Garciella nitratreducens gen. nov., sp. nov., an anaerobic, thermophilic, nitrate- and thiosulfate-reducing bacterium isolated from an oilfield separator in the Gulf of Mexico

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A novel Gram-positive, anaerobic and thermophilic bacterium, strain MET79^T, was isolated from an oil well located in the Gulf of Mexico. Cells were straight rods, motile by a subpolar flagellum. Spores were formed in old cultures. Inner gas vacuoles swelled the cells when exposed to air. The optimum growth conditions were 55 °C, pH 7.5 and 1 % NaCl. Yeast extract was required for growth. Strain MET79^T fermented several sugars, some organic acids and Casamino acids. Glucose was fermented into lactate, acetate, butyrate, H₂ and CO₂. Strain MET79^T reduced thiosulfate to hydrogen sulfide and nitrate to ammonium. The DNA G + C content was 30.9 mol%. The closest phylogenetic relative of strain MET79^T was *Caloranaerobacter azorensis* (88.7 % 16S rDNA sequence similarity). As strain MET79^T (= DSM 15102^T = CIP 107615^T) was physiologically and phylogenetically different from its closest relatives, it is assigned as the type strain of a novel species of a new genus, *Garciella nitratreducens* gen. nov., sp. nov.

INTRODUCTION

Oilfield environments are known to harbour an important anaerobic microbial community that includes members of domains *Bacteria* and *Archaea* (Magot *et al.*, 2000). Several authors have reported the isolation (microbiological studies) or presence (molecular ecological studies) of microaerophiles in such ecosystems (Gevertz *et al.*, 2000; Telang *et al.*, 1997; Voordouw *et al.*, 1996). Besides microaerophiles, manganese-, iron-, elemental sulfur-, sulfate-, carbon dioxide- and nitrate-reducing microorganisms inhabit oil reservoirs (Greene *et al.*, 1997; Huu *et al.*, 1999; Magot *et al.*, 2000; Myhr & Torsvik, 2000; Rees *et al.*, 1997). Most studies have dealt with methanogens, sulfate-reducers and heterotrophs, and little attention has been paid to other microbial communities originating from this subterrestrial ecosystem. However, the addition of nitrate in oil reservoirs has been shown to stimulate the activity of indigenous nitrate-reducing, sulfide-oxidizing microaerophiles, and also that of heterotrophic denitrifiers that outcompete sulfate-reducing bacteria for energy sources (Gevertz *et al.*, 2000; Telang *et al.*, 1997).

Amongst microaerophilic nitrate-reducing bacteria, Gevertz *et al.* (2000) reported the isolation of strains of *Thiomicrospira*, *Arcobacter* and *Campylobacter* (phylogenetically related micro-organisms) from brine extracted at the Coleville oilfield in Canada. Nitrate-reducing anaerobes or facultative anaerobes include (i) the thermophilic, manganese- and iron-reducing bacterium *Deferribacter thermophilus*, isolated from the Beatrice oilfield in the North Sea (Greene *et al.*, 1997), (ii) the thermophilic, citrate-fermenting bacterium *Anaerobaculum thermo-terrenum*, isolated from the Redwash oilfield in Utah (Rees *et al.*, 1997), (iii) the citrate- and succinate-oxidizing bacterium *Marinobacter aquaeolei*, isolated from a southern Vietnamese oil reservoir (Huu *et al.*, 1999), and (iv) the acetate-oxidizing bacterium *Denitrovibrio acetiphilus*, isolated from an oil reservoir model column (Myhr & Torsvik, 2000).

Here, we describe a novel thermophilic, heterotrophic, nitrate-reducing isolate from a water separator collecting fluids produced from different oil wells located in the SAMIII oilfield, Gulf of Mexico. It belongs to the order *Clostridiales*, cluster XII, and has a number of significant phenotypic, genotypic and phylogenetic differences from other members of cluster XII that justify its assignment to a

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain MET79^T is AY176772.

new genus and species, *Garciella nitratreducens* gen. nov., sp. nov.

METHODS

Sample and strain source. Several strains were isolated from a water sample collected from a separator tank in the SAMIII oilfield in Tabasco, Gulf of Mexico. The samples were collected in sterile plastic bottles and kept at room temperature until used. The *in-situ* temperature of the oil-bearing formation ranged from 35 to 100 °C. The temperature in the water separator was 30–35 °C and the salinity of the samples was 32–35 g l⁻¹.

Enrichment, isolation and growth conditions. Enrichment was performed in a medium containing (l⁻¹ distilled water) 0.3 g K₂HPO₄, 0.3 g KH₂PO₄, 0.2 g MgSO₄·7H₂O, 0.1 g CaCl₂, 0.1 g KCl, 30 g NaCl, 1 g NH₄Cl, 0.5 g cysteine hydrochloride, 0.2 g yeast extract (Difco), 10 ml trace mineral solution (Balch *et al.*, 1979) and 1 mg resazurin. The pH was adjusted to 7.0 with 10 M KOH and the medium was boiled under a stream of O₂-free N₂ gas and cooled to room temperature. The medium was dispensed into serum bottles (20 ml) and Hungate tubes (5 ml) under a stream of O₂-free N₂ gas, subsequently replaced by a mixture of N₂/CO₂ (80:20%, v/v). Vessels were autoclaved for 45 min at 110 °C and, prior to inoculation, Na₂S·9H₂O, NaHCO₃, peptone (Difco) and thiosulfate were added from sterile stock solutions to obtain respective final concentrations of 0.04, 0.2 and 0.5% and 20 mM. Hydrogen (2 bars) was added in the gas phase. For enrichment, a 2-ml oil-well-water sample was inoculated into 20 ml medium and incubated at 40 °C without agitation. Three enrichment series were performed in the same medium before isolation. Strains were isolated by repeated use of the roll-tube technique (Hungate, 1969), with medium solidified with 1.6% agar. The process of serial dilution in roll tubes was repeated at least twice in order to purify the cultures.

Determination of growth parameters. The basal medium used for characterization of the pH, temperature and NaCl ranges for growth of the isolate and resistance to antibiotics was similar to the enrichment medium with the following modifications: the NaCl concentration was decreased to 10 g l⁻¹, glucose was added at a concentration of 20 mM and 0.2 g MgSO₄·7H₂O l⁻¹ was replaced by 1 g MgCl₂·6H₂O l⁻¹. The culture medium was adjusted to different pH values by injecting 1 M HCl, NaHCO₃ or Na₂CO₃ from 10% (w/v) sterile anaerobic stock solutions. For studies on NaCl requirements, NaCl was weighed directly into tubes prior to dispensing the medium. The strain was subcultured at least once under the same experimental conditions prior to determination of the growth rate.

Substrate utilization tests. Substrates were tested at a final concentration of 20 mM in basal medium or 1% for cellulose, starch and xylan or 0.2% for peptone, bio-Trypticase and Casamino acids.

Electron acceptors and H₂S production. To test for electron acceptors, sodium thiosulfate (20 mM), sodium sulfate (20 mM), sodium fumarate (20 mM), sodium sulfite (5 mM), elemental sulfur (1%), sodium nitrate (10 mM) and sodium nitrite (5 mM) were added individually to the medium. The use of electron acceptors was evaluated by measuring OD₅₈₀ and H₂S, succinate, ammonium or nitrite production. Nitrate reduction was tested in the absence of ammonium chloride as nitrogen source.

Antibiotic susceptibility. The sensitivity of the isolate to ampicillin, chloramphenicol, kanamycin, rifampicin and vancomycin was tested at 10, 25, 50 and 100 µg ml⁻¹. Controls were performed with ethanol and DMSO (respectively solvents for chloramphenicol and rifampicin). Growth was monitored by measuring OD₅₈₀ and by microscopic observations.

Sporulation test and oxygen susceptibility. The presence of spores was analysed by phase-contrast microscopic observations of young and old cultures and pasteurization tests performed at 80, 90 and 100 °C for 10 and 20 min. The effect of O₂ on growth was determined in Hungate tubes containing basal medium supplemented with 20 mM glucose. After inoculation, 20 ml sterile air was added to the gas phase. The cultures were incubated at 55 °C under agitation (150 r.p.m.). Growth was monitored by turbidity measurements (OD₅₈₀). Experiments were conducted in duplicate and repeated at least twice.

Analytical techniques. Unless otherwise indicated, duplicate culture tubes were used throughout these studies. Growth was measured at 580 nm using a UV-Visible spectrophotometer 50 Scan (Varian). Sulfide was determined photometrically as colloidal CuS by using the method of Cord-Ruwisch (1985). Ammonium was detected by the Nessler method. Fermentation products were determined by HPLC using a pump (Spectra Series P100; Thermo Separation Products), an automatic sampler (Spectra Series AS100), an Aminex HPX 87H column (300 × 7.8 mm) (Bio-Rad), a differential refractometer detector (Spectra System RI-150) and an integrator (Azur Microsoft). An aliquot of 20 µl cell-free supernatant was injected into the column, which was maintained at 37 °C. A solution of 0.01 M H₂SO₄ was used as solvent at a flow rate of 0.6 ml min⁻¹. The presence of L-alanine as an end product of sugar metabolism was determined enzymically at 340 nm as described previously (Fardeau *et al.*, 1997).

Light and electron microscopy. Morphological characteristics of isolates were observed with a phase microscope (Nikon). For scanning electron microscopy studies, cells were stained with osmium tetroxide, prepared as described by Bozzola & Russell (1991) and observed in a JEOL-5900 low-vacuum electron microscope at an accelerating voltage of 10 kV. For transmission electron microscopy studies, cells were negatively stained with sodium phosphotungstate and prepared and observed as described by Koussémon *et al.* (2001).

Determination of G+C content and 16S rDNA sequence analysis. The G+C content of DNA was determined at the DSMZ by using HPLC as described by Mesbah *et al.* (1989). Non-methylated lambda DNA (Sigma) was used as the standard. The 16S rRNA gene of the isolate was amplified by adding 2.5 µl cell culture to a thermocycler microtube containing 5 µl 10 × Taq buffer (Promega), 3 µl 25 mM MgCl₂, 0.5 µl 25 mM dNTPs, 0.5 µl 100 nM primers, 37.5 µl sterile distilled water and 0.5 µl 5 U Taq polymerase µl⁻¹ (Promega). The universal primers Fd1 (5'-AGAGTTTGATCCTGGCTCAG-3', positions 8–28) and Rd1 (5'-AAGGAGGTGATCCAGCC-3', positions 1547–1531, MWG) (*Escherichia coli* numbering) were used to obtain the PCR product (Winker & Woese, 1991). PCR was performed by an initial denaturation at 96 °C for 3 min followed by 30 cycles of annealing at 57.1 °C for 30 s, extension at 72 °C for 2 min and denaturation at 96 °C for 30 s and, finally, an extension cycle of 72 °C for 7 min. The amplified fragment contained 1471 nucleotides. PCR products were purified with a QIAquick gel extraction kit. Direct sequencing of the PCR product was performed by Genome Express. The new sequence was aligned to a full-length consensus 16S rDNA sequence, assembled and checked for accuracy manually, using the alignment editor BioEdit version 5.0.9 (Hall, 1999). These were compared with other sequences in GenBank (Benson *et al.*, 1999) and the RDP (Maidak *et al.*, 2001) using BLAST (Altschul *et al.*, 1997) to identify the closest relatives. Positions of sequence and alignment ambiguity were omitted and pairwise evolutionary distances based on 1267 unambiguous nucleotides were computed by using the method of Jukes & Cantor (1969). A dendrogram was constructed using the neighbour-joining method (Saitou & Nei, 1987). Confidence in the tree topology was determined by using 100 bootstrapped trees (Felsenstein, 1985).

RESULTS AND DISCUSSION

Anaerobes have always been considered as the dominant indigenous micro-organisms of the oilfield ecosystem (Magot *et al.*, 2000). Amongst these, the occurrence of methanogenic, sulfate-reducing and heterotrophic bacteria belonging to the families *Thermotogaceae* and *Thermoanaerobiaceae* has been established from various oil reservoirs throughout the world (Magot *et al.*, 2000). Microaerophilic nitrate-reducing bacteria have been shown to be helpful in oil reservoirs because they oxidize sulfur-containing compounds and reduce H_2S production (Telang *et al.*, 1997). However, few studies have reported the isolation of such bacteria from oil production waters (Gevertz *et al.*, 2000). Besides microaerophilic nitrate-reducing bacteria, few studies have provided evidence of the existence of anaerobic nitrate-reducers in oil reservoirs (Greene *et al.*, 1997; Myhr & Torsvik, 2000; Rees *et al.*, 1997). Here, we report a novel anaerobic, nitrate-reducing bacterium isolated from an oilfield separator in the Gulf of Mexico. Enrichment cultures were positive after incubation at 40 °C for 15 days. Microscopic examination revealed the presence of motile, rod-shaped bacteria. Round, slightly black-coloured colonies (2 mm diameter) developed on roll tubes after 1 week. Single colonies were picked and serially diluted and reinoculated in roll tubes three times before cultures were considered pure. Several strains similar in morphology were isolated from the oil reservoir, and one strain, designated MET79^T, was used for further characterization.

Cells of strain MET79^T were straight rods, 0.5–0.7 × 1.4–2.8 µm in size (Fig. 1a, b), motile with a subpolar flagellum. Spherical and terminal spores appeared in old cultures. Irregular gas vacuoles were sometimes observed (Fig. 1a). Ultrathin sections of cells showed a Gram-positive-type cell wall composed of a thick (17 nm) and homogeneous S-layer (Fig. 1c). Strain MET79^T grew at temperatures ranging from 25 to 60 °C, with optimum growth at 55 °C. Growth occurred at initial pH values between 5.5 and 9.0 at 55 °C and the optimum was pH 7.5. The isolate grew in the presence of NaCl concentrations ranging from 0 to 10 %, with an optimum NaCl concentration of 1 % at pH 7.5 and 55 °C. Strain MET79^T grew in the presence of up to 25 µg kanamycin and chloramphenicol ml⁻¹ and 100 µg ampicillin ml⁻¹, but not in the presence of 10 µg vancomycin or rifampicin ml⁻¹. Yeast extract was required for growth. Vitamins or bio-Trypticase could not replace yeast extract. Strain MET79^T fermented the following compounds: cellobiose, fructose, galactose, glucose, lactose, maltose, mannose, ribose, sucrose, D-xylose, glycerol, mannitol, fumarate, lactate, malate, pyruvate and Casamino acids. One mol glucose was fermented into 1.5 mol lactate as the major end product, with minor amounts of acetate [0.03 mol (mol glucose consumed)⁻¹] and butyrate [0.2 mol (mol glucose consumed)⁻¹], and into H_2 [0.3 mol (mol glucose consumed)⁻¹] and CO_2 . The following compounds were not used: arabinose, raffinose, rhamnose, starch, cellulose, xylan, acetate,

Fig. 1. Micrographs of strain MET79^T. (a) Phase-contrast micrograph of cells grown in optimal conditions in reduced medium in the presence of air, showing gas vacuoles. Bar, 2 µm. (b) Scanning electron microscopy after thermal stress, showing spherical, terminal spores swelling the cells and a subpolar flagellum. Bar, 1 µm. (c) Electron micrograph of an ultrathin section showing a Gram-positive-type cell wall with a thick layer. Bar, 0.2 µm.

butyrate, propionate, methanol, ethanol, butanol, propanol, formate, methylamine, *p*-coumaric acid, ferulic acid, peptone, bio-Trypticase and H_2 . Strain MET79^T reduced thiosulfate to H_2S and nitrate to ammonium. The other electron acceptors tested were not used. Growth was enhanced in the presence of thiosulfate or nitrate. Strain MET79^T grew under anaerobic conditions. The G+C content of strain MET79^T was 30.9 mol%. 16S rDNA sequence analysis revealed that strain MET79^T was a member of the order *Clostridiales*, cluster XII, as defined

Fig. 2. Dendrogram based on 16S rDNA sequences indicating the position of strain MET79^T amongst members of cluster XII of *Clostridiales*. Accession numbers are given in parentheses. Bootstrap values from 100 replications are shown; only values above 95 were considered significant and are reported. Bar, 5 substitutions per 100 nucleotides.

by Collins *et al.* (1994), and its closest phylogenetic relative was *Caloranaerobacter azorensis* (Wery *et al.*, 2001), family *Clostridiaceae* (88.7% similarity). Fig. 2 presents a dendrogram generated by the neighbour-joining method (Saitou & Nei, 1987).

Three species belonging to the order *Clostridiales* have so far been isolated from oil wells. They include two mesophiles, *Dethiosulfovibrio peptidovorans*, family *Syntrophomonadaceae* (Magot *et al.*, 1997), and *Fusibacter paucivorans*, family *Peptostreptococcaceae* (Ravot *et al.*, 1999), and the thermophilic *Anaerobaculum thermoterrenum*, family *Syntrophomonadaceae* (Rees *et al.*, 1997). *Dethiosulfovibrio peptidovorans* is peripherally related to cluster V, consisting of the family *Thermoanaerobiaceae* (Magot *et al.*, 1997), whereas *Fusibacter paucivorans* grouped with members of cluster XI (Ravot *et al.*, 1999). Similarly to strain MET79^T, *Anaerobaculum thermoterrenum* also reduced nitrate, but the latter forms a branch that is approximately equidistant from *Dictyoglomus thermophilum* and members of *Thermoanaerobacter* (Rees *et al.*, 1997). In addition to phylogenetic differences between strain MET79^T and these three species, there are also numerous phenotypic and genotypic differences (Table 1). Two other anaerobes isolated from oilfield ecosystems were reported to reduce nitrate, *Deferribacter thermophilus* and *Denitrovibrio acetiphilus* (Greene *et al.*, 1997; Myhr & Torsvik, 2000). Neither bacterium belongs to the *Clostridiales*. The organism that is phylogenetically most closely related to *Deferribacter thermophilus* is *Flexistipes sinusarabici* (sequence similarity 88%), whereas the closest relative of *Denitrovibrio acetiphilus* is *Geovibrio ferrireducens* (sequence similarity 86.9%). In contrast to strain MET79^T, *Deferribacter thermophilus* and *Denitrovibrio acetiphilus* are specialized in the oxidation of organic acids and are unable to ferment sugars (Greene *et al.*, 1997; Myhr & Torsvik, 2000). Within cluster XII, strain MET79^T has *Caloranaerobacter azorensis* (Wery *et al.*, 2001) as its closest phylogenetic relative (sequence similarity 88.7%). Strain MET79^T differs from *Caloranaerobacter azorensis*, isolated from a deep-sea hydrothermal vent, by its cell-wall structure. Furthermore, *Caloranaerobacter azorensis* is a non-sporulating bacterium that does not reduce thiosulfate

or nitrate but reduces elemental sulfur. The end products of glucose metabolism by these two bacteria are also very different (Table 1). The two strains also differ in the ranges and optima of growth conditions, in the range of substrates used and in the G + C content of DNA (Table 1).

Interestingly, strain MET79^T reduced thiosulfate to sulfide, as do several other heterotrophic anaerobic isolates from the oilfield ecosystem (Fardeau *et al.*, 1997, 2000; Magot *et al.*, 1997, 2000; Ravot *et al.*, 1995a, b, 1999). Such activity might result in increasing biocorrosion processes *in situ* (Magot *et al.*, 1997). In light of its phenotypic, genotypic and phylogenetic characteristics, strain MET79^T represents a novel genus within cluster XII of the order *Clostridiales* that we propose to name *Garciella nitratreducens* gen. nov., sp. nov.

Description of *Garciella* gen. nov.

Garciella (Gar.ci.el'la. L. dim. ending -ella; N.L. fem. n. *Garciella* named in honour of the French microbiologist Jean-Louis Garcia, for his important contribution to the taxonomy of anaerobes).

Cells are straight rods with a Gram-positive-type cell wall. Terminal spores are formed. Growth is strictly anaerobic. Moderately thermophilic, fermentative member of the domain *Bacteria*, phylum *Firmicutes*, cluster XII of the order *Clostridiales*, family *Clostridiaceae*. Carbohydrates and organic acids serve as fermentable substrates. The type species is *Garciella nitratreducens*.

Description of *Garciella nitratreducens* sp. nov.

Garciella nitratreducens (ni.trati.re.du'cens. N.L. n. *nitratum* nitrate; L. v. *reduco* to draw backwards, bring back to a state or condition; N.L. part. adj. *nitratreducens* nitrate-reducing).

Displays the following properties in addition to those given in the genus description. Cells (0.5–0.7 × 1.4–2.8 µm) occur singly or in pairs and possess one subpolar flagellum. Terminal spores appear in old cultures. Round colonies (2 mm diameter) develop in roll tubes after 1 week of incubation at 55 °C. Chemo-organotrophic. The optimum temperature for growth is 55 °C at pH 7.5; temperature

Table 1. Characteristics that distinguish between strain MET79^T, *Caloranaerobacter azorensis* and other members of the *Clostridiales* isolated from oil wells

Strain/species: 1, strain MET79^T; 2, *Caloranaerobacter azorensis* (data from Wery *et al.*, 2001); 3, *Anaerobaculum thermoterrenum* (Rees *et al.*, 1997); 4, *Dethiosulfovibrio peptidovorans* (Magot *et al.*, 1997); 5, *Fusibacter paucivorans* (Ravot *et al.*, 1999). ND, Not determined.

Characteristic	1	2	3	4	5
Temperature for growth (°C):					
Range	25–60	45–65	28–60	20–45	20–45
Optimum	55	65	55	42	37
pH for growth:					
Range	5.5–9.0	5.5–9.0	5.5–8.6	5.5–8.8	5.7–8.0
Optimum	7.5	7.0	7.0–7.6	7.0	7.3
NaCl concentration for growth (%):					
Range	0–10	0.6–6.5	0–2	0.5–9.5	0–10
Optimum	1	2	1	3	0–3
DNA G + C content (mol%)	30.9	26–28	44	56	43
Reduction of S ⁰	–	+	+	+	+
Reduction of S ₂ O ₃ ^{2–}	+	–	+	+	+
Flagella	1, Subpolar	ND	–	Lateral	Peritrichous
Substrates used:					
Cellobiose	+	–	ND	–	+
Fructose	+	+	+	–	+
Galactose	+	+	ND	–	–
Glucose	+	+	+	–	+
Lactose	+	–	ND	–	–
Maltose	+	–	ND	–	–
Mannose	+	ND	+	–	–
Ribose	+	+	ND	–	+
Starch	–	+	+	–	–
Xylose	+	+	ND	–	–
Glycerol	+	ND	+	–	–
Mannitol	+	–	ND	–	+
Pyruvate	+	+	+	–	–
Xylan	–	+	ND	–	ND
Diagnostic fermentation products from glucose:					
Lactate	+	ND	–	–	–
Acetate	+	+	+	–	+
Butyrate	+	ND	–	–	+

range 25–60 °C. The optimum pH is 7.5; growth occurs between pH 5.5 and 9.0. Halotolerant, growing in the presence of up to 10 % NaCl with an optimum at 1 %. Yeast extract is required for growth and cannot be replaced by vitamins. Ferments cellobiose, fructose, galactose, glucose, lactose, maltose, mannose, ribose, sucrose, D-xylose, glycerol, mannitol, fumarate, lactate, malate, pyruvate and Casamino acids. Arabinose, raffinose, rhamnose, starch, cellulose, xylan, acetate, butyrate, propionate, methanol, ethanol, butanol, propanol, formate, methylamine, *p*-coumaric acid, ferulic acid, peptone, bio-Trypticase and H₂ are not used. Lactate, acetate, butyrate, H₂ and CO₂ are produced during glucose fermentation. Thiosulfate is reduced to sulfide and nitrate to ammonium; elemental sulfur, sulfite, sulfate,

fumarate and nitrite are not used as electron acceptors. Thiosulfate or nitrate enhance growth. Can grow in the presence of 25 µg kanamycin or chloramphenicol ml^{–1} and 100 µg ampicillin ml^{–1} but not in the presence of 10 µg vancomycin or rifampicin ml^{–1}. The G + C content of the DNA of the type strain is 30.9 mol% (HPLC).

The type strain, MET79^T (= DSM 15102^T = CIP 107615^T), was isolated from an oilfield separator in the Gulf of Mexico.

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REFERENCES

- Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Balch, W. E., Fox, G. E., Magrum, R. J., Woese, C. R. & Wolfe, R. S. (1979). Methanogens: reevaluation of a unique biological group. *Microbiol Rev* **43**, 260–296.
- Benson, D. A., Boguski, M. S., Lipman, D. J., Ostell, J., Ouellette, B. F. F., Rapp, B. A. & Wheeler, D. L. (1999). GenBank. *Nucleic Acids Res* **27**, 12–17.
- Bozzola, J. J. & Russell, L. D. (1991). Electron microscopy. In *Principles and Techniques for Biologists*, pp. 40–61. Boston: Jones & Bartlett.
- Collins, M. D., Lawson, P. A., Willems, A., Cordoba, J. J., Fernandez-Garayzabal, J., Garcia, P., Cai, J., Hippe, H. & Farrow, J. A. E. (1994). The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. *Int J Syst Bacteriol* **44**, 812–826.
- Cord-Ruwisch, R. (1985). A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria. *J Microbiol Methods* **4**, 33–36.
- Fardeau, M.-L., Ollivier, B., Patel, B. K. C., Magot, M., Thomas, P., Rimbault, A., Rocchiccioli, F. & Garcia, J.-L. (1997). *Thermotoga hypogea* sp. nov., a xylanolytic, thermophilic bacterium from an oil-producing well. *Int J Syst Bacteriol* **47**, 1013–1019.
- Fardeau, M.-L., Magot, M., Patel, B. K. C., Thomas, P., Garcia, J.-L. & Ollivier, B. (2000). *Thermoanaerobacter subterraneus* sp. nov., a novel thermophile isolated from oilfield water. *Int J Syst Evol Microbiol* **50**, 2141–2149.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Gevertz, D., Telang, A. J., Voordouw, G. & Jenneman, G. E. (2000). Isolation and characterization of strains CVO and FWKO B, two novel nitrate-reducing, sulfide-oxidizing bacteria isolated from oil field brine. *Appl Environ Microbiol* **66**, 2491–2501.
- Greene, A. C., Patel, B. K. C. & Sheehy, A. J. (1997). *Deferribacter thermophilus* gen. nov., sp. nov., a novel thermophilic manganese- and iron-reducing bacterium isolated from a petroleum reservoir. *Int J Syst Bacteriol* **47**, 505–509.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98 NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Hungate, R. E. (1969). A roll-tube method for the cultivation of strict anaerobes. *Methods Microbiol* **3B**, 117–132.
- Huu, N. B., Denner, E. B. M., Ha, D. T. C., Wanner, G. & Stan-Lotter, H. (1999). *Marinobacter aquaeolei* sp. nov., a halophilic bacterium isolated from a Vietnamese oil-producing well. *Int J Syst Bacteriol* **49**, 367–375.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 211–232. Edited by H. N. Munro. New York: Academic Press.
- Koussémon, M., Combet-Blanc, Y., Patel, B. K. C., Cayol, J.-L., Thomas, P., Garcia, J.-L. & Ollivier, B. (2001). *Propionibacterium microaerophilum* sp. nov., a microaerophilic bacterium isolated from olive mill wastewater. *Int J Syst Evol Microbiol* **51**, 1373–1382.
- Magot, M., Ravot, G., Campagnolle, X., Ollivier, B., Patel, B. K. C., Fardeau, M.-L., Thomas, P., Crolet, J.-L. & Garcia, J.-L. (1997). *Dethiosulfovibrio peptidovorans* gen. nov., sp. nov., a new anaerobic, slightly halophilic, thiosulfate-reducing bacterium from corroding offshore oil wells. *Int J Syst Bacteriol* **47**, 818–824.
- Magot, M., Ollivier, B. & Patel, B. K. C. (2000). Microbiology of petroleum reservoirs. *Antonie van Leeuwenhoek* **77**, 103–116.
- Maidak, B. L., Cole, J. R. & Lilburn, T. G. (2001). The RDP-II (Ribosomal Database Project). *Nucleic Acids Res* **29**, 173–174.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Myhr, S. & Torsvik, T. (2000). *Denitrovibrio acetiphilus*, a novel genus and species of dissimilatory nitrate-reducing bacterium isolated from an oil reservoir model column. *Int J Syst Evol Microbiol* **50**, 1611–1619.
- Ravot, G., Magot, M., Fardeau, M.-L., Patel, B. K. C., Prensier, G., Egan, A., Garcia, J.-L. & Ollivier, B. (1995a). *Thermotoga elfii* sp. nov., a novel thermophilic bacterium from an African oil-producing well. *Int J Syst Bacteriol* **45**, 308–314.
- Ravot, G., Ollivier, B., Magot, M., Patel, B. K. C., Crolet, J.-L., Fardeau, M.-L. & Garcia, J.-L. (1995b). Thiosulfate reduction, an important physiological feature shared by members of the order *Thermotogales*. *Appl Environ Microbiol* **61**, 2053–2055.
- Ravot, G., Magot, M., Fardeau, M.-L., Patel, B. K. C., Thomas, P., Garcia, J.-L. & Ollivier, B. (1999). *Fusibacter paucivorans* gen. nov., sp. nov., an anaerobic, thiosulfate-reducing bacterium from an oil-producing well. *Int J Syst Bacteriol* **49**, 1141–1147.
- Rees, G. N., Patel, B. K. C., Grassia, G. S. & Sheehy, A. J. (1997). *Anaerobaculum thermoterrenum* gen. nov., sp. nov., a novel, thermophilic bacterium which ferments citrate. *Int J Syst Bacteriol* **47**, 150–154.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Telang, A. J., Ebert, S., Foght, J. M., Westlake, D. W. S., Jenneman, G. E., Gevertz, D. & Voordouw, G. (1997). Effect of nitrate injection on the microbial community in an oil field as monitored by reverse sample genome probing. *Appl Environ Microbiol* **63**, 1785–1793.
- Voordouw, G., Armstrong, S. M., Reimer, M. F., Fouts, B., Telang, A. J., Shen, Y. & Gevertz, D. (1996). Characterization of 16S rRNA genes from oil field microbial communities indicates the presence of a variety of sulfate-reducing, fermentative, and sulfide-oxidizing bacteria. *Appl Environ Microbiol* **62**, 1623–1629.
- Wery, N., Moricet, J.-M., Cuffe, V., Jean, J., Pignet, P., Lesongeur, F., Cambon-Bonavita, M.-A. & Barbier, G. (2001). *Caloranaerobacter azorensis* gen. nov., sp. nov., an anaerobic thermophilic bacterium isolated from a deep-sea hydrothermal vent. *Int J Syst Evol Microbiol* **51**, 1789–1796.
- Winker, S. & Woese, C. R. (1991). A definition of the domain *Archaea*, *Bacteria* and *Eucarya* in terms of small subunit ribosomal RNA characteristics. *Syst Appl Microbiol* **13**, 161–165.