Dissimilatory reduction of ferric-iron oxides is considered to be an important process for the mineralization of organic matter in anoxic soils and sediments (Lovley, 1991, 1997; Thamdrup, 2000). During the last few years, numerous dissimilatory ferric-iron-reducing bacteria belonging to different phylogenetic groups have been isolated from various habitats (e.g. Boone et al., 1995; Lovley, 1997; Greene et al., 1997; Cummings et al., 1999; Kieft et al., 1999). Within the delta subclass of the Proteobacteria, members of the genera Desulfuromonas, Desulfuro- musa, Geobacter and Pelobacter form a monophyletic group and share the ability to reduce ferric iron and/or S⁰ (Lonergan et al., 1996).

Recently, it was recognized that ferrous iron can serve as an electron donor under anaerobic conditions for mesophilic, nitrate-reducing bacteria (Straub et al., 1996; Benz et al., 1998). During anoxic growth of nitrate-reducing bacteria on ferrous iron, poorly crystallized ferrihydrite was produced (Straub et al., 1998). This biologically produced ferrihydrite turned out to be a suitable electron acceptor for ferric-iron-reducing bacteria and was used to enrich and isolate strains Dfr¹T and Dfr²T (Straub et al., 1998). Analysis of cytochrome content and detailed physiological and phylogenetic characterization showed an affiliation of both strains to species of the genus Geobacter. In the present study, strains Dfr¹T and Dfr²T are described as two novel species of the genus Geobacter and the species names Geobacter bremensis sp. nov. and Geobacter pelophilus sp. nov. are proposed.

Strains Dfr¹T (= DSM 12179T = OCM 796T) and Dfr²T (= DSM 12255T = OCM 797T) were taken from subcultures that had been kept in our laboratory since the isolation of these bacteria. Both strains were originally isolated from sediment samples obtained from freshwater ditches in Bremen, Germany (Straub et al., 1998). Details of cultivation are given in the original description (Straub et al., 1998). For analysis of cytochromes, both strains were grown with 10 mM acetate and 40 mM fumarate in bicarbonate-buffered, freshwater medium; the medium was reduced with 2 mM cysteine added from a 250 mM stock solution (filter-sterilized, stored at 4°C in the dark under N₂).

Whole-cell suspensions and membrane fractions of strains Dfr¹T and Dfr²T were examined for the presence of cytochromes by recording redox difference
spectra with a Uvikon 930 spectrophotometer. Membrane fractions were obtained as described elsewhere (Galushko & Schink, 2000).

The results of comparative 16S rDNA sequence analysis showed an affiliation of strains Dfr\(^T\) and Dfr\(^{2T}\) with species of the family Geobacteraceae and, in particular, with members of the Geobacter cluster (Lonergan et al., 1996). The sequence identity between strains Dfr\(^{1T}\) and Dfr\(^{2T}\) was 92.5\%.

Further aspects of the phylogenetic affiliation of the two strains have been discussed before (Straub et al., 1998).

The physiological properties of strains Dfr\(^{1T}\) and Dfr\(^{2T}\) have been described in detail elsewhere (Straub et al., 1998). Like Geobacter metallireducens, both strains were able to grow with Fe(III), Mn(IV), S\(^{0}\), fumarate and malate as electron acceptor (Caccavo et al., 1994). With acetate as the electron donor, both strains reduced approximately 8 mM biologically produced ferrihydrite within 3 d completely to ferrous iron. A comparison of compounds used as electron donors showed differences between Geobacter metallireducens, G. sulfurireducens, strain Dfr\(^{1T}\) and strain Dfr\(^{2T}\) (Straub et al., 1998).

Pelobacter propionicus intermixes phylogenetically with members of the genus Geobacter (Lonergan et al., 1996). The original characterization of P. propionicus suggested that it could only grow fermentatively with a limited number of substrates (Schink, 1984). However, strains Dfr\(^{1T}\) and Dfr\(^{2T}\) were not able to grow by fermentation (Straub et al., 1998). Furthermore, the reduction of ferric iron by P. propionicus is only poorly documented and it is unclear whether the organism can grow by dissimilatory reduction of ferrihydrite (Lonergan et al., 1996).

The presence of c-type cytochrome(s) was shown by spectral analysis of whole-cell suspensions and membrane fractions of G. metallireducens and G. sulfurireducens (Lovley et al., 1993; Naik et al., 1993; Caccavo et al., 1994; Seeliger et al., 1998). In contrast, cells of P. propionicus lack c-type cytochromes (Schink, 1984). When strains Dfr\(^{1T}\) and Dfr\(^{2T}\) were grown with ferrihydrite as electron acceptor, iron minerals masked the colour of the cells. When cells were grown with fumarate as electron acceptor, cell suspensions were red in colour. This was the first indication of the presence of c-type cytochromes in both strains. Spectral analyses of whole-cell suspensions and membrane fractions of strains Dfr\(^{1T}\) and Dfr\(^{2T}\) confirmed the presence of c-type cytochromes: air-oxidized spectra showed absorption maxima at 410 nm, while dithionite-reduced minus air-oxidized differential spectra showed absorption maxima at 552, 523 and 423 nm (Figs 1 and 2).

**Fig. 1.** Spectra of membrane fractions from strain Dfr\(^{1T}\) (a) and strain Dfr\(^{2T}\) (b). Dashed lines, air-oxidized absorption spectra; solid lines, dithionite-reduced minus air-oxidized differential absorption spectra.

**Fig. 2.** Dithionite-reduced minus air-oxidized differential absorption spectra of membrane fractions of strain Dfr\(^{1T}\) (a) and strain Dfr\(^{2T}\) (b) showing \(\alpha\)- and \(\beta\)-bands at higher resolution.
Geobacter strains are described as two novel species of the genus Geobacter. On the basis of these characteristics, the two G.Dfr1 and Dfr2 can be differentiated from each other by their low 16S rDNA sequence identity (92%) and differences in physiological characteristics in comparison with other members of this cluster, strains Dfr1T and Dfr2T represent novel species. Strains Dfr1T and Dfr2T can be isolated from a freshwater ditch in Bremen, Germany. 

In conclusion, the results of the 16S rDNA sequence analyses showed the affiliation of strains Dfr1T and Dfr2T with species of the Geobacter cluster within the delta subclass of the Proteobacteria. On the basis of the low 16S rDNA sequence identity values (< 94%) and differences in physiological characteristics in comparison with other members of this cluster, strains Dfr1T and Dfr2T represent novel species. Strains Dfr1T and Dfr2T can be differentiated from each other by their low 16S rDNA sequence identity (92.5%), their G + C content and the range of substrates used for growth. On the basis of these characteristics, the two strains are described as two novel species of the genus Geobacter. *Geobacter bremenensis* sp. nov. for strain Dfr1T and *Geobacter pelophilus* sp. nov. for strain Dfr2T.

**Description of Geobacter bremenensis** *sp. nov.*

*Geobacter bremenensis* (bre.men’sis. N.L. n. Bremen Bremen, in northern Germany; L. masc. suffix -ensis indicating provenance; N.L. masc. adj. bremenensis from Bremen, where samples for enrichment cultures were taken).

Gram-negative, slightly curved rods, 1-8 µm long and 0.6 µm wide; the majority of the cells are non-motile and tend to form aggregates. No formation of spores. Multiplication by binary fission. The colour of the cells is red due to the presence of c-type cytochromes. Electron donors utilized are hydrogen, formate, acetate, propionate, butyrate, pyruvate, lactate, malate, succinate, fumarate, benzoate, ethanol, propanol and butanol. Electron acceptors utilized are Fe(III), Mn(IV), S0, fumarate and malate; strictly anaerobic. Optimal growth with ferrihydride as the electron acceptor at 30–32 °C and pH 6.7–7. No vitamins required. The G + C content of the DNA is 53 mol%.

The type strain, Dfr1T (= DSM 12179T = OCM 796T), was isolated from a freshwater ditch in Bremen, Germany.

**Description of Geobacter pelophilus** *sp. nov.*

*Geobacter pelophilus* (pe.lo’phi.lus. Gr. n. pelos mud; Gr. adj. philos loving; N.L. masc. adj. pelophilus mud-loving, as this species was isolated from freshwater mud).

Gram-negative, slightly curved rods, 1-5 µm long and 0.6 µm wide; the majority of the cells are non-motile and tend to form aggregates. No formation of spores. Multiplication by binary fission. The colour of the cells is red due to the presence of c-type cytochromes. Electron donors utilized are hydrogen, formate, acetate, propionate, pyruvate, malate, succinate, fumarate, ethanol and propanol. Electron acceptors utilized are Fe(III), Mn(IV), S0, fumarate and malate; strictly anaerobic. Optimal growth with ferrihydrite as the electron acceptor at 30–32 °C and pH 6.7–7. No vitamins required. The G + C content of the DNA is 53 mol%.

The type strain, Dfr2T (= DSM 12255T = OCM 797T), was isolated from a freshwater ditch in Bremen, Germany.

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**References**


