NOTE

Sulfurospirillum barnesii* sp. nov. and *Sulfurospirillum arsenophilum* sp. nov., new members of the *Sulfurospirillum* clade of the ϵ *Proteobacteria

John F. Stolz,¹ Debra J. Ellis,² Jodi Switzer Blum,³ Dianne Ahmann,⁴ Derek R. Lovley² and Ronald S. Oremland³

Author for correspondence: John F. Stolz. Tel: +1 412 396 6333. Fax: +1 412 396 5907.
e-mail: stolz@duq3.cc.duq.edu

¹ Department of Biological Sciences, Duquesne University, Pittsburgh, PA 15202-1521, USA

² Department of Microbiology, University of Massachusetts, Amherst, MA 01003, USA

³ Water Resources Division, US Geological Survey, 345 Middlefield Road, Menlo Park, CA 94025, USA

⁴ Nicholas School of the Environment, Duke University, Durham, NC 27708, USA

Two strains of dissimilatory arsenate-reducing vibrio-shaped bacteria are assigned to the genus *Sulfurospirillum*. These two new species, *Sulfurospirillum barnesii* strain SES-3^T and *Sulfurospirillum arsenophilum* strain MIT-13^T, in addition to *Sulfurospirillum* sp. SM-5, two strains of *Sulfurospirillum deleyianum*, and *Sulfurospirillum arcachonense*, form a distinct clade within the ϵ subclass of the *Proteobacteria* based on 16S rRNA analysis.

Keywords: arsenate reduction, selenate reduction, ϵ *Proteobacteria*, *Sulfurospirillum barnesi*, *Sulfurospirillum arsenophilum*

Two new species of bacteria have recently been identified by their ability to grow as a result of the dissimilatory reduction of arsenate to arsenite. The first, strain SES-3^T, was isolated from a selenate-contaminated freshwater marsh in western Nevada (Oremland *et al.*, 1989, 1994). The second, strain MIT-13^T, was isolated from arsenic-contaminated watershed sediments in eastern Massachusetts (Ahmann *et al.*, 1994). As these organisms were among the first to be described with this unique physiology, the genus name *Geospirillum* was proposed and several of the initial papers describing their physiology and biochemistry were published using that genus name (Lonergan *et al.*, 1996; Lovley *et al.*, 1997; Stolz *et al.*, 1997). It was later discovered that a bacterium with very similar morphology and several shared physiological characteristics had already been used to describe a new genus *Sulfurospirillum* (Schumacher *et al.*, 1992). This organism, *Sulfurospirillum deleyianum*, is the type species for the genus (Schumacher *et al.*, 1992). Recently, Finster and colleagues, in describing another new member of the genus, *Sulfurospirillum arcachonense*, suggested that SES-3^T should be included in the genus (Finster *et al.*, 1997). Here we have

used 16S rRNA and DNA–DNA hybridization analyses as well as comparison of other characteristics to clarify the taxonomic status of the arsenate-reducing strains SES-3^T and MIT-13^T. This is the first formal description of these bacteria.

The 16S rRNA gene from SES-3^T was amplified using PCR with the EubA and EubB primers (Giovannoni, 1991), cloned using the TA cloning system (Invitrogen), and sequenced using the methods of Sanger *et al.* (1977) with forward and reverse sequencing primers (Giovannoni, 1991; Lane, 1991). The 16S rRNA gene sequences from MIT-13^T and SM-5 were obtained as previously described (Lonergan *et al.*, 1996). Additional sequences were obtained from GenBank and the Ribosomal Database Projects (Maidak *et al.*, 1996; Van der Peer *et al.*, 1997). Alignment was done using CLUSTAL W (Higgins & Sharp, 1989). Maximum-parsimony and neighbour-joining trees were generated and sequence similarities were determined using a version of PHYLIP (Felsenstein, 1988). The ability of the type strain of *S. deleyianum* (ATCC 51133^T) to respire arsenate and selenate was tested using the medium described in Schumacher *et al.* (1992) with no cysteine and less potassium phosphate (140 mg l⁻¹). Formate, fumarate or hydrogen were used as electron donors with acetate as the carbon source. Stock cultures of *S. deleyianum* were maintained on medium containing

The GenBank accession number for the 16S rDNA sequence of strain SES-3^T is AF038843.

Table 1. Comparison of *Sulfurospirillum barnesii* and *S. arsenophilum* to the type species *S. deleyianum* and *S. arcachonense*

Character	<i>S. deleyianum</i>	<i>S. arcachonense</i>	<i>S. barnesii</i>	<i>S. arsenophilum</i>
Width	0.3–0.5 µm	0.3 µm	0.3 µm	0.1–0.3 µm
G + C (mol %)	40.6	32	40.8	40.9
Growth at:				
25 °C	+	+	+	+
30 °C	+	+	+	+
42 °C	–	–	–	–
pH optimum	7.0–7.1	7.0–7.4	7.5	ND
NaCl (%)	< 0.2	1.2–2.0	0.8	0.1
Oxidase	+	+	ND	ND
Catalase	–	+	ND	ND
Electron acceptors:				
Nitrate	+	–	+	+
Nitrite	+	–	+	+
Thiosulfate	+	–	+	+
TMAO	+	–	+	ND
Fe(III)	ND	–	+	ND
Sulfite	+	–	–	ND
DMSO	+	–	–	ND
Arsenate	–	ND	+	+
Selenate	–	ND	+	–
Elemental sulfur	+	+	+	+
Oxygen (microaerobic)	+	+	+	+
Electron donors:				
Lactate	–	+	+	+
Pyruvate	+	ND	+	+
Fumarate	+	+	+	+
Formate	+	ND	+	+
Fermentation:				
Fumarate	+	ND	+	+
Malate	+	–	ND	ND
Growth with 1 % glycerol	–	+	ND	ND

ND, Not determined.

fumarate (Schumacher *et al.*, 1992). G + C content determination (as determined by HPLC) and DNA–DNA hybridization analyses were done by the Deutsche Sammlung von Mikroorganismen und Zellkulturen.

The comparison of known species characteristics is shown in Table 1. In addition to its ability to respire arsenate and selenate, *Sulfurospirillum barnesii* can be distinguished from *S. deleyianum* by pH and salinity optima, the ability to use lactate as an electron donor, and the inability to use sulfite as an electron acceptor. *S. barnesii* SES-3^T can be distinguished from *Sulfurospirillum arsenophilum* MIT-13^T by size, salinity optimum, lactate oxidation to acetate (*S. arsenophilum* oxidized lactate completely to CO₂), and ability to use selenate as a terminal electron acceptor. It is also not closely related to another known selenate-respiring bacterium, *Thauera selenatis*, a member of the β *Proteobacteria* (Macy *et al.*, 1993). *S. arsenophilum* is distinguished from the type species by its size, the ability to use lactate, and arsenate respiration. Neither

S. barnesii nor *S. arsenophilum* are related to the known arsenate-reducing species *Desulfotomaculum auripigmentum*, which is a low G + C Gram-positive bacterium (Newman *et al.*, 1997) or *Chrysiogenes arsenatis* which is the sole member of a deeply branching lineage of the Bacteria (Macy *et al.*, 1996). The results of our phylogenetic analysis concurred with Finster *et al.* (1997) as it showed that *S. barnesii* is most closely related to two strains of *S. deleyianum* (Fig. 1) with a 98 % sequence similarity. *S. arsenophilum* also branched with *S. barnesii* and the *S. deleyianum* strains (Fig. 1), with a sequence similarity to all three of 97 %. The results of the sequence analysis also show that there are significant differences between the two strains of *S. deleyianum*, CCUG 13942 (DSM 806, also known as *Campylobacter* sp. Veldkamp) (Laanbroek & Veldkamp, 1979; Laanbroek *et al.*, 1977) and CCUG 23206^T (ATCC 51133^T, DSM 6946^T, also known as *Spirillum* 5176).

Additional molecular analysis was done because of the close sequence similarity of the 16S rRNA sequences

Fig. 1. Phylogenetic tree of *Sulfurospirillum* species based on 16S rRNA gene sequence (1250 bases) using maximum-parsimony analysis (Felsenstein, 1988). The species in the *Sulfurospirillum* clade (denoted by a bracket) are (with accession nos in parentheses) *S. deleyianum* DSM 6946^T (Y13671), *S. deleyianum* CCUG 13942 (L14632), *S. barnesii* strain SES-3^T (AF038843), *S. arsenophilum* strain MIT-13^T (U85964), *Sulfurospirillum* sp. strain SM-5 (U85965) and *S. arcachonense* DSM 9755^T (Y11561). Bootstrap numbers are given at the branch nodes.

of *S. barnesii*, *S. arsenophilum* and *S. deleyianum* DSM 6946^T. The G+C content was 40.8 mol% for *S. barnesii* and 40.9 mol% for *S. arsenophilum* as compared to 40.6 mol% for *S. deleyianum*. The percentage DNA–DNA hybridizations were as follows: *S. barnesii* to *S. deleyianum*, 55; *S. arsenophilum* to *S. deleyianum*, 30.7; *S. barnesii* to *S. arsenophilum*, 49.7. These results clearly show that *S. barnesii* and *S. arsenophilum* are arguably separate species and definitively distinct from the type strain.

The addition of *S. barnesii* and *S. arsenophilum*, as well as the uncharacterized isolate strain SM-5 (Coleman *et al.*, 1993), to the genus *Sulfurospirillum* further strengthens the suggestion of Finster *et al.* (1997) that the members of the genus form a distinct clade within the ϵ Proteobacteria distinct from the *Campylobacter*, *Arcobacter*, *Alvinella* and *Helicobacter* clades.

Description of *Sulfurospirillum barnesii* sp. nov.

Sulfurospirillum barnesii (barnes'i.i. M.L. *barnesii* of Barnes, named after our deceased colleague, Ivan Barnes, 1931–1989, of the US Geological Survey, in tribute to his recognition of the environmental significance of selenium).

Gram-negative. Vibrioid to spirillum-shaped cells, 0.3 μ m wide and 1–2 μ m in length. Motile by polar flagellum. Growth optimum 33 $^{\circ}$ C, pH 7.5 and 0.05% NaCl. Selenate, arsenate, thiosulfate, elemental sulfur, trimethylamine oxide, Fe(III), nitrate, fumarate, aspartate and manganese dioxide can all be used as a terminal electron acceptor. Capable of microaerobic growth. Selenate is reduced through selenite to elemental selenium. Nitrate is reduced to ammonium. Lactate and H₂ can serve as electron donors. When grown with hydrogen, acetate is required as the carbon source. The G+C content of the DNA is 40.8 mol%. Strain SES-3^T (= ATCC 700032^T) is the type strain.

Isolated from a selenium-contaminated freshwater marsh.

Description of *Sulfurospirillum arsenophilum* sp. nov.

Sulfurospirillum arsenophilum (ar.sen.o'phi.lum. Gr. n. *arsenikon* yellow orpiment, arsenic; Gr. adj. *-philos* loving; N.L. adj. *arsenophilum* arsenic-loving, named because of its ability to grow dissimilatorily on arsenate).

Gram-negative. Vibrioid to spirillum-shaped cells, 0.3 μ m wide and 1–2 μ m in length. Motile by single polar flagellum. Growth optima are 20 $^{\circ}$ C, pH 7.5 and 0.1% NaCl. Hydrogen and formate can serve as electron donors when acetate is used as the carbon source. Lactate, pyruvate and fumarate can be used as electron donors. Arsenate, nitrate and fumarate can serve as terminal electron acceptors. Nitrate is reduced to ammonia. Can grow fermentatively on fumarate. The G+C content of the DNA is 40.9 mol%. Strain MIT-13^T (= ATCC 700056^T) is the type strain. Isolated from arsenic-contaminated freshwater sediments.

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