Sulfurospirillum arcachonense sp. nov., a New Microaerophilic Sulfur-Reducing Bacterium

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The isolation of a new motile, gram-negative, heterotrophic, sulfur-reducing, microaerophilic, vibrioid bacterium, strain F1F6, from oxidized marine surface sediment (Arcachon Bay, French Atlantic coast) is described. Hydrogen (with acetate as the carbon source), formate (with acetate as the carbon source), pyruvate, lactate, α -ketoglutarate, glutarate, glutamate, and yeast extract supported growth with elemental sulfur under anaerobic conditions. Apart from H_2 and formate, the oxidation of the substrates was incomplete. Microaerophilic growth was supported with hydrogen (acetate as the carbon source), formate (acetate as the carbon source), acetate, propionate, pyruvate, lactate, α -ketoglutarate, glutamate, yeast extract, fumarate, succinate, malate, citrate, and alanine. The isolate grew fermentatively with fumarate, succinate being the only organic product. Elemental sulfur and oxygen were the only electron acceptors used. Vitamins or amino acids were not required. The isolate was oxidase, catalase, and urease positive. Comparative 16S rDNA sequence analysis revealed a tight cluster consisting of the validly described species *Sulfurospirillum deleyianum* and the strains SES-3 and CCUG 13942 as the closest relatives of strain F1F6 (level of sequence similarity, 91.7 to 92.4%). Together with strain F1F6, these organisms form a novel lineage within the epsilon subclass of proteobacteria clearly separated from the described species of the genera *Arcobacter*, *Campylobacter*, *Wolinella*, and *Helicobacter*. Due to the phenotypic characteristics shared by strain F1F6 and *S. deleyianum* and considering their phylogenetic relationship, we propose the inclusion of strain F1F6 in the genus *Sulfurospirillum*, namely, as *S. arcachonense* sp. nov. Based on the results of this study, an emended description of the genus *Sulfurospirillum* is given.

The ability to use elemental sulfur as an electron acceptor for organotrophic or lithotrophic growth has been reported from a relatively large number of metabolically versatile microbial groups of the domains *Bacteria* and *Archaea* (15, 21, 22, 24, 33, 37, 38, 47). Sulfur-reducing bacteria can be placed in two distinct physiological groups, which include genera belonging to different phylogenetic lineages. The first group (group 1) includes species of obligate anaerobic bacteria belonging to the genera *Desulfuromonas* (33), *Desulfurella* (2), *Desulfuromusa* (22), *Geobacter* (5), and *Pelobacter* (24). With the exception of those members of the genus *Pelobacter* that have been tested for the ability to grow with sulfur as an electron acceptor (24), all members of group 1 completely oxidize their substrates (complete oxidizers), releasing exclusively CO_2 and H_2S . A second group (group 2) includes members of the genera *Wolinella* (49), *Campylobacter* (37), *Shewanella* (27) (originally described as a member of the genus *Alteromonas* [30]), and *Sulfurospirillum* (37, 48), as well as isolates which have not been characterized taxonomically, such as free-living “*Campylobacter*-like organisms,” (DSM 806 = CCUG 13942) (19) and the dissimilatory selenate- and arsenate-reducing strain SES-3 (21, 31) that has been tentatively named “*Geospirillum barnesii*” (23, 41). These species have in common the abilities to grow at low oxygen concentrations and to oxidize organic substrates incompletely with elemental sulfur as the electron acceptor (incomplete oxidizers). Among the sulfate-reducing bacteria, in particular, members of the genera *Desulfovibrio* and *Desulfomicrobium* (1) have the ability to couple the reduction of sulfur to growth. Members of both genera are obligate

anaerobes, like the group 1 sulfur reducers. It is noteworthy that all of the bacterial groups under discussion belong to the *Proteobacteria*. However, the sulfate-reducing bacteria mentioned above and group 1 sulfur reducers, except *Desulfurella acetivorans* (34), form distinct lineages within the delta subclass of the *Proteobacteria*, whereas all microaerophilic Group 2 sulfur reducers are placed either in the gamma subclass (*Shewanella* spp.) or in the epsilon subclass of the *Proteobacteria* (*Campylobacter* spp., *Sulfurospirillum* spp. [results of this study], and *Wolinella* spp.).

In this communication, we report on the enrichment, isolation, and genotypic and phenotypic characterization of strain F1F6, a marine, microaerophilic, sulfur-reducing, gram-negative bacterium.

MATERIALS AND METHODS

Source of inoculum. Oxidized iron-rich surface sediment from a *Zostera noltii*-overgrown mud flat (Arcachon, French Atlantic Coast) served as the inoculum for a dilution series used to enumerate formate-consuming sulfur reducers. Six strains were isolated from the highest dilution in which growth occurred (10^6). When it became apparent that the isolates closely resembled each other phenotypically, only one strain, designated F1F6, was studied in detail.

Medium and cultivation. The defined mineral medium used was prepared and handled as described by Finster and Bak (11). Substrates were added from sterile stock solutions with glass pipettes prior to inoculation. Elemental sulfur was sterilized as described by Pfennig and Biebl (33). Amorphous Fe(III)-oxyhydroxide was prepared by titration of an acidic $FeCl_3$ solution with NaOH to pH 7. The suspension was sterilized by autoclaving. Organic and inorganic substrates were sterilized by either autoclaving or filtration through sterile 0.2- μ m-pore-size filters into sterile glass bottles. Cultures were incubated at 26°C in the dark.

Enrichment cultures and isolation. On the basis of the physical and chemical measurements carried out to characterize the natural environment under study, it was shown that the following environmental conditions prevailed: (i) high iron content, (ii) positive redox potential, and (iii) no free hydrogen sulfide (17). We therefore prepared dilution series containing 8 ml of defined marine minimal medium, highly purified elemental sulfur, 1 ml of a 0.3 M suspension of amorphous Fe(III)-oxyhydroxide, formate (20 mM), and acetate (1 mM). The test

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tubes were incubated at 20°C in the dark and examined daily for growth. Sulfur-reducing bacteria were isolated by repeated application of deep agar dilutions (30). Fe(III)-oxyhydroxide-containing solid media in test tubes were prepared as described by Finster et al. (12).

In addition to microscopic examination, isolates were checked for purity in complex medium containing yeast extract (0.1%), fumarate (5 mM), glucose (5 mM), and pyruvate (5 mM), as well as on blood agar plates under oxic and microoxic (3% O₂) conditions.

Substrate tests. The spectrum of electron donors and electron acceptors utilized by strain F1F6 was determined in 20-ml screw-cap test tubes supplemented with the appropriate substrate combination and inoculated with bacteria precultured on fumarate. The inoculum was 1% (vol/vol). In electron donor tests, either sulfur or oxygen (3% in the gas phase) served as the electron acceptor. Test medium with elemental sulfur also contained 1 ml of Fe(III)-oxyhydroxide. The reduction of elemental sulfur was monitored by observing blackening of the iron precipitate. Growth was monitored by microscopic examination. Experiments with oxygen as the electron acceptor were carried out with 120-ml serum bottles sealed with rubber stoppers. They contained 20 ml of inoculated medium and were incubated on a bottle roller (120 rpm). Inoculated test tubes and serum bottles containing elemental sulfur or oxygen, respectively, but no electron donor served as controls. Lactate, pyruvate, malate, fumarate, maleate, glutamate, oxalacetate, and aspartate (all at 5 mM) were also tested without an electron acceptor. Inoculated test tubes without electron donors and acceptors served as controls. The ability to grow by fermentation of glucose, mannitol, inositol, rhamnose, sucrose, melibiose, amygdalin, and arabinose was tested with API 20E test kits (Biomérieux, Marcy-l'Etoile, France) under oxygen-free conditions. Electron acceptors were tested with formate (20 mM) and acetate (1 mM) as the electron donor and carbon source, respectively. Inoculated test tubes without an electron acceptor served as controls. The incubations were carried out at 26°C in the dark. All tests were carried out in duplicate, and tubes were incubated for 4 weeks and periodically examined for sulfide production and growth.

Growth parameters. Carbon sources, the vitamin requirement, and the pH, salt, and temperature tolerance of the isolate were studied by growth tests in which the change in optical density of the culture medium or the accumulation of hydrogen sulfide was monitored. All experiments were carried out in duplicate. The temperature range for growth of the isolate was determined by incubating inoculated test tubes in an insulated aluminium temperature gradient block with a minimum temperature of 4°C and a maximum temperature of 50°C. Incubation was stopped after a maximum of 6 weeks. Generation times were calculated from the increase in the optical density of the culture over time. When the strain was grown with formate, acetate, and elemental sulfur, the generation time was derived from a semilogarithmic plot of the sulfide concentrations as a function of time. A straight line was interpreted as exponential growth of the bacterial population. The presence of catalase was assayed by addition of a few drops of a 3% H₂O₂ solution to a grown culture of strain F1F6. Cytochrome oxidase was assayed with prefabricated test strips by following the instructions of the supplier (Merck, Darmstadt, Germany).

Extraction of respiratory lipoquinones and polar lipids. Respiratory lipoquinones and polar lipids were extracted from 100 mg of freeze-dried cell material by using the two-stage method described by Tindall (44, 45). Polar lipids were extracted by adjusting a methanol-0.3% aqueous NaCl phase to give a chloroform-methanol-0.3% aqueous NaCl mixture (1:2:0.8, vol/vol/vol). The extraction solvent was stirred, and the cell debris was pelleted by centrifugation. Polar lipids were recovered in the chloroform phase by adjusting the chloroform-methanol-0.3% aqueous NaCl mixture to a ratio of 1:1:0.9 (vol/vol/vol).

Analysis of respiratory lipoquinones. Respiratory lipoquinones were separated into their different classes (menaquinones and ubiquinones) by thin-layer chromatography on silica gel (Macherey-Nagel no. 805 023) with hexane-*tert*-butylmethylether (9:1, vol/vol) as the solvent. UV-absorbing bands corresponding to menaquinones or ubiquinones were removed from the plate and further analyzed by high-performance liquid chromatography (HPLC). This step was carried out on an LDC Analytical (Thermo Separation Products) HPLC apparatus fitted with a reverse-phase column (2 by 125 mm, 3 μ m, RP₁₈; Macherey-Nagel) with methanol as the eluant. Respiratory lipoquinones were detected at 269 nm.

Analysis of polar lipids. Polar lipids were separated by two-dimensional silica gel thin-layer chromatography (Macherey-Nagel no. 818 135). The first direction was developed in chloroform-methanol-water (65:25:4, vol/vol/vol), and the second was developed in chloroform-methanol-acetic acid-water (80:12:15:4, vol/vol/vol/vol). Total lipid material and specific functional groups were detected by using dodecamolybdophosphoric acid (total lipids), Zinzadze reagent (phosphate), ninhydrin (free amino groups), periodate-Schiff (α -glycols), Dragendorff's test (quaternary nitrogen), and anisaldehyde-sulfuric acid (glycolipids).

Fatty acid analysis. Fatty acids were analyzed as the methyl ester derivatives prepared from 10 mg of dry cell material. Cells were subjected to differential hydrolysis to detect ester-linked and non-ester-linked (amide-bound) fatty acids (45a). Fatty acid methyl esters were analyzed by gas chromatography with using a nonpolar capillary column (0.2 μ m by 25 m) and a flame ionization detector. The run conditions were an injection and detector port temperature of 300°C, an inlet pressure of 60 kPa, a split ratio of 50:1, an injection volume of 1 μ l, and a temperature program ranging from 130 to 310°C at a rate of 4°C/min.

Chemical analysis. Volatile fatty acids, lactate, succinate, and fumarate were analyzed by ion-exclusion chromatography with an HPLC system as described recently (12). Sulfide was measured by the methylene blue method as described by Cline (6).

G+C content of genomic DNA. Determination of the G+C content of the DNA was carried out at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany, by using HPLC separation and UV detection (43). Calibration and determination of G+C content were performed as described by Mesbah et al. (26).

Comparative 16S rDNA sequence analysis. DNA isolation, PCR-mediated amplification of the almost complete 16S rRNA gene, and sequence analysis were done as described previously (22). In addition to that of strain F1F6, the 16S rDNA sequence of *Sulfurospirillum deleyianum* 5175 (DSM 6946^T) was determined for reference. The 16S rDNA sequences were added to a database of about 6,000 complete or partial 16S rRNA primary structures of bacteria (25, 35, 46) by using the automatic alignment tool of the ARB program package (42). The alignment was checked and, if necessary, manually corrected. The phylogenetic positions of strain F1F6 and *S. deleyianum* were deduced by comparing their 16S rDNA sequences with reference sequences from members of the epsilon subclass of the *Proteobacteria* for which at least 1,400 determined nucleotide positions were available (103 sequences). The overall tree topology was evaluated for a sequence stretch ranging from positions 28 through 1475 (*Escherichia coli* 16S rRNA numbering [4]) by using distance matrix and maximum-parsimony methods (PHYLIP; reference [10]). In addition, different subsets of about 30 sequences were used to reconstruct phylogenetic trees by applying the maximum-likelihood method (fastDNAm1 [25]). These sets of data varied with respect to the reference sequences used, as well as the alignment positions included. To avoid possible treeing artifacts caused by nucleotide sequence positions that are subject to multiple mutational changes and/or not to align unambiguously, only those positions which contained identical residues in at least 50% of the alignment positions were used for phylogenetic analyses. The usefulness of this invariance criterion for the inclusion and exclusion, respectively, of nucleotide sequence positions from the treeing analysis has been discussed in detail by Friedrich et al. (13). The variabilities of individual alignment positions were determined by using the appropriate tool of the ARB package. The base frequencies were calculated based on the complete set of available epsilon proteobacterial sequences and based on different subsets comprising equivalent numbers of reference sequences belonging to the major subgroups of the epsilon subclass. This resulted in the use of 1323 to 1352 nucleotide sequence positions for reconstruction of the phylogenetic trees. The statistical significance of interior branching points was tested in bootstrap analyses by using the maximum-parsimony (PHYLIP; 100 data resamplings) and neighbor-joining (ARB; 1,000 data resamplings) methods. All phylogenetic analyses were done by using software in the ARB package.

Nucleotide sequence accession numbers. The 16S rDNA sequences of strain F1F6 and *S. deleyianum* 5175 (DSM 6946^T) have been deposited in the EMBL, GenBank, and DDBJ nucleotide sequence databases under accession numbers Y11561 and Y13671, respectively. All of the reference sequences used are part of the ARB software package (16S rRNA database: tree_1400_feb97).

RESULTS

Enrichment and isolation. After an incubation period of 3 weeks, in all tubes the red Fe(III)-oxyhydroxide had reacted to black iron sulfide. The majority of the highly motile curved rods which were dominant in all dilutions of the most-probable-number series was present in the black precipitate. The number of free-swimming cells was increased after particles of the precipitate were crushed under the coverslip. The highest positive dilution was chosen to inoculate agar dilution series. Well-separated, whitish, slightly filamentous colonies from the fifth dilution step were withdrawn and grown in iron-containing liquid medium. The procedure was repeated until culture purity was obtained. Interestingly, a different colony morphology and colony pigmentation appeared when the agar deeps were supplemented with either fumarate as the sole substrate or succinate and oxygen (3% in the gas phase). In both cases, the colonies were lens shaped with entire edges and had yellow-brown pigmentation. Large colonies were surrounded by a halo consisting of less densely packed cells. No difference, however, was observed at the cellular level. Colonies transferred from one of the three agar media to liquid medium containing either fumarate, succinate, and oxygen (3% in the gas phase) or formate, acetate, sulfur, and Fe(III)-oxyhydroxide grew equally well compared to colonies that were transferred from agar medium to liquid medium identical in com-

FIG. 1. Phase-contrast photomicrograph of *S. arcachonense* F1F6 grown with fumarate. Bar, 5 μ m.

position. Anaerobic or microaerophilic (3% oxygen in the gas phase) growth occurred neither on agar plates containing basal medium and fumarate, on blood agar plates, nor in liquid McConkey medium.

Morphology. Cells of strain F1F6 were vibrioid, 1.0 to 2.5 μ m long, and 0.3 μ m wide (Fig. 1). In growing cultures, spirilla consisting of two or more single cells were often observed. Cells were highly motile by a polar or subpolar flagellum. The cells stained gram negative.

Carbon sources and electron donors. (i) **Growth with elemental sulfur.** The substrate concentration used was 5 mM if not indicated otherwise. Strain F1F6 was able to grow on hydrogen or formate in the presence of acetate or another organic carbon source (see below for carbon sources), lactate, pyruvate, α -ketoglutarate, glutarate, glutamate, and yeast extract (0.15 mM) when elemental sulfur served as the electron acceptor. The following electron donors were not used with elemental sulfur as the electron acceptor: acetate, propionate, butyrate, valerate, myristate (2 mM), benzoate (1 mM), trimethoxybenzoate (1 mM), cyclohexanecarboxylate (2 mM), vanillin (2 mM), pyrogallol (2 mM), pyrimidine (2 mM), phenylpropionate (2 mM), *p*-cresol (1 mM), succinate, fumarate, malate, maleate (2 mM), pimelate, adipate, methanol, ethanol, *n*-propanol, glycerol, glycolate (2 mM), glyoxylate (2 mM), acetone, ethylene glycol, oxalate (1 mM), formaldehyde (1 mM), betaine, cholinchloride, alanine, aspartate, mannitol, glucose, gluconate (2 mM), and starch (0.1%). The strain was not able to grow autotrophically with elemental sulfur and Fe(III)-oxyhydroxide, nor was the strain able to disproportionate elemental sulfur.

Of all the substrates tested, only fumarate was fermented. Succinate was the sole organic product. The following substrates were not fermented (concentrations in API 20E test kit are unknown): lactate, pyruvate, malate, aspartate, maleate, glutamate, oxalacetate, glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, and arabinose.

(ii) **Growth with oxygen.** In the presence of oxygen (3% in the headspace) as the electron acceptor, the following compounds sustained growth: H_2 (acetate as the carbon source), acetate, propionate, succinate, pyruvate, lactate, α -ketoglutarate, glutamate, citrate, malate, alanine, and yeast extract. Growth with formate (with acetate as the carbon source) was

observed only with 1% or less oxygen in the headspace. At higher oxygen concentrations, growth was inhibited.

In contrast, oxygen concentrations of up to 15% in the gas phase supported growth with succinate as the organic substrate in agitated cultures. Culture growth was initiated by clump formation by the inoculated cells. After some days, the turbidity of the medium increased, indicating more free-swimming cells, as verified by microscopy. In nonagitated culture bottles, strain F1F6 grew with succinate as the substrate under an atmosphere containing 20% oxygen.

When bacteria were grown with fumarate in the presence of oxygen, succinate was produced as an intermediate. Acetate, propionate, lactate, fumarate, and succinate were oxidized completely with oxygen as the electron acceptor. The degradation of the other substrates was not studied in detail. The following substrates did not sustain growth with oxygen (3%) in the gas phase: glutarate, propionate, butyrate, methanol, ethanol, glycerol, starch, and betaine.

The strain was not able to utilize sulfate, thiosulfate, sulfite (2 mM), dimethyl sulfoxide, trimethylamine *N*-oxide, nitrate, nitrite (2 mM), Fe(III)-oxyhydroxide, Fe-citrate, malate, or aspartate as an electron acceptor. The generation times of strain F1F6 (measured at 22°C) with different substrate combinations were as follows: formate (acetate as the carbon source) plus elemental sulfur, 33 h; fumarate, 16 h; fumarate plus oxygen (3% in the gas phase), 12 h; succinate plus oxygen (3% in the gas phase), 32 h.

(iii) **Organic carbon sources.** Strain F1F6 was not able to grow anaerobically by sulfur reduction with formate and CO_2 alone but required an additional organic carbon source. Acetate could be replaced with propionate, citrate, succinate, malate, or alanine. Glucose, glycerol, or ethanol was not used as a carbon source. Cultures with acetate, citrate, or succinate grew equally well, and signs of growth (blackening of the iron phase and clumping of the precipitate) were observed 3 days after inoculation. A lag phase of 7 days was observed in cultures grown on propionate, while cultures with malate or alanine as the carbon source started to grow after 16 days.

pH, salt, and temperature ranges and optima. Strain F1F6 grew within a pH range of 6.1 to 8.2 with an optimum between 7.0 and 7.4. The lowest temperature at which growth occurred was 8°C, and the highest was 30°C. The optimum temperature was 26°C.

Strain F1F6 required both NaCl and $MgCl_2 \cdot 6H_2O$ for growth. Growth was inhibited with less than 0.6% or more than 4% NaCl in the medium. The optimum NaCl concentration was between 1.2 and 2%. At least 0.1% $MgCl_2 \cdot 6H_2O$ was required. The maximum concentration was not determined. Growth was only slightly inhibited with 2.1% $MgCl_2 \cdot 6H_2O$ compared to the optimum concentration (0.1 to 0.3%). Vitamins were not required for optimal growth. Strain F1F6 was oxidase and catalase positive.

G+C content of the genomic DNA. The G+C ratio of the DNA of strain F1F6 was 32.0 mol%.

Quinones and lipids. The only respiratory lipoquinones detected were naphthoquinones, as determined by thin-layer chromatography. Two bands were evident on the thin-layer plates, with R_f values suggesting the presence of a menaquinone and a monomethylmenaquinone. This was confirmed by HPLC separation of the naphthoquinones, resulting in the detection of two compounds only, a menaquinone with six isoprenoid units (MK-6; ~83%) and a monomethylmenaquinone with six isoprenoid units (MMK-6; ~17%).

Polar lipids. Only phospholipids were detected, and they comprised two major components, phosphatidylglycerol and phosphatidylethanolamine, together with a smaller amount of

FIG. 2. 16S rDNA-based phylogenetic dendrogram reconstructed for strain F1F6, *S. deleyianum* 5175, and 84 reference organisms comprising the major subgroups within the epsilon subclass of the Proteobacteria and *E. coli* as an outgroup reference. The tree topology was derived by performing distance matrix analyses (calculation of the distance matrix, Jukes-Cantor equation [18]; tree construction, neighbor-joining method [36]) and corrected in accordance with the results of maximum-parsimony and maximum-likelihood analyses. The presence of four major subgroups characterized by *S. deleyianum*, strain SES-3 (*G. barnesii*), and strain F1F6 (I); *Campylobacter* spp. (II); *Arcobacter* spp. (III); and *Helicobacter* spp. plus *Wolinella succinogenes* (IV) was confirmed by bootstrap values as high as 95 through 99 (percentage of outcome) in neighbor-joining tests and 100 in maximum-parsimony tests for the respective interior branching points (arrows). The triangles indicate the phylogenetic depth of the genera *Arcobacter*, *Campylobacter*, and *Helicobacter* plus related organisms, respectively. Each number in parentheses is the number of reference sequences of the respective genus used to reconstruct the phylogenetic tree. The scale bar represents the estimated number of base changes per nucleotide sequence position.

a third amino- and phosphate-positive lipid, with an R_f value similar to that of lysophosphatidylethanolamine.

Fatty acids. Analysis of the fatty acid pattern revealed as predominant compounds 14:0 (2.7%), 14:1 (3.2%), 16:0 (31.0%), 16:1 (40.8%), 18:0 (20.8%), and 18:1 (0.6%). Trace amounts of 15:0, 17:0, and 3OH 14:0 were also detected. All major fatty acids appeared to be ester linked. The 3OH 14:0 was amide linked. Particularly interesting, however, was the failure to detect significant quantities of hydroxy fatty acids (<2% in strain F1F6).

Phylogenetic analysis. Phenotypic characteristics shared by strain F1F6 and *S. deleyianum* 5175 suggested that it would be appropriate to include the 16S rDNA sequence of the latter organism in this study. Integration of these two sequences into the available database of bacterial 16S rRNA sequences showed that both strains belong to the epsilon subclass of the Proteobacteria. All treeing analyses not only confirmed the phylogenetic affiliation of both organisms but also indicated that they form, together with strains SES-3 and CCUG 13942, a novel lineage clearly separated from members of the genera *Arcobacter*, *Campylobacter*, and *Helicobacter* plus *Wolinella* which comprises the major subgroups of cultured organisms within the epsilon subclass of proteobacteria (Fig. 2). Within the phylogenetic radiation of the *Sulfurospirillum* subgroup, strain F1F6 forms a slightly separate branch whereas the other three organisms represent a tight cluster with overall 16S rRNA similarity values above 97.5%. The corresponding values between strain F1F6 and *S. deleyianum*, strain SES-3, and strain CCUG 13942 are 92.0, 91.7, and 92.5%, respectively. In contrast, the level of sequence similarity between strain F1F6 and representatives of the other major lineages within the epsilon subclass of proteobacteria ranges between 82.9 and 88.2%.

DISCUSSION

Based on a detailed polyphasic approach which included physiological, chemotaxonomic, and phylogenetic investigations, the new isolate, strain F1F6, was identified as a true member of the epsilon subclass of proteobacteria. *S. deleyianum* 5175 represents its closest validly described relative. The overall 16S rRNA dissimilarity value of 8.0% between both microorganisms suggests the taxonomic rank of a new genus for strain F1F6. However, due to the common phenotypic characteristics in conjunction with their phylogenetic affiliation, we describe strain F1F6 as the type strain of a new species of the genus *Sulfurospirillum*, namely, as *S. arcachonense* sp. nov.

Another interesting result of this study is the finding of a close relationship between *S. deleyianum* and strain SES-3. The 16S rRNA sequences of both microorganisms share an overall similarity of 99% and thus can be considered as phylogenetic twins. In recent publications, the dissimilatory Fe(III)-, selenate-, and arsenate-reducing strain SES-3 has tentatively been named "*Geosprillum barnesii*" (23, 41). However, the genus name "*Geosprillum*" is in conflict with the validly described taxon *Sulfurospirillum* (16, 37), which should be considered in the further taxonomic description of strain SES-3.

Chemotaxonomy. Although menaquinones and monomethylmenaquinones have been reported in members of the genus *Shewanella* (29), members of this genus also contain ubiquinones. The only organisms known to produce exclusively menaquinones and monomethylmenaquinones are members of the genus *Thermoplasma* and members of the genera *Arcobacter*, *Campylobacter*, *Helicobacter*, and *Wolinella* (7, 8, 28). Members of the genus *Thermoplasma* produce MK-7 and MMK-7, whereas the majority of those members of the genera *Arcobacter*, *Campylobacter*, *Helicobacter*, and *Wolinella* examined to date produce MK-6 and MMK-6. Exceptions within the members of the *Campylobacter*-*Helicobacter*-*Arcobacter* complex include some species which produce a menaquinone with an unknown structural modification in the side chain (28). *S. deleyianum* has also been shown to produce MK-6 and MMK-6 in proportions similar to those found in strain F1F6 (9). Thus, in the absence of ether-linked lipids, an organism found to contain only menaquinones and monomethylmenaquinones has to be tentatively assigned to the *Campylobacter*-*Helicobacter*-*Arcobacter* complex.

The fatty acid pattern of strain F1F6 showed a number of features which appear to be shared by the limited number of strains belonging to the *Campylobacter*-*Helicobacter*-*Arcobacter* complex which have been studied with respect to their fatty acid composition (3, 14, 20). All of the organisms examined contained 14:0, 16:0, 18:0, and 18:1 ω 7c, a combination which appears to be distinctive within the Proteobacteria. Various subgroups are defined by the presence of fatty acids such as 12:0 14:1 ω 7c, and 16:1 ω 7c. While the majority of strains examined in previous studies also contained 3-hydroxy fatty acids, various subgroups included those organisms containing 3-OH 12:0 and 3-OH 16:0, only 3-OH 14:0, 3-OH 14:0 and 3-OH 16:0, or 3-OH 18:0, indicating the potential for using fatty acids in the taxonomy of this group. Significantly, strain F1F6 contained all of the fatty acids determined to be characteristic of the *Campylobacter*-*Helicobacter*-*Arcobacter* complex but was also distinctive in containing the fatty acid 14:1 (a fatty acid not commonly found in those species examined) and contained no significant quantities of 3-hydroxy fatty acids. The presence of trace amounts of 3-OH 14:0 was indicative of the vestiges of a compound which is otherwise fairly widely distrib-

uted within this group of organisms. No data on the fatty acid composition of *S. deleyianum* is available.

Practically no data on the polar lipid composition of members of the *Campylobacter-Helicobacter-Arcobacter* complex is available, making any comparisons difficult.

In conclusion, the results of the respiratory lipoquinone, polar lipid, and fatty acid analysis of strain F1F6 indicate that it is chemically similar in many respects to other members of the *Campylobacter-Helicobacter-Arcobacter* complex studied to date. This provides independent evidence in support of the 16S rDNA sequence. While the chemical data available on members of the *Campylobacter-Helicobacter-Arcobacter* complex is rather scant, it supports the inclusion of strain F1F6 in the same phyletic group as members of the genera *Campylobacter*, *Helicobacter*, *Arcobacter*, and *Wolinella*. However, some of the chemical features of strain F1F6 (particularly the fatty acid pattern) indicate that it belongs to a distinct phyletic group within the *Campylobacter-Helicobacter-Arcobacter* complex.

Phenotypic comparison with *S. deleyianum* 5175 and strain SES-3. The relatively close evolutionary affiliation of strain F1F6 with *S. deleyianum* is also reflected by a number of common phenotypic characteristics (37): (i) curved-to-spiral morphology, (ii) the ability to use elemental sulfur for organotrophic or lithotrophic growth, (iii) the ability to use oxygen as an electron acceptor, (iv) the ability to reduce fumarate to succinate, (v) the inability to use sulfate as an electron acceptor, (vi) the requirement for an organic carbon source, (vii) the inability to ferment glucose, (viii) a mesophilic temperature range, and (ix) oxidase activity.

Strain F1F6, however, differs from *S. deleyianum* in the following traits: (i) the lower G+C content of the DNA (38 to 40 mol% for *S. deleyianum* versus 32 mol% for strain F1F6) (this is still in a range which is not in conflict with the proposal to place both organism in the same genus) (40), (ii) the presence of catalase, (iii) the inability to use nitrate or nitrite and sulfite, thiosulfate, or DMSO as an electron acceptor, (iv) the inability to ferment malate, (v) the ability to use lactate, (vi) the ability to use H₂, acetate, or propionate with oxygen as an electron donor, and (vii) the ability to grow in the presence of 1% glycine and 3.5% NaCl.

With "*G. barnesii*" SES-3, strain F1F6 shares (21, 31) (i) the curved morphology, (ii) the ability to use elemental sulfur, fumarate, and oxygen for organotrophic growth, and (iii) the inability to use sulfate. In contrast to "*G. barnesii*" SES-3, strain F1F6 was unable to use nitrate, nitrite, thiosulfate, trimethylamine *N*-oxide, and Fe(III). Growth of strain F1F6 by arsenate and selenate reduction was not tested.

Taxonomy. On the basis of phenotypic and genotypic data, we propose that strain F1F6 be included in the genus *Sulfurospirillum* as the type strain of a new species, for which we propose the name *S. arcachonense*, with *S. deleyianum* as the closest validly described relative.

Description of *Sulfurospirillum arcachonense* sp. nov. *Sulfurospirillum arcachonense* (ar.ca.cho.nen' se. M. L. adj. *arcachonense*, pertaining to the city of Arcachon, French Atlantic coast, the locality from which the strain was isolated). Curved cells are 0.3 µm wide and 1.0 to 2.5 µm long. Gram negative. Motile by polar flagellum. Colonies in deep agar cultures are whitish, round, and filamentous with elemental sulfur as an electron acceptor. With oxygen or fumarate, colonies are lens shaped and yellow-brownish.

The pH range is 6.1 to 8.2; optimum, 7.0 to 7.4. The temperature range is 8 to 30°C; growth optimum, 26°C. The salinity range is 0.6 to 4.0% NaCl; optimum growth occurs in the presence of 1.2 to 2.0% NaCl. At least 0.1% MgCl₂ · 6H₂O was required.

Microaerophilic growth: in agitated cultures growth at 15% oxygen and in nonagitated cultures growth at 20% oxygen in the gas phase with succinate as the electron donor; no growth at >1% oxygen in the gas phase with formate as the electron donor.

Oxygen or elemental sulfur served as an electron acceptor; fumarate was fermented. Nitrate, nitrite, thiosulfate, sulfate, Fe(III) oxyhydroxide, and dimethyl sulfoxide are not used as electron acceptors. Formate, acetate, propionate, succinate, fumarate, lactate, α-ketoglutarate, glutamate, citrate, malate, alanine, and yeast extract are oxidized with oxygen as the electron acceptor. With elemental sulfur as the electron acceptor, hydrogen, formate, lactate, pyruvate, α-ketoglutarate, glutarate, glutamate, and yeast extract serve as electron donors. An organic carbon source is required. Vitamins are not required.

MK-6 and MMK-6 are the sole quinones, with MK-6 predominating. The fatty acids comprise 14:0 (2.7%), 14:1 (3.2%), 16:0 (31.0%), 16:1 (40.8%), 18:0 (20.8%), and 18:1 (0.6%), with trace amounts of 15:0, 17:0, and 3OH 14:0. The polar lipids comprised phosphatidylglycerol and phosphatidylethanolamine, together with a third phospholipid tentatively identified as lysophosphatidylethanolamine. The G+C content of the DNA is 32.0 mol%.

Isolated from oxidized surface sediment of a *Z. noltii*-overgrown intertidal mud flat near Arcachon, France.

Strain F1F6 (DSMZ 9755) is the type strain.

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