Geobacter bemidjiensis sp. nov. and *Geobacter psychrophilus* sp. nov., two novel Fe(III)-reducing subsurface isolates

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Fe(III)-reducing isolates were recovered from two aquifers in which Fe(III) reduction is known to be important. Strain Bem^T was enriched from subsurface sediments collected in Bemidji, MN, USA, near a site where Fe(III) reduction is important in aromatic hydrocarbon degradation. Strains P11, P35^T and P39 were isolated from the groundwater of an aquifer in Plymouth, MA, USA, in which Fe(III) reduction is important because of long-term inputs of acetate as a highway de-icing agent to the subsurface. All four isolates were Gram-negative, slightly curved rods that grew best in freshwater media. Strains P11, P35^T and P39 exhibited motility via means of monotrichous flagella. Analysis of the 16S rRNA and *nifD* genes indicated that all four strains are δ-proteobacteria and members of the *Geobacter* cluster of the *Geobacteraceae*. Differences in phenotypic and phylogenetic characteristics indicated that the four isolates represent two novel species within the genus *Geobacter*. All of the isolates coupled the oxidation of acetate to the reduction of Fe(III) [iron(III) citrate, amorphous iron(III) oxide, iron(III) pyrophosphate and iron(III) nitrilotriacetate]. All four strains utilized ethanol, lactate, malate, pyruvate and succinate as electron donors and malate and fumarate as electron acceptors. Strain Bem^T grew fastest at 30 °C, whereas strains P11, P35^T and P39 grew equally well at 17, 22 and 30 °C. In addition, strains P11, P35^T and P39 were capable of growth at 4 °C. The names *Geobacter bemidjiensis* sp. nov. (type strain Bem^T = ATCC BAA-1014^T = DSM 16622^T = JCM 12645^T) and *Geobacter psychrophilus* sp. nov. (strains P11, P35^T and P39; type strain P35^T = ATCC BAA-1013^T = DSM 16674^T = JCM 12644^T) are proposed.

INTRODUCTION

Fe(III) is an abundant electron acceptor in subsurface environments (Lovley, 1991). A wide variety of bacteria and archaea are able to couple the oxidation of organic matter to the reduction of Fe(III) minerals (Lovley *et al.*, 2004). The temperature at which micro-organisms are known to reduce Fe(III) ranges from 4 to 121 °C (Finneran *et al.*, 2003; Kashefi & Lovley, 2003; Holmes *et al.*, 2004c; Lovley *et al.*, 2004). Most previously described Fe(III)-reducing micro-organisms grow fastest at temperatures in the range 20–30 °C (Lovley *et al.*, 2004), and a number of Fe(III)-reducing hyperthermophiles and thermophiles have been described (Vargas *et al.*, 1998; Kashefi *et al.*, 2004). However, only two Fe(III)-reducing bacteria have been show to grow with Fe(III) as electron acceptor at 4 °C in pure culture, *Rhodoferax ferrireducens*, a freshwater facultative organism capable of reducing soluble, chelated iron(III) nitrilotriacetate (NTA) (Finneran *et al.*, 2003), and *Geopsychrobacter electrodiphilus*, a marine anaerobe capable of amorphous iron(III) oxide reduction (Holmes *et al.*, 2004c). *Geopsychrobacter* species are members of the family *Geobacteraceae*, the largest group of Fe(III)-reducing bacteria, within the '*Deltaproteobacteria*' (Lovley *et al.*, 2004). Members of this family have many distinguishing characteristics other than their ability to reduce Fe(III), among them nitrogen fixation and the ability to oxidize acetate and other multi-carbon organic substrates to carbon dioxide (Bazylinski *et al.*, 2000; Coppi *et al.*, 2001; Holmes *et al.*, 2004b; Lovley *et al.*, 2004).

Members of the family *Geobacteraceae* also play an

Abbreviations: AQDS, anthraquinone 2,6-disulfonate; NTA, nitrilotriacetate.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *gyrB*, *fusA*, *nifD*, *rpoB* and *recA* gene sequences of strain Bem^T are AY187307, AY547335, AY188890, AY186994, AY186914 and AY186883 and those for the 16S rRNA, *fusA* and *nifD* gene sequences of strain P35^T are AY653548, AY653550 and AY795909.

Similarity matrices for 16S rRNA and *nifD* gene sequences of the novel isolates and related strains are available as supplementary material in IJSEM Online.

important role in the *in-situ* bioremediation of uranium-contaminated aquifers (Anderson *et al.*, 2003; Istok *et al.*, 2004; Vrionis *et al.*, 2005) and in other contaminated subsurface environments, including aquifers contaminated with petroleum (Rooney-Varga *et al.*, 1999; Snoeyenbos-West *et al.*, 2000) or landfill leachate (Roling *et al.*, 2001) as well as lake sediments contaminated with heavy metals (Cummings *et al.*, 2003). *Geobacter* species are also found in a wide variety of pristine environments (Snoeyenbos-West *et al.*, 2000; Stein *et al.*, 2001; Ikenaga *et al.*, 2003; Petrie *et al.*, 2003; Helms *et al.*, 2004; Holmes *et al.*, 2004a). One reason for this is that members of the *Geobacteraceae* can oxidize acetate, which is the primary intermediate in the anaerobic degradation of organic matter in sedimentary systems (Lovley & Chapelle, 1995). Furthermore, members of the *Geobacteraceae* have a highly effective strategy for localizing iron(III) oxides and directly transferring electrons to the iron(III) oxide surface (Nevin & Lovley, 2000a, 2002a, b; Childers *et al.*, 2002).

Despite the environmental significance of dissimilatory Fe(III) reduction and the important role that *Geobacter* species play in Fe(III) reduction in the environment, relatively few *Geobacter* species have been fully characterized. The purpose of this study was to recover organisms with 16S rRNA gene sequences similar to those that predominate in subsurface environments, in which Fe(III) reduction is important, in order to understand better the role that these organisms play in Fe(III) reduction. Here we describe three isolates from a site in Massachusetts and an isolate from a site in Minnesota; all four isolates are members of *Geobacter*.

METHODS

Enrichment and isolation. Strict anaerobic techniques for the culturing of Fe(III)-reducing micro-organisms (Lovley, 2000, 2005) were used throughout. Sediment was collected as previously described from the background section of an aquifer in Bemidji, MN, USA, as part of ongoing research to study Fe(III) reduction and aromatic hydrocarbon oxidation in the subsurface (Anderson & Lovley, 1999, 2000). Sediment was transferred to the laboratory, incubated under anoxic conditions and amended with 5 mM acetate and 100 μM anthraquinone 2,6-disulfonate (AQDS) to promote Fe(III) reduction in the sediments, as described previously (Nevin & Lovley, 2000b). Once active Fe(III) reduction had been established, sediments were serially diluted in a bicarbonate-buffered freshwater medium (Lovley & Phillips, 1988) with acetate (20 mM) as the electron donor and poorly crystalline iron(III) oxide (100 mmol l^{-1}) as the electron acceptor. The 10^{-3} dilution, which actively reduced Fe(III), was transferred (10 % inoculum) into fresh medium three times and then streaked onto basal salts solid plate medium with fumarate (40 mM) as the electron acceptor and acetate (20 mM) as the electron donor. The isolate was obtained from a single colony and grown up in basal salts liquid medium before transfer to iron(III) citrate media for further characterization.

The Plymouth isolates (strains P11, P35^T and P39) were obtained from groundwater from a highway runoff recharge pool located adjacent to State Route 25 (SR25) in Plymouth, MA, USA. The pool was constructed to collect runoff generated by SR25, which opened in August 1987 (Church *et al.*, 1996). The Massachusetts Department of Environmental Protection enacted restrictions on this area requiring the use of non-chloride de-icing agents along a 1900 m section of highway impacting nearby cranberry bogs. Since opening, the primary road de-icing agent used on this stretch of highway has been calcium magnesium acetate (CMA). The unconfined aquifer underlying the study site is part of the Wareham Outwash Plain, consisting of fine- to coarse-grained sand. The concentration of acetate in the groundwater varies between 0 and 5 mM (Ostendorf, 1997–2004). This site serves as an analogue for the microbiology likely to be found in extended long-term acetate injection into the subsurface for *in-situ* uranium bioremediation. Groundwater (1 ml) was added to the same acetate-iron(III) oxide medium described above. After 15 consecutive transfers (10 % inoculum), roll tubes containing iron(III) oxide and acetate were made. Colonies were picked anaerobically from roll tubes with a bent Pasteur pipette and placed in 2 ml of the same liquid medium.

Light and electron microscopy. Cells were routinely examined by phase-contrast microscopy. Electron microscopy was done in the microscopy facility at the University of Massachusetts, Amherst.

Characterization of anaerobic growth and electron donor and acceptor utilization. Strain Bem^T was incubated at 30 °C and strains P11, P35^T and P39 were incubated at 17 °C for all growth and donor/acceptor utilization experiments. Acetate (10 mM) was the electron donor for all evaluations of electron acceptor utilization. For electron donor utilization experiments, iron(III) citrate (55 mM) was the electron acceptor for the studies with strain Bem^T and iron(III) oxide (100 mM) was the acceptor for studies with strains P11, P35^T and P39. Iron(III) oxide reduction was monitored as production of magnetite (Lovley *et al.*, 1987); all other electron acceptors were determined visually by observing precipitation [iron(III) citrate, iron(III) pyrophosphate and iron(III) NTA], colour change [AQDS, iron(III) citrate, iron(III) pyrophosphate, iron(III) NTA and manganese(IV) oxide] or increase in optical density (fumarate, nitrate and malate). A graphite electrode serving as the sole electron acceptor was evaluated in an anoxic dual-chambered fuel cell as described previously (Bond *et al.*, 2002; Bond & Lovley, 2003).

Temperature, pH and salt tolerance. Temperature and pH tests for strain Bem^T were performed in media containing iron(III) citrate (55 mM) and acetate (10 mM).

Strains P11, P35^T and P39 were tested for temperature, pH and salt tolerance in media containing iron(III) oxide (100 mmol l^{-1}) and acetate (10 mM). Strains P11, P35^T and P39 were also tested for growth in media containing salt concentrations ranging from 10 to 50 g NaCl l^{-1}. Salt tolerance was also tested by transferring cells back to medium containing no NaCl after two exposures to these various NaCl concentrations. Fe(II) was assayed with ferrozine as described previously (Lovley & Phillips, 1987).

Cytochrome content, G+C content and DNA–DNA hybridization. Cytochrome analysis was performed on the four isolates and *Geobacter metallireducens* using cells grown in either iron(III) citrate or iron(III) oxide medium. Oxalate was used to dissolve Fe(II) (Lovley & Phillips, 1986). Three millilitres of culture was re-suspended in 20 mM pH 7 PIPES/NaOH and spectra were obtained as described previously (Caccavo *et al.*, 1994) on a Shimadzu UV2401-PC dual-beam spectrophotometer. G+C content and DNA–DNA hybridization analyses (Cashion *et al.*, 1977; De Ley *et al.*, 1970; Huß *et al.*, 1983) were performed by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen.

DNA extraction and PCR amplification of 16S rRNA and *nifD* genes. Cells (10 ml) were collected by centrifugation and genomic DNA was extracted using the BIO 101 FastDNA Spin kit following the manufacturer's instructions.

16S rRNA gene sequences for strains P35^T, P11, P39 and Bem^T were amplified with primers 8 forward (Eden *et al.*, 1991) and 1492 reverse (Amann *et al.*, 1990; Achenbach & Woese, 1995) as described previously (Holmes *et al.*, 2004b). Gene fragments from the *nifD* genes in strains P35^T and Bem^T were amplified with primers NIFD883F/NIFD1337R (Ueda *et al.*, 1995) as described previously (Holmes *et al.*, 2004b). PCR products were purified from agarose gel with the QIAquick gel extraction kit (Qiagen) and ligated into the TOPO TA cloning kit, version K2 (Invitrogen) according to the manufacturers' instructions. Plasmid inserts were then amplified with M13 forward and reverse primers (Invitrogen) and PCR products were prepared for sequencing with a Qiaquick PCR purification kit (Qiagen).

Phylogenetic analysis of strains P35^T, P39, P11 and Bem^T. The 16S rRNA and *nifD* gene fragments from strains P35^T, P11, P39 and Bem^T were compared to the GenBank nucleotide and protein databases using the BLASTN and BLASTX algorithms (Altschul *et al.*, 1990). Nucleotide and amino acid sequences were manually aligned in the Genetic Computer Group (GCG) sequence editor (Wisconsin Package version 10). Aligned sequences were imported into PAUP 4.0b 4a (Swofford, 1998), where phylogenetic trees were inferred. Identical branching orders were observed with maximum-parsimony, maximum-likelihood and distance-based algorithms when 16S rRNA gene sequences were compared (not shown). Similar branching orders were obtained with these three algorithms when *nifD* gene sequences were compared (not shown). Bootstrap values were calculated by all three analyses and 1280 and 456 nucleotide positions, respectively, were considered for 16S rRNA and *nifD* gene comparisons.

The similarity matrix program (Maidak *et al.*, 2001) available on the Ribosomal Database Project II website, LFASTA version 3.2 (Pearson, 1990) and CLUSTAL W (Thompson *et al.*, 1997) were used to generate similarity matrices considering 1280 nucleotides from the 16S rRNA gene and 151 amino acid positions from the translation product of the *nifD* gene fragment.

RESULTS AND DISCUSSION

Morphology

Cells of strains Bem^T, P11, P35^T and P39 were rod-shaped, curved, Gram-negative, non-sporulating and slightly wrinkly (when viewed by electron microscopy) in appearance. Cells of strains P11, P35^T and P39 were approx. 2·5–3 × 0·8 μm and were motile by means of a monotrichous flagellum two to three times the length of the cell (Fig. 1a). Cells of strain Bem^T were approx. 2·5–4 × 0·5 μm and motility and flagella were not observed (Fig. 1b). Cells from all strains appeared as single cells or in chains of two to three cells. Strains P11, P35^T and P39 formed aggregates when growing on some electron acceptors, including iron(III) citrate and fumarate.

Electron donors and acceptors utilized

Compounds used by the strains as electron donors are indicated in Table 1 and those used as electron acceptors are indicated in Table 2.

Temperature and pH and salt tolerance

Strain Bem^T grew fastest at 30 °C, but also grew at 15, 20, 25, 30, 32 and 37 °C. The pH for fastest growth of strain Bem^T was 7. The pH and temperature ranges of strain Bem^T are typical of previously described *Geobacter* species (Lovley *et al.*, 2004).

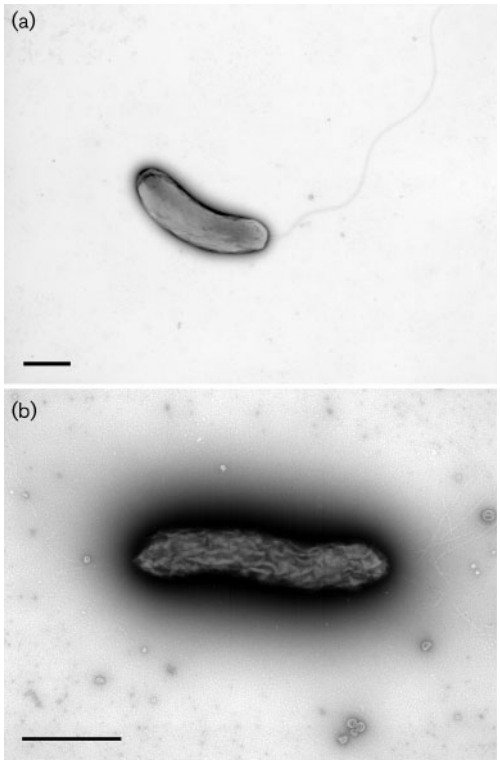

Fig. 1. (a) Transmission electron micrograph of strain P35^T grown on medium with poorly crystalline iron(III) oxide (100 mmol l^{-1}) provided as the electron acceptor and acetate (10 mM) as the electron donor. (b) Scanning electron micrograph of a cell of strain Bem^T grown on medium with iron(III) citrate (50 mM) provided as the electron acceptor and acetate (10 mM) as the electron donor. Bars, 1 μm.

Strains P11, P35^T and P39 had a broad temperature growth range; similar growth rates were observed at 17, 22 and 30 °C (Fig. 2). They also grew at 4 and 10 °C, but not at 37 °C (Fig. 2). No previously described *Geobacter* species are capable of growth at 4 °C (Lovley *et al.*, 2004); however, other members of the family *Geobacteraceae*, the marine strains of *Geopsychrobacter electrodiphilus*, grow at 4 °C (Holmes *et al.*, 2004c). *Geopsychrobacter electrodiphilus* and *Rhodoferax ferrireducens* are the only other bacteria that are known to grow at 4 °C while using Fe(III) as terminal electron acceptor. At 4 °C, strains P11, P35^T and P39 reduce Fe(III) two to three times faster than has been reported for the other Fe(III)-reducers capable of growth at this temperature (Finneran *et al.*, 2003; Holmes *et al.*, 2004c). Other iron(III)-citrate-reducing bacteria [*Shewanella gelidimarina* ACAM 456^T, *Shewanella frigidimarina* ACAM 591^T (Bowman *et al.*, 1997), *Shewanella pealeana* ANG-SQI1^T (Leonardo *et al.*, 1999), *Shewanella livingstonensis* NF22^T (Bozal *et al.*, 2002), *Desulfofrigus oceanense* ASv26^T,

Table 1. Growth of strains Bem^T, P11, P35^T and P39, *Geobacter chapellei* 172^T and *Geobacter bremensis* Dfr1^T on various electron donors

Iron(III) citrate (strain Bem^T) or iron(III) oxide (strains P11, P35^T and P39) was provided as the electron acceptor. Data for reference species were taken from Coates *et al.* (1996, 2001) (*Geobacter chapellei* 172^T) and from Straub *et al.* (1998) and Straub & Buchholz-Cleven (2001) (*Geobacter bremensis* Dfr1^T). Acetate, ethanol and lactate (all at 10 mM) tested positive for all strains shown. The following were tested and proved negative for strains Bem^T, P11, P35^T and P39: acetoin (1 g l^{-1}), arginine (5 mM), caproate (10 mM), Casamino acids (1 g l^{-1}), ferulate (0·5 mM), fructose (10 mM), gallic acid (0·68 g l^{-1}), glucose (5 mM), glycerol (6 mM), mannitol (10 mM), nicotinate (0·5 mM), proline (10 mM), serine (10 mM) and syringate (0·5 mM). The following were also tested and proved negative for strain Bem^T: benzaldehyde (0·5 mM), benzyl alcohol (0·5 mM), glutamine (10 mM), isopropanol (10 mM), methanol (20 mM), naphthalene (0·5 mM), *o*-hydroxybenzoate (0·5 mM), *p*-hydroxybenzaldehyde (0·5 mM), *p*-hydroxybenzoate (0·5 mM), *p*-hydroxybenzyl alcohol (0·5 mM), phenol (0·5 mM), salicylic acid (10 mM) and toluene (1, 5 and 10 mM). NT, Not tested.

Donor	Bem^T	P11	P35^T	P39	172^T	Dfr1^T
Benzoate (2 mM)	+	−	−	−	−	+
Butanol (10 mM)	+	+	+	+	NT	+
Butyrate (10 mM)	+	−	−	−	NT	+
Formate (10 mM)	−	+	+	+	+	+
Hydrogen (130 kPa)*	+	−	−	−	−	+
Isobutyrate (10 mM)	+	−	−	−	NT	NT
Malate (10 mM)	+	+	+	+	NT	+
Propionate (10 mM)	+	−	−	−	−	+
Pyruvate (10 mM)	+	+	+	+	NT	+
Succinate (10 mM)	+	+	+	+	NT	+
Tryptone (1 g l^{-1})	−	+	−	−	NT	NT
Valerate (10 mM)	+	−	−	−	NT	−
Yeast extract (0·5 g l^{-1})	−	−	−	+	NT	NT

*Acetate (0·1 mM) was provided as carbon source for growth on hydrogen.

Desulfofrigus fragile LSv21^T, *Desulfotalea psychrophila* Lsv54^T and *Desulfotalea arctica* LSv514^T (Knoblauch *et al.*, 1999)] that are capable of growth at 4 °C were not tested for Fe(III) reduction at 4 °C, nor are they able to reduce insoluble forms of Fe(III), which are the predominant forms of Fe(III) in most environments.

Strains P11, P35^T and P39 were capable of growth and magnetite production at pH values ranging from 6·0 to 9·0, with fastest growth at pH 7·0. No growth was observed at pH 5·0. The pH ranges of strains P11, P35^T and P39 are typical of other *Geobacter* species (Lovley *et al.*, 2004).

Strains P11, P35^T and P39 were tested for salt tolerance, because NaCl, in the form of road salt, periodically impacts the sediment from which the strains were isolated. Strains P11, P35^T and P39 were capable of growth and production of magnetite in the presence of 10 g NaCl l^{-1}. Additionally, they were tolerant of 10, 20 and 30 g NaCl l^{-1}.

Table 2. Growth of strains Bem^T, P11, P35^T and P39, *Geobacter chapellei* 172^T and *Geobacter bremensis* Dfr1^T on various electron acceptors

Acetate (10 mM) was provided as the electron donor. Data for reference species were taken from Coates *et al.* (1996, 2001) (*Geobacter chapellei* 172^T) and from Straub *et al.* (1998) and Straub & Buchholz-Cleven (2001) (*Geobacter bremensis* Dfr1^T). NT, Not tested. Fumarate (40 mM) and iron(III) oxide (100 mM) tested positive for all strains shown; nitrate (5 mM), sulfate (10 mM) and thiosulfate (10 mM) tested negative for all strains shown.

Acceptor	Bem^T	P11	P35^T	P39	172^T	Dfr1^T
AQDS (5 mM)	+	+	+	−	+	NT
Elemental sulfur (20 g l^{-1})	−	−	−	−	NT	+
Graphite electrode	−	NT	+	NT	NT	NT
Iron(III) citrate (55 mM)	+	−	+	+	−	NT
Iron(III) pyrophosphate (10 mM)	+	+	+	+	NT	NT
Iron(III) NTA (5 mM)	+	+	+	+	+	NT
Malate (10 mM)	+	+	+	+	−	+
Manganese(IV) oxide (20 g l^{-1})	+	−	+	+	+	+

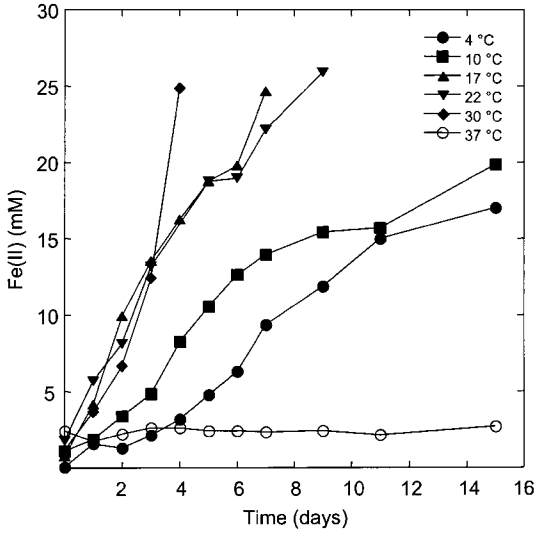

Fig. 2. Effect of temperature on the growth rate of strain P35^T. Cells were grown in medium with iron(III) oxide (100 mmol l^{-1}) provided as the electron acceptor and acetate (5 mM) as the electron donor. Data presented are means of triplicate incubations.

Cytochrome content, G+C content and DNA–DNA hybridization

The dithionite-reduced minus air-oxidized difference spectrum of all tested *Geobacter* species had peaks consistent with the presence of *c*-type cytochromes. Strain Bem^T had absorbance peaks at 422 and 555 nm and a shoulder at 522 nm. Strain P11 had absorbance peaks at 420 and 551 nm and a shoulder at 521 nm. Strain P35^T had absorbance peaks at 420 and 551 nm and a shoulder at 521 nm. Strain P39 had absorbance peaks at 420 and 554 nm and a shoulder at 526 nm. A similar spectrum was obtained from the control, *Geobacter metallireducens*, with peaks at 420 and 552 nm and a shoulder at 526 nm.

The G+C content of strain Bem^T was 60·9 mol% and that of strain P35^T was 63·8 mol%. The G+C contents of *Geobacter chapellei* 172^T, *Geobacter pelophilus* Dfr2^T, *Geobacter metallireducens* GS-15^T, *Geobacter grbiciae* TACP-2^T, *Geobacter hydrogenophilus* H-2^T *Geobacter bremensis* Dfr1^T and *Geobacter sulfurreducens* PCA^T are respectively 50·2, 53, 56·6, 57·4, 58·4, 60 and 60·9 mol% (Lovley *et al.*, 1993; Straub *et al.*, 1998; Coates *et al.*, 2001; Methé *et al.*, 2003).

DNA–DNA hybridization of *Geobacter bremensis* Dfr1^T and strain Bem^T yielded a DNA–DNA relatedness value of 63·5 % (repeated measurement 55·8 %). *Geobacter bremensis* Dfr1^T and strain Bem^T are not related at the species level when a threshold value of 70 % DNA–DNA relatedness for definition of bacterial species is considered (Wayne *et al.*, 1987).

Phylogenetic analysis of strains P35^T and Bem^T

Strain Bem^T was included in a comprehensive phylogenetic study of the *Geobacteraceae* (Holmes *et al.*, 2004b). When the nucleotide and amino acid sequences from six conserved genes, 16S rRNA, *rpoB*, *recA*, *gyrB*, *fusA* and *nifD*, from strain Bem^T were analysed, it was apparent that strain Bem^T fell within the *Geobacter* clade of the family *Geobacteraceae* in the class '*Deltaproteobacteria*'. According to 16S rRNA gene nucleotide and *nifD* amino acid sequence comparisons, strain Bem^T was most similar to *Geobacter bremensis* Dfr1^T and '*Geobacter humireducens*' (99 and 95 % identical; see Supplementary Table S1 available in IJSEM Online).

Phylogenetic analysis of the 16S rRNA gene from strains P35^T, P39 and P11 also indicated that these organisms belong to the genus *Geobacter* within the family *Geobacteraceae* (Fig. 3). Phylogeny of the *nifD* gene supports the placement of these strains as members of *Geobacter* (Fig. 3). The 16S rRNA nucleotide and *nifD* amino acid sequences from strain P35^T were most similar to those of *G. chapellei* 172^T (96 and 82 % identical; Supplementary Table S1).

Description of *Geobacter bemidjiensis* sp. nov.

Geobacter bemidjiensis (be.mid.ji.en′sis. N.L. masc. adj. *bemidjiensis* from Bemidji, MN, USA, where

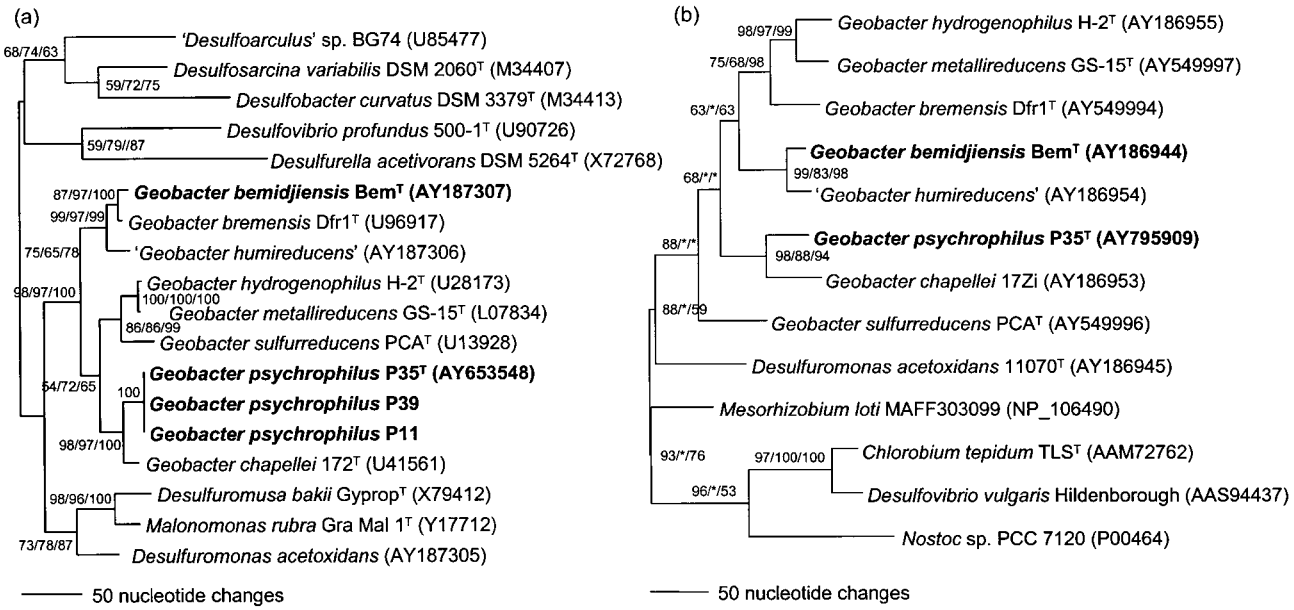

Fig. 3. Phylogenetic trees comparing 16S rRNA gene sequences (a) and *nifD* sequences (b) from species of the *Geobacteraceae*. '*Desulfoarculus*' sp. BG74, *Desulfosarcina variabilis*, *Desulfobacter curvatus*, *Desulfovibrio profundus* and *Desulfurella acetivorans* served as outgroups for construction of the 16S rRNA gene tree, while *Desulfovibrio vulgaris*, *Mesorhizobium loti*, *Chlorobium tepidum* and *Nostoc* sp. PCC 7120 were outgroups for the *nifD* tree. Bootstrap values obtained from 100 replicates by maximum-parsimony/maximum-likelihood/distance-based analyses are shown; asterisks indicate values below 50.

sediment samples were taken from which the type strain was isolated).

Non-motile, Gram-negative, curved rods, approximately 2·5–4 μm in length and 0·5 μm in diameter. Can couple the reduction of Fe(III) to the oxidation of acetate, benzoate, butanol, butyrate, ethanol, hydrogen, isobutyrate, malate, lactate, propionate, pyruvate, succinate and valerate. No growth when acetoin, arginine, benzaldehyde, benzyl alcohol, caproate, Casamino acids, ferulate, fructose, formate, gallic acid, glucose, glycerol, glutamine, isopropanol, mannitol, methanol, naphthalene, nicotinate, *o*-hydroxybenzoate, *p*-hydroxybenzaldehyde, *p*-hydroxybenzoate, *p*-hydroxybenzyl alcohol, phenol, proline, salicylic acid, serine, syringate, tryptone, toluene or yeast extract is provided as the electron donor. This species can utilize Fe(III), fumarate, AQDS, malate and manganese(IV) oxide as electron acceptors. No growth when elemental sulfur, nitrate, sulfate, thiosulfate or a graphite electrode is provided as the electron acceptor. Growth occurs at temperatures between 15 and 37 °C (optimum temperature is approximately 30 °C). *c*-Type cytochromes are abundant. The G+C content of the DNA is 60·9 mol%.

The type strain is strain Bem^T (=ATCC BAA-1014^T=DSM 16622^T=JCM 12645^T). The 16S rRNA (AY187307), *gyrB* (AY547335), *fusA* (AY188890), *nifD* (AY186994), *rpoB* (AY186914) and *recA* (AY186883) gene sequences of the type strain have been deposited in GenBank.

Description of *Geobacter psychrophilus* sp. nov.

Geobacter psychrophilus (psy.chro′phil.us. Gr. adj. *psychros* cold; Gr. adj. *philos* liking, loving; N.L. masc. adj. *psychrophilus* cold-loving).

Motile (monotrichous flagella), Gram-negative curved rods, approximately 2·5–3 μm in length and 0·8 μm in diameter. Can couple the reduction of Fe(III) to the oxidation of acetate, butanol, ethanol, formate, lactate, malate, pyruvate and succinate as the electron donor. No growth when acetoin, arginine, benzoate, butyrate, caproate, Casamino acids, ferulate, fructose, gallic acid, glycerol, hydrogen, isobutyrate, mannitol, nicotinate, proline, propionate, serine, syringate, tryptone, valerate or yeast extract is provided as electron donor. This species can utilize AQDS, iron(III) citrate, iron(III) oxide, iron(III) pyrophosphate, iron(III) NTA, fumarate, malate, manganese(IV) oxide and graphite electrodes as electron acceptors. No growth when elemental sulfur, nitrate, sulfate or thiosulfate is provided as electron acceptor. Growth occurs at temperatures between 4 and 30 °C (optimum temperature range is 17–30 °C). *c*-Type cytochromes are abundant. The G+C content of the DNA of the type strain is 63·8 mol%.

The type strain is strain P35^T (=ATCC BAA-1013^T=DSM 16674^T=JCM 12644^T). The 16S rRNA (AY653548), *fusA* (AY653550) and *nifD* (AY795909) gene sequences have been deposited in GenBank.

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