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

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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; Höfte & 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.
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 RB2256T 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 successful 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 0.2 × 10⁶ 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 RB2256T-specific probe (Schut, 1994).

Upon first cultivation, strain RB2256T 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 RB2256T has been studied extensively because of its interesting physiological properties. The organism fulfills 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 0.1 μm³) (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 RB2256T (LMG 18877T) 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 subbranches 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 parapaucimobils, 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 RB2256T 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 10⁻⁴–10⁻⁶ dilutions of seawater sample no. 1, LMG 18871 and LMG 18877T 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

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

^T LMG, BCCM/LMG Culture Collection Laboratorium voor Microbiologie, University of Gent, Gent, Belgium.

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 DNA 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 rDNA 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 calculating pairwise using an open-gap penalty of 100% and a unit-gap penalty of 0%. A similarity matrix was created from the sequence of strain LMG 18877 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 maximum 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.
constitute a homogeneous cluster (dendrogram not shown). The major fatty acids are 17:1ω6c (33.2±3.4%) and 18:1ω7c (26.5±2.6%). Additionally, smaller (but significant) amounts of the following fatty acids are present: 14:0 2-0H, 15:0, 15:0 2-0H, 16:0, 16:0 2-0H, 16:1ω5c, 17:0, 17:1ω8c, 18:1ω7c 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 (RB2256), S. macrogoltabidus LMG 17324 and S. terrae LMG 17326, and of the type strain of the type species of the genus S. paucimobilis LMG 1227 were also determined. The fatty acid content of S. terrae LMG 17326 is very similar to the pattern of the marine strains: the dominant fatty acids are 17:1ω6c (39.8%) and 18:1ω7c (20.1%), and minor amounts of the fatty acids 14:0 2-0H, 15:0, 15:0 2-0H, 16:0, 16:1ω5c, 17:0, 17:1ω8c, 18:1ω7c 11 methyl and ‘Summed feature 4’ are present. S. macrogoltabidus LMG 17324 is easily differentiated by the absence of 15:0, 15:0 2-0H, 17:0, 17:1ω6c, 17:1ω8c and the presence of significantly higher amounts of 18:1ω7c (42.2%) and ‘Summed feature 4’ (34.8%). A major fatty acid in S. paucimobilis LMG 1227 is 18:1ω7c (73.1%), and smaller (but significant) amounts of the fatty acids 14:0, 14:0 2-0H, 17:0, 18:1ω5c 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) 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 (LMG 18877), 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

<table>
<thead>
<tr>
<th>Species (no. strains)</th>
<th>14:0</th>
<th>14:0 2-OH</th>
<th>15:0</th>
<th>15:0 2-OH</th>
<th>16:0</th>
<th>16:0 2-OH</th>
<th>16:1 α5c</th>
<th>SF4*</th>
<th>17:0</th>
<th>17:1 α6c</th>
<th>17:1 α8c</th>
<th>18:1 α5c</th>
<th>18:1 α7c</th>
<th>18:1 11 methyl</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. alaskensis</em> (7)</td>
<td>tr</td>
<td>13 (0.3)</td>
<td>28 (0.2)</td>
<td>46 (0.8)</td>
<td>73 (0.7)</td>
<td>14 (0.4)</td>
<td>11 (0.2)</td>
<td>8.2 (1.4)</td>
<td>27 (0.6)</td>
<td>3.2 (1.4)</td>
<td>76 (1.0)</td>
<td>tr</td>
<td>26.5 (2.6)</td>
<td>17 (0.2)</td>
</tr>
<tr>
<td><em>S. macrogoltabidus</em> (1)</td>
<td>tr</td>
<td>2.9</td>
<td>ND</td>
<td>ND</td>
<td>132</td>
<td>18</td>
<td>2.1</td>
<td>3.48</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>42.2</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td><em>S. terrae</em> (1)</td>
<td>ND</td>
<td>1.4</td>
<td>34</td>
<td>55</td>
<td>49</td>
<td>tr</td>
<td>1.4</td>
<td>9.6</td>
<td>2.8</td>
<td>39.8</td>
<td>76</td>
<td>tr</td>
<td>20.1</td>
<td>1.1</td>
</tr>
<tr>
<td><em>S. pacimonilis</em> (1)</td>
<td>1.1</td>
<td>8.7</td>
<td>ND</td>
<td>ND</td>
<td>72</td>
<td>tr</td>
<td>3.1</td>
<td>ND</td>
<td>2.7</td>
<td>ND</td>
<td>3.5</td>
<td>73.1</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

* 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, 16:1 ω7c, 17:0 11 methyl

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

<table>
<thead>
<tr>
<th>Test</th>
<th><em>S. alaskensis</em> (n = 7)</th>
<th><em>S. macrogoltabidus</em> LMG 17324†</th>
<th><em>S. terrae</em> LMG 17326†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis of aesculin</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Urease</td>
<td>v (1)*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Assimilation of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adipate</td>
<td>v (5)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Caprate</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D-Cellobiose</td>
<td>v (3)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>v (5)*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>β-Gentiobiose</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>–</td>
<td>+†</td>
<td>–</td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

* The result for the type strain is positive.
† The result was negative with the API 20NE system, but positive when the API 50CH gallery was used.

The aim of the present study was to determine whether LMG 18877T (= RB2256T) 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 18877T 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 µm³ to 0.09 µm³, 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 18877ᵀ (98.8%). As for LMG 18877ᵀ, 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 63.0 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 µm³ (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 \( \beta \text{-galactosidase} \). 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-cellulbiose 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, \( \beta \text{-gentiobiose} \), gluconate, 2-ketogluconate, 5-keto-gluconate, N-acetylglucosamine, methyl \( \alpha \text{-D-glucoside} \), glycerol, glycogen, inositol, inulin, lactose, D-lyxose, mannitol, D-mannose, methyl \( \alpha \text{-D-mannoside} \), D-melezitose, D-melibiose, D-raffinose, L-rhamnose, D-ribose, salicin, sorbitol, L-sorbose, sucrose, D-tagatose, D-turanose, xylitol, D-xyllose, L-xyllose and methyl-\( \beta \text{-xyloside} \). 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-\( \text{OCH}_3 \), 17:0 \( \text{OHc} \), 17:1 \( \text{Oc} \), 18:1 \( \text{Oc} \), 18:1 \( \text{Oc} \) 11 methyl and ‘Summed feature 4’. The G+C content is 65 mol\%. Isolated from seawater from Alaska. The type strain is LMG 18877\(^T\) (= \text{DSMZ} 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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REFERENCES


