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NOTE

Geobacter bremensis sp. nov. and *Geobacter pelophilus* sp. nov., two dissimilatory ferric-iron-reducing bacteria

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Two strictly anaerobic, dissimilatory ferric-iron-reducing bacteria, strains Dfr1^T and Dfr2^T, were isolated from freshwater mud samples with ferrihydrite as electron acceptor. Both strains also grew by reducing Mn(IV), S^0 and fumarate. Electron donors used by strains Dfr1^T and Dfr2^T for growth with ferric iron as electron acceptor included hydrogen, formate, acetate, pyruvate, succinate, fumarate and ethanol. An affiliation with the family *Geobacteraceae* was revealed by comparative analysis of 16S rRNA gene sequences. Strains Dfr1^T and Dfr2^T shared 92·5% sequence identity and their closest known relative was *Geobacter sulfurreducens*, with approximately 93% sequence identity. Cultures and colonies of strains Dfr1^T and Dfr2^T were intensely red in colour, due to the presence of *c*-type cytochromes. On the basis of physiological and phylogenetic data, strain Dfr1^T (= DSM 12179^T = OCM 796^T) is described as *Geobacter bremensis* sp. nov. and strain Dfr2^T (= DSM 12255^T = OCM 797^T) as *Geobacter pelophilus* sp. nov.

Keywords: *Geobacter bremensis* sp. nov., *Geobacter pelophilus* sp. nov., ferric iron reduction, ferrihydrite, *c*-type cytochromes

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*, *Desulfuromusa*, *Geobacter* and *Pelobacter* form a monophyletic group and share the ability to reduce ferric iron and/or S^0 (Lonergan *et al*., 1996).

Recently, it was recognized that ferrous iron can serve as an electron donor under anoxic 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 Dfr1^T and Dfr2^T (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 Dfr1^T and Dfr2^T 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 Dfr1^T (= DSM 12179^T = OCM 796^T) and Dfr2^T (= DSM 12255^T = OCM 797^T) 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 N_2).

Whole-cell suspensions and membrane fractions of strains Dfr1^T and Dfr2^T were examined for the presence of cytochromes by recording redox difference

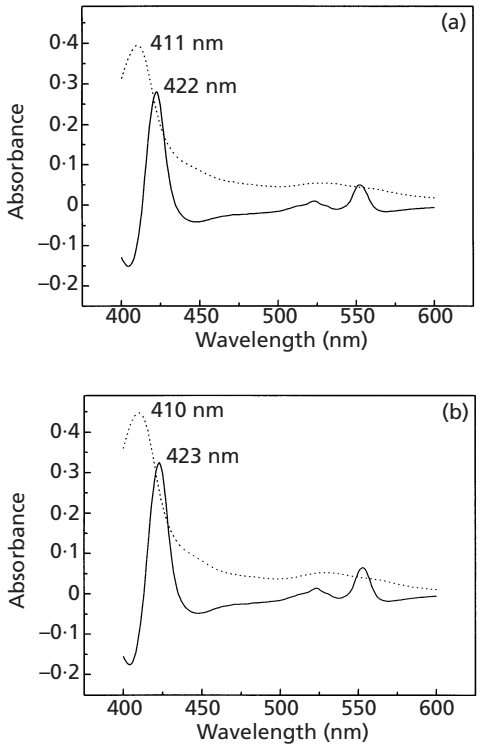

Fig. 1. Spectra of membrane fractions from strain Dfr1^T (a) and strain Dfr2^T (b). Dashed lines, air-oxidized absorption spectra; solid lines, dithionite-reduced minus air-oxidized differential absorption spectra.

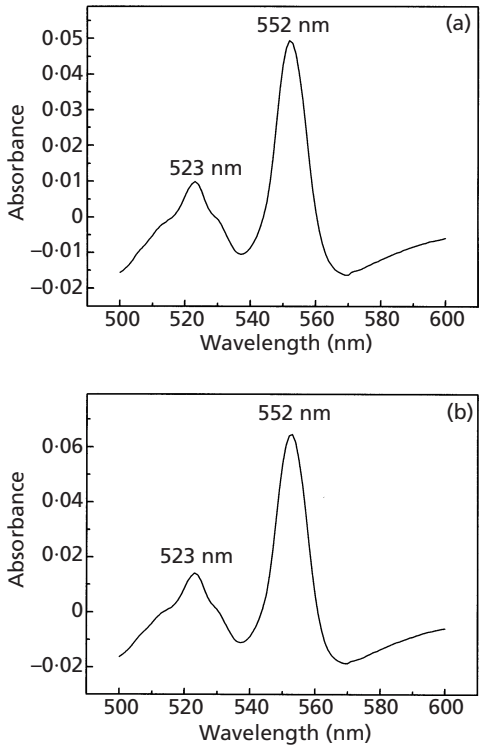

Fig. 2. Dithionite-reduced minus air-oxidized differential absorption spectra of membrane fractions of strain Dfr1^T (a) and strain Dfr2^T (b) showing α- and β-bands at higher resolution.

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 Dfr1^T and Dfr2^T with species of the family *Geobacteraceae* and, in particular, with members of the *Geobacter* cluster (Lonergan *et al.*, 1996). The sequence identity between strains Dfr1^T and Dfr2^T was 92·5%. Sequence identities of strains Dfr1^T and Dfr2^T to other species of the *Geobacter* cluster ranged between 92·3 and 93·7%. Further aspects of the phylogenetic affiliation of the two strains have been discussed before (Straub *et al.*, 1998).

The physiological properties of strains Dfr1^T and Dfr2^T have been described in detail elsewhere (Straub *et al.*, 1998). Like *Geobacter sulfurreducens*, 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. sulfurreducens*, strain Dfr1^T and strain Dfr2^T (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 Dfr1^T and Dfr2^T 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. sulfurreducens* (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 Dfr1^T and Dfr2^T 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 Dfr1^T and Dfr2^T 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).

Other taxonomically relevant physiological features of strains Dfr1^T and Dfr2^T are summarized in the species descriptions below.

In conclusion, the results of the 16S rDNA sequence analyses showed the affiliation of strains Dfr1^T and Dfr2^T 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 Dfr1^T and Dfr2^T represent novel species. Strains Dfr1^T and Dfr2^T 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 bremensis* sp. nov. for strain Dfr1^T and *Geobacter pelophilus* sp. nov. for strain Dfr2^T.

Description of *Geobacter bremensis* sp. nov.

Geobacter bremensis (bre.men′sis. N.L. n. *Brema* Bremen, in northern Germany; L. masc. suffix *-ensis* indicating provenance; N.L. masc. adj. *bremensis* 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), S^0, fumarate and malate; strictly anaerobic. Optimal growth with ferrihydrite as the electron acceptor at 30–32 °C and pH 5·5–6·7. No vitamins required. The G+C content of the DNA is 60 mol %.

The type strain, Dfr1^T (= DSM 12179^T = OCM 796^T), 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), S^0, 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, Dfr2^T (= DSM 12255^T = OCM 797^T), was isolated from a freshwater ditch in Bremen, Germany.

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