Ludwig & Strunk, 1996). On the basis of this work, the 16S rDNA sequences were compared with the existing 16S rDNA database for members of the phyletic group defined as members of the genera *Sphingomonas*, *Novosphingobium*, *Sphingobium* and *Sphingopyxis*. A phylogenetic tree was constructed as described previously (Hezayen *et al.*, 2001; Rehm, 2001). A preliminary comparison against the GenBank database indicated that the strain is closely related

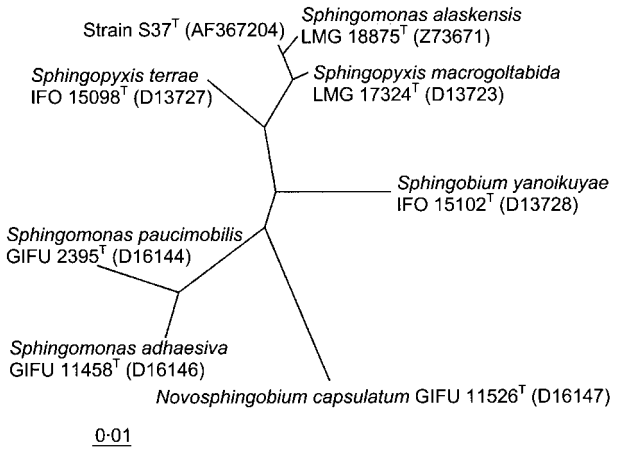

Fig. 1. Phylogenetic analysis of 16S rDNA sequences of the novel isolate strain S37^T and selected *Sphingomonas* and *Sphingopyxis* species as well as the type species of the genera *Sphingobium* and *Novosphingobium*. The tree was constructed by using the neighbour-joining method. Bar, 0·01 expected changes per site.

to members of the genera *Sphingopyxis* and *Sphingomonas*. The sequence of strain S37^T showed high similarity to sequences of *Sphingomonas alaskensis* LMG 18877^T (98·8 %) and *Sphingopyxis macrogoltabida* LMG 17324^T (98·2 %). Similarities of 92 and 95 % were found to the 16S rDNA sequences of *Sphingomonas adhaesiva* GIFU 11458^T and *Sphingopyxis terrae* IFO 15098^T, respectively. Construction of a 16S rDNA sequence-based phylogenetic tree indicated a phylogenetic position between *Sphingomonas alaskensis* and *Sphingopyxis macrogoltabida* (Fig. 1). For DNA base composition and DNA–DNA hybridization, cells were grown on trypticase soy agar (TSA; BBL) for 24 h at 28 °C. Isolation of genomic DNA and spectrophotometric determination of the G+C content were carried out according to Vancanneyt *et al.* (2001). The G+C content of the genomic DNA of strain S37^T was 65·5 mol%. This value is within the range observed for members of the genus *Sphingopyxis* (Yabuuchi *et al.*, 1990; Takeuchi *et al.*, 2001). DNA–DNA hybridization was carried out by fluorometric hybridization in microdilution wells using biotinylated DNA (Ezaki *et al.*, 1989). For the fluorescence measurements, an HTS7000 Bio Assay reader (Perkin-Elmer) was used. The hybridization temperature was 52 °C. The DNA–DNA reassociation values between strain S37^T and *Sphingomonas alaskensis* LMG 18877^T and *Sphingopyxis macrogoltabida* LMG 17324^T were respectively 50 and 38 %. These values are below the threshold value (approx. 70 %) for possible relatedness at the species level (Wayne *et al.*, 1987).

Cellular fatty acids

Cultures were grown on TSA for 24 h at 28 °C. Fatty acids were extracted, separated and identified according to the Microbial Identification System as described previously (Vandamme *et al.*, 1992). The whole-cell fatty acid compositions of strain S37^T and related *Sphingopyxis* reference strains are given in Table 2. The major whole-cell fatty acids in strain S37^T were octadecenoic acid (18 : 1ω7*c*), heptadecenoic acid (17 : 1ω6*c*), hexadecanoic acid (16 : 0) and summed feature 4 (see Table 2). Minor amounts of the 2-hydroxy fatty acids 14 : 0 2-OH, 15 : 0 2-OH and 16 : 0 2-OH were also present. The absence of 3-OH fatty acids and the presence of 2-OH myristic acid, hexadecenoic acid (16 : 1ω5*c*) and octadecenoic acid (18 : 1ω7*c*) as the major fatty acids and octadecenoic acid (18 : 1ω7*c* 11-methyl) as a minor fatty acid in strain S37^T are features characteristic of members of the genus *Sphingopyxis* (Table 2).

Analysis of PHA

To promote accumulation of PHA, strain S37^T and the type strains of *Sphingomonas adhaesiva*, *Sphingopyxis macrogoltabida* and *Sphingomonas alaskensis* were cultivated in mineral salts medium (Schlegel *et al.*, 1961) containing 0·05 % (w/v) NH_4Cl and 0·1 M glucose at 25 °C. For analysis of PHA, 5 mg lyophilized cells was subjected to methanolysis in the presence of a solution containing 1 ml chloroform, 0·85 ml methanol and 0·15 ml sulfuric acid for 5 h at 100 °C. The resulting methyl esters were analysed

Table 2. Major fatty acids of strain S37^T and related reference strains

Strains: 1, strain S37^T; 2, *Sphingomonas alaskensis* LMG 18877^T; 3, *Sphingopyxis macrogoltabida* LMG 17324^T; 4, *Sphingomonas paucimobilis* LMG 1227^T. Tr, Trace (less than 1·0 %); ND, not detected.

Compound	1	2	3	4
Saturated fatty acids				
14 : 0	Tr	Tr	Tr	1
15 : 0	1	3	ND	ND
16 : 0	9	7	13	7
17 : 0	1	3	ND	ND
Unsaturated fatty acids				
16 : 1ω5*c*	1	1	2	Tr
17 : 1ω6*c*	18	33	Tr	3
17 : 1ω8*c*	3	8	ND	ND
18 : 1ω5*c*	Tr	Tr	Tr	4
18 : 1ω7*c*	34	27	42	73
18 : 1ω7*c* 11-methyl	2	1	1	ND
Hydroxy fatty acids				
14 : 0 2-OH	4	1	3	9
15 : 0 2-OH	4	5	ND	ND
16 : 0 2-OH	2	1	2	ND
Summed feature 4*	18	8	35	3

*Represents one or more of the following fatty acids, which could not be separated by the Microbial Identification System: 15 : 0 iso 2-OH, 16 : 1ω7*c*, 16 : 1ω7*t*.

by GC (Agilent 6850 series GC system) (Brandl *et al.*, 1988). For qualitative analysis of methyl esters, a coupled GC/MS analysis was performed using an HP 6890 gas chromatograph with a model 5973 mass-selective detector (Hewlett Packard). The mass spectra obtained were compared with the NIST '98 mass spectrum library with the Windows Search program (version 1.6; National Institute of Standards and Technology, US Department of Commerce). Experiments with strain S37^T, *Sphingomonas adhaesiva*, *Sphingopyxis macrogoltabida* and *Sphingomonas alaskensis* cultivated on mineral medium with 0·1 M glucose showed that these strains accumulated PHAs consisting of 3-hydroxybutyric acid and 3-hydroxyvaleric acid (Table 3). The amount of PHA accumulated was variable among the species studied. The strongest PHA accumulation was obtained with *Sphingopyxis macrogoltabida*, in which it contributed 70·2 % of cellular dry weight. *Sphingomonas alaskensis* and strain S37^T accumulated similar amounts, PHAs contributing 26·0 and 24·3 % of cellular dry weight, respectively, whereas PHA accumulation in *Sphingomonas adhaesiva* contributed only 2·9 % of cellular dry weight. Except for *Sphingomonas adhaesiva*, the major constituent of the PHA was 3-hydroxybutyric acid (range 97·2–99 mol%), and 3-hydroxyvaleric acid was the minor constituent (range 0·9–2·8 mol%).

According to the classification proposed by Takeuchi *et al.* (2001) and the phylogenetic and phenotypic description given by Vancanneyt *et al.* (2001), *Sphingomonas alaskensis* should be reclassified in the genus *Sphingopyxis*. Phylogenetic data from the present study (Fig. 1) confirm this. We therefore propose the reclassification of *Sphingomonas alaskensis* within the genus *Sphingopyxis* as *Sphingopyxis alaskensis* comb. nov. This polyphasic study also clearly demonstrates that strain S37^T represents a novel species within the genus *Sphingopyxis*, for which we propose the name *Sphingopyxis chilensis* sp. nov.

Table 3. Composition of PHAs isolated after cultivation on mineral medium with 0·1 M glucose

3HB, 3-Hydroxybutyric acid; 3HV, 3-hydroxyvaleric acid.

Strain	PHA content (%)*	PHA composition (mol%)	
		3HB	3HV
Strain S37^T	24·3	97·2	2·8
Sphingomonas adhaesiva LMG 10922^T	2·9	66·7	33·2
Sphingopyxis macrogoltabida LMG 17324^T	70·2	99·1	0·9
Sphingomonas alaskensis LMG 18877^T	26·0	98·1	1·9

*Total PHAs as percentage (w/w) of cellular dry weight.

Description of *Sphingopyxis alaskensis* comb. nov.

Basonym: *Sphingomonas alaskensis* Vancanneyt *et al.* 2001.

The description of this species is given by Vancanneyt *et al.* (2001). The type strain is strain RB2256^T (=CCUG 45028^T=CIP 106977^T =DSM 13593^T =LMG 18877^T).

Description of *Sphingopyxis chilensis* sp. nov.

Sphingopyxis chilensis (chi.len′sis. N.L. adj. *chilensis* referring to Chile, where the type strain was isolated).

Cells are Gram-negative, motile, non-sporulating rods. Colonies are circular, low and convex with entire margins, yellow and 2 mm in diameter after 3 days incubation. The following enzyme activities are present: catalase, oxidase, hydrolysis of aesculin. No reactions are obtained for urease, production of indole, arginine dihydrolase, reduction of nitrate, hydrolysis of gelatin or lactose utilization. Glucose, D-mannose, maltose, malate and fumarate are assimilated. The following compounds are not assimilated: L-arabinose, D-mannitol, *N*-acetylglucosamine, caprate, citrate, phenylacetate, propionate, butyrate, glutarate, gluconate, valerate, hexanoate, octanoate and decanoate. The major fatty acids are 18 : 1ω7*c*, 17 : 1ω6*c*, 16 : 0 and summed feature 4; the presence of the 2-hydroxy fatty acids 14 : 0 2-OH, 15 : 0 2-OH and 16 : 0 2-OH is characteristic. The G+C content is 65·5 mol%. PHAs consisting of 3-hydroxybutyric acid and 3-hydroxyvaleric acid are accumulated in the presence of glucose as carbon source.

The type strain is S37^T (=LMG 20986^T =DSM 14889^T), isolated from superficial sediment of a river contaminated with chlorophenolic compounds in Concepción, Chile.

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References

Aranda, C., Godoy, F., González, B., Homo, J. & Martínez, M. (1999). Effects of glucose and phenylalanine upon 2,4,6-trichlorophenol degradation by *Pseudomonas paucimobilis* S37^T cells in non-growth state. *Microbios* **100**, 73–82.

Brandl, H., Gross, R. A., Lenz, R. W. & Fuller, R. C. (1988). *Pseudomonas oleovorans* as a source of poly(β-hydroxyalkanoates) for potential applications as biodegradable polyester. *Appl Environ Microbiol* **54**, 1977–1982.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Godoy, F., Zenteno, P., Cerda, F., González, B. & Martínez, M. (1999). Tolerance to trichlorophenols in microorganism from pristine and polluted zone of the Biobío river (central Chile). *Chemosphere* **38**, 655–662.

Hezayen, F. F., Rehm, B. H. A., Tindall, B. J. & Steinbüchel, A. (2001). Transfer of *Natrialba asiatica* B1T to *Natrialba taiwanensis* sp. nov. and description of *Natrialba aegyptiaca* sp. nov., a novel extremely halophilic, aerobic, non-pigmented member of the *Archaea* from Egypt that produces extracellular poly(glutamic acid). *Int J Syst Evol Microbiol* **51**, 1133–1142.

Kessler, B. & Palleroni, N. J. (2000). Taxonomic implications of synthesis of poly-β-hydroxybutyrate and other poly-β-hydroxyalkanoates by aerobic pseudomonads. *Int J Syst Evol Microbiol* **50**, 711–713.

Ludwig, W. & Strunk, O. (1996). ARB: a software environment for sequence data. Munich: Technische Universität München. http://www.mikro.biologie.tu-muenchen.de/

Rainey, F. A. & Stackebrandt, E. (1993). 16S rDNA analysis reveals phylogenetic diversity among the polysaccharolytic clostridia. *FEMS Microbiol Lett* **113**, 125–128.

Rehm, B. H. A. (2001). Bioinformatic tools for DNA/protein sequence analysis, functional assignment of genes and protein classification. *Appl Microbiol Biotechnol* **57**, 579–592.

Schlegel, H. G., Kaltwasser, H. & Gottschalk, G. (1961). Ein Submersverfahren zur Kultur wasserstoffoxidierender Bakterien: Wachstumsphysiologische Untersuchungen. *Arch Mikrobiol* **38**, 209–222.

Takeuchi, M., Hamana, K. & Hiraishi, A. (2001). Proposal of the genus *Sphingomonas sensu stricto* and three new genera, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*, on the basis of phylogenetic and chemotaxonomic analyses. *Int J Syst Evol Microbiol* **51**, 1405–1417.

Vancanneyt, M., Schut, F., Snauwaert, C., Goris, J., Swings, J. & Gottschal, J. C. (2001). *Sphingomonas alaskensis* sp. nov., a dominant bacterium from a marine oligotrophic environment. *Int J Syst Evol Microbiol* **51**, 73–79.

Vandamme, P., Vancanneyt, M., Pot, B. & 10 other authors (1992). Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. *Int J Syst Bacteriol* **42**, 344–356.

Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

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.

Yeber, M. C., Freer, J., Martínez, M. & Mansilla, H. D. (2000). Bacterial response to photocatalytic degradation of 6-chlorovanillin. *Chemosphere* **41**, 1257–1261.