Sporolituus thermophilus gen. nov., sp. nov., a citrate-fermenting thermophilic anaerobic bacterium from geothermal waters of the Great Artesian Basin of Australia

Christopher D. Ogg and Bharat K. C. Patel

Correspondence

Bharat K. C. Patel

b.patel@griffith.edu.au

Microbial Gene Research and Resources Facility, School of Biomolecular and Physical Sciences, Griffith University, Brisbane, QLD 4111, Australia

A strictly anaerobic, sluggishly motile, spore-forming, thermophilic bacterium, designated strain AeG^T, was isolated from microbial mats colonizing a runoff channel formed by free-flowing thermal waters of a bore well (New Lorne Bore; registered number 17263) in the Great Artesian Basin, Australia. Cells of strain AeG^T were curved rods (2.0–10.0×0.8–1.0 µm) and stained Gram-negative. The strain grew optimally in tryptone-yeast extract-citrate medium at 55 °C (range for growth between 45 and 60 °C) and pH 7.0 (range for growth between pH 6.5 and 8.0). Citrate and malate, but no other organic acids, carbohydrates or amino acids could be used in the presence of up to 0.1 % yeast extract. Although yeast extract and/or tryptone were required for growth on citrate, they did not support growth as sole carbon sources. Strain AeG^T reduced thiosulfate and sulfite in the presence of 0.2 % yeast extract, but not Fe(III), Mn(IV), sulfate, elemental sulfur, nitrate or nitrite. Growth was inhibited by chloramphenicol, streptomycin, tetracycline, penicillin and ampicillin and in the presence of NaCl concentrations >1 %. The DNA G + C content was 55.4 ± 1.0 mol% as determined by the thermal denaturation method. 16S rRNA gene sequence analysis indicated that strain AeG^T was a member of the family *Veillonellaceae*, class '*Clostridia*', phylum '*Firmicutes*' and was most closely related to members of the genus *Propionispora* (mean 16S rRNA gene sequence similarity value to type strains was 90.8 %). Based on these results, strain AeG^T is considered to represent a novel species in a new genus, for which the name *Sporolituus thermophilus* gen. nov., sp. nov. is proposed. The type strain of the type species is AeG^T (=JCM 15556^T=KCTC 5668^T).

The Great Artesian Basin (GAB) underlies more than one-fifth of the Australian continent (1.7 × 10⁶ km²) and, with a water-storage capacity of 8.7 × 10¹² m³, is the world's largest geothermal subsurface aquifer (Habermehl, 1980). The GAB was formed between 100 and 250 million years ago and is composed of multiple alternating layers of water-bearing permeable sandstone aquifers and non-water-bearing impermeable shale strata. The water flows from the recharge areas at the edge of the basin to the discharge areas in central Australia as mound springs, at an estimated rate of 1–5 m year⁻¹. The Earth's magma heats these slow-flowing subsurface waters up to 100 °C depending on the depth of the water-bearing layer. The high temperatures and the geological formations surrounding the aquifer affect the chemistry and local chemical composition of the bicarbonate-, chloride-, sulfate- or

iron-rich GAB groundwaters. Some 5000 free-flowing bores tap the GAB and provide a vital water supply to outback communities for agricultural, industrial and domestic purposes. The bores can be 3000 m deep and source temperatures can range between 30 and 100 °C, depending on the bore depth. The chemistry, temperature and slow flow rate of the deep subsurface and the surface groundwaters in the runoff channels formed from the free-flowing bores provide a conducive environment for a diverse range of micro-organisms to thrive. For example, culture-independent analyses of four microbial mat communities colonizing the New Lorne bore (registered bore number 17263) runoff channel at temperatures ranging from 52 to 75 °C identified species spanning the full spectrum of the domain '*Bacteria*', including a diverse range of currently uncultured novel organisms (Spanevello, 2001). Similarly, culture-dependent studies have isolated numerous novel thermophilic and mesophilic micro-organisms, including sulfate-reducers (Love *et al.*, 1993; Redburn & Patel, 1994), carbohydrate-fermenters

Abbreviation: GAB, Great Artesian Basin.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AeG^T is FJ169187.

(Andrews & Patel, 1996), strict aerobes (Kanso & Patel, 2003; Spanevello *et al.*, 2002) and metal-reducers (Ogg & Patel, 2009a, b, c; Kanso *et al.*, 2002). Most culture-dependent studies of subsurface environments, including ours, have focused largely on the isolation of carbohydrate-fermenting and metal- or sulfur-compound-reducing organisms, perhaps due to the potential commercial value of their thermostable enzymes, and their potential applications in microbial-enhanced oil recovery and bioremediation processes (Haki & Rakshit, 2003; Head *et al.*, 2003; Lazar *et al.*, 2007; Lovley, 2001). In this report, the properties of a novel thermophilic, citrate-fermenting, strictly anaerobic bacterium, designated strain AeG^T, were investigated. An isolate with an almost identical 16S rRNA gene sequence (>99 %) was also cultured from the outflow of another GAB bore (registered bore number 22981).

A sample of a green microbial mat thriving at 57 °C was collected from New Lorne Bore, situated near Blackall, some 970 km north-west of Brisbane, Queensland, Australia (24° 54' 48" S 145° 08' 18" E) (Spanevello *et al.*, 2002) and water samples from another bore (registered bore number 22981), situated at Mitchell, approximately 570 km west-north-west of Brisbane and 400 km south-west of Blackall were also collected. Bore 22981 is 403 m deep and has a source temperature of 46 °C. Sulfate-reducers, carbohydrate-fermenters and species of the genus *Meiothermus* have been isolated previously from samples from both bores (Spanevello & Patel, 2004).

Enrichments for iron-reducers were performed in an aerobic medium designated APL medium. APL medium contained (per 1 deionized water): 1 g NH₄Cl, 0.6 g K₂HPO₄, 0.3 g KH₂PO₄, 0.1 g MgCl₂·6H₂O, 0.1 g CaCl₂·2H₂O, 1.0 g NaCl, 12.0 g HEPES, 1 ml vitamin solution (Wolin *et al.*, 1963), 1 ml trace element solution (Zeikus *et al.*, 1979), 1 ml 0.2 % resazurin, 0.2 g yeast extract and 2.0 g ammonium iron (III) citrate (brown in colour). The pH of the medium was adjusted to 7.2 with 1 M NaOH and 1 M HCl. APL medium (9.5 ml) was distributed in McCartney bottles and autoclaved for 30 min at 121 °C and a pressure of 1.0–1.5 kg cm⁻² (98–147 kPa). APL medium that was rendered anoxic by heating for 10 min in an autoclave at 121 °C and 1.0–1.5 kg cm⁻² to remove dissolved oxygen and subsequently cooled under a stream of oxygen-free nitrogen gas was designated PL medium. Vitamins and trace elements were added and 9.3 ml medium was dispensed into Hungate tubes under oxygen-free nitrogen gas and sterilized for 20 min at 121 °C and 1.0–1.5 kg cm⁻².

Enrichment was performed by adding a 500 µl sample to 9.5 ml APL medium amended with yeast extract (0.2 %) followed by incubation at 50 °C. After 3 days incubation, a slimy surface layer was observed in the upper portion of the APL medium suggesting aerobic growth and the medium, which was originally reddish-brown in colour due to the presence of iron (III) oxide, was colourless and a dark precipitate [Fe(II)] had formed which settled to the bottom,

inferring Fe(III) reduction. On inversion of the enrichment culture, the medium became oxygenated and the colourless medium changed to pink due to the presence of resazurin. This enrichment was then subcultured several times in PL media that had been fortified with a high concentration of yeast extract (0.2 %) or contained a low concentration of yeast extract (0.02 %). The enrichments were subsequently serially diluted in PL media with the respective high and low yeast extract concentrations and incubated under the same conditions; the tube containing the lowest serial dilution was selected and the procedure was repeated at least three times. An isolate purified by the serial end-point dilution method in PL medium amended with low concentrations of yeast extract was designated strain AeG^T. The purity of strain AeG^T was confirmed by microscopy and 16S rRNA gene sequencing and the culture was stored in a glycerol/PL medium (50:50) mixture at -20 °C. Pure cultures from PL medium containing high yeast extract enrichments were obtained using the agar shake method. For this, PL medium fortified with 0.2 % yeast extract and 1 % Gelrite was inoculated with a 0.1 ml sample of the lowest serial dilution enrichment culture. Colonies that had developed in PL medium containing high yeast extract after 3 days incubation at 50 °C were picked and an isolate designated 142 was selected for further studies. Subsequent 16S rRNA gene sequencing and sequence comparisons of 1467 nt of strain 142 revealed an almost identical (similarity value >99 %) sequence to that of strain AeG^T. Strain 142 also exhibited identical morphological characteristics to strain AeG^T and so no further characterization tests were performed on this strain. A third isolate, obtained by streaking medium D agar plates (Brock & Freeze, 1969), from a sample taken from the surface layer of the initial enrichment culture was designated strain Md-G. 16S rRNA gene sequence analyses and phenotypic studies identified strain Md-G as a species of the genus *Anoxybacillus*; a report on the characterization of strains 142 and Md-G will be presented elsewhere. The characterization of strain AeG^T is presented in this report.

In parallel, an attempt to enrich for aromatic-oxidizing, Fe(III)-reducing thermophiles was made by inoculating 700 µl water from bore 22981 into 9.3 ml PL medium amended with 4 mM cinnamate followed by incubation at 50 °C. After 4 days incubation, Fe(III) reduction was observed and the culture was purified by the repeat end-point serial dilution method as described above; the pure culture was designated strain 27. Although 16S rRNA gene sequencing and gene analysis of 1501 nt of strain 27 revealed an almost identical (similarity value >99 %) 16S rRNA gene sequence to that of strain AeG^T, further characterization of this strain has yet to be carried out.

Morphology of cells of strain AeG^T was determined by phase-contrast microscopy and electron microscopy (Kanso & Patel, 2003). Cells were sluggishly motile, peritrichously flagellated, curved rods (2.0–10.0 × 0.8–1.0 µm), which existed singly and in short chains and readily formed spores in all media tested. Cells of strain

AeG^T stained Gram-negative and electron micrographs of thin sections confirmed a Gram-negative cell-wall ultra-structure consisting of an inner electron-dense layer adjacent to the cell membrane and an outer electron-light layer consisting of regular protein subunits (Fig. 1).

Unless otherwise indicated, all growth experiments were conducted at least twice and cultures were subcultured at least once in the same medium prior to the start of the experiment. Growth studies were performed in a modified anaerobic tryptone-yeast extract-glucose (TYEG) medium, designated TYECit medium, which consisted of low-phosphate-buffered salts (LPBS) amended with 0.2 % each of tryptone, yeast extract and citrate (in the place of glucose). TYECit medium was prepared anaerobically as described previously (Patel *et al.*, 1985a, b). Growth was measured at 580 nm by inserting Hungate tubes directly into a modified cuvette holder of a Novaspec LKB spectrophotometer (Pharmacia Biotech). Growth of strain AeG^T was tested at 26–70 °C and pH 5.0–10.0. Strain AeG^T grew optimally in TYECit medium at 55 °C (range: 45–60 °C) and pH 7.0 (range: pH 6.5 and 8.0).

To investigate the effect of each specific energy substrate present in TYECit medium (yeast extract, tryptone and citrate) on growth, each carbon source was added to LPBS medium individually and in combination and growth was monitored. No growth of strain AeG^T was observed in LPBS amended with 0.2 % tryptone (medium T), 0.2 % yeast extract (medium YE), 0.2 % each of tryptone and yeast extract (medium TYE), or on 0.2 % citrate as the sole carbon source. Growth only occurred in LPBS medium containing 0.2 % each of citrate and tryptone (TCit medium) and LPBS medium containing 0.2 % each of citrate and yeast extract (YECit medium), where optical densities after 25 h incubation reached approximately 75 and 83 %, respectively, of those obtained in TYECit medium (OD_{580 nm} of 0.19 taken as 100 %). When yeast extract concentrations were lowered in YECit medium to

0.02 % and 0.05 %, approximately 35 % and 20 % less growth was observed, respectively, after 25 h incubation and approximately equal growth was observed with 0.1 % yeast extract compared with 0.2 % yeast extract (OD₅₈₀ of 0.16 taken as 100 %). This suggests that yeast extract increases the growth rate of strain AeG^T in the presence of citrate in a dose-dependent manner up to 0.1 %. Strain AeG^T was unable to grow under aerobic conditions in medium D (Brock & Freeze, 1969), medium D amended with citrate (0.2 %) or in aerobic YECit medium.

Substrate utilization tests were performed in LPBS medium supplemented with 0.02 % and 0.1 % yeast extract (YE medium). Soluble substrates were added from sterile anaerobic stock solutions to a final concentration of 0.2 %. Insoluble substrates were weighed directly into Hungate tubes and the medium was dispensed and then sterilized. Growth (OD₅₈₀) was measured three times a day until stationary phase was reached. Cultures amended with citrate were used as positive controls and cultures lacking any additional substrates were used as negative controls. Growth was observed in 0.02 % and 0.1 % YE medium amended with citrate and malate, but not with glucose, galactose, mannose, lactose, cellobiose, rhamnose, xylose, sucrose, ribose, fructose, arabinose, maltose, raffinose, mannitol, inositol, dextrin, starch, pectin, chitin, peptone, glycerol, ethanol, acetate, propionate, succinate, pyruvate, aspartate, formate, Casamino acids or amyl media (Research Achievement).

Sodium thiosulfate (20 mM), sodium sulfate (20 mM), sodium sulfite (10 mM), elemental sulfur (1 %) and sodium nitrate (20 mM) were tested as electron acceptors in YE medium lacking the reductant Na₂S as described by Ramamoorthy *et al.* (2006) and Ogg & Patel (2009a). The reduction of FeCl₃, amorphous iron (III) oxyhydroxide (Lovley & Phillips, 1986) and Mn(IV) (Lovley & Phillips, 1988) were tested at concentrations of 2 g l⁻¹ in PL medium lacking ammonium iron (III) citrate. Fe(III)

Fig. 1. Electron micrographs of cells of strain AeG^T. (a) Negatively stained cell showing curved cell structure and flagella (F); bar, 500 nm. (b) Transmission electron micrograph of a thin section showing cell-wall ultrastructure revealing the presence of a cytoplasmic membrane (CM), an electron-dense wall layer adjacent to the membrane (CWi) and an outer cell-wall layer (CWo); bar, 100 nm.

reduction was determined using the ferrozine method (Sørensen, 1982). For this, 0.1 ml sample was mixed with 3 ml ferrozine reagent and, after 1 min, the absorbance at 562 nm was determined. Mn(IV) reduction was inferred by a clearing of the media and was further confirmed using the leuco crystal violet manganese oxide detection technique (Spratt *et al.*, 1994). In the presence of yeast extract (0.2 %), strain AeG^T was able to reduce thiosulfate and sulfite, but not sulfate, sulfur, nitrate, FeCl₃, amorphous iron (III) oxyhydroxide or Mn(IV). The inability of strain AeG^T to reduce FeCl₃ and amorphous iron (III) oxyhydroxide suggests that citrate is utilized as a carbon source and, therefore, the observed Fe(III) reduction is a by-product of this metabolism. Growth of strain AeG^T in TYECit medium without added ammonium iron (III) citrate supported this conclusion.

Antibiotic sensitivity was determined by adding antibiotics from filter-sterilized stock solutions to sterilized TYECit media to final concentrations of 10 and 100 µg ml⁻¹. Growth inhibition (%) of each antibiotic was calculated from final turbidity measurements of cultures that had been grown in antibiotic-free TYECit medium. Strain AeG^T was completely inhibited (100 % inhibition) in the presence of ampicillin, streptomycin, tetracycline, penicillin and chloramphenicol (all at 10 µg ml⁻¹). To test the effect of NaCl on growth of strain AeG^T, NaCl was weighed directly into Hungate tubes and TYECit medium was dispensed and then sterilized. Strain AeG^T was unable to grow at NaCl concentrations higher than 1 %.

The DNA G + C content of strain AeG^T was determined by the thermal denaturation method (Marmur & Doty, 1962)

in a Cintra20 spectrophotometer (GBC Scientific Equipment) with DNA that had been amplified using a TempliPhi Amplification kit (Amersham Biosciences) essentially as described by Ogg & Patel (2009a). The genomic DNA G + C content was calculated to be 55.4 ± 1.0 mol%.

16S rRNA gene amplification and sequencing were carried out using the method of Ogg & Patel (2009a). Phylogenetic analysis of the consensus 16S rRNA gene sequence comprising 1501 nt indicated that strain AeG^T was a member of the family Veillonellaceae, class 'Clostridia', phylum 'Firmicutes', and was most closely related to *Propionispora vibrioides* DSM 13305^T (90.7 % sequence similarity to type strain) and *Propionispora hippei* DSM 15287^T (90.8 % sequence similarity to type strain) (Fig. 2).

This report describes a modified enrichment strategy aimed at creating a microaerophilic Fe(III)-reducing environment from which a citrate-fermenting bacterium, designated strain AeG^T, was isolated. The isolation of strain 27 from the Mitchell bore sample, which had an almost identical 16S rRNA gene sequence (>99 % similarity) to that of strain AeG^T, suggests that AeG^T is perhaps a dominant citrate reducer and is widely dispersed within the GAB subsurface environment. Strain AeG^T and the two most closely related species based on 16S rRNA gene sequence analysis, i.e. *Propionispora vibrioides* (Abou-Zeid *et al.*, 2004) and *Propionispora hippei* (Biebl *et al.*, 2000), isolated from compost and sewage sludge, respectively, are Gram-negative curved rods that readily form terminal endospores and grow at near-neutral pH levels. However, strain AeG^T could be differentiated from both species of the genus

Fig. 2. A dendrogram showing the phylogenetic position of strain AeG^T and its closest relatives. Bootstrap values >95 % are shown. Bar, 5 nt changes per 100 nt. GenBank accession numbers are given in parentheses. The triangle represents *Sporomusa* species including: *Sporomusa rhizae* DSM 16652^T (GenBank accession no. AM158322); *Sporomusa aerivorans* DSM 13326^T (AJ506191); *Sporomusa ovata* DSM 2662^T (AJ279800); *Sporomusa silvacetica* DSM 10669^T (Y09976); *Sporomusa termitida* DSM 4440^T (M61920); *Sporomusa malonica* DSM 5090^T (AJ279799); *Sporomusa acidovorans* DSM 3132^T (AJ279798); and *Sporomusa sphaeroides* DSM 2875^T (AJ279801).

Table 1. Differential characteristics of strain AeG^T and members of the genus *Propionispora*

Taxa: 1, *Sporolituus thermophilus* sp. nov. AeG^T; 2, *Propionispora hippei* (Biebl *et al.*, 2000); 3, *Propionispora vibrioides* (Abou-Zeid *et al.*, 2004). +, Positive; –, negative; ND, not determined.

Characteristic	1	2	3
Source of isolation	Microbial mats from a thermal aquifer bore outflow	Sewage sludge, from a municipal treatment plant	Compost
Morphology	Curved rods	Vibrio to slightly curved rods	Vibrio to slightly curved rods
Length (µm)	2.0–10.0	2.0–10.0	2.2–6.0
Width (µm)	0.8–1.0	0.6–1.0	0.6
Growth temperature (°C)			
Optimum	55	37	37
Range	45–60	20–50	25–40
Carbohydrate fermentation	–	+	+
Organic acid fermentation	+	ND	–
DNA G + C content (mol%)	55.4*	42.3†	48.5†
Similarity to strain AeG ^T (%)‡	100	90.8	90.7

*Determined by the thermal denaturation method.

†Determined by HPLC.

‡Similarity based on 16S rRNA gene sequence analysis of type strains.

Propionispora by its thermophilic nature, higher DNA G + C content and its inability to ferment substrates other than citrate and malate (Table 1). Based on phenotypic differences and the large phylogenetic distance separating strain AeG^T from other members of the family *Veillonellaceae*, it is proposed that the novel species *Sporolituus thermophilus* gen. nov., sp. nov., should be created to accommodate this newly described strain.

Description of *Sporolituus* gen. nov.

Sporolituus (Spo.ro.li'tu.us. Gr. fem. n. *spora* a seed and, in biology, a spore; L. masc. n. *lituus* a curved rod, crook; N.L. masc. n. *Sporolituus* a spore-forming, curved rod).

Cells are strictly anaerobic, thermophilic, spore-forming, curved rods that stain Gram-negative. Sluggishly motile. Cells possess peritrichous flagella. 16S rRNA gene sequence analysis indicates that the genus is a member of the family *Veillonellaceae* and most closely related to the genus *Propionispora*. The type species is *Sporolituus thermophilus*.

Description of *Sporolituus thermophilus* sp. nov.

Sporolituus thermophilus (ther.mo.phi'lus. Gr. fem. n. *thermê* heat; Gr. adj. *philos* loving; N.L. masc. adj. *thermophilus* heat-loving).

Has the following characteristics in addition to those given for the genus above. Cells are 2.0–10.0 × 0.8–1.0 µm. Growth occurs between 45 and 60 °C (optimum temperature 55 °C) and at pH 6.5–8.0 (optimum pH 7.0). Utilizes citrate and malate, but not yeast extract, tryptone, peptone, glucose, galactose, mannose, lactose, cellobiose, rhamnose, xylose, sucrose, ribose, fructose, arabinose, maltose,

raffinose, mannitol, inositol, dextrin, starch, pectin, chitin, glycerol, ethanol, acetate, propionate, succinate, pyruvate, aspartate, formate, Casamino acids or amyl media. Thiosulfate and sulfite are reduced, but not sulfate, sulfur, nitrate, Fe(III) or Mn(IV). Sensitive to chloramphenicol, streptomycin, tetracycline, penicillin and ampicillin. Sensitive to NaCl concentrations >1 %.

The type strain, AeG^T (=JCM 15556^T=KCTC 5668^T), was isolated from a microbial mat sample from the outflow of a GAB bore (New Lorne Bore) in Queensland, Australia. The genomic DNA G + C content of the type strain is 55.4 ± 1.0 mol%.

References

- Abou-Zeid, D. M., Biebl, H., Spröer, C. & Müller, R. (2004). *Propionispora hippei* sp. nov., a novel Gram-negative, spore-forming anaerobe that produces propionic acid. *Int J Syst Evol Microbiol* **54**, 951–954.
- Andrews, K. T. & Patel, B. K. C. (1996). *Fervidobacterium gondwanense* sp. nov., a new thermophilic anaerobic bacterium isolated from nonvolcanically heated geothermal waters of the Great Artesian Basin of Australia. *Int J Syst Bacteriol* **46**, 265–269.
- Biebl, H., Schwab-Hanisch, H., Spröer, C. & Lünsdorf, H. (2000). *Propionispora vibrioides*, nov. gen., nov. sp., a new gram-negative, spore-forming anaerobe that ferments sugar alcohols. *Arch Microbiol* **174**, 239–247.
- Brock, T. D. & Freeze, H. (1969). *Thermus aquaticus* gen. n. and sp. n., a nonsporulating extreme thermophile. *J Bacteriol* **98**, 289–297.
- Habermehl, M. A. (1980). The Great Artesian Basin, Australia. *BMR J Aust Geol Geophys* **5**, 9–38.
- Haki, G. D. & Rakshit, S. K. (2003). Developments in industrially important thermostable enzymes: a review. *Bioresour Technol* **89**, 17–34.

- Head, I. M., Jones, D. M. & Larter, S. R. (2003). Biological activity in the deep subsurface and the origin of heavy oil. *Nature* **426**, 344–352.
- Kanso, S. & Patel, B. K. C. (2003). *Microvirga subterranea* gen. nov., sp. nov., a moderate thermophile from a deep subsurface Australian thermal aquifer. *Int J Syst Evol Microbiol* **53**, 401–406.
- Kanso, S., Greene, A. C. & Patel, B. K. C. (2002). *Bacillus subterraneus* sp. nov., an iron- and manganese-reducing bacterium from a deep subsurface Australian thermal aquifer. *Int J Syst Evol Microbiol* **52**, 869–874.
- Lazar, I., Petrisor, I. G. & Yen, T. F. (2007). Microbial enhanced oil recovery (MEOR). *Pet Sci Technol* **25**, 1353–1366.
- Love, C. A., Patel, B. K. C., Nichols, P. D. & Stackebrandt, E. (1993). *Desulfotomaculum australicum*, sp. nov., a thermophilic sulfate-reducing bacterium isolated from the Great Artesian Basin of Australia. *Syst Appl Microbiol* **16**, 244–251.
- Lovley, D. R. (2001). Bioremediation. Anaerobes to the rescue. *Science* **293**, 1444–1446.
- Lovley, D. R. & Phillips, E. J. P. (1986). Organic matter mineralization with reduction of ferric iron in anaerobic sediments. *Appl Environ Microbiol* **51**, 683–689.
- Lovley, D. R. & Phillips, E. J. P. (1988). Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Appl Environ Microbiol* **54**, 1472–1480.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Ogg, C. D. & Patel, B. K. C. (2009a). *Caloramator australicus* sp. nov., a thermophilic, anaerobic bacterium from the Great Artesian Basin of Australia. *Int J Syst Evol Microbiol* **59**, 95–101.
- Ogg, C. D. & Patel, B. K. C. (2009b). *Thermotalea metallivorans* gen. nov., sp. nov., a thermophilic, anaerobic bacterium from the Great Artesian Basin of Australia aquifer. *Int J Syst Evol Microbiol* **59**, 964–971.
- Ogg, C. D. & Patel, B. K. C. (2009c). *Fervidicola ferrireducens* gen. nov., sp. nov., a thermophilic anaerobic bacterium from geothermal waters of the Great Artesian Basin, Australia. *Int J Syst Evol Microbiol* **59**, 1100–1107.
- Patel, B. K. C., Morgan, H. W. & Daniel, R. M. (1985a). A simple and efficient method for preparing and dispensing anaerobic media. *Biotechnol Lett* **7**, 277–288.
- Patel, B. K. C., Morgan, H. W. & Daniel, R. M. (1985b). *Fervidobacterium nodosum* gen. nov. and spec. nov., a new chemo-organotrophic, caldoactive, anaerobic bacterium. *Arch Microbiol* **141**, 63–69.
- Ramamoorthy, S., Sass, H., Langner, H., Schumann, P., Kroppenstedt, R. M., Spring, S., Overmann, J. & Rosenzweig, R. F. (2006). *Desulfosporosinus lacus* sp. nov., a sulfate-reducing bacterium isolated from pristine freshwater lake sediments. *Int J Syst Evol Microbiol* **56**, 2729–2736.
- Redburn, A. C. & Patel, B. K. C. (1994). *Desulfovibrio longreachii* sp. nov., a sulfate-reducing bacterium isolated from the Great Artesian Basin of Australia. *FEMS Microbiol Lett* **115**, 33–38.
- Sørensen, J. (1982). Reduction of ferric iron in anaerobic, marine sediment and interaction with reduction of nitrate and sulfate. *Appl Environ Microbiol* **43**, 319–324.
- Spanevello, M. D. (2001). *The phylogeny of prokaryotes associated with Australia's Great Artesian Basin*. PhD thesis, School of Biomolecular and Physical Science, Griffith University, Brisbane, Australia.
- Spanevello, M. D. & Patel, B. K. C. (2004). The phylogenetic diversity of *Thermus* and *Meiothermus* from microbial mats of an Australia subsurface aquifer runoff channel. *FEMS Microbiol Ecol* **50**, 63–73.
- Spanevello, M. D., Yamamoto, H. & Patel, B. K. C. (2002). *Thermaerobacter subterraneus* sp. nov., a novel aerobic bacterium from the Great Artesian Basin of Australia, and emendation of the genus *Thermaerobacter*. *Int J Syst Bacteriol* **52**, 795–800.
- Spratt, H. G., Jr, Siekmann, E. C. & Hodson, R. E. (1994). Microbial manganese oxidation in salt marsh surface sediments using a leuco crystal violet manganese oxide detection technique. *Est Coast Shelf Sci* **38**, 91–112.
- Wolin, E. A., Wolin, M. J. & Wolfe, R. S. (1963). Formation of methane by bacterial extracts. *J Biol Chem* **238**, 2882–2886.
- Zeikus, J. G., Hegge, P. W. & Anderson, M. A. (1979). *Thermoanaerobium brockii* gen. nov. and sp. nov., a new chemoorganotrophic, caldoactive, anaerobic bacterium. *Arch Microbiol* **122**, 41–48.