Prolixibacter bellariivorans gen. nov., sp. nov., a sugar-fermenting, psychrotolerant anaerobe of the phylum *Bacteroidetes*, isolated from a marine-sediment fuel cell

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A Gram-negative, non-motile, filamentous, rod-shaped, non-spore-forming bacterium (strain F2^T) was isolated from the surface of an electricity-harvesting electrode incubated in marine sediments. Strain F2^T does not contain *c*-type cytochromes, flexirubin or carotenoids. It is a facultative anaerobe that can ferment sugars by using a mixed acid fermentation pathway and it can grow over a wide range of temperatures (4–42 °C). The DNA G + C (44.9 mol%) content and chemotaxonomic characteristics (major fatty acids, a-15:0 and 15:0) were consistent with those of species within the phylum *Bacteroidetes*. Phylogenetic analysis of the 16S rRNA nucleotide and elongation factor G amino acid sequences indicated that strain F2^T represents a unique phylogenetic cluster within the phylum *Bacteroidetes*. On the basis of 16S rRNA gene sequence phylogeny, the closest relative available in pure culture, *Alkaliflexus imshenetskii*, is only 87.5 % similar to strain F2^T. Results from physiological, biochemical and phylogenetic analyses showed that strain F2^T should be classified as a novel genus and species within the phylum *Bacteroidetes*, for which the name *Prolixibacter bellariivorans* gen. nov., sp. nov. is proposed. The type strain is F2^T (= ATCC BAA-1284^T = JCM 13498^T).

Electricity can be produced by sediment fuel cells placed in anoxic sediments: these cells contain natural populations of micro-organisms that transfer electrons to current-harvesting electrodes (Bond *et al.*, 2002; Holmes *et al.*, 2004b; Reimers *et al.*, 2001; Tender *et al.*, 2002). These electrons then flow to the cathode in the overlying aerobic water, where they react with oxygen. Several organisms that are able to oxidize organic acids, such as acetate, to CO₂ and utilize an electrode as the sole electron acceptor have already been isolated (Bond *et al.*, 2002; Bond & Lovley, 2003, 2005; Chaudhuri & Lovley, 2003; Holmes *et al.*, 2004d). However, there has been very little emphasis on the role that fermentative organisms might play within the microbial community of the sediment fuel cell.

Molecular analyses of marine-sediment fuel cells have led to the suggestion that fermentative organisms account for approximately 10 % of the microbial community present on the surfaces of current-harvesting anodes (Bond *et al.*, 2002; Holmes *et al.*, 2004b; Tender *et al.*, 2002). In addition, micro-organisms with 16S rRNA gene sequences most similar to those of fermentative micro-organisms have been detected on anodes from microbial fuel cells powered by other fuel sources, such as organic wastewater (Kim *et al.*, 2004), activated sludge (Lee *et al.*, 2003) and oligotrophic river water (Phung *et al.*, 2004). In fact, when wastewater from a corn-processing plant was provided as the electron donor in a microbial fuel cell powered by activated sludge, up to 19 % of the 16S rRNA gene sequences detected were most similar to known fermentative micro-organisms from the phylum *Bacteroidetes* (Kim *et al.*, 2004).

Although fermentative micro-organisms are important members of the electrode community, they are unlikely to be directly involved in current production by the microbial fuel cell. Several micro-organisms with fermentative types of metabolism have been able to grow and produce current in microbial fuel cells; however, electron transfer to the electrode performed by these organisms was very inefficient

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Abbreviation: PFLA, phospholipid fatty acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *fusA* gene sequences of strain F2^T are AY918928 and AY918929, respectively.

The phospholipid fatty acid content (%) of strain F2^T and a 16S rRNA gene sequence similarity matrix for strain F2^T and other *Bacteroidetes* species are available in supplementary tables in IJSEM Online.

(Park *et al.*, 2001; Pham *et al.*, 2003). For example, when *Clostridium butyricum* was grown in an anodic chamber with glucose as the electron donor, only 0.04 % of the electrons available in glucose were transferred to the electrode surface (Park *et al.*, 2001) and less than 0.02 % of the electrons available from yeast extract (calculated from the chemical oxygen demand, 250 mg l⁻¹) were transferred to an electrode by *Aeromonas hydrophila* (Pham *et al.*, 2003). In addition, only ~25 % of the electrons available from the incomplete oxidation of pyruvate, lactate and propionate were transferred to the electrode surface by *Desulfobulbus propionicus*, via a mixed fermentative metabolism (Holmes *et al.*, 2004a).

Dissimilatory metal-reducing micro-organisms, on the other hand, are able to transfer 80–95 % of the electrons available from the complete oxidation of an electron donor to a current-harvesting electrode (Bond & Lovley, 2003; Chaudhuri & Lovley, 2003; Holmes *et al.*, 2004d). On the basis of these findings, it is likely that fermentative micro-organisms that can convert complex organic matter in anaerobic sediments to fermentation products such as acetate are involved in the initial steps of power production by the microbial fuel cell. These side-products are then completely oxidized by dissimilatory metal-reducing micro-organisms that are able to transfer electrons directly to the current-harvesting anode and produce a current.

Information concerning the role of fermentative micro-organisms on electrodes will help in the optimization of microbial fuel cells capable of harvesting electricity from waste biomass produced by agricultural, municipal and industrial sources in which energy is primarily stored in the form of carbohydrates. Here we report the characterization of a novel strain of bacteria, strain F2^T, isolated from the surface of an electricity-harvesting electrode incubated in marine sediments, that is likely to be a member of the fermentative niche within the microbial community on such electrodes.

Strain F2^T was isolated from a marine-sediment fuel cell that was constructed in the laboratory with sediments collected from Boston Harbour (MA, USA) near the World's End peninsula, at a water depth of 5 m, as described previously (Bond *et al.*, 2002; Holmes *et al.*, 2004b). After incubation and energy harvesting at 15 °C for 6 months, the anode was pulled from the sediment, washed with sterile anaerobic marine medium lacking electron donors or acceptors and containing Na₂S (1.0 mM) as a reducing agent. The anode surface was then scraped with a sterile razor blade into a slightly modified version of APW medium (Coates *et al.*, 1995), referred to as A1 medium (Holmes *et al.*, 2004d). Serial dilutions in A1 medium containing poorly crystalline Fe(III) oxide (100 mM) provided as the electron acceptor and benzoate (0.5 mM) as the electron donor yielded an enrichment culture comprising three organisms: a *Geopsychrobacter* species, a *Fusibacter* species and strain F2^T. In order to separate this tri-culture and isolate strain F2^T, eight 10-fold serial dilutions, with glucose (5 mM) provided as

the fermentation substrate, were performed at 22 °C. The highest dilutions that were able to grow were diluted to extinction three more times and were then transferred to solidified medium (2 % agar) with glucose (5 mM). Isolated colonies were then selected and grown in A1 medium supplemented with glucose (5 mM).

For morphological and phenotypic characterization, strain F2^T was cultivated under anoxic conditions (N₂/CO₂; 80:20, v/v) in A1 medium with galactose (6 mM) as the fermentation substrate. Growth on an electrode was tested as previously described (Bond *et al.*, 2002; Bond & Lovley, 2003) and utilization of the various electron donors and acceptors was only considered positive after four transfers (5 % inoculum). The end-products of glucose fermentation were analysed with a high-pressure liquid chromatograph (series 1100; Hewlett Packard) on a Fast Acid Analysis column (Bio-Rad) with an eluant of 5 mM H₂SO₄ and absorbance detection at 210 nm. Strain F2^T exhibited a mixed acid fermentative metabolism when grown on glucose; the end products included acetate, succinate and propionate.

Temperature, pH and salinity ranges were determined as described previously (Nevin *et al.*, 2005). For salinity characterization tests, media contained NaCl without any divalent salts. There was no requirement for seawater. The API 20NE test (bioMérieux) was used according to the manufacturer's directions, with cells grown anaerobically with galactose (5–6 mM) as the fermentation substrate. Strain F2^T was positive for hydrolysis of aesculin and gelatin, indole production, β -galactosidase activity and arabinose assimilation and was negative for reduction of nitrate and nitrite, glucose fermentation, arginine dihydrolase, urease and the assimilation of glucose, mannose, mannitol, *N*-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Growth on urease agar, MacConkey agar and DNase agar, acid production from sucrose and glucose, indole production, capnophilic metabolism and gliding motility were assessed using standard tests. Strain F2^T was found to be negative for growth on urease, DNase agar and MacConkey agar, and negative for acid production from sucrose and glucose, capnophilic metabolism and gliding motility. The strain was sensitive to penicillin. Pigment production was determined by using two methods: for flexirubin, the KOH method (Reichenbach *et al.*, 1974) was used; for carotenoids, an acetone extraction method was used to produce an absorption spectrum (Denger *et al.*, 2002). For cytochrome determinations, whole-cell suspensions were analysed using dithionite-reduced and air-oxidized spectra (350–700 nm) by using a UV2401-PC dual-beam spectrophotometer (Shimadzu). No peaks were observed in the dithionite-reduced minus air-oxidized difference spectrum of strain F2^T, a result that is consistent with the absence of *c*-type cytochromes. The results from these tests are shown, in comparison with those for genera within the phylum *Bacteroidetes*, in Table 1. The physiology

Table 1. Phenotypic characteristics of strain F2^T and members of the phylum *Bacteroidetes*

Taxa: 1, Strain F2^T; 2, *Alkaliflexus imshenetskii* DSM 15055^T; 3, *Anaerophaga thermohalophila* DSM 12881^T; 4, *Marinilabilia salmonicolor* DSM 6480^T; 5, *Cytophaga fermentans* DSM 9555^T; 6, *Pedobacter cryoconitis* DSM 14825^T. All of the taxa shown utilized cellobiose (2.5 mM), glucose (5 mM), mannose (2 mM), starch (1 g l⁻¹) and xylose (2 mM). NT, Not tested.

Characteristic	1	2	3	4	5	6
Origin	Marine-sediment fuel cell	Soda lake	Oilfield	Marine	Marine	Glacier cryoconite
Cell size (µm)	0.33 × 10.5–12.5	0.25–0.4 × 4–10	0.3 × 4–8	0.3–0.5 × 2–6	0.5–0.7 × 2–10	0.7–0.9 × 1.5–3.0
Motility	Not observed	Gliding	Not observed	Gliding	Gliding	Gliding
Colour	White	Pink	Orange–red	Yellow to salmon pink	Bright yellow	Light yellow
DNA G+C content (mol%)	44.9	44.3	41.8	37	39	43.4
Pigment	None detected	Carotenoid	Carotenoid + flexirubin	NT	NT	NT
O ₂ metabolism	Facultatively anaerobic	Anaerobic; low O ₂ tolerance	Strictly anaerobic	Facultatively anaerobic	Facultatively anaerobic	Aerobic
pH for growth:						
Optimum	7.0	8.5	6.8	7.0–7.5	NT	6–7
Range	5.0–9.0	7.2–10.2	NT	NT	NT	5–8
Temperature for growth (°C):						
Optimum	22	35	55	28–37	30	20
Range	4–42	10–45	37–55	NT	NT	1–25
NaCl for growth (g l ⁻¹):						
Optimum	20	20	20–60	NT	NT	NT
Range	5–80	0.8–53	20–120	10–30	< 30	NT
Catalase	–	+	–	+	+	+
Substrates:						
Arabinose (10 mM)	–	+	+	+	+	–
Agar (1 g l ⁻¹)	–	–	NT	–	+	+
Cellulose (1 g l ⁻¹)	–	–	–	NT	–	NT
Fructose (2 mM)	–	–	+	+	+	+
Galactose (2, 6 and 10 mM)	+	+	+	+	–	+
Lactose (10 mM)	+	–	+	+	+	+
Maltose (5 mM)	+	+	NT	+	+	+
Trehalose (2 mM)	+	+	+	+	–	+
References	This study	Zhilina <i>et al.</i> (2004)	Denger & Schink (1995); Denger <i>et al.</i> (2002)	Reichenbach (1992); Suzuki <i>et al.</i> (1999)	Reichenbach (1992)	Margesin <i>et al.</i> (2003)

Fig. 1. Scanning electron micrograph of cells of strain F2^T grown on medium with glucose (5 mM) as the fermentative substrate. Bar, 1 µm.

of strain F2^T was most similar to that of *Alkaliflexus imshenetskii*.

Cell morphology was observed under a Nikon Eclipse E600 microscope. Electron microscopic observations were performed in the microscopy facility at the University of Massachusetts, Amherst (Fig. 1).

The phospholipid fatty acid (PLFA) composition (Pinkart *et al.*, 2002), the double-bond geometry and the positions of monounsaturated PLFAs were determined by using previously described techniques (Dunkelblum *et al.*, 1985). Seventeen major PLFAs and 15 trace PLFAs were observed in strain F2^T (see Supplementary Table S1 available in IJSEM Online). The major PLFAs were as follows: a-15:0 (23.29 %), 15:0 (20.73 %), i-15:0 (13.65 %), 17:0 (8.78 %), 16:0 (5.63 %), i-16:0 (4.51 %), 17:1 ω 8 (3.40 %), i-17:1 ω 8a (3.16 %), a-17:0 (2.83 %), i-14:0 (2.45 %), i-16:1 ω 7 (2.40 %), a-17:1 ω 8 (1.80 %), i-17:0 (1.49 %), a-15:1 ω 8 (1.03 %), 15:1 ω 6 (0.78 %), i-17:1 ω 8b (0.75 %) and 16:1 ω 7c (0.72 %). PFLA analysis showed that 35.1 % of the fatty acids in strain F2^T were normal saturated, 48.2 % were terminally branched saturated, 9.14 % were branched monounsaturated and 4.90 % were monounsaturated. The fatty acid composition of strain F2^T was similar to those of other species within the phylum *Bacteroidetes* (*Alkaliflexus imshenetskii*, *Marinilabilia salmonicolor* and *Anaerophaga thermohalophila*) (Table 2).

Analysis of the DNA G + C content (Cashion *et al.*, 1997) was performed by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). Genomic DNA for the 16S rRNA gene and *fusA*, the gene that encodes elongation factor G, sequence analysis was extracted using the FastDNA spin kit (BIO 101) according to the manufacturer's instructions. The 16S rRNA gene sequence for strain F2^T was amplified with primers 8 forward (Lane *et al.*, 1985; Lane, 1991) and 1492 reverse (Achenbach & Woese, 1995; Amann *et al.*, 1990) as

Table 2. Fatty acid contents (%) of strain F2^T and three related *Bacteroidetes* species

Taxa: 1, strain F2^T; 2, *Alkaliflexus imshenetskii* DSM 15055^T; 3, *M. salmonicolor* DSM 6480^T; 4, *Anaerophaga thermohalophila* DSM 12881^T. Data for taxa 2–4 are from Zhilina *et al.*, 2004.

Fatty acid	1	2	3	4
Straight chain				
13:0	0.09	1.1	–	–
14:0	0.20	–	–	1.8
15:0	20.73	39.0	19.5	4.7
16:0	5.63	1.0	12.2	9.5
17:0	8.78	–	3.4	–
18:0	0.06	–	4.6	3.3
15:1	0.78	4.1	–	–
16:1	0.98	–	–	–
17:1	3.40	–	–	–
18:1	–	–	5.4	1.9
18:2	–	–	4.6	–
Branched chain				
i-13:0	0.12	1.4	–	–
i-14:0	2.45	1.1	–	1.9
i-15:0	13.65	29.4	11.3	30.2
i-16:0	4.51	1.0	2.1	2.9
i-17:0	1.49	–	–	–
i-15:1	0.41	–	–	–
i-16:1	2.64	–	–	–
i-17:1	3.91	–	–	–
a-13:0	0.03	–	–	–
a-15:0	23.29	11.6	31.7	21.2
a-17:0	2.83	–	2.0	–
a-15:1	1.03	–	–	–
a-17:1	1.80	3.5	–	–
br-14:1	0.02	–	–	–
br-18:1	0.35	–	–	–
17:0cy	–	4.0	–	–
3-Hydroxyl				
3-OH-15:0	0.45	2.4	–	5.1
3-OH-16:0	0.32	–	–	3.4
3-OH-i-16:0	–	–	–	2.3
3-OH-i-17:0	–	–	3.3	7.8

described previously (Holmes *et al.*, 2004c). A gene fragment from the *fusA* gene in strain F2^T was amplified with primers fusAf and fusAr (Berchet *et al.*, 2000) as described previously (Holmes *et al.*, 2004c). The 16S rRNA and *fusA* gene fragments were compared with the GenBank nucleotide and protein databases using the BLASTN and BLASTX algorithms (Altschul *et al.*, 1990). Nucleotide and amino acid sequences were manually aligned and hypervariable regions were masked in the Genetic Computer Group (GCG) sequence editor (Wisconsin Package, version 10). Aligned sequences were imported into PAUP 4.0b 4a (Swofford, 1998) in which the phylogenetic trees were inferred. The branching order was determined and compared with those obtained using

maximum-parsimony, maximum-likelihood and distance-based algorithms. Similarity matrices were generated using ALIGN (Pearson, 1990).

Phylogenetic analysis of 16S rRNA and *fusA* gene sequences indicated that strain F2^T clusters within the phylum *Bacteroidetes* in the *Bacteria* (Fig. 2). Strain F2^T is unique in that the most similar 16S rRNA gene sequence from an organism that is available in pure culture (*Alkaliflexus imshenetskii*) has a similarity of only 87.5 % (see Supplementary Table S2 available in IJSEM Online). The amino acid sequence of elongation factor G from strain F2^T was also only 71 % similar to that of another species within the *Bacteroidetes*, *Porphyromonas gingivalis*.

Although organisms phylogenetically related to strain F2^T are not available in pure culture, the 16S rRNA gene sequence from an uncultured organism detected in 2-bromophenol-dehalogenating and phenol-degrading consortia (Knight *et al.*, 1999) was 97.2 % similar to that of strain F2^T. These results are interesting, as sediments previously collected from Boston Harbour were contaminated with polycyclic aromatic hydrocarbons (Hayes & Lovley, 2002; Hayes *et al.*, 1999; McGroddy & Farrington, 1995; McGroddy *et al.*, 1996; Rothermich *et al.*, 2002; Rudnick & Chen, 1998; Wang *et al.*, 2001). Other uncultured 16S rRNA gene sequences closely related to that of strain F2^T have been detected in permanently cold marine environments, such as sediments from the Arctic Ocean (Ravenschlag *et al.*, 1999), the Japan Trench land slope (Li *et al.*, 1999) and Antarctic

sea ice (Brown & Bowman, 2001). Again, this is not very surprising, as strain F2^T is capable of growth at temperatures as low as 4 °C. On the basis of physiological, biochemical and phylogenetic data, strain F2^T represents a novel genus and species within the phylum *Bacteroidetes*, for which the name *Prolixibacter bellariivorans* gen. nov., sp. nov. is proposed.

Description of *Prolixibacter* gen. nov.

Prolixibacter [Pro.lix'i.bac.ter. L. adj. *prolixus* -a -um long, extended; N.L. masc. n. *bacter* (from Gr. n. *bakterion*) a rod; N.L. masc. n. *Prolixibacter* a long rod].

Comprises long, filamentous, curved rods that occur as single, non-motile cells. Cells do not have pili or flagella and do not form spores. Cell-wall structure is typical of a Gram-negative bacterium. Cells do not contain *c*-type cytochromes and are oxidase- and catalase-negative. Organisms in this genus can grow at temperatures as low as 4 °C and consist of facultative anaerobes that can ferment sugars by a mixed acid fermentation pathway. The genus *Prolixibacter* is in the phylum *Bacteroidetes* in the *Bacteria*. The type species is *Prolixibacter bellariivorans*.

Description of *Prolixibacter bellariivorans* sp. nov.

Prolixibacter bellariivorans [bell.ar.ii'vorans. L. pl. n. *bellaria* sweets, dessert; L. part. adj. *vorans* (from L. v. *vorare*)

Fig. 2. Phylogenetic trees comparing (a) 16S rRNA gene sequences and (b) elongation factor G amino acid sequences from various species. The phylogenetic tree comparing 16S rRNA gene sequences was constructed by using Jukes–Cantor distance-based analysis with *Pedobacter cryoconitis*, *Arcicella aquatica* and *Aquiflexum balticum* as the outgroups. The phylogenetic tree comparing the elongation factor G amino acid sequences was constructed by using maximum-parsimony analysis with *Thermotoga maritima* as the outgroup. CFB, *Cytophaga*–*Flavobacterium*–*Bacteroides*. Bars, (a) 0.05 nucleotide substitutions per site, (b) 0.05 amino acid changes.

devouring; N.L. part. adj. *bellariivorans* sweet-devouring, consuming sweet things].

Exhibits the following properties in addition to those given in the genus description. Cells are approximately 10.5–12.5 µm in length and 0.33 µm in diameter. Growth is observed when atmospheric oxygen is provided as the electron acceptor, with galactose as the electron donor. Cannot utilize nitrate, Fe(III)-pyrophosphate, Fe(III)-citrate, Fe(III)-oxide, Fe(III)-nitrilotriacetic acid, sulfate, thiosulfate or a graphite electrode poised at +200 mV as an electron acceptor. Under anaerobic conditions, can ferment arbutin (5 mM), cellobiose, aesculin (1 g l⁻¹), galactose (6 mM), lactose, maltose, mannose, melezitose (2.5 mM), melibiose (2.5 mM), methyl D-glucoside (5 mM), raffinose (2 mM), salicin (10 mM), starch (1 g l⁻¹), sucrose (5 mM), trehalose (2 mM), turanose (2.5 mM), xylose and yeast extract (0.5 g l⁻¹). Substrates tested but not utilized include acetate (10 mM), acetoin (1 g l⁻¹), adonitol (5 mM), alginate (1 g l⁻¹), arginine (5 mM), benzoate (2 mM), 2,3-butanediol (0.9 g l⁻¹), butyrate (10 mM), caproate (10 mM), carrageenan (1 g l⁻¹), Casamino acids (1 g l⁻¹), casein (10 mM), citrate (10 mM), ethanol (10 mM), ferulate (10 mM), formate (10 mM), fucose (2.5 mM), fumarate (10 mM), gallic acid (0.5 g l⁻¹), gelatin (1 g l⁻¹), glycerol (6 mM), β-hydroxybutyrate (1 and 4 mM), hydrogen (130 kPa) with 0.1 mM acetate provided as a carbon source for growth, inulin (1 g l⁻¹), isobutyrate (10 mM), lactate (10 mM), malate (10 mM), malonate (10 mM), mannitol (10 mM), methyl α-D-mannoside (5 mM), nicotine (10 mM), polyethylene glycol (1 g l⁻¹), proline (10 mM), propionate (10 mM), pyrogallol (0.5 g l⁻¹), pyruvate (10 mM), rhamnose (10 mM), ribose (10 mM), serine (10 mM), sorbitol (5 mM), sorbose (10 mM), succinate (10 mM), syringate (10 mM), tryptone (1 g l⁻¹), valerate (10 mM), nitrate (5 mM) plus Fe(III)-pyrophosphate (20 mM) [with acetate (10 mM), citrate (10 mM), galactose (2.5 mM) or lactate (10 mM)], atmospheric oxygen with [acetate (10 mM), citrate (10 mM) or lactate (10 mM)], and a graphite electrode with [acetate (10 mM) or galactose (6 mM)]. Growth occurs at temperatures between 4 and 42 °C, with an optimum at 22 °C. The optimal pH is 7.0.

The type strain, F2^T (=ATCC BAA-1284^T=JCM 13498^T), was isolated from the surface of an electricity-harvesting electrode incubated in marine sediments.

References

- Achenbach, L. & Woese, C. (1995). 16S and 23S rRNA-like primers. In *Archaea: a Laboratory Manual*, pp. 201–203. Edited by F. T. Robb, A. R. Place, K. R. Sowers, H. J. Schreier, S. DasSarma & E. M. Fleischmann. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- Amann, R. I., Binder, B. J., Olson, R. J., Chisholm, S. W., Devereux, R. & Stahl, D. A. (1990). Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Appl Environ Microbiol* **56**, 1919–1925.
- Berchet, V., Thomas, T., Cavicchioli, R., Russell, N. J. & Gounot, A. M. (2000). Structural analysis of the elongation factor G protein from the low-temperature-adapted bacterium *Arthrobacter globiformis* SI55. *Extremophiles* **4**, 123–130.
- Bond, D. R. & Lovley, D. R. (2003). Electricity production by *Geobacter sulfurreducens* attached to electrodes. *Appl Environ Microbiol* **69**, 1548–1555.
- Bond, D. R. & Lovley, D. R. (2005). Evidence for involvement of an electron shuttle in electricity generation by *Geothrix fermentans*. *Appl Environ Microbiol* **71**, 2186–2189.
- Bond, D. R., Holmes, D. E., Tender, L. M. & Lovley, D. R. (2002). Electrode-reducing microorganisms that harvest energy from marine sediments. *Science* **295**, 483–485.
- Brown, M. V. & Bowman, J. P. (2001). A molecular phylogenetic survey of sea-ice microbial communities (SIMCO). *FEMS Microbiol Ecol* **35**, 267–275.
- Cashion, P., Holder-Franklin, M., McCully, J. & Franklin, M. (1997). A rapid method for the base determination of bacterial DNA. *Anal Biochem* **81**, 461–466.
- Chaudhuri, S. K. & Lovley, D. R. (2003). Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. *Nat Biotechnol* **21**, 1229–1232.
- Coates, J. D., Lonergan, D. J., Philips, E. J., Jenter, H. & Lovley, D. R. (1995). *Desulfuromonas palmitatis* sp. nov., a marine dissimilatory Fe(III) reducer that can oxidize long-chain fatty acids. *Arch Microbiol* **164**, 406–413.
- Denger, K. & Schink, B. (1995). New halo- and thermotolerant fermenting bacteria producing surface-active compounds. *Appl Environ Microbiol* **52**, 173–178.
- Denger, K., Warthmann, R., Ludwig, W. & Schink, B. (2002). *Anaerophaga thermohalophila* gen. nov., sp. nov. a moderately thermophilic, strictly anaerobic fermentative bacterium. *Int J Syst Evol Microbiol* **52**, 173–178.
- Dunkelblum, E., Tan, S. H. & Silk, P. J. (1985). Double-bond location in monounsaturated fatty acids by dimethyl disulfide derivatization and mass spectrometry: application to analysis of fatty acids in pheromone glands of four *Lepidoptera*. *J Chem Ecol* **11**, 265–277.
- Hayes, L. A. & Lovley, D. R. (2002). Specific 16S rDNA sequences associated with naphthalene degradation under sulfate-reducing conditions in harbor sediments. *Microb Ecol* **43**, 134–145.
- Hayes, L. M., Nevin, K. P. & Lovley, D. R. (1999). Role of prior exposure on anaerobic degradation of naphthalene and phenanthrene in marine harbor sediments. *Org Geochem* **30**, 937–945.
- Holmes, D. E., Bond, D. R. & Lovley, D. R. (2004a). Electron transfer by *Desulfobulbus propionicus* to Fe(III) and graphite electrodes. *Appl Environ Microbiol* **70**, 1234–1237.
- Holmes, D. E., Bond, D. R., O'Neil, R. A., Reimers, C. E., Tender, L. R. & Lovley, D. R. (2004b). Microbial communities associated with electrodes harvesting electricity from a variety of aquatic sediments. *Microb Ecol* **48**, 178–190.
- Holmes, D. E., Nevin, K. P. & Lovley, D. R. (2004c). Comparison of 16S rRNA, *nifD*, *recA*, *rpoB* and *fusA* genes within the family *Geobacteraceae* fam. nov. *Int J Syst Evol Microbiol* **54**, 1591–1599.
- Holmes, D. E., Nicoll, J. S., Bond, D. R. & Lovley, D. R. (2004d). Potential role of a novel psychrotolerant member of the family *Geobacteraceae*, *Geopsychrobacter electrodiphilus* gen. nov., sp. nov., in electricity production by a marine sediment fuel cell. *Appl Environ Microbiol* **70**, 6023–6030.

- Kim, B. H., Park, H. S., Kim, H. J., Kim, G. T., Chang, I. S., Lee, J. & Phung, N. T. (2004). Enrichment of microbial community generating electricity using a fuel-cell-type electrochemical cell. *Appl Microbiol Biotechnol* **63**, 672–681.
- Knight, V. K., Kerkhof, L. J. & Häggblom, M. M. (1999). Community analyses of sulfidogenic 2-bromophenol-dehalogenating and phenol-degrading microbial consortia. *FEMS Microbiol Ecol* **29**, 137–147.
- Lane, D. L. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.
- Lane, D. L., Pace, B., Olsen, G. J., Stahl, D., Sogin, M. L. & Pace, N. R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analysis. *Proc Natl Acad Sci U S A* **82**, 6955–6959.
- Lee, J. Y., Phung, N. T., Chang, I. S., Kim, B. H. & Sung, H. C. (2003). Use of acetate for enrichment of electrochemically active microorganisms and their 16S rDNA analyses. *FEMS Microbiol Lett* **223**, 185–191.
- Li, L., Kato, C. & Horikoshi, K. (1999). Microbial diversity in sediments collected from the deepest cold-seep area, the Japan Trench. *Mar Biotechnol* **1**, 391–400.
- Margesin, R., Sproer, C., Schumann, P. & Schinner, F. (2003). *Pedobacter cryoconitis* sp. nov., a facultative psychrophile from alpine glacier cryoconite. *Int J Syst Evol Microbiol* **53**, 1291–1296.
- McGroddy, S. E. & Farrington, J. W. (1995). Sediment porewater partitioning of polycyclic aromatic hydrocarbons in three cores from Boston Harbor, Massachusetts. *Environ Sci Technol* **29**, 1542–1550.
- McGroddy, S. E., Farrington, J. W. & Gschwend, P. M. (1996). Comparison of the *in situ* and desorption sediment-water partitioning of polycyclic aromatic hydrocarbons and polychlorinated biphenyls. *Environ Sci Technol* **30**, 172–177.
- Nevin, K. P., Holmes, D. E., Woodard, T. L., Hinlein, E. S., Ostendorf, D. W. & Lovley, D. R. (2005). *Geobacter bemidjensis* sp. nov. and *Geobacter psychrophilus* sp. nov., two novel Fe(III)-reducing subsurface isolates. *Int J Syst Evol Microbiol* **55**, 1667–1674.
- Park, H. S., Kim, B. H., Kim, H. S., Kim, H. J., Kim, G. T., Kim, M., Chang, I. S., Park, Y. K. & Chang, H. I. (2001). A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to *Clostridium butyricum* isolated from a microbial fuel cell. *Anaerobe* **7**, 297–306.
- Pearson, W. R. (1990). Rapid and sensitive sequence comparisons with FASTP and FASTA. *Methods Enzymol* **183**, 63–98.
- Pham, C. A., Jung, S. J., Phung, N. T., Lee, J., Chang, I. S., Kim, B. H., Yi, H. & Chun, J. (2003). A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to *Aeromonas hydrophila*, isolated from a microbial fuel cell. *FEMS Microbiol Lett* **223**, 129–134.
- Phung, N. J., Lee, J., Kang, K. H., Chang, I. S., Gadd, G. M. & Kim, B. H. (2004). Analysis of microbial diversity in oligotrophic microbial fuel cells using 16S rDNA sequences. *FEMS Microbiol Lett* **233**, 77–82.
- Pinkart, H. C., Ringelberg, D. B., Piceno, Y. M., Macnaughton, S. J. & White, D. C. (2002). *Manual of Environmental Microbiology*, 2nd edn. Washington, DC: American Society for Microbiology.
- Ravenschlag, K., Sahm, K., Pernthaler, J. & Amann, R. (1999). High bacterial diversity in permanently cold marine sediments. *Appl Environ Microbiol* **65**, 3982–3989.
- Reichenbach, H. (1992). The Order *Cytophagales*. In *The Prokaryotes*, pp. 3631–3675. Edited by A. Balows, H. G. Truper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer.
- Reichenbach, H., Kleinig, H. & Achenbach, H. (1974). The pigments of *Flexibacter elegans*. Novel and chemosystematically useful compounds. *Arch Microbiol* **101**, 131–134.
- Reimers, C. E., Tender, L. M., Fertig, S. & Wang, W. (2001). Harvesting energy from the marine sediment-water interface. *Environ Sci Technol* **35**, 192–195.
- Rothermich, M. M., Hayes, L. A. & Lovley, D. R. (2002). Anaerobic, sulfate-dependent degradation of polycyclic aromatic hydrocarbons in petroleum-contaminated harbor sediment. *Environ Sci Technol* **36**, 4811–4817.
- Rudnick, S. M. & Chen, R. F. (1998). Laser-induced fluorescence of pyrene and other polycyclic aromatic hydrocarbons (PAH) in seawater. *Talanta* **47**, 907–919.
- Suzuki, M., Nakagawa, Y., Harayama, S. & Yamamoto, S. (1999). Phylogenetic analysis of genus *Marinilabilia* and related bacteria based on the amino acid sequences of *gyrB* and emended description of *Marinilabilia salmonicolor* with *Marinilabilia agarovorans* as its subjective synonym. *Int J Syst Bacteriol* **49**, 1551–1557.
- Swofford, D. L. (1998). PAUP*: Phylogenetic analysis using parsimony (* and other methods), version 4. Sunderland, MA: Sinauer Associates.
- Tender, L. M., Reimers, C. E., Stecher, H. A., Holmes, D. E., Bond, D. R., Lowy, D. A., Pilobello, K., Fertig, S. J. & Lovley, D. R. (2002). Harnessing microbially generated power on the seafloor. *Nat Biotechnol* **20**, 821–825.
- Wang, X. C., Zhang, Y. X. & Chen, R. F. (2001). Distribution and partitioning of polycyclic aromatic hydrocarbons (PAHs) in different size fractions in sediments from Boston Harbor, United States. *Mar Pollut Bull* **42**, 1139–1149.
- Zhilina, T. N., Appel, R., Probian, C., Brossa, E. L., Harder, J., Widdel, F. & Zavarzin, G. A. (2004). *Alkaliflexus imshenetskii* gen. nov. sp. nov., a new alkaliphilic gliding carbohydrate-fermenting bacterium with propionate formation from a soda lake. *Arch Microbiol* **182**, 244–253.