**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 F{1}F{6}, 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, glutamate, glutamine, and yeast extract supported growth with elemental sulfur under anaerobic conditions. Apart from H2 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 urase 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 F{1}F{6} (level of sequence similarity, 91.7 to 92.4%). Together with strain F{1}F{6}, these organisms form a novel lineage within the epsilon 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 (*Wolinella* 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 F{1}F{6}, 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 F{1}F{6}, 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 Pfenning and Biebl (33). Amorphous Fe(III)-oxyhydroxide was prepared by titration of an acidic FeCl3 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-μm-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
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 (20, 40, 80, and 160 dilution steps). The spectrum of electron donors and electron acceptors served as controls. Fatty acids were analyzed as the methyl ester derivatives by using the two-stage method described by Römisch et al. (44, 45). The ability to grow by fermentation of glucose, maleate, glutamate, oxalacetate, and aspartate (all at 5 mM) was also tested with the two-stage method described previously (22). In addition to that of strain F1F6, the 16S rDNA sequences of strain F1F6 and S. deleyianum 5175 (DSM 6946T) 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 rDNA database: tree-1400-feb97).

RESULTS

Enrichment and isolation. After an incubation period of 3 weeks, in all tubes the red Fe(III)-or 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 sodium pyruvate (5 mM) and pyruvate (5 mM) and pyruvate (5 mM), as well as on blood agar plates under oxic and microaerobic conditions (3% O2-containing atmosphere). Growth was monitored by microscopic examination. Cells were subjected to differential hydrolysis to detect ester-linked and non-ester-linked (amide-bound) fatty acids by using the two-stage method described by Römisch et al. (44, 45). The use of different subsets 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 1523 to 1552 nucleotide sequence positions for construction of the phylogenetic trees. The significance of the interior branching points was tested in bootstrap analyses by using the maximum-likelihood method (fastDNAml [25]) and the parsimony method (PHYLIP; reference [10]). In addition, different subsets of about 30 sequences were used to reconstruct phylogenetic trees by applying the maximum-likelihood method (fastDNAmML [25]). These sets of data varied with respect to the reference sequences used, as well as the alignment positions identified to avoid possible artifacts caused by nucleotide 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 use of different rates of sequence evolution 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 1523 to 1552 nucleotide sequence positions for construction of the phylogenetic trees. The significance of the interior branching points was tested in bootstrap analyses by using the maximum-likelihood method (fastDNAml: 100 data resamplings) and neighbor-joining (ARB: 10,000 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 6946T) have been deposited in the EMLB, 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 rDNA database: tree-1400_feb97).

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 Hedges et al. (12).

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 analysis (43). Calibration determination of G+C content were performed as described by Moshah et al. (26).

Comparative 16S rDNA sequence analysis. DNA isolation, PCR-mediated amplification of the almost complete 16S rDNA 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 6946T) was determined for reference. The 16S rDNA sequences were added to a database of about 6,000 complete or partial 16S rDNA primary structures of bacteria (25, 55, 66) 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 rDNA 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 (fastDNAmML [25]). These sets of data varied with respect to the reference sequences used, as well as the alignment positions identified to avoid possible artifacts caused by nucleotide 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 use of different rates of sequence evolution 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 1523 to 1552 nucleotide sequence positions for construction of the phylogenetic trees. The significance of the interior branching points was tested in bootstrap analyses by using the maximum-likelihood method (fastDNAml: 100 data resamplings) and neighbor-joining (ARB: 10,000 data resamplings) methods. All phylogenetic analyses were done by using software in the ARB package.

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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 μm long, and 0.3 μm 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), benzate (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), methoxybenzoate (1 mM), cyclohexanecarboxylate (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(II1)-oxyhydroxide, nor was the strain able to disproportionate 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. (ii) Growth with oxygen. Strain F1F6 was not able to grow anaerobically by sulfur reduction with formate and CO2 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 MgCl2 · 6H2O 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% MgCl2 · 6H2O was required. The maximum concentration was not determined. Growth was only slightly inhibited with 2.1% MgCl2 · 6H2O 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 Rp 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
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FIG. 2. 16S rDNA-based phylogenetic dendrogram reconstructed for strain F1F6, S. deliyanum 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. deliyanum, strain SES-3 (G. barnesi), 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-likelihood 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 Rf 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 amided 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. deliyanum 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. deliyanum, 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. deliyanum 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. deliyanum 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 “Geospirillum barnesii” (23, 41). However, the genus name “Geospirillum” 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. deliyanum 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, 18:1ω7c, a combination which appears to be distinctive within the Proteobacteria. Various subgroups are defined by the presence of fatty acids such as 12:0 14:0, and 16:1ω7c. 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 lipquinone, 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 phylogenetic 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 sulfate, 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. barnesi*" 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. barnesi*" SES-3, strain F1F6 was unable to use nitrate, nitrite, thiosulfate, trimethyamine N-oxide, and Fe(III). Growth of strain F1F6 by amanate 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 *Arcobacter arcachonense*, with *S. deleyianum* as the closest validly described relative.

**Description of Sulfurospirillum arcachonense sp. nov. Sulfurospirillum arcachonense** (arc.ca.cho. nen’ se. M. L. adj. arca-
chonense, 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-brown.

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) oxhydroxide, and dimethyl sulfide were 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.

**References**


