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Sphingomonas alaskensis sp. nov., a dominant bacterium from a marine oligotrophic environment

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Seven Gram-negative strains, isolated in 1990 from a 10^6-fold dilution series of seawater from Resurrection Bay, a deep fjord of the Gulf of Alaska, were identified in a polyphasic taxonomic study. Analysis of 16S rDNA sequences and DNA-homology studies confirmed the phylogenetic position of all strains in the genus *Sphingomonas* and further indicated that all of the strains constitute a single homogeneous genomic species, distinct from all validly described *Sphingomonas* species. The ability to differentiate the species, both phenotypically and chemotaxonomically, from its nearest neighbours justifies the proposal of a new species name, *Sphingomonas alaskensis* sp. nov., for this taxon. Strain LMG 18877^T (= RB2256^T = DSM 13593^T) was selected as the type strain.

Keywords: *Sphingomonas alaskensis* sp. nov., identification, polyphasic taxonomy, marine ultramicrobacterium

INTRODUCTION

Microbiologists have been intrigued by the phenomenon of 'unculturability' for over half a century, especially with respect to bacteria in the open ocean (MacLeod, 1985). This notion has now virtually reached the status of a dogma but is still based primarily on the common observation that often numerically dominant marine bacteria cannot be grown on agar surfaces (Austin, 1988; Giovannoni *et al.*, 1995; Jannasch & Jones, 1959; Kogure *et al.*, 1979, 1980; Poindexter & Leadbetter, 1986; Roszak & Colwell, 1987; Schut *et al.*, 1997a, b; Van Es & Meyer-Reil, 1982). These studies all point in the same direction and indicate that by using agar-plate counts and isolation procedures usually less than 0·1 % of the total community that is observed by direct microscopy can be cultured. As a logical consequence of this situation, most of our knowledge on the physiological properties of marine bacteria is based on those species that are readily obtained in culture but which mostly belong to a minority of the total community.

Culture-independent molecular techniques are now widely used to obtain a thorough understanding of the identity and nature of the bacteria comprising marine heterotrophic communities, because of their apparent unculturability. This approach has indeed indicated the existence of numerous possibly 'new' and unusual uncultured bacteria (Amann *et al.*, 1995; Britschgi & Giovannoni, 1991; Fuhrman *et al.*, 1992, 1993; Giovannoni *et al.*, 1990, 1995; Höfle & Brettar, 1996; Mullins *et al.*, 1995; Schmidt *et al.*, 1991). The developments in the use of molecular probes for the detection of natural bacterial populations and for the analysis of genetic diversity within communities have revolutionized our approach in microbial ecology so much that they may create the impression that isolation and cultivation of naturally occurring bacterial strains might soon cease to be required. However, the phylogenetic position that can be inferred from molecular sequence data still tells us very little about the physiological properties of uncultured and unknown strains. For this reason, the need for isolation of bacteria from nature will remain essential in the foreseeable future (Schut *et al.*, 1997a, b). Most interestingly, in a limited number of studies, in which both molecular and culture-dependent techniques were

Abbreviations: FAS, filtered-autoclaved seawater; FAMEs, fatty acid methyl esters.

The EMBL accession numbers for the sequences determined in this work are AF145752 (1280 bp), AF145753 (1416 bp) and AF145754 (1428 bp), respectively.

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used, there were indications that a small number of cultured bacteria in a marine environment did appear to account for up to 20% of the total bacterial community (Fuhrman *et al.*, 1994; Moran *et al.*, 1995; Rehnstam *et al.*, 1993).

Strain RB2256^T was isolated from Resurrection Bay (a deep fjord of the Gulf of Alaska) after a million-fold dilution of an original seawater sample and a succesful enrichment using the dilution to extinction technique (Button *et al.*, 1993; Schut *et al.*, 1993). This indicates that the organism was a numerically dominant member of the indigenous community that amounts to approximately $0{\cdot}2 \times 10^6$ bacteria ml^{-1}. This was subsequently supported by a Southern hybridization of PCR-amplified eubacterial 16S rDNA sequences from extracted community DNA from Resurrection Bay seawater by using a strain RB2256^T-specific probe (Schut, 1994).

Upon first cultivation, strain RB2256^T was obligately oligotrophic and could be grown only in liquid seawater medium containing less than 1 mg dissolved organic carbon per litre. Only after prolonged starvation did the organism become culturable on nutrient-enriched agar plates and corresponding liquid media, being described as facultatively oligotrophic (Schut *et al.*, 1993, 1997a, b; Schut, 1994).

Since its isolation in 1990, strain RB2256^T has been studied extensively because of its interesting physiological properties. The organism fulfils all of the criteria for a 'model oligotroph'. The strain possesses high-affinity substrate-uptake systems, the ability to take up mixed substrates simultaneously, and is very small in size ('ultramicro'; less than $0{\cdot}1\ \mu m^3$) (Schut *et al.*, 1993, 1997a; Schut, 1994; Eguchi *et al.*, 1996). The cells have a DNA content of approximately 25% of that of a single *Escherichia coli* genome (Schut *et al.*, 1993) and appear to be resistant to various stress-inducing agents (Eguchi *et al.*, 1996; Schut *et al.*, 1997a; Joux *et al.*, 1999). Taken together, these characteristics have led some authors to define the strain as an 'oligotrophic ultramicrobacterium' (Eguchi *et al.*, 1996; Schut *et al.*, 1997a, b; Fegatella *et al.*, 1998).

In the course of these previous studies, the 16S rDNA sequence of strain RB2256^T (LMG 18877^T) was determined and deposited in the EMBL database as Z73631. It was shown that the isolate belongs to the α-*Proteobacteria* and, more particularly, to the genus *Sphingomonas* (Schut, 1994).

The genus *Sphingomonas* is phylogenetically heterogeneous and currently comprises 23 validly described species. At least the following four phylogenetic sub-branches are recognized (Van Bruggen *et al.*, 1993; Balkwill *et al.*, 1997; Kämpfer *et al.*, 1997; Denner *et al.*, 1999; Yabuuchi *et al.*, 1999). (1) The *Sphingomonas sensu stricto* group contains nine species (including the type species of the genus): *Sphingomonas adhaesiva*, *Sphingomonas asaccharolytica*, *Sphingomonas echinoides*, *Sphingomonas mali*, *Sphingomonas parapaucimobilis*, *Sphingomonas paucimobilis*, *Sphingomonas pruni*, *Sphingomonas sanguinis* and *Sphingomonas trueperi*. (2) The *Sphingomonas yanoikuyae* group comprises six species: *Sphingomonas chlorophenolica*, *Sphingomonas herbicidovorans*, *S. yanoikuyae* and, at a deeper level, the recently reclassified species *Sphingomonas suberifaciens*, *Sphingomonas natatoria* and *Sphingomonas ursincola*. (3) The *Sphingomonas capsulata*-group contains six species: *Sphingomonas aromaticivorans*, *S. capsulata*, *Sphingomonas subterranea*, *Sphingomonas stygia*, *Sphingomonas subarctica* and *Sphingomonas rosa*. (4) A fourth cluster comprises the species *Sphingomonas macrogoltabidus* and *Sphingomonas terrae*.

In the present study, it was our aim to identify and characterize strain RB2256^T and to determine, in a polyphasic taxonomic study, its relatedness to six analogous isolates from seawater samples from the same habitat. The results of 16S rDNA sequencing studies of these strains indicated that they all belong to the genus *Sphingomonas*. Determination of the DNA base ratios, DNA–DNA hybridizations, and cellular fatty acid analyses indicated that the seven strains studied constitute, genotypically as well as chemotaxonomically, a homogeneous taxon that is different from all other *Sphingomonas* species. An extensive phenotypic analysis was performed in order to describe the new species and to differentiate it from the other *Sphingomonas* species. The name *Sphingomonas alaskensis* sp. nov. is proposed.

METHODS

Isolation conditions. Seawater samples were taken from a depth of 10 m below the surface near the centre of Resurrection Bay of the Gulf of Alaska (60° E 03′ N, 149° E 25′ W) and collected in acid-washed, thoroughly rinsed Niskin bottles with fired (550 °C) glassware on 24 March 1990. Samples were diluted 10^5–10^6-fold into fired glass tubes with filtered-autoclaved seawater (FAS). During incubation of these tubes, growth was monitored over a period of up to 2 months by flow cytometry or epifluorescence (Schut, 1994). Schut *et al.* (1993) demonstrated that none of the cultures enriched and isolated via this dilution culture could be cultivated directly onto nutrient-rich agar media. Growth was observed only when FAS or synthetic seawater medium containing less than approximately 1 mg carbon per litre was used as the medium. However, storage of stationary-phase cultures for at least 6–12 months at 5–8 °C triggered a (so far, unexplained) process that resulted in the development of the ability to grow on high-nutrient laboratory media. All experiments in the present study were performed with subcultures of the latter strains.

Bacterial strains and growth conditions. Data on the origin of the isolates and reference strains studied are listed in Table 1. The seven marine isolates originate from three different seawater samples from Resurrection Bay. Strains LMG 18872, LMG 18875 and LMG 18876 were isolated from separate 10^5–10^6 dilutions of seawater sample no. 1, LMG 18871 and LMG 18877^T of sample no. 2 and LMG 18873 and LMG 18874 of sample no. 3.

During the present study, all strains were grown and maintained on trypticase soy agar (TSA, catalogue no.

Table 1. *Sphingomonas* strains studied in this work

Taxon	Strain no.*	Other strain designation	Source and place of isolation
S. alaskensis	LMG 18871	RB255	Seawater; Resurrection Bay, Alaska; 1990
S. alaskensis	LMG 18872	RB2515	Seawater; Resurrection Bay, Alaska; 1990
S. alaskensis	LMG 18873	RB2109	Seawater; Resurrection Bay, Alaska; 1990
S. alaskensis	LMG 18874	RB2108	Seawater; Resurrection Bay, Alaska; 1990
S. alaskensis	LMG 18875	RB2510	Seawater; Resurrection Bay, Alaska; 1990
S. alaskensis	LMG 18876	RB2519	Seawater; Resurrection Bay, Alaska; 1990
S. alaskensis	LMG 18877^T	RB2256^T	Seawater; Resurrection Bay, Alaska; 1990
S. macrogoltabidus	LMG 17324^T		Soil; Kyoto, Japan
S. paucimobilis	LMG 1227^T		Hospital respirator; London, UK
S. terrae	LMG 17326^T		Activated sludge; Nagoya, Japan

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11768; BBL) and incubated aerobically at 28 °C, unless indicated otherwise. Bacteriological purity was checked by plating and examination of living and Gram-stained cells.

16S rDNA sequence analysis. Cultures LMG 18871, LMG 18872 and LMG 18875 were grown on Marine Agar 2216 (Difco), harvested and suspended in 200 µl TE (10 mM Tris, 1 mM EDTA). DNA extraction was performed as described by Schut (1994). A fragment of the 16S rDNA was amplified with a PCR using the conserved primers F8 and R1492 (F, forward primer; R, reverse primer; *E. coli* 16S rRNA gene-sequencing numbering) (Amann *et al.*, 1995; Weisburg *et al.*, 1991). Sequencing was performed using an ABI 310 automated DNA sequencer (Perkin-Elmer) with the Big Dye terminator cycle sequencing ready reaction kit according to the instructions of the supplier (van der Maarel *et al.*, 1998). The sequencing primers used were F8, F519, F907, R1392, R926 and R536.

Phylogenetic analysis was performed using the software package GENECOMPAR (Applied Maths) after including the consensus sequence in an alignment of small ribosomal-subunit sequences collected from the international nucleotide sequence library EMBL. This alignment was calculated pairwise using an open-gap penalty of 100% and a unit-gap penalty of 0%. A similarity matrix was created using a homology calculation with a gap penalty of 0% and after masking ambiguous and unknown bases. The resulting tree was constructed using the neighbour-joining method (Saitou & Nei, 1987).

Preparation of high-molecular-weight DNA. High-molecular-weight native DNA was prepared as described previously (Vandamme *et al.*, 1992).

DNA base compositions. DNA was enzymically degraded into nucleosides as described by Mesbah *et al.* (1989). The nucleoside mixture was separated by HPLC using a Waters SymmetryShield C8 column thermostated at 37 °C. The solvent was 0·02 M $NH_4H_2PO_4$ (pH 4·0) with 1·5% acetonitrile. Non-methylated lambda-phage DNA (Sigma) was used as the calibration reference.

DNA–DNA hybridization experiments. DNA–DNA hybridizations were performed with photobiotin-labelled probes in microplate wells as described by Ezaki *et al.* (1989), using an HTS7000 Bio Assay Reader (Perkin-Elmer) for the fluorescence measurements. The hybridization temperature was 52 °C.

Fatty acid methyl ester analysis. All strains were grown for 24 h on Petri dishes under the growth conditions described above. Preparation, separation, identification and numerical comparison of the fatty acid methyl esters (FAMEs) was performed on a loopful of cells according to the Microbial Identification System (Microbial ID) as described previously (Vandamme *et al.*, 1992).

Phenotypic characterization. All strains studied were characterized biochemically using the following two commercial galleries: API 20NE and API 50CH (bioMérieux). For these tests, cells were precultivated on nutrient agar (CM3; Oxoid) plates for 24 h at 28 °C. The inoculation procedure, incubation, and visual reading of the galleries were performed according to the instructions of the manufacturers.

RESULTS

16S rDNA sequence analysis

Representative strains from the FAME analysis were selected for further 16S rDNA sequence analysis. In previous work, the 16S rDNA sequence of strain RB2256^T (= LMG 18877^T) was determined, indicating that the strain belongs to the genus *Sphingomonas* (Schut, 1994). In the present study, the 16S rDNA sequences of three additional isolates, LMG 18871, LMG 18872 and LMG 18875, obtained from the same marine environment, were determined. Phylogenetic analysis (Fig. 1) showed that all three sequences are very similar (> 99·4% sequence similarity) and cluster at a similarity level of 98·8% with the sequence of strain LMG 18877^T (Z73631). The sequences show the highest similarities (97·4%) with *S. macrogoltabidus* LMG 17324^T (D13723) and with the type strain of *S. terrae* LMG 17326^T (95·4%). A sequence similarity of only 91–92% was obtained with the type species of the genus *S. paucimobilis*. An analogous grouping was obtained using maxium-parsimony analysis (data not shown).

DNA base compositions

Determination of the DNA base composition of six of the marine isolates (not LMG 18872) yielded G+C contents between 65·0 and 65·1 mol%. For the type strain of *S. macrogoltabidus*, a value of 64·6 mol% was obtained.

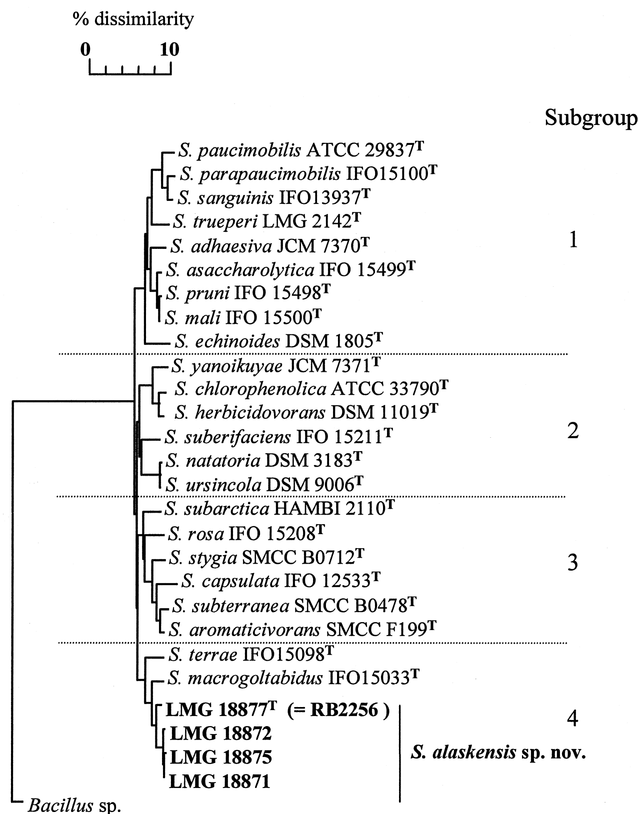

Fig. 1. Distance-matrix tree showing the phylogenetic relationships between *Sphingomonas alaskensis* and other species of the genus *Sphingomonas*. Sequences of the following *Sphingomonas* reference strains were extracted from the EMBL database (accession nos in parentheses): *S. adhaesiva* JCM 7370^T (X72720), *S. alaskensis* LMG 18871 (AF145752), LMG 18872 (AF145753), LMG 18875 (AF145754) and LMG 18877^T (Z73631), *S. aromaticivorans* SMCC F199^T (U20756), *S. asaccharolytica* IFO 15499^T (Y09639), *S. capsulata* IFO 12533^T (D16147), *S. chlorophenolica* ATCC 33790^T (X87161), *S. echinoides* DSM 1805^T (AJ012461), *S. herbicidovorans* DSM 11019^T (AB022428), *S. macrogoltabidus* IFO15033^T (D13723), *S. mali* IFO15500^T (Y09638), *S. natatoria* DSM 3183^T (AB024288), *S. parapaucimobilis* IFO 15100^T (D13724), *S. paucimobilis* ATCC 29837^T (U37337), *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. suberifaciens* IFO 15211^T (D13737), *S. subterranea* SMCC B0478^T (U20773), *S. terrae* IFO 15098^T (D13727), *S. trueperi* LMG 2142^T (X97776), *S. ursincola* DSM 9006^T (AB024289), *S. yanoikuyae* JCM 7371^T (X72725).

DNA–DNA hybridizations

DNA–DNA hybridization data revealed that isolates LMG 18871 to LMG 18877^T (LMG 18872 was not studied) exhibited DNA binding values of between 89 and 100 %. No significant DNA relatedness (13–30 %) was measured between the marine isolates and *S. macrogoltabidus* LMG 17324^T.

Cellular fatty acids (FAMEs)

The fatty acid patterns of all seven marine isolates were compared by numerical analysis and were found to constitute a homogeneous cluster (dendrogram not shown). The major fatty acids are 17:1ω6*c* (33·2 ± 3·4 %) and 18:1ω7*c* (26·5 ± 2·6 %). Additionally, smaller (but significant) amounts of the following fatty acids are present: 14:0 2-OH, 15:0, 15:0 2-OH, 16:0, 16:0 2-OH, 16:1ω5*c*, 17:0, 17:1ω8*c*, 18:1ω7*c* 11 methyl and 'Summed feature 4' (Table 2). For comparison, the fatty acid compositions of the type strains of the nearest phylogenetic neighbours of LMG 18877^T (= RB2256^T), *S. macrogoltabidus* LMG 17324^T and *S. terrae* LMG 17326^T, and of the type strain of the type species of the genus *S. paucimobilis* LMG 1227^T were also determined. The fatty acid content of *S. terrae* LMG 17326^T is very similar to the pattern of the marine strains: the dominant fatty acids are 17:1ω6*c* (39·8 %) and 18:1ω7*c* (20·1 %), and minor amounts of the fatty acids 14:0 2-OH, 15:0, 15:0 2-OH, 16:0, 16:1ω5*c*, 17:0, 17:1ω8*c*, 18:1ω7*c* 11 methyl and 'Summed feature 4' are present. *S. macrogoltabidus* LMG 17324^T is easily differentiated by the absence of 15:0, 15:0 2-OH, 17:0, 17:1ω6*c*, 17:1ω8*c* and the presence of significantly higher amounts of 18:1ω7*c* (42·2 %) and 'Summed feature 4' (34·8 %). A major fatty acid in *S. paucimobilis* LMG 1227^T is 18:1ω7*c* (73·1 %), and smaller (but significant) amounts of the fatty acids 14:0, 14:0 2-OH, 17:0, 18:1ω5*c* and 'Summed feature 4' are found.

Phenotypic characterization

A phenotypic characterization using two different API galleries (API 20NE, API 50CH) yielded a number of characteristic phenotypic features useful for distinguishing the marine isolates from taxonomically related reference strains (Table 3; see below). The strains do not have an obligate requirement for high salt concentrations (isolation conditions); in addition, good growth was obtained with 0·5 % NaCl (TSA).

DISCUSSION

It is often assumed that the strikingly small size (< 0·1 μm^3) of the bacterial cells of marine bacterioplankton communities (Amy & Morita, 1983; Hood & MacDonell, 1987; Lee & Fuhrman, 1987; MacDonell & Hood, 1982; Morita, 1997; Tabor *et al.*, 1981) is due to the fact that they represent starved forms of known and unknown bacteria. However, it has gradually become apparent that these so-called 'ultramicrobacteria' (Torella & Morita, 1981) are mostly metabolically active and growing (Button & Robertson, 1989; Cho & Azam, 1988; Ishida & Kadota, 1981; Kaprelyants *et al.*, 1993; Kirchman, 1993; Schut *et al.*, 1993, 1997a).

One of these so-called 'unculturable' ultramicrobacteria, strain RB2256^T (= LMG 18877^T), was successfully cultured and has been studied in recent years in considerable detail (see below). The strain was preliminary characterized phylogenetically as belonging to the genus *Sphingomonas*, but its exact relationships with existing species of this genus were not

Table 2. Fatty acid composition (mean percentage of total) of *S. alaskensis* and related reference strains

All of the strains listed in Table 1 were investigated. Fatty acids that accounted for less than 1·0% of the total fatty acids in all of the strains studied are not shown. tr, Trace (less than 1·0%); ND, not detected. Standard deviations are shown in parentheses.

Species (no. strains)	14:0	14:0 2-OH	15:0	15:0 2-OH	16:0	16:0 2-OH	16:1 ω5c	SF4*	17:0	17:1 ω6c	17:1 ω8c	18:1 ω5c	18:1 ω7c	18:1 ω7c 11 methyl
S. alaskensis (7)	tr	1·3 (0·3)	2·8 (0·2)	4·6 (0·8)	7·3 (0·7)	1·4 (0·4)	1·1 (0·2)	8·2 (1·4)	2·7 (0·6)	33·2 (3·4)	7·6 (1·0)	tr	26·5 (2·6)	1·7 (0·2)
S. macrogoltabidus (1)	tr	2·9	ND	ND	13·2	1·8	2·1	34·8	ND	tr	ND	tr	42·2	1·4
S. terrae (1)	ND	1·4	3·4	5·5	4·9	tr	1·4	9·6	2·8	39·8	7·6	tr	20·1	1·1
S. paucimobilis (1)	1·1	8·7	ND	ND	7·2	ND	tr	3·1	ND	2·7	ND	3·5	73·1	ND

* SF4, Summed feature 4 consisted of 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* and 16:1ω7*t*.

Table 3. Phenotypic characteristics of *S. alaskensis* and related reference strains

All of the strains listed in Table 1 were investigated. Using the API 20NE or the API 50CH system, none of the strains assimilated the following carbohydrates: phenyl-acetate, adonitol, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, arbutin, citrate, dulcitol, erythritol, D-fructose, D-fucose, L-fucose, gluconate, 2-ketogluconate, 5-ketogluconate, *N*-acetylglucosamine, methyl α-D-glucoside, glycerol, glycogen, inositol, inulin, lactose, D-lyxose, mannitol, methyl α-D-mannoside, D-melezitose, D-melibiose, D-raffinose, L-rhamnose, D-ribose, salicin, sorbitol, L-sorbose, sucrose, D-tagatose, D-turanose, xylitol, D-xylose, L-xylose and methyl β-xyloside. All of the strains shown in the table below gave the same results in the following tests: nitrate reduction (−); indole formation (−); gelatin liquefaction (−); arginine dihydrolase (−); acidification of D-glucose (−); oxidase (+); catalase (+); and assimilation of D-glucose (+), L-malate (+) and trehalose (+). +, Positive test result; MIN , negative test result; V, variable feature (the number of strains with a positive reaction is shown in parentheses).

Test	*S. alaskensis* (*n* = 7)	*S. macrogoltabidus* LMG 17324^T	*S. terrae* LMG 17326^T
Hydrolysis of aesculin	+	+	−
β-Galactosidase	+	+	−
Urease	V (1)*	−	−
Assimilation of:			
Adipate	V (5)	−	
Caprate	−	+	−
D-Cellobiose	V (3)	+	−
D-Galactose	V (5)*	−	−
β-Gentiobiose	−	+	−
Maltose	+	−	+
D-Mannose	−	+†	−
Starch	+	−	+

* The result for the type strain is positive.

† The reaction was negative with the API 20NE system, but positive when the API 50CH gallery was used.

determined. The aim of the present study was to determine whether LMG 18877^T (= RB2256^T) belongs to one of the described species of the genus or instead represents a separate species. In addition, the taxonomic position of six analogous isolates from the same habitat was studied.

A physiological property common to all seven marine isolates of the present study is that none of them could be cultivated, after isolation, directly on nutrient-rich agar media. Growth was observed only when synthetic seawater medium containing less than approximately 1 mg carbon per litre was used as the medium; this indicates that these organisms might be obligately oligotrophic. However, storage of stationary-phase cultures for several months at 5–8 °C resulted in their ability to grow on high-nutrient laboratory media. In other words, in the course of this process the cultures may have been transformed into facultative oligotrophs (Schut *et al.*, 1993).

The physiological properties of strain LMG 18877^T have been determined in much more detail (Cavicchioli *et al.*, 1999; Eguchi *et al.*, 1996; Fegatella *et al.*, 1998; Schut *et al.*, 1993, 1997a, b; Schut, 1994). The results from these studies may be briefly summarized as follows. When cultivated on low-carbon liquid media as well as highly enriched media such as Marine agar 2216 and Luria broth, very little variation in the remarkably small cell volume has been observed. Volumes vary from 0·05 μm^3 to 0·09 μm^3, with cell diameters ranging from 0·2 to 0·5 μm and cell lengths

from 0·5 to 3·0 µm. However, on TSA only, somewhat larger and elongated cells (diameter, 0·8 µm; length, 2–3 µm) have been observed. This was not observed in earlier studies on this organism (Schut *et al.*, 1993; Schut 1994; Eguchi *et al.*, 1996) and it may indicate that the 'ultramicro' size is either prone to some variation on very rich media or that some adaptation to prolonged maintenance in laboratory culture has occurred. The DNA content of this strain is only 1·0–1·7 fg $cell^{-1}$ and it contains only one single copy of the rRNA operon. A fairly high protein content (> 800 mg ml^{-1} cell volume) has been observed. Because of the constitutive presence of a binding-protein-dependent, relatively unspecific amino-acid-uptake system and an alanine-uptake system with extremely high affinity, significant growth rates may be possible even at ambient substrate concentrations in the marine environment. The organism appears to lack a typical starvation-survival response and has appeared to be unusually stress resistant both during growth and during starvation (Eguchi *et al.*, 1996; Joux *et al.*, 1999). The combination of these properties has not been reported for any other marine isolates known to date and this very combination seems to make the organism well adapted to a life in the severely nutrient-limited environment of the open ocean.

To obtain further information on the relatedness and phylogenetic position of all the marine isolates from the present study, the three strains LMG 18871, LMG 18872 and LMG 18875 were selected for 16S rDNA sequence analysis. Numerical analysis (Fig. 1) demonstrates that all of them belong to the genus *Sphingomonas* and further indicates that the three strains are highly related to each other (similarity values higher than 99·4 %) and to strain LMG 18877^T (98·8 %). As for LMG 18877^T, strains LMG 18871, LMG 18872 and LMG 18875 showed the highest 16S rDNA relatedness with *S. macrogoltabidus*, having a similarity value of 97·4 % – a value which may indicate relatedness at the species level. No significant similarities (values higher than 97·0 %) with other *Sphingomonas* type strains were found, excluding relatedness at the species level (Stackebrandt & Goebel, 1994).

Six of the seven marine isolates and the type strain of *S. macrogoltabidus* were selected for DNA–DNA hybridization experiments. Among the marine isolates, DNA binding values of 89–100 % were obtained (LMG 18872 was not studied). No significant DNA relatedness (less than 30 %) was found between the marine strains and the type strain of *S. macrogoltabidus*. These data clearly demonstrate that the marine strains represent a new and separate genomic species. The DNA base composition of the six strains used for DNA hybridization experiments varied between 65·0 and 65·1 mol % G+C, a value which is very similar to the value (64·6 %) that was obtained for *S. macrogoltabidus*.

Fatty acid analysis was used as a chemotaxonomic approach to characterize strains of the newly delineated genomic taxon. For comparison, the type strains of *S. macrogoltabidus*, *S. terrae* and *S. paucimobilis* were included (Table 2). The overall fatty acid content, in particular the significant amounts of 14:0 2-OH (Kämpfer *et al.*, 1997), clearly confirms that all marine isolates belong to the genus *Sphingomonas*. A very similar fatty acid content was observed for all seven marine isolates but was clearly different from the pattern obtained for *S. macrogoltabidus*. The data obtained in this study are both qualitatively and quantitatively very similar to the data from Kämpfer *et al.* (1997). Because of a lack of quantitative data in the studies of Takeuchi *et al.* (1993) and Balkwill *et al.* (1997), only a qualitative congruency could be confirmed.

For the phenotypic characterization, all seven marine isolates were included in a comparative study using three different commercial galleries. With the API 20NE and the API 50CH galleries, a number of features discriminated the new taxon from its closest relatives, *S. macrogoltabidus* and *S. terrae*. The strains were differentiated from *S. macrogoltabidus* by their ability to assimilate maltose and starch and by their inability to assimilate caprate, D-mannose and β-gentiobiose. Features that distinguish the new taxon from *S. terrae* are hydrolysis of aesculin and β-galactosidase activity. A more detailed phenotypic profile of the marine isolates is given below (Table 3). These data are in general agreement with the data given by Takeuchi *et al.* (1993), Balkwill *et al.* (1997) and Kämpfer *et al.* (1997).

Our polyphasic approach has demonstrated that the marine strains belong to the genus *Sphingomonas* and constitute a separate species, for which the name *S. alaskensis* is proposed. The species name reflects the geographic location at which all of the strains thus far classified in this new species were isolated and at which they constitute a dominant population of the indigenous bacterioplankton. The species description given below is based on data from Eguchi *et al.* (1996), Schut (1994) and the present study.

Description of *Sphingomonas alaskensis* sp. nov.

Sphingomonas alaskensis (a.las.ken′sis. M.L. adj. *alaskensis* referring to Alaska, the source of the type strain).

The cells are Gram-negative, non-sporulating and motile small rods. When cultivated on low-carbon liquid media as well as highly enriched media such as Marine agar 2216 and Luria broth, very little variation in the remarkably small cell volume has been observed. Volumes vary from 0·05 to 0·09 $µm^3$ (the diameter ranges from 0·2 to 0·5 µm and the length from 0·5 to 3 µm). On TSA, somewhat larger and elongated cells (diameter, 0·8 µm; length, 2–3 µm) have been observed. Although the strains are isolated at low temperatures (4–8 °C), they all grow aerobically at 28 °C on most common bacteriological media. The optimal growth temperature for the type strain is approximately 37 °C but growth is possible between 44

and 48 °C. Strains grow at NaCl concentrations ranging from 0·5% (TSA) to at least 3% (isolation conditions). Colonies on TSA are circular, yellow- to beige-pigmented and convex with entire margins. No acidification of D-glucose occurs. Indole is not produced. Nitrates are not reduced. The following enzyme activities are present: oxidase, catalase, hydrolysis of aesculin, and β-galactosidase. The urease activity is variable. Gelatinase and arginine dihydrolase activities are absent. In the API 20NE and API 50CH system, all strains assimilate D-glucose, L-malate, maltose, starch and trehalose. Assimilation of the following carbohydrates is strain-dependent: adipate, D-cellobiose and D-galactose. None of the strains assimilate phenylacetate, adonitol, amygdalin, D-arabinose, L-arabinose, D-arabitol, L-arabitol, arbutin, caprate, citrate, dulcitol, erythritol, D-fructose, D-fucose, L-fucose, β-gentiobiose, gluconate, 2-ketogluconate, 5-ketogluconate, *N*-acetylglucosamine, methyl α-D-glucoside, glycerol, glycogen, inositol, inulin, lactose, D-lyxose, mannitol, D-mannose, methyl α-D-mannoside, D-melezitose, D-melibiose, D-raffinose, L-rhamnose, D-ribose, salicin, sorbitol, L-sorbose, sucrose, D-tagatose, D-turanose, xylitol, D-xylose, L-xylose and methyl-β-xyloside. The dominant fatty acid content consists of the fatty acids 14:0 2-OH, 15:0, 15:0 2-OH, 16:0, 16:0 2-OH, 16:1ω5*c*, 17:0, 17:1ω6*c*, 17:1ω8*c*, 18:1ω7*c*, 18:1ω7*c* 11 methyl and 'Summed feature 4'. The G+C content is 65 mol%. Isolated from seawater from Alaska. The type strain is LMG 18877^T (= RB2256^T = DSM 13593^T). All strains have been deposited in the BCCM/LMG Bacteria Collection (Laboratorium voor Microbiologie Gent, Gent, Belgium) and only the type strain has been deposited in the DSMZ Culture Collection (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany).

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