Geobacter daltonii sp. nov., an Fe(III)- and uranium(VI)-reducing bacterium isolated from a shallow subsurface exposed to mixed heavy metal and hydrocarbon contamination

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An Fe(III)- and uranium(VI)-reducing bacterium, designated strain FRC-32^T, was isolated from a contaminated subsurface of the USA Department of Energy Oak Ridge Field Research Center (ORFRC) in Oak Ridge, Tennessee, where the sediments are exposed to mixed waste contamination of radionuclides and hydrocarbons. Analyses of both 16S rRNA gene and the *Geobacteraceae*-specific citrate synthase (*gltA*) mRNA gene sequences retrieved from ORFRC sediments indicated that this strain was abundant and active in ORFRC subsurface sediments undergoing uranium(VI) bioremediation. The organism belonged to the subsurface clade of the genus *Geobacter* and shared 92–98% 16S rRNA gene and 75–81% *rpoB* gene sequence similarities with other recognized species of the genus. In comparison to its closest relative, *Geobacter uraniireducens* Rf4^T, according to 16S rRNA gene sequence similarity, strain FRC-32^T showed a DNA–DNA relatedness value of 21%. Cells of strain FRC-32^T were Gram-negative, non-spore-forming, curved rods, 1.0–1.5 µm long and 0.3–0.5 µm in diameter; the cells formed pink colonies in a semisolid cultivation medium, a characteristic feature of the genus *Geobacter*. The isolate was an obligate anaerobe, had temperature and pH optima for growth at 30 °C and pH 6.7–7.3, respectively, and could tolerate up to 0.7% NaCl although growth was better in the absence of NaCl. Similar to other members of the *Geobacter* group, strain FRC-32^T conserved energy for growth from the respiration of Fe(III)-oxyhydroxide coupled with the oxidation of acetate. Strain FRC-32^T was metabolically versatile and, unlike its closest relative, *G. uraniireducens*, was capable of utilizing formate, butyrate and butanol as electron donors and soluble ferric iron (as ferric citrate) and elemental sulfur as electron acceptors. Growth on aromatic compounds including benzoate and toluene was predicted from preliminary genomic analyses and was confirmed through successive transfer with fumarate as the electron acceptor. Thus, based on genotypic, phylogenetic and phenotypic differences, strain FRC-32^T is considered to represent a novel species of the genus *Geobacter*, for which the name *Geobacter daltonii* sp. nov. is proposed. The type strain is FRC-32^T (=DSM 22248^T=JCM 15807^T).

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Abbreviations: ANI, average nucleotide identity; DOE, Department of Energy; NCBI, National Center for Biotechnology Information; OD, optical density; ORFRC, Oak Ridge Field Research Center; *rpoB*, β-subunit of the DNA-directed RNA polymerase gene; SEM, scanning electron microscopy.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *rpoB* gene sequences of strain FRC-32^T are EU660516 and NC_011979, respectively.

Figures showing a neighbour-joining tree of complete *rpoB* gene sequences of *Geobacter* species, a scanning electron micrograph of cells of strain FRC-32^T and graphs showing Fe(III) reduction and growth of strain FRC-32^T with acetate as the electron donor and Fe(III)-oxyhydroxide as the sole electron acceptor are available as supplementary material with the online version of this paper.

Dissimilatory Fe(III) reduction is a critical electron-accepting process coupled to the terminal decomposition of organic matter in aquatic and marine sediments (Kostka *et al.*, 1999; Thamdrup, 2000; Canfield *et al.*, 2005) and may also impact global biogeochemical cycles by controlling the release of inorganic phosphate sequestered in Fe(III) oxide minerals and by suppressing methane emission (Roden & Wetzel, 1996). In contaminated subsurface environments, Fe(III)-reducing micro-organisms have been shown to degrade or remove organic and inorganic contaminants and play a central role in remediation strategies for the protection of groundwater resources (Lovley, 2006).

When molecular techniques have been used to assess microbial community composition in environments where metal reduction is an important process, members of the *Geobacteraceae* in the *Desulfuromonadales* are often detected (Lovley, 2006). Studies of freshwater sediments (Stein *et al.*, 2001), petroleum hydrocarbon-contaminated aquifers (Rooney-Varga *et al.*, 1999), and radionuclide-contaminated subsurface sediments (Holmes *et al.*, 2002; Anderson *et al.*, 2003; North *et al.*, 2004) have all revealed an abundance of sequences affiliated with members of the *Geobacteraceae* and specifically from the genus *Geobacter*.

Members of the genus *Geobacter* are Gram-negative, non-spore-forming, motile or non-motile, curved rod-shaped bacteria belonging to the family *Geobacteraceae*, order *Desulfuromonadales* and class *Deltaproteobacteria* of the phylum *Proteobacteria* (Nevin *et al.*, 2005; Shelobolina *et al.*, 2008; Coates *et al.*, 2001; Straub & Buchholz-Cleven, 2001; Shelobolina *et al.*, 2007; Nevin *et al.*, 2005, 2007). At the time of writing, 13 species of the genus *Geobacter* with validly published names had been reported (<http://www.bacterio.cict.fr/g/geobacter.html>). Phenotypic features shared by members of the genus *Geobacter* include an obligately anaerobic lifestyle, complete oxidation of carbon substrates to carbon dioxide, and conservation of energy for growth from the respiration of Fe(III)-oxyhydroxide coupled to the oxidation of acetate (Lovley *et al.*, 2004).

Uranium contamination in subsurface environments is a widespread problem that threatens drinking water resources across the globe and especially in areas within the USA Department of Defense weapons complex managed by the USA Department of Energy (DOE; Abdelouas *et al.*, 1999; Istok *et al.*, 2004; Riley & Zachara, 1992). Members of the family *Geobacteraceae* are substantial contributors to the reductive immobilization of uranium in contaminated subsurface sediments and are important for the long-term stability of reduced uranium (Anderson *et al.*, 2003; North *et al.*, 2004; Vrionis *et al.*, 2005). In addition, members of the *Geobacter* group have been shown to degrade aromatic hydrocarbons in the shallow subsurface (Lovley & Anderson, 2000). However, no members of the *Geobacter* subsurface clade (Holmes *et al.*, 2007) have been shown to be capable of the oxidation of aromatic contaminants. In this study, we hypothesized

that *Geobacter* species are well adapted for the extremes of subsurface sediments exposed to mixed waste contamination and thus the objective of our isolation effort was to isolate an Fe(III)-reducing micro-organism that was capable of both uranium(VI) reduction and the degradation of aromatic hydrocarbons. Strain FRC-32^T was isolated from stable enrichment cultures initiated from highly contaminated subsurface sediment exposed to mixed heavy metal and hydrocarbon wastes using Fe(III)-oxyhydroxide as the electron acceptor and acetate as an electron donor (Petrie *et al.*, 2003). Using structural and functional gene analysis, strain FRC-32^T was detected in abundance in uranium-contaminated subsurface sediments (Akob *et al.*, 2008) and is thought to be an important metal-reducing organism in the subsurface of contaminated DOE sites undergoing remediation. Here we show that strain FRC-32^T has a wide range of metabolic capabilities consistent with such an environment, and the capacity for this organism to oxidize aromatic hydrocarbons is both an indication of the environmental significance and the novelty of this organism, as close relatives are not known to have these capabilities. Because of its origin and data relating it to ongoing remediation efforts at the Oak Ridge Field Research Center (ORFRC), strain FRC-32^T could be an ideal model organism for the development of bioremediation technologies for contaminants at DOE sites.

For the isolation of Fe(III)-reducing bacteria, contaminated subsurface sediment was collected from the USA Department of Energy Environmental Remediation Sciences Programs ORFRC located at the Y-12 complex Department of Defense on the Oak Ridge Reservation, Oak Ridge, Tennessee. Sediment cores were sampled on 16 February 2001 with an Acker Drill Co. (TBD-II) Hologator track drill equipped with polyurethane sleeves lining the corer. After sampling, cores (3.75 cm in diameter, 182 cm in length) were transferred immediately to a Coy anaerobic chamber and subsampled using strictly anoxic and aseptic techniques. Samples were sealed under argon and transported overnight to Florida State University on blue ice packs.

For enrichment, a bicarbonate-buffered freshwater minimal medium (Widdel & Bak, 1992) was prepared in a Widdel flask and dispensed into Hungate tubes using anoxic and aseptic techniques. Before dispensing, the medium was saturated with N₂/CO₂ (80:20 mix) and the headspace of the tubes was maintained using the same gas mixture to obtain the desired pH (6.9). Amorphous Fe(III)-oxyhydroxide was prepared as described by Lovley & Phillips (1986) and used as an electron acceptor at a final concentration of 50 mM. FeCl₂ was used as a mild reductant whereas acetate was used as a carbon source at a final concentration of 10 mM. Enrichments were inoculated with 10% sediment (w/v) and incubated in the dark at 30 °C for 10 months. Active Fe(III) reduction was monitored by using visual screening and by a colorimetric assay for ferrous iron (Petrie *et al.*, 2003). Tubes that developed a black colour due to the reduction

of Fe(III) were selected for further isolation work. Isolation and purification of strains was performed using gellan gum (Gelrite; Sigma) shakes and serial dilutions of the enrichment. Pink-coloured colonies formed in the Gelrite dilution series, presumably as a result of high levels of *c*-type cytochromes present in the constituent organisms (Nevin *et al.*, 2007). Individual pink colonies were picked with a sterile pasture pipette, repurified by using serial dilution in Gelrite and screened for Fe(III) reduction in a liquid medium, as described above. A pure culture of an Fe(III)-reducing bacterium was obtained from this study and designated strain FRC-32^T. The purity of the culture was checked by using microscopy and by streaking onto agar plates containing acetate and fumarate as electron donor and acceptor, respectively. Stocks of this strain were stored in glycerol at -80 °C, and working cultures were maintained at 4 °C.

Genomic DNA from harvested cells was extracted using a Mo Bio UltraClean Microbial DNA isolation kit (Mo Bio laboratories), according to the manufacturer's instructions. For 16S rRNA gene sequences, extracted DNAs were amplified by PCR using the 27F and 1492R universal bacterial primers, as described by Lane (1991). The amplified PCR product was purified using a GenCatch PCR Cleanup kit (Epoch Biolabs) and sequencing was performed using the DNA sequencing facility of Florida State University. The raw sequence data were screened and low quality data were removed. Three sequencing reactions were conducted to generate a near-full-length 16S rRNA gene sequence. The reactions were trimmed and assembled using the software package SEQUENCHER (Gene Codes Corporation). An almost-complete 16S rRNA gene sequence (1424 bases) was obtained for strain FRC-32^T and submitted to GenBank. A database search using the basic local alignment sequence tool (BLAST) at the National Center for Biotechnology Information (NCBI) was performed (Altschul *et al.*, 1997), and the 16S rRNA gene sequence that appeared to be most similar to that of strain FRC-32^T was that recovered from *Geobacter uraniireducens* Rf4^T. For phylogenetic analyses, 16S rRNA gene sequences of the type species of the genus *Geobacter* and other *Geobacter* species were retrieved from the NCBI database together with that of *Pelobacter acidigallici* Ma Gal 2^T, an outgroup member of the order *Desulfuromonadales*. Selected sequences were aligned using the software package 'Greengenes' (DeSantis *et al.*, 2006). Aligned sequences were imported into the MEGA 4.0 software package (Tamura *et al.*, 2007) and neighbour-joining phylogenetic trees were constructed using the maximum composite likelihood substitution model with pairwise deletion of gapped positions. The robustness of inferred tree topologies was evaluated by 1000 bootstrap resamplings of the data. In addition, Bayesian analyses were performed on the sequence data (MrBayes v. 3.1; Ronquist & Huelsenbeck, 2003) by running four simultaneous chains (three heated, one cold) for ten million generations, sampling every 1000 generations. The model selected was the general time

reversible (GTR) using empirical base frequencies, and estimating the shape of the gamma distribution and proportion of invariant sites from the data. A resulting 50% majority-rule consensus tree (after discarding the burn-in of 25% of the generations) was determined to calculate the posterior probabilities for each node. The split differential between the two runs was below 0.01 after the completion of the run. Evaluation of the tree topology indicated that strain FRC-32^T formed a monophyletic clade with *G. uraniireducens* Rf4^T and the isolate *Geobacter* sp. TMJ1, and clustered with *Geobacter bemidjensis* Bem^T, '*Geobacter humireducens*' JW3 and *Geobacter* sp. M21, consistent with the subsurface *Geobacter* clade 1 defined by Holmes *et al.* (2007) (Fig. 1). The highest 16S rRNA gene sequence similarity was observed with *G. uraniireducens* Rf4^T (98.1% similarity), whereas similarities in the range 92–96% were observed for the other recognized species of the genus *Geobacter*.

The level of 16S rRNA gene sequence similarity between *G. uraniireducens* Rf4^T and strain FRC-32^T (98.1%) was above the threshold generally employed for the delineation of novel species (i.e. 97% similarity or below; Clarridge, 2004; Stackebrandt & Goebel, 1994; Vandamme *et al.*, 1996). However, such thresholds are by no means definitive and we note that the previously described, physiologically distinct species *Geobacter metallireducens* GS-15^T, *Geobacter grbiciae* TACP-2^T and *Geobacter hydrogenophilus* H-2^T all share greater than 99% sequence similarity. Thus, 16S rRNA gene sequence similarity thresholds have limited use as phylogenetic markers for the delineation of *Geobacter* species (Coates *et al.*, 2001). To demonstrate that *G. uraniireducens* Rf4^T and strain FRC-32^T represent distinct species, we performed a series of additional genetic analyses, including DNA–DNA hybridization, *in silico* genome–genome comparison and phylogenetic analysis of the β -subunit of the DNA-direct RNA polymerase gene (*rpoB*).

For DNA–DNA hybridization analysis, genomic DNA was isolated from 3 g *G. uraniireducens* Rf4^T and strain FRC-32^T cells using a French pressure cell (Thermo Spectronic) and was purified by using chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was performed by the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) as described by De Ley *et al.* (1970) under consideration of the modifications described by Huß *et al.* (1983), using a model Cary 100 Bio UV-VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with *in situ* temperature probe (Varian). The DNA–DNA relatedness of strain FRC-32^T with *G. uraniireducens* Rf4^T was determined to be 21%. The recommended DNA–DNA hybridization threshold for delineating bacterial species is 70%, and thus strain FRC-32^T is considered to represent a novel species according to the criterion of the Ad hoc Committee for the re-evaluation of the species definition in bacteriology (Wayne *et al.*, 1987).

In addition to DNA–DNA hybridization analysis, the complete genome sequences of strain FRC-32^T and *G. uraniireducens* Rf4^T were compared directly according to the methodology described by Goris *et al.* (2007). The genome of strain FRC-32^T was cut *in silico* into 1000 base fragments, and these fragments were compared by using BLAST analysis with the intact genome of *G. uraniireducens* Rf4^T. This analysis generated two values: (i) average nucleotide identity (ANI; mean identity of regions of similarity identified by BLAST searches); and (ii) conserved DNA percentage (per cent of strain FRC-32^T genome that shares >90 % nucleotide identity to *G. uraniireducens* Rf4^T). This *in silico* comparison revealed highly dissimilar genomes with an ANI value of 73.84 % and a conserved DNA percentage of 0.27 %. According to the regression analysis conducted by Goris *et al.* (2007) these genome similarities correlated with DNA–DNA hybridization values of below 20 %.

Analysis of *rpoB* gene sequences, coding for the β -subunit of the DNA-direct RNA polymerase, has been suggested as a supplementary tool for determining novel species designation (Adékambi *et al.*, 2008). Complete gene sequences of *rpoB* were acquired from the genome sequences of eight dissimilatory Fe(III)-reducing taxa, six different *Geobacter* species and two outgroup *Shewanella* species (USA Department of Energy Joint Genome Institute; <http://www.jgi.doe.gov/>). These gene sequences were aligned using the online software package CLUSTALW 2 (Thompson *et al.*, 1994; www.ebi.ac.uk/clustalw/). Phylogenetic and molecular evolutionary analyses were conducted on the aligned sequence data using MEGA version 4 (Tamura *et al.*, 2007). A bootstrapped (1000 iterations)

neighbour-joining tree was generated using 4022 informational DNA sequence positions. Complete deletion of gaps and missing data were implemented, along with a maximum composite likelihood nucleotide substitution model. The *rpoB* gene of strain FRC-32^T was most similar to that of *G. uraniireducens* strain Rf4^T, with a similarity of 81.1 % across the entire gene (Supplementary Fig. S1, available in IJSEM Online). This level of similarity was substantially below the 97.7 % threshold suggested by Adékambi *et al.* (2008) and was well below the level of similarity between *G. metallireducens* GS-15^T, *G. grbicium* TACP-2^T and *G. hydrogenophilus* H-2^T (95 % similarity, across approximately 500 bp of sequence information; Holmes *et al.*, 2004). These data are consistent with the conclusion that strain FRC-32^T represents a novel species of genus *Geobacter*.

Phenotypic characteristics were determined according to previously described methods under comparable laboratory conditions (Lovley *et al.*, 1993; Coates *et al.*, 2001; Straub & Buchholz-Cleven, 2001; Shelobolina *et al.*, 2007, 2008). Out of 13 recognized species of the genus *Geobacter* at the time of writing, five species were selected for phenotypic comparison. These species showed highest similarity with strain FRC-32^T of the 13 *Geobacter* species based on 16S rRNA gene sequence similarity, and also clustered together to form the subsurface clade of the genus *Geobacter*. Unless otherwise stated, an anaerobic bicarbonate-buffered freshwater medium sparged with a N₂/CO₂ gas mixture (80:20, v/v) and pre-reduced with 2.0 mM cysteine hydrochloride was used for all growth and physiological tests. In order to test the optimum temperature for growth of strain FRC-32^T, the bacterium was inoculated in freshwater medium with 10 mM acetate and

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of members of the *Geobacteraceae* in the *Deltaproteobacteria*, including strain FRC-32^T isolated from the subsurface of the ORFRC (indicated by bold type). Nodes supported by bootstrap values greater than 70 % are indicated by numeric values. Nodes supported by Bayesian analysis with posterior probability values greater than 70 % (open circles) or 95 % (filled circles) are indicated. Bar, 0.01 substitutions per nucleotide position.

10 mM fumarate as electron donor and acceptor respectively, and incubated in a series of temperatures (10, 20, 30, 35 and 40 °C). Growth at each temperature was monitored by using optical density (OD) measurements at a wavelength of 600 nm. Growth in a range of pH values was performed using the same medium, but with acetate and fumarate, and supplemented with organic buffers (homopipes, pH 4–5; MES, pH 5.5–6.5; and HEPES, pH 6.8–8.2). Likewise, growth with NaCl was examined by inoculating strain FRC-32^T into tubes with freshwater medium with acetate and fumarate, and a series of NaCl concentrations of 0–1.0 % at intervals of 0.1 %. All growth experiments were conducted in triplicate and the tubes were inoculated and incubated at optimum temperature, and growth was monitored by using OD at 600 nm.

For the morphological analysis, strain FRC-32^T was grown as described above and fixed for scanning electron microscopy (SEM) using 2 % glutaraldehyde. Fixed samples were filtered through a 0.45 µm filter, dehydrated through an ethanol series (25, 50, 75, 2 × 100 %, v/v) and then critically point dried using an Autosamdri-815 critical point drier (Tousimis). Dried samples were mounted on aluminium stubs, platinum coated and imaged at 5 kv on a Leo-1540XB Field Emission Gun-SEM. SEM revealed that strain FRC-32^T is a curved rod-shaped bacterium, morphologically similar to other members of the genus *Geobacter* (Supplementary Fig. S2, in IJSEM Online).

A comparison of phenotypic characteristics between strain FRC-32^T and closely affiliated *Geobacter* species is given in Table 1. Strain FRC-32^T conserved energy for growth by coupling the respiration of Fe(III)-oxyhydroxide to the oxidation of acetate, as indicated by the production of Fe²⁺ concurrent with cell growth (Supplementary Fig. S3, in IJSEM Online). Respiration of solid phase iron is a characteristic feature of members of the *Geobacteraceae*. In contrast to its closest relative (*G. uraniireducens* Rf4^T), strain FRC-32^T grew with ferric citrate and elemental sulfur as electron acceptors. Similarly, strain FRC-32^T could use formate, butyrate and butanol as electron donors whereas *G. uraniireducens* Rf4^T does not (Table 1). To test the uranium-reducing activity of strain FRC-32^T, a resting cell suspension study was performed according to previous methods (Lovley *et al.*, 1991; Gorby & Lovley, 1992; Shelobolina *et al.*, 2007). The U(VI)-reducing activity of strain FRC-32^T was shown to be comparable to that of *Geobacter sulfurreducens* PCA^T and *G. uraniireducens* Rf4^T (Shelobolina *et al.*, 2007, 2008). Little to no uranium reduction was observed in uninoculated controls or in control cultures to which fumarate was added as a competing electron acceptor.

The complete genome of strain FRC-32^T was 4 304 501 basepairs in length and is approximately 0.8 Mb shorter than that of *G. uraniireducens* strain Rf4^T. Preliminary screening of the genome sequence revealed that strain FRC-32^T contained the genes for aromatic hydrocarbon degradation. To our knowledge, *G. metallireducens* GS-

Table 1. Physiological characteristics of *Geobacter daltonii* strain FRC-32^T and closely related species of the genus *Geobacter*

All tests were performed under comparable conditions. Strains: 1, strain FRC-32^T (*Geobacter daltonii* sp. nov.; data from this study); 2, *G. uraniireducens* Rf4^T (Shelobolina *et al.*, 2008); 3, '*G. humireducens*' JW3 (Coates *et al.*, 2001); 4, *G. bremensis* Dfr1^T (Straub *et al.*, 1998; Straub & Buchholz-Cleven, 2001); 5, *G. bemidjiensis* Bem^T (Nevin *et al.*, 2005); 6, *G. metallireducens* GS-15^T (Lovley *et al.*, 1993). +, Positive; –, negative; ND, no data.

Characteristic	1	2	3	4	5	6
DNA G + C content (mol%)	53	54	ND	60	60.9	56.6
Optimal temperature (°C)	30	32	30	30–32	30	30–35
Electron acceptor usage						
Ferric citrate	+	–	+	ND	+	+
Elemental sulfur	+	–	+	+	–	–
Nitrate	–	–	+	–	–	+
Malate	+	+	–	+	+	ND
Fumarate	+	+	+	+	+	–
Electron donor usage						
Lactate	–	+	+	+	+	–
H ₂	–	–	+	+	+	–
Formate	+	–	+	+	–	–
Propionate	–	–	–	+	+	+
Butyrate	+	–	–	+	+	+
Valerate	ND	–	ND	–	+	+
Succinate	–	–	–	+	+	ND
Butanol	+	–	ND	+	+	+

15^T is the only cultivated *Geobacter* strain that has been demonstrated to utilize aromatic compounds (benzoate, toluene) as an electron donor (Lovley, 2006). Thus, we initiated preliminary physiological testing of strain FRC-32^T on monoaromatic hydrocarbon compounds. Active cultures of strain FRC-32^T grown on acetate were transferred into a medium containing 5 mM benzoate or 1 mM toluene as the electron donor and 10 mM fumarate as the electron acceptor. After prolonged incubation only cultures showing clear turbidity were further transferred into the medium containing different substrates over five passages. With fumarate as the electron acceptor, strain FRC-32^T grew in successive transfers with both benzoate and toluene as the electron donor. Monoaromatic hydrocarbons are among the most common water-soluble groundwater contaminants of subsurface aquifers used for drinking water resources in the USA and in other industrialized countries (Rooney-Varga *et al.*, 1999; Lovley & Anderson, 2000). Strain FRC-32^T is the first organism from the subsurface clade of genus *Geobacter* (Holmes *et al.*, 2007) that has been shown to be capable of growth on aromatic hydrocarbons and provides an ideal model organism for further study of this important process.

The DNA G + C content of strain FRC-32^T was 53 mol%, determined by using genome sequence analysis. Strain

FRC-32^T showed optimal growth at 30 °C, with no growth below 10 °C or above 40 °C. Strain FRC-32^T grew at pH 6–8, with optimum growth at pH 6.7–7.3. NaCl was observed to be inhibitory for growth, although strain FRC-32^T tolerated up to 0.7 % (w/v) NaCl.

The physiological capabilities of strain FRC-32^T, particularly the capacity to utilize a distinct range of electron acceptors and donors, were the most significant characteristics that differentiate the novel strain from the most closely related members of the genus *Geobacter* (Table 1). In addition, complete genome–genome comparison of strain FRC-32^T and *G. uraniireducens* strain Rf4^T was definitive in differentiating the two organisms. In addition to a significant divergence in total genome size, the organisms shared few genes other than the rRNA genes that have greater than 90 % sequence similarity. Similarly, DNA regions shared by the two organisms had a low mean nucleotide similarity consistent with the DNA–DNA hybridization value of 21 %. The phylogenetic reconstruction of the *rpoB* gene of strain FRC-32^T with closely related, recognized species was consistent with the isolation of a novel species of *Geobacter*. These data suggest that DNA–DNA hybridization data are redundant for species delineation when molecular data other than rRNA gene sequences indicate low genome similarity. With the drastically reduced effort now required for genome sequencing, it is clear that genome sequence data will be more readily accessible than DNA–DNA hybridization comparisons.

In combination, the genetic analyses revealed the robustness of direct sequence-based comparison for the characterization of novel species. Furthermore, although the genetic analyses demonstrate that strain FRC-32^T represents a novel species and, on a genome-scale significantly divergent from the closest recognized species, phenotypic analyses indicate that strain FRC-32^T shares many common features with other members of the genus *Geobacter*. Thus, phenotypic as well as genotypic results of the current study indicate that strain FRC-32^T can be sufficiently delineated from its closest relative *G. uraniireducens* Rf4^T and thus a novel species of genus *Geobacter* is proposed with the name *Geobacter daltonii* sp. nov.

Description of *Geobacter daltonii* sp. nov.

Geobacter daltonii (dal.to'ni.i. N.L. masc. gen. n. *daltonii* of Dalton, named after Dava Dalton who performed the initial isolation of the strain and passed away shortly thereafter).

Cells are Gram-negative, non-spore-forming, slightly curved rods, 1.0–1.5 µm long and 0.3–0.5 µm in diameter, and form pink colonies when growing on acetate and fumarate. Growth with Fe(III)-oxyhydroxide as electron acceptor is observed with acetate as electron donor and carbon substrate. Has 21 % DNA–DNA relatedness with *G. uraniireducens* Rf4^T. Growth occurs at 20–35 °C, with optimal growth at 30 °C. Growth occurs at pH 6.0–8.0,

with optimum growth at pH 6.7–7.3. Optimum growth occurs in the absence of NaCl, but can tolerate up to 0.7 % NaCl. Ferric citrate, elemental sulfur, malate and fumarate are used as electron acceptors; nitrate is not reduced. Formate, butanol and butyrate, benzoate and toluene are utilized as electron donors with fumarate as an electron acceptor; lactate, hydrogen, propionate and succinate are not used as electron donors. The DNA G + C content of the type strain is 53 mol%.

The type strain, FRC-32^T (=DSM 22248^T=JCM 15807^T), was isolated from a shallow subsurface of the ORFRC, Oak Ridge, Tennessee, where the sediments are exposed to mixed waste contamination including high levels of uranium(VI) and aromatic hydrocarbons.

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