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Fe(III) and S^0 Reduction by *Pelobacter carbinolicus*

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There is a close phylogenetic relationship between *Pelobacter* species and members of the genera *Desulfuromonas* and *Geobacter*, and yet there has been a perplexing lack of physiological similarities. *Pelobacter* species have been considered to have a fermentative metabolism. In contrast, *Desulfuromonas* and *Geobacter* species have a respiratory metabolism with Fe(III) serving as the common terminal electron acceptor in all species. However, the ability of *Pelobacter* species to reduce Fe(III) had not been previously evaluated. When a culture of *Pelobacter carbinolicus* that had grown by fermentation of 2,3-butanediol was inoculated into the same medium supplemented with Fe(III), the Fe(III) was reduced. There was less accumulation of ethanol and more production of acetate in the presence of Fe(III). *P. carbinolicus* grew with ethanol as the sole electron donor and Fe(III) as the sole electron acceptor. Ethanol was metabolized to acetate. Growth was also possible on Fe(III) with the oxidation of propanol to propionate or butanol to butyrate if acetate was provided as a carbon source. *P. carbinolicus* appears capable of conserving energy to support growth from Fe(III) respiration as it also grew with H_2 or formate as the electron donor and Fe(III) as the electron acceptor. Once adapted to Fe(III) reduction, *P. carbinolicus* could also grow on ethanol or H_2 with S^0 as the electron acceptor. *P. carbinolicus* did not contain detectable concentrations of the *c*-type cytochromes that previous studies have suggested are involved in electron transport to Fe(III) in other organisms that conserve energy to support growth from Fe(III) reduction. These results demonstrate that *P. carbinolicus* may survive in some sediments as an Fe(III) or S^0 reducer rather than growing fermentatively on rare substrates or syntrophically as an ethanol-oxidizing acetogen. These studies also suggest that the ability to use Fe(III) as a terminal electron acceptor may be an important unifying feature of the *Geobacter-Desulfuromonas-Pelobacter* branch of the delta *Proteobacteria*.

The genus *Pelobacter* encompasses a unique group of fermentative microorganisms in the delta *Proteobacteria* (54). The original characterization of these organisms suggested that they could grow only by fermentation of a limited range of substrates that are relatively rare in most anaerobic environments (52–56). Fermentable substrates include 2,3-butanediol and acetoin, which most *Pelobacter* species (*Pelobacter carbinolicus*, *Pelobacter venetianus*, *Pelobacter acetylenicus*, and *Pelobacter propionicus*) can ferment. Some *Pelobacter* species can also ferment ethylene glycol (*P. carbinolicus* and *P. venetianus*), polyethylene glycol (*P. venetianus*), acetylene (*P. acetylenicus*), or gallic acid and related compounds (*Pelobacter acidigallici*). Sugars and other common fermentable substrates are not metabolized. *P. carbinolicus*, *P. venetianus*, and *P. acetylenicus* can also grow in coculture with H_2-consuming microorganisms by oxidizing ethanol, propanol, or butanol to the corresponding acid with the production of H_2 (52, 53, 56). None of the *Pelobacter* species were found to use any of the potential physiological electron acceptors typically considered at that time. Thus, it was assumed that these organisms could conserve energy only through substrate-level phosphorylation.

Phylogenetic analyses based on 16S rRNA oligonucleotide cataloging (57) or sequencing (16, 26) have indicated that the members of the genus *Pelobacter* are phylogenetically intertwined with members of the genus *Desulfuromonas*. Some *Pelobacter* species are more closely related to *Desulfuromonas* species than to other *Pelobacter* species. Yet members of the genus *Desulfuromonas* are respiratory organisms which couple the oxidation of simple organic compounds to the reduction of S^0 (60). That two apparently different physiological groups would be so phylogenetically interrelated is surprising and unprecedented (57) and has been difficult to justify in terms of biochemical evolution (57, 60).

After the discovery of the *Pelobacter* species, anaerobic microorganisms which could conserve energy to support growth by coupling the oxidation of organic compounds to the reduction of Fe(III) were discovered (35, 39). The first of these organisms, *Geobacter metallireducens*, is closely related to *Desulfuromonas acetoxidans* (30). Although *G. metallireducens* cannot grow via S^0 reduction, it was found that *D. acetoxidans* can grow via Fe(III) reduction (50). In fact, at the time, *D. acetoxidans* was only the second organism known to be able to couple the complete oxidation of multicarbon compounds to the reduction of Fe(III). These results with *D. acetoxidans* illustrated the potential for phylogenetic analysis based on 16S rRNA sequences to predict previously unsuspected physiological traits. Since then, a number of other acetate-oxidizing, Fe(III)-reducing microorganisms which are closely related to *G. metallireducens* and *D. acetoxidans* have been isolated. These include *Geobacter sulfurreducens*, isolated from a drainage ditch (8); *Geobacter hydrogenophilus*, isolated from a petroleum-contaminated aquifer (10); *Desulfuromonas palmitatis*, isolated from marine sediments (11); and *Geobacter chapellei*, isolated from the deep subsurface (10). Although some *Geobacter* and *Desulfuromonas* species can grow via S^0, nitrate, or fumarate reduction, not all can. One trait that all *Geobacter* and *Desulfuromonas* species do share is the ability to use Fe(III) as a terminal electron acceptor.

Thus, we hypothesized that the capacity for Fe(III) respiration might be the central physiological characteristic held in common among the *Geobacter*, *Desulfuromonas*, and *Pelobacter* species. To further investigate this, the ability of *P. carbinolicus* to use Fe(III) as an electron acceptor was evaluated. This species of *Pelobacter* was chosen because it is one of a group of three pelobacters (including *P. acetylenicus* and *P. venetianus*) that, according to 16S rRNA analysis and DNA-DNA homologies (16, 57), are most closely related to *D. ace-*

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toxidans. Furthermore, oligonucleotide cataloging (57) has indicated that *P. carbinolicus* is the closest known relative of a freshwater strain of *Desulfuromonas*, *Desulfuromonas acetexigens* (19), which can also conserve energy to support growth from Fe(III) reduction (11, 50). Here we report that *P. carbinolicus* can use Fe(III) as a terminal electron acceptor and that, once adapted to Fe(III) reduction, it was also capable of reducing S^0. In addition to expanding the potential environmental significance of *P. carbinolicus*, these findings are of physiological interest because *P. carbinolicus* appears to lack the *c*-type cytochromes (52) that previously (1, 13, 30, 44, 46, 50) have been considered to be involved in energy-conserving electron transport in dissimilatory Fe(III)-reducing bacteria.

MATERIALS AND METHODS

Culturing techniques. Strict anaerobic culturing techniques (4, 22, 43) were used throughout as previously described (35). Two cultures of *P. carbinolicus* (DSM 2380) and a culture of *D. acetexigens* (DSM 1397) were purchased from the German Collection of Microorganisms (DSM), Braunschweig, Germany. *G. metallireducens* was from our culture collection.

D. acetexigens was grown in the medium suggested by the DSM for culturing this organism with fumarate as the electron acceptor. *G. metallireducens* was grown in acetate-Fe(III) citrate medium as previously described (35). *P. carbinolicus* was grown in a modified version of the medium suggested for this organism by the DSM. The medium contained (in grams per liter of water) $NaHCO_3$ (2.5), NH_4Cl (0.25), KCl (0.5), KH_2PO_4 (0.2), NaCl (20), $MgCl_2 \cdot 6H_2O$ (2), and $CaCl_2 \cdot 2H_2O$ (0.15), as well as a trace metal solution (32). The major difference in the medium from the one suggested by DSM was that there was nitrilotriacetic acid (NTA) and tungstate in the trace metal mixture. The medium was dispensed into pressure tubes (10 ml) or 160-ml serum bottles (100 ml), bubbled with N_2-CO_2 (80:20) for 5 min (pressure tubes) or 15 min (serum bottles), and then sealed with thick butyl rubber stoppers. The medium was sterilized by autoclaving. The medium pH was ca 6.8.

All other additions were made to the cooled, sterile medium immediately prior to inoculation from anoxic sterile aqueous stocks. Except where noted, sodium sulfide (1.7 mM) was added as a reductant. $FeCl_2$ (2.5 mM) was added as a reductant in the initial studies on growth on colloidal S^0. The following electron donors were added where noted at a final concentration of 10 mM: 2,3-butanediol, ethanol, propanol, butanol, or formate. H_2 was added at 60 kPa. Acetate (5 mM) was added as a carbon source for growth on H_2, formate, propanol, and butanol. Fe(III) forms evaluated included poorly crystalline Fe(III) oxide (100 mmol/liter) prepared as previously described (33), Fe(III)-NTA (20 mM) (50), and Fe(III)-citrate (10 mM). S^0 forms evaluated were colloidal S^0 (5) (ca. 3 mM) and sublimed sulfur (ca. 20 g/liter). Cultures were incubated at 30°C in the dark.

For the cell suspension study, cells were grown on 2,3-butanediol in serum bottles and then harvested under N_2-CO_2 (80:20) by centrifugation in sealed bottles. The cells were resuspended in an anoxic marine salts, bicarbonate buffer that contained (in grams per liter) $NaHCO_3$ (2.5), NaCl (20), $MgCL_2 \cdot 6H_2O$ (2), KCl (0.5), and $CaCl_2$ (0.1). The cells were again collected by centrifugation and resuspended in the buffer. An aliquot of the concentrated cells (3.5 mg of protein) was added to anoxic bicarbonate buffer (10 ml in 25-ml serum bottles) amended with Fe(III)-NTA (20 mM). One set of cells was preincubated at 80°C for 20 min and then added to buffer and incubated at 80°C in order to inhibit enzymatic activity. Fe(II) production was monitored by removing aliquots over time and analyzing for Fe(II) as outlined below.

Growth experiments were carried out in 10-ml cultures in anaerobic pressure tubes. For growth on H_2, the tubes were incubated horizontally to promote gas transfer. Aliquots were removed over time and analyzed for cell numbers, Fe(II) or sulfide, or electron donor and product concentrations.

Analytical techniques. Concentrations of HCl-extractable Fe(II) in the cultures were monitored with ferrozine as previously described (35). For the cell suspension study, the samples were not treated with HCl but were added directly to buffer containing ferrozine. Sulfide was analyzed colorimetrically with the methylene blue method (9). Cell numbers were determined with epifluorescent microscopy (21). H_2 concentrations in the headspace of cultures were measured with a reduction gas analyzer (31). Acetate, ethanol, propanol, butanol, and 2,3-butanediol were analyzed in samples filtered through a 0.2-μm-pore-diameter filter (Gelman Acrodisc) with high-pressure liquid chromatography on an Aminex HPX-87H column (Bio-Rad) with 0.01 N H_2SO_4 as the eluant and a differential refractometer detector. Concentrations of protein in cell suspensions were determined by digesting the samples in 1 N NaOH for 5 min at 100°C and measuring protein with the Lowry method (41).

Cytochrome content. Dithionite-reduced minus air-oxidized difference spectra of washed suspensions of cells that had been grown fermentatively on butanediol or with ethanol or H_2 as the electron donor and Fe(III) or S^0 as the electron acceptor were measured as previously described (30).

16S rRNA gene sequencing. Nucleic acids were isolated as previously described (2) and treated with RNase. The genes coding for 16S rRNA of *P. carbinolicus* were amplified by using eubacterial primer 50F (5′-AACACATG CAAGTCGAACG-3′) (23) and universal primer 1492R (5′-GGTTACCTTGT TACGACTT-3′) (15, 58). The genes coding for 16S rRNA of *D. acetexigens* were amplified by using 50F and universal primer 1391R (24). The PCR products of genes coding for 16S rRNA were purified with a Wizard PCR Prep System (Promega Corp., Madison, Wis.) and resuspended in sterile water. Both strands of the purified PCR products were sequenced by automated dye dideoxy terminator sequencing at Michigan State University Sequencing Facility with a 373A DNA sequencing system (Applied Biosystems, Foster City, Calif.). Oligonucleotides complementary to the conserved regions of the eubacterial 16S rRNA were chosen to prime the sequencing reactions and were synthesized on either a model 394 DNA-RNA synthesizer or a model 380B DNA synthesizer (Applied Biosystems). Sequence alignments were either performed manually or obtained from the Ribosomal Data Base Project (25). Phylogenetic trees were inferred by the maximum likelihood method (17, 25, 48). Statistical significance of the branching pattern was determined by bootstrap analysis.

Nucleotide sequence accession number. The accession numbers for GenBank and EMBL sequences used in the phylogenetic analysis are as follows: *D. acetexigens*, U23140; *D. acetoxidans*, M26634; *Desulfuromusa bakii*, X79412; *Desulfuromusa kysingii*, X79414; *Desulfuromusa succinoxidans*, X79415; *Desulfovibrio desulfuricans*, M34113; *Desulfovibrio vulgaris*, M34399; *G. metallireducens*, L07834; *G. sulfurreducens*, U13928; *P. acetylenicus*, X70955; *P. acidigallici*, X77216; *P. carbinolicus*, U23141; *P. propionicus*, X70954; *Shewanella alga*, X81621; and *Shewanella putrefaciens*, X81623.

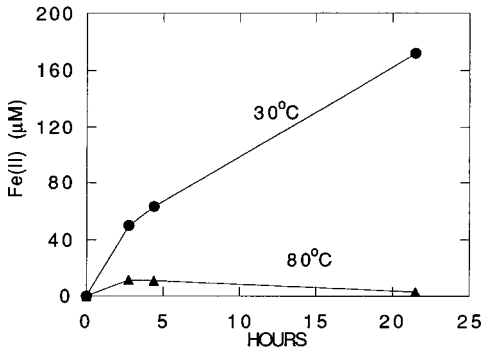

FIG. 1. Fe(II) production over time when washed cell suspensions of *P. carbinolicus* (3.5 mg of protein) were suspended in 10 ml of a marine salts, bicarbonate buffer with Fe(III)-NTA (20 mM) as the potential electron acceptor. The buffer composition was (in grams per liter) $NaHCO_3$ (2.5), NaCl (20), $MgCl_2 \cdot 6H_2O$ (2), KCl (0.5), and $CaCl_2$ (0.1).

RESULTS

Fe(III) reduction with 2,3-butanediol, alcohols, and H_2. Washed cell suspensions of *P. carbinolicus* reduced Fe(III)-NTA, and this activity appeared to be enzymatic as it was inhibited by incubating the cells at 80°C (Fig. 1). Fe(III) was also reduced when *P. carbinolicus* grown on 2,3-butanediol was inoculated into 2,3-butanediol growth medium amended with Fe(III) (Fig. 2A). In the presence of Fe(III), less ethanol and more acetate were produced than in 2,3-butanediol medium without Fe(III) (Fig. 2B).

P. carbinolicus grew in medium with ethanol as the sole electron donor and Fe(III) as the electron acceptor (Fig. 3). Cell growth coincided with Fe(III) reduction. Ethanol was converted to acetate. The ratio of Fe(II) produced to acetate produced was 3.8, which suggests that ethanol was oxidized according to the following: $CH_3CH_2OH + 4Fe(III) + H_2O \rightarrow CH_3COO^- + 4Fe(II) + 5\ H^+$. *P. carbinolicus* also oxidized propanol and butanol to the corresponding fatty acids with Fe(III) serving as the electron acceptor, but acetate was required as a carbon source for growth on these substrates. In addition to growing in the medium at marine salinity, *P. carbinolicus* also grew with ethanol as the electron donor and Fe(III) as the electron acceptor in freshwater medium. With ethanol

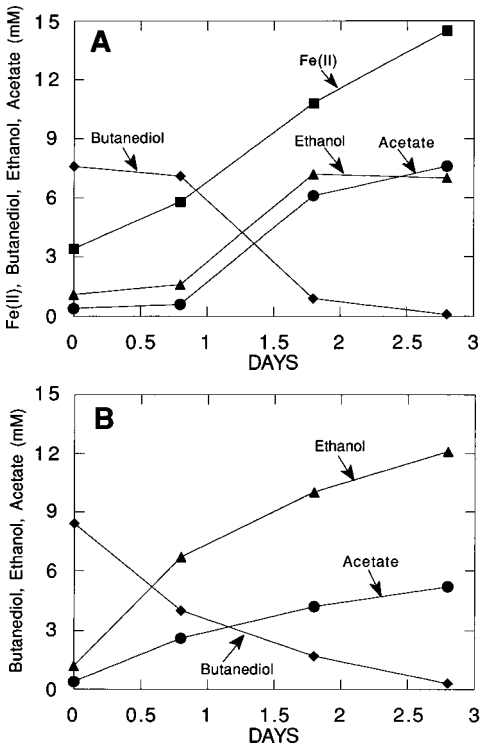

FIG. 2. Metabolism of 2,3-butanediol in the presence (A) and absence (B) of Fe(III)-NTA. As no two replicate cultures have the exact same lag time, in this and subsequent figures the data from one representative culture for each treatment are shown. The ca. 3 mM Fe(II) at time zero in this and subsequent experiments results from the addition of the sulfide reductant which chemically reduces Fe(III).

as the electron donor, *P. carbinolicus* reduced poorly crystalline Fe(III) oxide but not Fe(III) citrate.

P. carbinolicus also grew in medium in which H_2 was provided as an electron donor with acetate as the carbon source (Fig. 4). In five replicate cultures, the stoichiometery of Fe(III) reduced to H_2 consumed was 1.9 ± 0.2 (mean ± standard deviation). This suggests that *P. carbinolicus* could conserve energy to support growth by the reaction $H_2 + 2\ Fe(III) \rightarrow 2\ H^+ + 2\ Fe(II)$. Formate also served as an electron donor for Fe(III) reduction (29).

S^0 reduction. Since many dissimilatory Fe(III) reducers can also reduce S^0 (28), the possibility of S^0 reduction was evaluated. When *P. carbinolicus* grown on 2,3-butanediol was inoculated into 2,3-butanediol medium that also contained sublimed S^0, the initial 10 mM 2,3-butanediol was metabolized down to 3 mM within 2 days and down to 0.3 mM in 12 days. There was no detectable accumulation of sulfide over this period. Thus, *P. carbinolicus* was fermenting the 2,3-butanediol without significant S^0 reduction.

However, when *P. carbinolicus* that had been grown on ethanol-Fe(III) was inoculated into medium in which the Fe(III) was replaced with colloidal S^0 and Fe(II) was added as a reductant instead of sulfide, the medium turned black, indicating that sulfide was produced. S^0 reduction was also observed with H_2, formate, butanol, or propanol when acetate was provided as a carbon source. S^0 continued to be reduced (as visually evidenced from the disappearance of S^0) when the ethanol-S^0 culture was transferred into ethanol-S^0 medium without added Fe(II). S^0 reduction was associated with cell growth (Fig. 5). Once adapted to the ethanol-colloidal S^0 medium, *P. carbinolicus* also grew with sublimed S^0 as the electron acceptor (Fig. 5).

The studies of Fe(III) and S^0 reduction were repeated with a fresh culture of *P. carbinolicus* from DSM with the same results.

Cytochrome content. The cytochrome content of the organism was tested because *P. carbinolicus* does not contain *c*-type cytochromes (52) but all other organisms which can conserve

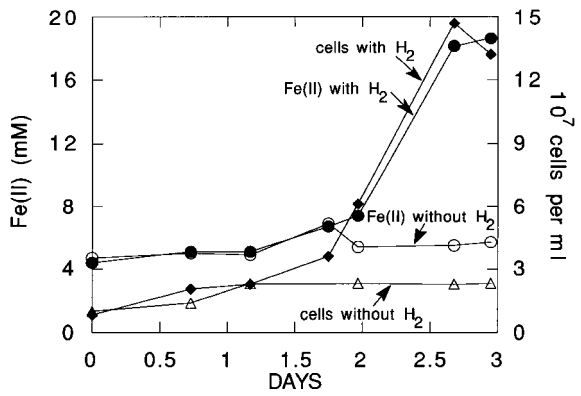

FIG. 4. Growth of *P. carbinolicus* with H_2 as the electron donor and Fe(III)-NTA as the electron acceptor.

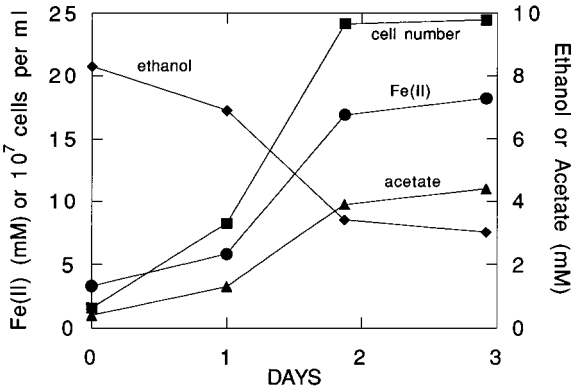

FIG. 3. Growth of *P. carbinolicus* with ethanol as the electron donor and Fe(III)-NTA as the electron acceptor.

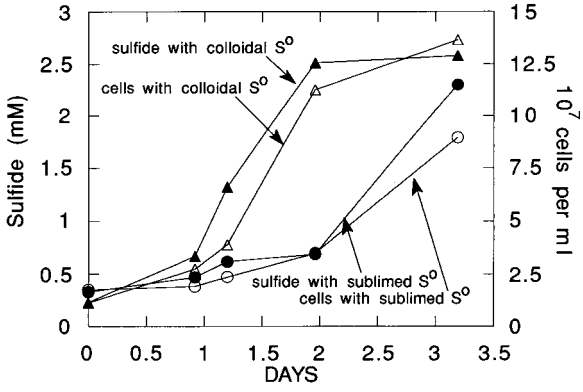

FIG. 5. Growth and sulfide production by *P. carbinolicus* with ethanol as the electron donor and colloidal S^0 or sublimed S^0 as the electron acceptor.

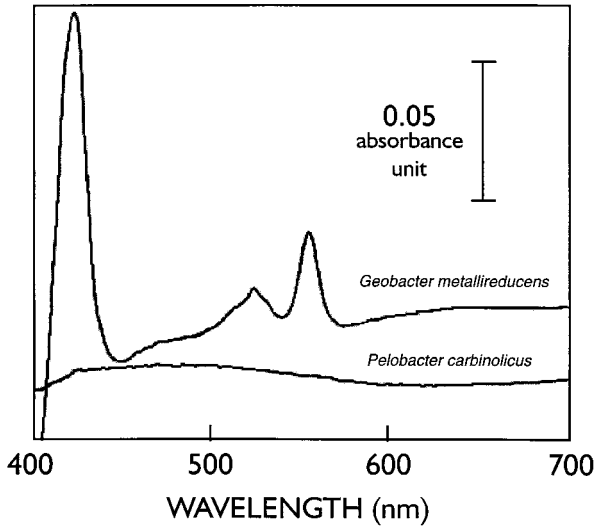

FIG. 6. Dithionite-reduced minus air-oxidized spectra of equivalent amount of cell protein (0.2 mg/ml) of Fe(III)-grown *G. metallireducens* and *P. carbinolicus*.

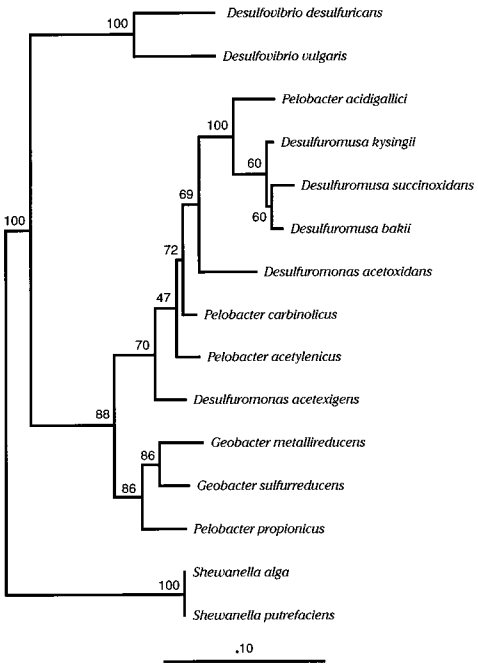

FIG. 7. Phylogenetic tree inferred from 16S rRNA sequences. Bootstrap values from 100 bootstrap analyses are given at branch nodes. A total of 1,161 positions were considered. Bar length represents 1% sequence difference.

energy to support growth from Fe(III) reduction do (28). Dithionite-reduced minus air-oxidized spectra of Fe(III)-grown cells gave no indication of *c*-type cytochromes whereas *c*-type cytochromes were readily apparent in a cell suspension containing an equivalent amount of *G. metallireducens* protein (Fig. 6). There was also no evidence of *c*-type cytochromes in S^0-grown cells of *P. carbinolicus* (29).

16S rRNA sequence. In order to further confirm that the organism being studied was *P. carbinolicus*, the 16S rRNA gene of the original culture used in these investigations was sequenced. The 16S rRNA gene of the Fe(III)- and S^0-reducing microorganism *D. acetexigens* was also sequenced for reference purposes. Analysis of the sequences indicated that, in agreement with previous phylogenetic placement of *P. carbinolicus* based on 16S rRNA oligonucleotide cataloging (57), the organism was in the delta *Proteobacteria*, closely related to *D. acetexigens* and *P. acetylenicus* (Fig. 7). Another nearly complete 16S rRNA sequence of *P. carbinolicus* has recently been reported (26) and is virtually identical, with only seven unambiguous differences in the 1,417 positions that could be considered.

DISCUSSION

This study demonstrates that, in contrast to its previous designation as a solely fermentative organism (52), *P. carbinolicus* can use Fe(III) or S^0 as a terminal electron acceptor. Thus, there are some physiological similarities between *P. carbinolicus* and organisms in the closely related *Geobacter* and *Desulfuromonas* genera. It seems likely that the common ancestor of these organisms was an Fe(III)- and S^0-reducing microorganism. The results further emphasize the potential of 16S rRNA-based phylogeny to predict previously unsuspected physiological capabilities.

There are several reasons why the ability of *P. carbinolicus* to reduce Fe(III) and S^0 was not observed previously. Despite growing awareness of dissimilatory Fe(III) reduction as an environmentally important process, Fe(III) is not routinely tested as an electron acceptor in many characterization studies. There was little reason to evaluate the potential for *P. carbinolicus* to reduce Fe(III) when it was isolated in the early 1980s, as the capacity for microorganisms to conserve energy to support growth by coupling the oxidation of organic compounds to the reduction Fe(III) was not yet known. The previously reported lack of S^0 reduction is in accordance with the studies reported here in that when *P. carbinolicus* is transferred from a medium in which it is growing fermentatively to the same medium with sublimed S^0, it continues to grow fermentatively. Reduction of S^0 after direct transfer from fermentative medium is observed only with colloidal S^0. This form of S^0 is not typically used in screening electron acceptors of novel organisms. Once adapted to S^0 reduction with colloidal S^0, *P. carbinolicus* readily reduces sublimed S^0.

Electron transport to Fe(III). Fe(III) reduction by *P. carbinolicus* appears to be enzymatically catalyzed. The finding that *P. carbinolicus* could also reduce S^0 raised the issue of whether *P. carbinolicus* might be indirectly reducing Fe(III) because S^0 was present in the Fe(III) medium as the result of the Fe(III) oxidizing the sulfide that was added to the medium as a reductant. Thus, it seemed possible that *P. carbinolicus* was reducing the S^0 in the Fe(III) medium to sulfide which then chemically reduced Fe(III) with the regeneration of S^0. However, subsequent studies have indicated that such cycling is not an effective mechanism for Fe(III) reduction in our culturing system because when organisms which reduce S^0 but not Fe(III) are incubated in medium in which Fe(III) is provided as the electron acceptor and sulfide is added as a reductant there is not sustained Fe(III) reduction (29). Furthermore, as shown here, washed cell suspensions of *P. carbinolicus* reduced Fe(III) in sulfur-free buffer, demonstrating that Fe(III) is still reduced when the potential for sulfide acting as the Fe(III) reductant is eliminated.

The ability of *P. carbinolicus* to conserve energy to support growth by oxidizing ethanol to acetate with the reduction of Fe(III) may not be that surprising given a previous report (52) that *P. carbinolicus* can grow on ethanol in syntrophic associ-

ation with H_2-consuming microorganisms. In that metabolism, *P. carbinolicus* disposes of electrons derived from ethanol oxidation by reducing H^+ to H_2. The H_2-consuming microorganisms are necessary in order to maintain H_2 concentrations low enough for H^+ reduction to be thermodynamically favorable. In a similar manner, *P. carbinolicus* oxidizes propanol and butanol to propionate and butyrate, respectively, if acetate is provided as a carbon source (52). Energy to support growth of *P. carbinolicus* during syntrophic growth on alcohols is assumed to come from substrate-level phosphorylation. Fe(III) could merely be substituting for H^+ in alcohol metabolism, even potentially being reduced by the same enzyme. Thus, the ability of *P. carbinolicus* to grow via ethanol oxidation coupled to Fe(III) reduction does not necessarily indicate that *P. carbinolicus* can conserve energy to support growth from electron transport to Fe(III).

In contrast, substrate-level phosphorylation is unlikely to be the mechanism for energy conservation during growth of *P. carbinolicus* via H_2 oxidation coupled to Fe(III) reduction. The ability of microorganisms to conserve energy to support growth by oxidizing H_2 with the reduction of Fe(III) is well known (3, 7, 8, 37, 51). However, the electron transport pathway from H_2 to Fe(III) in these organisms is yet to be elucidated.

Numerous studies have suggested that *c*-type cytochromes are involved in electron transport to Fe(III) in H_2-oxidizing Fe(III) reducers as well as in Fe(III) reducers which conserve energy to support growth by oxidizing acetate with the reduction of Fe(III) (1, 13, 30, 44, 46, 50). However, in all of these studies, the evidence for cytochrome involvement in electron transport to Fe(III) is indirect and circumstantial. For example, *c*-type cytochromes were implicated in electron transport to Fe(III) in *G. metallireducens* and *D. acetoxidans* based on the finding that the addition of Fe(III) to cell suspensions oxidized the *c*-type cytochromes (30, 50). However, such an oxidation may not reflect physiological electron transport as oxygen, which does not support growth of these organisms, had a similar effect. The c_3 cytochrome from *Desulfovibrio vulgaris* has been identified as a U(VI) and Cr(VI) reductase as well as a possible Fe(III) reductase, but this organism cannot grow with metals as the sole electron acceptor (27, 36, 40).

The finding that *P. carbinolicus* can conserve energy to support growth from Fe(III) reduction and yet lacks detectable levels of *c*-type cytochromes suggests that *c*-type cytochromes are not always necessary for energy-conserving electron transport to Fe(III). Given the close phylogenetic relationship of *P. carbinolicus* to the Fe(III)-reducing *Geobacter* and *Desulfuromonas* species, it is conceivable that the *c*-type cytochromes that are prevalent in the *Geobacter* and *Desulfuromonas* species are not directly involved in electron transport to Fe(III).

Comparison of *P. carbinolicus* with *Geobacter*, *Desulfuromonas*, and *Desulfuromusa* species. In addition to differences in cytochrome content, *P. carbinolicus* is unlike all known Fe(III)-reducing *Geobacter* and *Desulfuromonas* species in its inability to completely oxidize acetate to carbon dioxide with Fe(III) as the electron acceptor. Furthermore, the *Geobacter* and *Desulfuromonas* species that metabolize ethanol oxidize it completely to carbon dioxide (35, 49). Although *Desulfuromonas* species (59) and the first species of *Geobacter* isolated, *G. metallireducens* (30), cannot use H_2 as an electron donor, three subsequently isolated *Geobacter* species are similar to *P. carbinolicus* in that they can use H_2 as an electron donor for Fe(III) reduction (8, 10). Furthermore, a *Desulfuromonas* species which can oxidize H_2 with the reduction of Fe(III) has been isolated (11). None of the *Geobacter* species that have been evaluated have been found to have the capacity to ferment 2,3-butanediol or to oxidize ethanol in coculture with a H_2-consuming methanogen (10). Although *D. acetoxidans* can grow fermentatively with malate, fumarate, or betaine (20, 49), none of the *Desulfuromonas* species that have been evaluated have the capacity to ferment any of the substrates fermented by *Pelobacter* species (57).

Since the completion of the studies reported here, the new genus *Desulfuromusa*, which is closely related to the *Geobacter-Desulfuromonas-Pelobacter* group (Fig. 7), has been described (26). Each of the three *Desulfuromusa* species is capable of S^0 reduction. One of the species, *Desulfuromusa kysingii*, can reduce Fe(III)-citrate, but data demonstrating growth with Fe(III) as the electron acceptor were not presented or discussed (18, 26). The other two species, *Desulfuromusa bakii* and *Desulfuromusa succinoxidans*, cannot reduce Fe(III)-citrate. However, although some Fe(III) reducers can use Fe(III)-citrate as an Fe(III) source (8, 35, 37), not all can. As reported here, *P. carbinolicus* did not reduce Fe(III) citrate and neither did *D. acetoxidans* (50), *D. acetexigens* (29), *Desulfovibrio desulfuricans* (38), nor a recently isolated *Geobacter* species (10). It seems likely that, when other Fe(III) forms are evaluated, all of the species in the *Desulfuromusa* group will be found to be able to conserve energy to support growth from Fe(III) reduction.

Ecological considerations. These findings extend the known potential biogeochemical contributions of *P. carbinolicus* in sedimentary environments. The substrates on which *P. carbinolicus* can grow fermentatively, acetoin, 2,3-butanediol, and ethylene glycol, are not likely to be abundant in aquatic sediments. Thus, the primary role of *P. carbinolicus* in sediments was considered to be as an ethanol-oxidizing organism living syntrophically with H_2 consumers (54). However, the results presented here demonstrate that *P. carbinolicus* could also be living in some sediments as a dissimilatory Fe(III) and/or S^0 reducer. H_2 is generally considered to be a much more significant intermediate than ethanol in the anaerobic metabolism of organic matter in sediments and thus would be the most likely electron donor for growth of *P. carbinolicus* on Fe(III) and/or S^0.

Until recently, the most commonly isolated H_2-oxidizing Fe(III)-reducing microorganisms were *Shewanella* species (7, 37, 51). These facultative anaerobes which are in the gamma *Proteobacteria* (42, 51) can readily be recovered from sedimentary environments (14, 45, 47). However, it remains to be determined whether *Shewanella* and other facultative Fe(III)-reducing microorganisms (3) are the predominant H_2-oxidizing Fe(III) reducers in most sediments or whether this process is catalyzed primarily by strict anaerobes such as several *Geobacter* species (8, 10), *Desulfovibrio* species (12, 38), and *P. carbinolicus.*

P. carbinolicus is the first example of an organism that can conserve energy to support growth from Fe(III) reduction that can also grow syntrophically with H_2-consuming microorganisms. This finding has implications for the mechanisms for inhibition of methane production in the presence of Fe(III). The ultimate factor limiting methane production under steady-state conditions in Fe(III)-containing sediments is the ability of H_2- and acetate-oxidizing microorganisms to maintain the concentrations of H_2 and acetate too low for methanogens to metabolize (34). However, the ability of H_2-producing acetogens to switch from H_2 production to Fe(III) reduction when Fe(III) becomes available suggests that another mechanism limiting methanogenesis might be lower rates of H_2 production as the result of H^+-reducing organisms switching to Fe(III) reduction. Other organisms which are likely to respond in a similar manner are *Desulfovibrio* species which can also grow

syntrophically via H_2 production (6) and can reduce Fe(III) (12, 38).

In summary, this study adds only the second marine microorganism (after *D. acetoxidans* [50]) to the short but growing list of strict anaerobic microorganisms capable of conserving energy to support growth from Fe(III) reduction. Another marine Fe(III) reducer, *D. palmitatis*, was recently directly isolated from marine sediments with Fe(III) as the electron acceptor (11). Preliminary results indicate that *P. acetylenicus* and *P. venetianus*, the two species most closely related to *P. carbinolicus* (57), can also reduce Fe(III) (29). Thus, the ability to use Fe(III) as a terminal electron acceptor appears to be a highly conserved characteristic within the *Geobacter-Desulfuromonas-Pelobacter* group. The fact that Fe(III) reducers from this branch of the delta *Proteobacteria* have been isolated from such a wide variety of aquatic sediments and aquifers with different enrichment and isolation techniques, including different electron acceptors, suggests that the organisms within this tight phylogenetic assemblage are important components of many sedimentary environments.

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REFERENCES

1. **Arnold, R. G., T. J. DiChristina, and M. R. Hoffmann.** 1986. Inhibitor studies of dissimilative Fe(III) reduction by *Pseudomonas* sp. strain 200 ("*Pseudomonas ferrireductans*"). Appl. Environ. Microbiol. **52:**281–289.
2. **Ausubel, F., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1992. Short protocols in molecular biology. John Wiley and Sons, New York.
3. **Balashova, V. V., and G. A. Zavarzin.** 1980. Anaerobic reduction of ferric iron by hydrogen bacteria. Microbiology **48:**635–639.
4. **Balch, W. E., G. E. Fox, L. J. Magrum, C. R. Woese, and R. S. Wolfe.** 1979. Methanogens: reevaluation of a unique biological group. Microbiol. Rev. **43:**260–296.
5. **Blumentals, I. I., M. Itoh, G. J. Olson, and R. M. Kelly.** 1990. Role of polysulfides in reduction of elemental sulfur by the hyperthermophilic archaebacterium *Pyrococcus furiosus*. Appl. Environ. Microbiol. **56:**1255–1262.
6. **Bryant, M. P., L. L. Campbell, C. A. Reddy, and M. R. Crabill.** 1977. Growth of *Desulfovibrio* in lactate or ethanol media low in sulfate in association with H_2-utilizing methanogenic bacteria. Appl. Environ. Microbiol. **33:**1162–1169.
7. **Caccavo, F., Jr., R. P. Blakemore, and D. R. Lovley.** 1992. A hydrogen-oxidizing, Fe(III)-reducing microorganism from the Great Bay Estuary, New Hampshire. Appl. Environ. Microbiol. **58:**3211–3216.
8. **Caccavo, F., Jr., D. J. Lonergan, D. R. Lovley, M. Davis, J. F. Stolz, and M. J. McInerney.** 1994. *Geobacter sulfurreducens* sp. nov., a hydrogen- and acetate-oxidizing dissimilatory metal-reducing microorganism. Appl. Environ. Microbiol. **60:**3752–3759.
9. **Cline, J. D.** 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. **14:**454–458.
10. **Coates, J. D.** Unpublished data.
11. **Coates, J. D., D. J. Lonergan, H. L. Jenter, and D. R. Lovley.** *Desulfuromonas palmitatis* sp. nov., a long-chain fatty acid oxidizing Fe(III) reducer from marine sediments. Submitted for publication.
12. **Coleman, M. L., D. B. Hedrick, D. R. Lovley, D. C. White, and K. Pye.** 1993. Reduction of Fe(III) in sediments by sulphate-reducing bacteria. Nature (London) **361:**436–438.
13. **DiChristina, T. J., R. G. Arnold, M. E. Lidstrom, and M. R. Hoffmann.** 1988. Dissimilative iron reduction by the marine eubacterium *Alteromonas putrefaciens* strain 200. Water Sci. Technol. **20:**69–79.
14. **DiChristina, T. J., and E. F. DeLong.** 1993. Design and application of rRNA-targeted oligonucleotide probes for dissimilatory iron- and manganese-reducing bacterium *Shewanella putrefaciens*. Appl. Environ. Microbiol. **59:**4152–4160.
15. **Eden, P. E., T. M. Schmidt, R. P. Blakemore, and N. R. Pace.** 1991. Phylogenetic analysis of *Aquaspirillum magnetotacticum* using polymerase chain reaction-amplified 16S rRNA-specific DNA. Int. J. Syst. Bacteriol. **41:**324–325.
16. **Evers, S., M. Weizenegger, W. Ludwig, B. Schink, and K.-H. Schleifer.** 1993. The phylogenetic positions of *Pelobacter acetylenicus* and *Pelobacter propionicus*. Syst. Appl. Microbiol. **16:**216–218.
17. **Felstein, J.** 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. J. Mol. Evol. **17:**368–376.
18. **Finster, K., and F. Bak.** 1993. Complete oxidation of propionate, valerate, succinate, and other organic compounds by newly isolated types of marine, anaerobic, mesophilic, gram-negative, sulfur-reducing eubacteria. Appl. Environ. Microbiol. **59:**1452–1460.
19. **Finster, K., F. Bak, and N. Pfennig.** 1994. *Desulfuromonas acetexigens* sp. nov., a dissimilatory sulfur-reducing eubacterium from anoxic freshwater sediments. Arch. Microbiol. **161:**328–332.
20. **Heijthuijsen, J. H. F. G., and T. A. Hansen.** 1989. Betaine fermentation and oxidation by marine *Desulfuromonas* strains. Appl. Environ. Microbiol. **55:** 965–969.
21. **Hobbie, J. E., R. J. Daley, and S. Jasper.** 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. **33:**1225–1228.
22. **Hungate, R. E.** 1969. A roll tube method for cultivation of strict anaerobes. Methods Microbiol. **3B:**117–132.
23. **Lane, D. J., K. G. Field, G. J. Olsen, and N. R. Pace.** 1988. Reverse transcriptase sequencing of ribosomal RNA for phylogenetic analysis. Methods Enzymol. **167:**138–144.
24. **Lane, D. L., B. Pace, G. J. Olsen, D. Stahl, M. L. Sogin, and N. R. Pace.** 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analysis. Proc. Natl. Acad. Sci. USA **82:**6955–6959.
25. **Larson, N., G. J. Olsen, B. L. Maidak, M. J. McCaughey, R. Overbeek, T. Macke, T. L. Marsh, and C. R. Woese.** 1993. The ribosomal database project. Nucleic Acids Res. **21:**3021–3023.
26. **Liesack, W., and K. Finster.** 1994. Phylogenetic analysis of five strains of gram-negative, obligately anaerobic, sulfur-reducing bacteria and description of *Desulfuromusa* gen. nov., including *Desulfuromusa kysingii* sp. nov., *Desulfuromusa bakii* sp. nov., and *Desulfuromusa succinoxidans* sp. nov. Int. J. Syst. Bacteriol. **44:**753–758.
27. **Lovley, D. R.** 1993. Dissimilatory metal reduction. Annu. Rev. Microbiol. **47:**263–290.
28. **Lovley, D. R.** 1994. Microbial reduction of iron, manganese, and other metals. Adv. Agron. **54:**175–231.
29. **Lovely, D. R.** Unpublished data.
30. **Lovley, D. R., S. J. Giovannoni, D. C. White, J. E. Champine, E. J. P. Phillips, Y. A. Gorby, and S. Goodwin.** 1993. *Geobacter metallireducens* gen. nov. sp. nov., a microorganism capable of coupling the complete oxidation of organic compounds to the reduction of iron and other metals. Arch. Microbiol. **159:**336–344.
31. **Lovley, D. R., and S. Goodwin.** 1988. Hydrogen concentrations as an indicator of the predominant terminal electron accepting reactions in aquatic sediments. Geochim. Cosmochim. Acta **52:**2993–3003.
32. **Lovley, D. R., R. C. Greening, and J. G. Ferry.** 1984. Rapidly growing rumen methanogenic organism that synthesizes coenzyme M and has a high affinity for formate. Appl. Environ. Microbiol. **48:**81–87.
33. **Lovley, D. R., and E. J. P. Phillips.** 1986. Organic matter mineralization with reduction of ferric iron in anaerobic sediments. Appl. Environ. Microbiol. **51:**683–689.
34. **Lovley, D. R., and E. J. P. Phillips.** 1987. Competitive mechanisms for inhibition of sulfate reduction and methane production in the zone of ferric iron reduction in sediments. Appl. Environ. Microbiol. **53:**2636–2641.
35. **Lovley, D. R., and E. J. P. Phillips.** 1988. Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. Appl. Environ. Microbiol. **54:**1472–1480.
36. **Lovley, D. R., and E. J. P. Phillips.** 1994. Reduction of chromate by *Desulfovibrio vulgaris* and its c_3 cytochrome. Appl. Environ. Microbiol. **60:**726–728.
37. **Lovley, D. R., E. J. P. Phillips, and D. J. Lonergan.** 1989. Hydrogen and formate oxidation coupled to dissimilatory reduction of iron or manganese by *Alteromonas putrefaciens*. Appl. Environ. Microbiol. **55:**700–706.
38. **Lovley, D. R., E. E. Roden, E. J. P. Phillips, and J. C. Woodward.** 1993. Enzymatic iron and uranium reduction by sulfate-reducing bacteria. Mar. Geol. **113:**41–53.
39. **Lovley, D. R., J. F. Stolz, G. L. Nord, and E. J. P. Phillips.** 1987. Anaerobic production of magnetite by a dissimilatory iron-reducing microorganism. Nature (London) **330:**252–254.
40. **Lovley, D. R., P. K. Widman, J. C. Woodward, and E. J. P. Phillips.** 1993. Reduction of uranium by cytochrome c_3 of *Desulfovibrio vulgaris*. Appl. Environ. Microbiol. **59:**3572–3576.
41. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
42. **MacDonell, M. T., and R. R. Colwell.** 1985. A phylogeny for the Vibrionaceae, and recommendation for two new genera, Listonella and Shewanella. Syst. Appl. Microbiol. **6:**171–182.
43. **Miller, T. L., and M. J. Wolin.** 1974. A serum bottle modification of the Hungate technique for cultivating obligate anaerobes. Appl. Microbiol. **27:** 985–987.
44. **Myers, C. R., and J. M. Myers.** 1992. Localization of cytochromes to the outer membrane of anaerobically grown *Shewanella putrefaciens* MR-1. J. Bacteriol. **174:**3429–3438.
45. **Myers, C. R., and K. H. Nealson.** 1988. Bacterial manganese reduction and

growth with manganese oxide as the sole electron acceptor. Science **240:** 1319–1321.
46. **Obuekwe, C. O., and D. W. S. Westlake.** 1982. Effects of medium composition on cell pigmentation, cytochrome content, and ferric iron reduction in a *Pseudomonas* sp. isolated from crude oil. Can. J. Microbiol. **28:**989–992.
47. **Obuekwe, C. O., D. W. S. Westlake, and F. D. Cook.** 1981. Effect of nitrate on reduction of ferric iron by a bacterium isolated from crude oil. Can. J. Microbiol. **27:**692–697.
48. **Olsen, G. J., H. Matsuda, R. Hagstrom, and R. Overbeek.** 1994. fastDNAml: a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. Comput. Appl. Biosci. **10:**41–48.
49. **Pfennig, N., and H. Biebl.** 1976. *Desulfuromonas acetoxidans* gen. nov. and sp. nov., a new anaerobic, sulfur-reducing, acetate-oxidizing bacterium. Arch. Microbiol. **110:**3–12.
50. **Roden, E. E., and D. R. Lovley.** 1993. Dissimilatory Fe(III) reduction by the marine microorganism, *Desulfuromonas acetoxidans*. Appl. Environ. Microbiol. **59:**734–742.
51. **Rossello-Mora, R. A., F. J. Caccavo, K. Osterlehner, N. Springer, S. Spring, D. Schuler, W. Ludwig, R. Amann, M. Vannacanneyt, and K. H. Schleifer.** 1994. Isolation and taxonomic characterization of a halotolerant, facultatively iron-reducing bacterium. Syst. Appl. Microbiol. **17:**569–573.
52. **Schink, B.** 1984. Fermentation of 2,3-butanediol by *Pelobacter carbinolicus* sp. nov. and *Pelobacter propionicus* sp. nov., and evidence for propionate formation from C_2 compounds. Arch. Microbiol. **137:**33–41.
53. **Schink, B.** 1985. Fermentation of acetylene by an obligate anaerobe, *Pelobacter acetylenicus* sp. nov. Arch. Microbiol. **142:**295–301.
54. **Schink, B.** 1992. The genus *Pelobacter*, p. 3393–3399. *In* A. Balows, H. G. Truper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), The procaryotes. Springer-Verlag, New York.
55. **Schink, B., and N. Pfennig.** 1982. Fermentation of trihydroxybenzenes by *Pelobacter acidigallici* gen. nov. sp. nov., a new strictly anaerobic, non-sporeforming bacterium. Arch. Microbiol. **133:**195–201.
56. **Schink, B., and M. Stieb.** 1983. Fermentative degradation of polyethylene glycol by a strictly anaerobic, gram-negative, nonsporeforming bacterium, *Pelobacter venetianus* sp. nov. Appl. Environ. Microbiol. **45:**1905–1913.
57. **Stackebrandt, E., U. Wehmeyer, and B. Schink.** 1989. The phylogenetic status of *Pelobacter acidigallici*, *Pelobacter venetianus*, and *Pelobacter carbinolicus*. Syst. Appl. Microbiol. **11:**257–260.
58. **Weisburg, W. G., S. M. Barns, D. A. Pelletier, and D. J. Lane.** 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. **173:**697–703.
59. **Widdel, F.** 1988. Microbiology and ecology of sulfate- and sulfur-reducing bacteria, p. 469–585. *In* A. J. B. Zehnder (ed.), Biology of anaerobic microorganisms. John Wiley & Sons, New York.
60. **Widdel, F., and N. Pfennig.** 1992. The genus *Desulfuromonas* and other gram-negative sulfur-reducing eubacteria, p. 3379–3392. *In* A. Balows, H. G. Truper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), The prokaryotes. Springer-Verlag, New York.