Caloramator mitchellensis sp. nov., a thermoanaerobe isolated from the geothermal waters of the Great Artesian Basin of Australia, and emended description of the genus Caloramator

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A strictly thermophilic anaerobe, designated strain VF08T, was isolated from a water sample collected from a Great Artesian Basin bore (registered bore number 22981) situated at Mitchell, QLD, Australia. Cells of isolate VF08T were slightly curved, non-sporulating rods (1.5–3.5×0.4–0.8 μm), which stained Gram-negative but possessed a Gram-positive cell-wall ultrastructure. The strain grew optimally in tryptone-yeast extract-glucose (TYEG) medium at 55 °C (temperature growth range between 37 and 60 °C) and a pH of 7 (pH growth range, 6.0–9.0). Yeast extract or tryptone was required for growth on glucose, fructose, xylose, maltose, sucrose, raffinose, cellobiose, ribose, pyruvate, tryptone, peptone, Casamino acids, amyl media and serine, but could also support growth as the sole carbon source. End products from glucose fermentation were acetate, ethanol, CO2 and H2. The strain reduced vanadium(V), but not iron(III), manganese(IV), elemental sulfur, sulfate, thiosulfate, sulfite, nitrate or nitrite in the presence of 0.2 % yeast extract, peptone, tryptone, glucose, sucrose and Casamino acids, but an increase in the growth rate or cell yield was not observed. Growth was inhibited by chloramphenicol, streptomycin, tetracycline, penicillin, ampicillin and ≤2 % NaCl (w/v). The G+C content of the DNA was 38.4±0.8 mol% as determined by the thermal denaturation (Tm) method. 16S rRNA gene sequence analysis revealed that isolate VF08T was a member of the genus Caloramator with Caloramator australicus and Caloramator fervidus (formerly Clostridium fervidus) being the closest relatives with similarity values of 85.0 and 86.1 %, respectively, when helix 6 nucleotides were included in the analysis, and 95.2 % and 94 %, respectively, when these nucleotides were masked from the analysis. Further analysis revealed that strain VF08T formed an individual cluster (cluster II) within the genus Caloramator and could be distinguished from other species within the genus Caloramator (clusters I, III and IV) on the basis of signature nucleotides and differences in phenotypic traits. These data suggest that strain VF08T is a novel species of the genus Caloramator, for which the name Caloramator mitchellensis sp. nov. is proposed. The type strain is VF08T (=JCM 15828T=KCTC 5735T). An emended description of the genus Caloramator is also provided.

The Great Artesian Basin (GAB) underlies more than one-fifth of the Australian continent, an area of over 1.7×106 km2. With a water storage capacity estimated to be 8.7×1012 m3, the GAB is considered to be the world’s largest geothermal subsurface aquifer (Habermehl, 1980). A thriving microbial community spanning the entire spectrum of the domains Bacteria and Archaea has been reported from culture-independent studies of the GAB environment (Spanevello, 2001; Kimura et al., 2005) and many new taxa of thermophilic and mesophilic strict aerobes (Kanso & Patel, 2003; Spanevello et al., 2002), sulfate-reducers (Love et al., 1993; Redburn & Patel, 1994), metal-reducers (Kanso et al., 2002; Ogg & Patel, 2009a, b, c; Ogg et al., 2010) and fermenters (Andrews & Patel, 1996; Ogg & Patel, 2009d) have also been isolated. Our recent studies have focussed on thermophiles that reduce the transition metal vanadium, a contaminant widely distributed in the Earth’s crust and aquatic environments (Rehder, 1992). Vanadium can exist in oxidation states ranging from −2 to +5, though at neutral pH vanadium commonly exists as V(V) (vanadate) and V(IV) (vanadyl),

Abbreviations: GAB, Great Artesian Basin; LPBS, low phosphate buffered salts; TYEG, tryptone-yeast extract-glucose.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain VF08T is GU723692.

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of which the former is the most toxic and mobile form (Ortiz-Bernad et al., 2004). Vanadium(V)-reducing microbes that have been reported to date include species of the genus *Pseudomonas* (Lyalkova & Yurkova, 1992) and the metal-reducing organisms *Shewanella oneidensis* (Carpentier et al., 2003) and *Geobacter metallireducens* (Ortiz-Bernad et al., 2004). In this paper, we describe a strictly anaerobic, thermophilic bacterium designated strain VF08<sup>T</sup>, which reduces vanadium(V) and represents a novel species of the genus *Caloramator*. The presence of the sporulation gene spo0A in the members of the genus *Caloramator* is also discussed and we propose the division of the genus *Caloramator* into four subclusters based on 16S rRNA gene signature nucleotides.

Strain VF08<sup>T</sup> was isolated from a bore-water sample collected at the source of a free-flowing GAB bore (registered number 22981), situated in Mitchell, a town located approximately 570 km west-north-west of Brisbane, QLD, Australia. The bore well was 403 m deep and had a temperature of 46 °C at the bore source. Sulfate-reducers, carbohydrate-fermenters and species of the genus *Meiothermus* have previously been isolated from this bore sample (unpublished data). The bore-water sample was collected directly in sterile glass vessels which were filled to the brim, the vessels were then capped, transported to the laboratory and stored at room temperature until used.

Enrichment and isolation were performed in a modified anaerobic Patel Laboratory (PL) medium, which contained (L<sup>-1</sup> deionized water): 1 g NH<sub>4</sub>Cl, 0.6 g K<sub>2</sub>HPO<sub>4</sub>, 0.3 g KH<sub>2</sub>PO<sub>4</sub>, 0.1 g MgCl<sub>2</sub>, 0.1 g NaCl, 12.0 g HEPES and 0.2 g yeast extract. The pH of the medium was adjusted to 7.2 and the medium was heated in an autoclave at 121 °C and 1–1.5 kg cm<sup>-2</sup> pressure for 10 min to remove as much dissolved oxygen as possible. The medium was subsequently cooled under a stream of oxygen-free nitrogen gas and 1 ml each of a vitamin solution (Wolin et al., 1963) and a trace-element solution (Zeikus et al., 1979) were added per litre of medium. The medium was then dispensed into Hungate tubes under oxygen-free nitrogen gas and sterilized for 20 min at 121 °C and 1–1.5 kg cm<sup>-2</sup> pressure. After sterilization, vanadium(V) was added from a filter-sterilized 200 mM anaerobic stock solution to a final concentration of 4 mM. When vanadium(V) was added, this medium was designated V(V)-PL medium.

Enrichments were performed in 96 deep-well (1.2 ml) U-bottom plates (Sarstedt) that contained different growth substrates in each well. For this, 20 μl organic acids and aromatic compounds were dispensed from sterile stock solutions. The substrates were dried in the plates at 50 °C for 1 h and the plates stored inside an anaerobic chamber for at least 24 h to remove oxygen. Enrichments were initiated by dispensing a 1 ml aliquot of an inoculum that had been prepared by mixing V(V)-PL medium and a water sample from the Mitchell bore (RN 22981A) (90:10 v/v) into each well. Thus, the final concentration of organic acids was 0.2% and that of the aromatic compounds was between 1 and 4 mM. The plates were sealed, placed inside an anaerobic jar and incubated at 50 °C. After 3 days incubation, a number of wells had turned from a clear to a green colour, suggestive of vanadium(V)-reduction, and were scored as enrichment positive. At this point, the plate was removed from the anaerobic jar and cooled inside an anaerobic chamber for 1 h. All positive enrichment wells were subcultured into Hungate tubes containing 9 ml V(V)-PL medium amended with the corresponding electron donor. In this manuscript, the results from a culture isolated from a well (well F08), containing the substrate 3-hydroxybenzoate (4 mM) are reported. This culture was subcultured and a light green colour was observed after 5 days incubation at 50 °C. This culture was serially diluted, incubated under the same conditions and the tube containing the lowest serial dilution (10<sup>-3</sup>) selected. The procedure for isolation was repeated twice before a strain designated VF08<sup>T</sup> was regarded as a pure culture. Culture purity was subsequently confirmed by microscopy, subcultures onto substrates including glucose and repeat 16S rRNA gene sequencing. A pure culture was preserved in a glycerol-V(V)-PL medium (50:50) mixture at −20 °C.

Cell morphology of strain VF08<sup>T</sup> was determined by phase-contrast microscopy and by electron microscopy (Kanso & Patel, 2003). Cells of strain VF08<sup>T</sup> were peritrichously flagellated, slightly curved rods (1.5–3.5 x 0.4–0.8 μm) which existed singly, in pairs and in short chains. Cells of strain VF08<sup>T</sup> stained Gram-negative but electron micrographs of thin sections revealed a cell wall composed of a single electron-dense peptidoglycan layer adjacent to electron-light protein subunits that were typical of a Gram-positive cell-wall ultrastructure.

The presence of the spo0A sporulation gene in strain VF08<sup>T</sup>, *Caloramator proteoclasticus* DSM 10124<sup>T</sup>, *Caloramator coolhaasii* DSM 12679<sup>T</sup>, *Caloramator viterbiensis* DSM 13723<sup>T</sup>, *Caloramator australicus* KCTC 5601<sup>T</sup> and *Caloramator fervidus* ATCC 43204<sup>T</sup> was detected with a forward primer GGI(A/G)TICCICICA(C/T)AT(A/T/C)AA(A/G)GGITA and a reverse primer CATIGC(G/A/T)AT(A/G)AA(C/T)TCIG(G/C)(A/T)(A/G)TGTIGGC(T/C)TT (Brown et al., 1994) using the method described by Ogg & Patel (2009a). *Caloramator proteoclasticus* DSM 10124<sup>T</sup>, *Caloramator coolhaasii* DSM 12679<sup>T</sup> and *Caloramator viterbiensis* DSM 13723<sup>T</sup> were purchased from the DSMZ, *Caloramator fervidus* ATCC 43204<sup>T</sup> was supplied by Professor Hugh Morgan, Waikato University, Hamilton, New Zealand, and *Caloramator australicus* KCTC 5601<sup>T</sup> was obtained from our own culture collection, the Microbial Gene Research and Resources Facility. These primers are known to amplify the spo0A gene in members of family *Clostridiaceae* including *Thermobrachium celere* DSM 8682<sup>T</sup> (Onyenwoke et al., 2004) [Wiegel (2009) suggested that this species should be reclassified as ‘*Caloramator celere*’]. As expected, a fragment of approximately 270 nt was amplified from all six strains. Sequencing and subsequent sequence analysis using tblastx confirmed that the amplified
products were spo0A genes. Further analysis revealed that the closest matches for the putative spo0A gene from strain VF08T were the spo0A gene sequences from Clostridium tetani E88 (GenBank accession no. AE015927) and Clostridium novyi strain NT (CP000382) with similarity values of 80.3 and 79.1 %, respectively. However, microscopic examination of cultures of strain VF08T that had been grown under suboptimal conditions (pH and temperature), or in the presence of 0.2 % glucose or 0.2 % xylose, and of a culture that had been grown to stationary phase in the presence of 0.2 % glucose and subsequently exposed to 4 °C for 3 days, failed to reveal the presence of spores. Furthermore, cells of strain VF08T heated at 95 °C for 10 min failed to subculture in TYEG medium, suggesting the absence of heat-resistant bodies such as spores. The detection of spo0A genes in Caloramator fervidus DSM 43204T, Caloramator proteoclasticus DSM 10124T and T. celere DSM 8682T confirmed previous reports of the presence of spores in these cultures. However, the lack of congruence between the phenotypic characteristic in which spores were absent and molecular data in which spore genes were detected for strain VF08T, Caloramator coohaasi DSM 12679T, Caloramator australicus KCTC 5601T and Caloramator viterbiensis DSM 13723T was not surprising as the presence of spore-specific genes has also been reported for members of the genera Streptococcus, Enterococcus, Lactococcus and Listeria but none of these are known to produce endospores (Onyenwoke et al., 2004).

Unless otherwise indicated, all growth experiments were conducted at least twice and cultures were subcultured at least once in the same medium prior to conducting the experiments. Growth studies were performed in anaerobic TYEG medium which consisted of low phosphate buffered salts (LPBS) amended with 0.2 % each of tryptone, yeast extract and glucose. TYEG medium was prepared anaerobically by adding glucose to LB medium (0.2 % yeast extract and 0.2 % glucose) with growth reaching 65 % compared with growth in TYEG. When the yeast extract concentration in YEG medium was lowered to 0.1 and 0.05 %, growth of approximately 22 % and 19 %, respectively, was obtained, suggesting that yeast extract increased glucose utilization in a dose-dependent manner. In addition, growth was not observed in LPBS medium containing only 0.2 % glucose as the sole carbon source, confirming that yeast extract and/or tryptone were required for growth on glucose. End products from glucose fermentation were determined in a culture grown in YEG medium as previously described (Chrisostomos et al., 1996; Ogg & Patel, 2009a) and were identified as acetate, ethanol, CO₂ and H₂.

Substrate utilization tests were performed in LPBS medium supplemented with 0.1 % yeast extract. For this, substrates were added from sterile anaerobic stock solutions to a final concentration of 0.2 % with the exception of amino acids, which were tested at a final concentration of 5 mM. Growth was measured hourly at OD₅₈₀ until stationary phase was reached or for up to 4 days when no visible turbidity was observed. Growth was observed with glucose, fructose, xylose, maltose, sucrose, raffinose, cellobiose, ribose, pyruvate, tryptone, peptone, Casamino acids, amy media and serine, but not with galactose, mannose, mannitol, inositol, ethanol, glycerol, acetate, lactate, propionate, citrate, valine, arginine, alanine, glutamate, isoleucine or methionine. The ability of strain VF08T, Caloramator proteoclasticus DSM 10124T, Caloramator coohaasi DSM 12679T, Caloramator australicus KCTC 5601T and Caloramator fervidus DSM 43204T and Caloramator viterbiensis DSM 13723T to use various electron acceptors was tested. For this, sodium thiosulfate (20 mM), sodium sulfate (20 mM), sodium sulfite (5 mM), elemental sulfur (1 %), sodium nitrate (20 mM) and sodium nitrite (5 mM) were tested in YE medium as described by Ramamoorthy et al. (2006) and Ogg & Patel (2009a). The reduction of 0.2 % soluble ammonium Fe(III) citrate, 0.3 % amorphous Fe(III) oxyhydroxide (Lovley & Phillips, 1986) and 0.2 % amorphous Mn(IV) dioxide (Lovley & Phillips, 1988) was tested in PL medium amended with yeast extract (0.2 %) as the electron donor. Vanadium(V) reduction was determined in V(V)-PL medium amended with an additional amount of yeast extract (0.2 %). Cultures were incubated at optimum temperatures for growth for up to 14 days. Fe(III)-reduction was inferred if the reddish-brown colour of the Fe(III) was transformed to a dark precipitate [Fe(III)] and a clearing of the medium was observed. Reduction was further confirmed by using the ferrozine method (Sørensen, 1982). 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). Vanadium(V)-reduction was inferred when the colourless medium changed to green and/or a
green precipitate was formed. Vanadium(V)-reduction was confirmed using the vanadate assay as described by Carpentier et al. (2003). For this, 250 μl 1 % (w/v) diphenylcarbazide in acetone was added to 250 μl 2 M H2SO4 and 500 μl diluted sample was added. The absorbance was measured at 320 nm after 15 min. The analysis revealed that strain VF08T reduced vanadium(V) only, and did not reduce Fe(III), manganese(IV), elemental sulfur, sulfate, thiosulfate, sulfite, nitrate or nitrite. The analysis also revealed that the range of electron acceptors used was species-specific and therefore could be used as a phenotypic tool for the differentiation of species of the genus Caloramator (Table 1).

The reduction of vanadium(V) in the presence of a range of electron donors was also examined for strain VF08T using the method described above. Vanadium(V) was not reduced in the control uninoculated media but a slow rate of reduction was noted in media containing trace amounts of yeast extract (0.02 %). Strong vanadium(V)-reduction was observed in the presence of yeast extract, peptone, tryptone, glucose, sucrose and Casamino acids, but not with glycerol, ethanol, acetate, propionate, lactate, pyruvate or the aromatic compounds (4 mM) benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, 2-methoxybenzoate and 3-chlorobenzoate (2 mM). These findings suggest that the vanadium(V)-reduction detected in the primary enrichment culture and subsequent subcultures was probably due to the trace concentrations of yeast extract (0.02 %) rather than 3-hydroxybenzoate (4 mM) acting as an electron donor.

The effect of vanadium(V) on the growth of strain VF08T was tested in V(V)-PL medium amended with 0.2 % yeast extract, and was compared with the growth of the strain in the same medium minus vanadium(V). Cell numbers were determined periodically over a four day period by the most-probable-number technique (Greenberg et al., 1992) using TYEG medium. Results from experiments conducted in triplicate showed that the reduction of vanadium(V) (up to 5 mM) did not increase the biomass or the growth rate of strain VF08T, suggesting that it was not involved in energy generation. To the authors’ knowledge, strain VF08T therefore represents the first species of the genus Caloramator that is known to reduce vanadium(V) but in which vanadium(V) is not required as an obligate respiratory electron acceptor for growth. Our results are similar to the report of Ledbetter et al. (2007) in which they showed that Caloramator strain YeAs, an isolate from a terrestrial hydrothermal system, reduced arsenate(V), but this was not obligately required for growth. Interestingly, neither of

Table 1. The reduction of vanadium(V), Fe(III) and Mn(IV) by strain VF08T and various members of the genus Caloramator in the presence of 0.2 % yeast extract

<table>
<thead>
<tr>
<th>Electron acceptor</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>V(V)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Fe(III) oxhydroxide</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Fe(III) citrate</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Mn(IV)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

*Determined by Ogg & Patel (2009a).*
the nearest phylogenetic neighbours of strain YeAs, *Thermobrachium celere* and *Caloramator coolhaasii* reduce arsenate (Ledbetter et al., 2007). Thus, phylogenetic relatedness is a poor predictor of metabolic metal-transforming capabilities in species of the genus *Caloramator*.

Antibiotic sensitivity was determined by adding antibiotics from filter-sterilized stock solutions to sterilized TYEG media to a final concentration of 10 or 100 μg ml⁻¹. Strain VF08ᵀ was completely inhibited in the presence of 10 μg ml⁻¹ ampicillin, streptomycin, tetracycline, penicillin and chloramphenicol. The effect of NaCl on the growth of strain VF08ᵀ was tested in TYEG medium. For this, NaCl was weighed directly into the Hungate tube, the medium was then dispensed and sterilized. Strain VF08ᵀ was unable to grow at NaCl concentrations of ≥ 2 % (w/v).

High molecular weight DNA of strain VF08ᵀ was amplified as described by Ogg & Patel (2009a) using a TempliPhi Amplification kit (Amersham Biosciences) and the DNA G+C content was determined by the thermal denaturation method (Marmur & Doty, 1962) in a Cintra20 spectrophotometer (GBC Scientific Equipment). The G+C content of the genomic DNA was calculated to be 38.4 ± 0.9 mol% (mean ± SD from three determinations).

16S rRNA gene amplification, sequencing and analysis were performed using the method of Ogg & Patel (2009a). Phylogenetic analysis of the 1605 nt 16S rRNA gene sequence indicated that strain VF08ᵀ was a member of the genus *Caloramator*, family *Clostridiaceae*, class *Clostridia*, phylum *Firmicutes*, with the closest relatives being *Caloramator australicus* and *Caloramator fervidus* (Fig. 1). Further analysis revealed that strain VF08ᵀ possessed a 158 nt-long helix 6, as opposed to 43 nt in *Caloramator australicus* and 45 nt in *Caloramator fervidus* (*Escherichia coli* positions 61–106 according to Winker & Woese, 1991) (Fig. 2). The 16S rRNA gene sequence similarity values between strain VF08ᵀ and *Caloramator australicus* and *Caloramator fervidus* were 85.0 % and 86.1 % when the helix 6 nucleotides were included in the analysis. When the helix 6 nucleotides were masked from the analysis, the values were 95.2 % and 94 %, respectively.

Further comparisons of the 16S rRNA gene sequences supported the division of the genus *Caloramator* into four subclusters: subcluster I contained *Thermobrachium celere* DSM 8682ᵀ (GenBank accession no. X99238), *Caloramator indicus* ACM 3982ᵀ (X75788), *Caloramator uzoniensis* JW/VK-KU2 (AF489534), *Caloramator proteoelasticus* DSM 10124ᵀ (X90488) and *Caloramator coolhaasii* DSM 12679ᵀ (AF104215); subcluster II contained strain VF08ᵀ; subcluster III contained *Caloramator australicus* KCTC 5601ᵀ (EU409943) and *Caloramator fervidus* ATCC 43204ᵀ (L09187), and subcluster IV contained *Caloramator viterbiensis* DSM 13723ᵀ (AF181848) (Tables 2 and 3). In addition, the significant phylogenetic differences observed in the helix 6, 11 and 18 sequences [ *E. coli* helix numbering according to Neefs et al. (1993)] amongst members of the genus *Caloramator* also supported the inclusion of strain VF08ᵀ as a distinct species of the genus *Caloramator* and as the lone member of subcluster II.
In this paper, it is concluded that members of the genus *Caloramator* can be differentiated phylogenetically by their nucleotide signatures and helix structures (Tables 2 and 3), and on the basis of their ability to reduce the electron acceptors V(V), Fe(III) oxyhydroxide, Fe(III) citrate and Mn(IV) (Table 1). In addition, strain VF08T could also be differentiated from its nearest phylogenetic neighbours, *Caloramator australicus* KCTC 5601T and *Caloramator fervidus* ATCC 43204T, by its sensitivity to heat shock, growth temperature optima and range, DNA G+C content, the substrate utilization profile and its inability to reduce elemental sulfur (Table 4). Furthermore, strain VF08T could be differentiated from *Caloramator australicus* as it stained Gram-negative, was able to ferment serine but not galactose and was unable to reduce Fe(III) or Mn(IV). Strain VF08T could be differentiated from *Caloramator fervidus* by its inability to form spores and its ability to ferment maltose and Casamino acids. Based on the phenotypic and phylogenetic differences that distinguished strain VF08T from *Caloramator australicus*, *Caloramator fervidus* and other members of the genus *Caloramator*, it is suggested that strain VF08T represents a novel species in this genus for which the name *Caloramator mitchellensis* sp. nov. is proposed.

### Emended description of the genus *Caloramator* Collins et al. 1994

The description of the genus *Caloramator* (Collins et al., 1994; Baena & Patel, 2009) is emended as follows. Vanadium(V), Fe(III), Mn(IV) and elemental sulfur may or may not be reduced.

### Emended description of *Caloramator fervidus* (Patel et al. 1987) Collins et al. 1994

The description of *Caloramator fervidus* (Patel et al., 1987) is emended as follows. Ribose is not fermented. Elemental sulfur, but not vanadium(V), Fe(III), Mn(IV), sulfate, thiosulfate, sulfate, nitrate and nitrite, are reduced in the presence of 0.2 % yeast extract.

### Emended description of *Caloramator proteoclasticus* Tarlera et al. 1997

The description of *Caloramator proteoclasticus* (Tarlera et al., 1997) is emended as follows. Vanadium(V), soluble Fe(III) citrate, amorphous Fe(III) oxyhydroxide and Mn(IV) are reduced in the presence of 0.2 % yeast extract.

#### Table 2. 16S rRNA signature nucleotides in the proposed *Caloramator* subclusters I–IV

Subcluster I is composed of *Thermobrachium celere* DSM 8682T (X99238), *C. indicus* ACM 3982T (X75788), ‘*C. uzoniensis*’ JW/VK-KU2 (AF489534), *C. proteoclasticus* DSM 10124T (X90488) and *C. coolhaasii* DSM 12679T (AF104215); subcluster II is composed of strain VF08T (GU723692); subcluster III is composed of *C. australicus* KCTC 5601T (EU409943) and *C. fervidus* ATCC 43204T (L09187); and subcluster IV is composed of *C. viterbiensis* DSM 13723T (AF181848). Pur, purine; Pyr, pyrimidine.

<table>
<thead>
<tr>
<th>E. coli positions*</th>
<th>E. coli positions*</th>
<th>Signature nucleotide(s) in the genus <em>Caloramator</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of nucleotides in helix 6† (61–106)†</td>
<td>45</td>
<td>Subcluster I (n=5)</td>
</tr>
<tr>
<td>Helix 11† (198–219)$</td>
<td>43$</td>
<td>U–A</td>
</tr>
<tr>
<td>1262</td>
<td>C</td>
<td>Pur</td>
</tr>
<tr>
<td>1278</td>
<td>C</td>
<td>Pyr</td>
</tr>
<tr>
<td>1285 and 1285.2</td>
<td>C</td>
<td>CA</td>
</tr>
<tr>
<td>1436</td>
<td>U</td>
<td>C</td>
</tr>
</tbody>
</table>

*E. coli* numbering according to Winkler & Woese (1991).
†E. coli helix numbering according to Neefs et al. (1993).
§Refer to Fig. 2.
||Analysis does not include *T. celere* as the type strain has been only sequenced between *E. coli* nucleotide positions 98 and 1469 (Engle et al., 1996).

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[Table 2. 16S rRNA signature nucleotides in the proposed *Caloramator* subclusters I–IV](#)
Emended description of *Caloramator coolhaasii* Plugge *et al.* 2000

The description of *Caloramator coolhaasii* (Plugge *et al.*, 2000) is emended as follows. Vanadium(V) and soluble Fe(III) citrate, but not amorphous Fe(III) oxyhydroxide, arsenate and Mn(IV), are reduced in the presence of 0.2 % yeast extract.

Emended description of *Caloramator viterbiensis* Seyfried *et al.* 2002

The description of *Caloramator viterbiensis* (Seyfried *et al.*, 2002) is emended as follows. Amorphous Fe(III) oxyhydroxide and Mn(IV), but not vanadium(V) or soluble Fe(III) citrate, are reduced in the presence of 0.2 % yeast extract.

Emended description of *Caloramator australicus* Ogg and Patel 2009

The description of *Caloramator australicus* (Ogg & Patel, 2009a) is emended as follows. Casamino acids are fermented. Vanadium(V) is reduced in the presence of 0.2 % yeast extract.

Description of *Caloramator mitchellensis* sp. nov.

*Caloramator mitchellensis* (mit.chell.en’sis. N.L. masc. adj. *mitchellensis* pertaining to the Mitchell bore in QLD, Australia, from which the strain was isolated).

Table 3. 16S rRNA gene sequences of helix 11 (*E. coli* positions 198–219) and helix 18 (*E. coli* positions 451–482) within the genus *Caloramator*

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Helix 11 (<em>E. coli</em> positions 198–219)</th>
<th>Helix 18 (<em>E. coli</em> positions 451–482)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>GAGGGGGA CCU U CGG GCCUCU</td>
<td>U AAGGGAGTAAAGTTA ATA CCTTGCTCAT TGA</td>
</tr>
<tr>
<td>Subcluster I</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. celere</em> DSM 8682&lt;sup&gt;T&lt;/sup&gt; (X99238)</td>
<td>GGGGUCUUAGCCA A AGG UUAUU</td>
<td>A TAA TGA</td>
</tr>
<tr>
<td><em>C. indicus</em> ACM 3982&lt;sup&gt;T&lt;/sup&gt; (X75788)</td>
<td>GGGGUCUUAGCCA A AGG UUAUU</td>
<td>A TAA TGA</td>
</tr>
<tr>
<td><em>C. uzoniensis</em> JW/VK-KU2 (AF489534)</td>
<td>GGGGUCUUAGCCA A AGG UUAUU</td>
<td>A TAA TGA</td>
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<td><em>C. proteoclasticus</em> DSM 10124&lt;sup&gt;T&lt;/sup&gt; (X90488)</td>
<td>GGGGUCUUAGCCA A AGG UUAUU</td>
<td>A TAA TGA</td>
</tr>
<tr>
<td><em>C. coolhaasii</em> DSM 12679&lt;sup&gt;T&lt;/sup&gt; (AF104215)</td>
<td>GGGGUCUUAGCCA A AGG UUAUU</td>
<td>A TAA TGA</td>
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<tr>
<td>Subcluster I consensus sequence</td>
<td>GGGGUCUUAGCCAAPurGG UUA*A&lt;sup&gt;†&lt;/sup&gt;U&lt;sup&gt;†&lt;/sup&gt;</td>
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<tr>
<td>Subcluster II</td>
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<tr>
<td>Strain VF08&lt;sup&gt;T&lt;/sup&gt; (GU723692)</td>
<td>GGGAUGAUAGCC AAAAGG AGAAA</td>
<td>U AAG AGA TGA</td>
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<td>Subcluster III</td>
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<tr>
<td><em>C. australicus</em> KCTC 5601&lt;sup&gt;T&lt;/sup&gt; (EU409943)</td>
<td>GAUAACAUAGCCA A AGG AGGA</td>
<td>U AAG AGA TGA</td>
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<tr>
<td><em>C. fervidus</em> ATCC 43204&lt;sup&gt;T&lt;/sup&gt; (L09187)</td>
<td>GCUCUUGGUAGCCA A AGG AGGCAUAGCU AAG</td>
<td>GAAG TGA</td>
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<td>Subcluster IV</td>
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<tr>
<td><em>C. viterbiensis</em> DSM 13723&lt;sup&gt;T&lt;/sup&gt; (AF181848)</td>
<td>GAGGCGUUAGCCA A AGG AGCAA</td>
<td>U A TAA TGA</td>
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The type strain, VF08\(^T\) (JCM 15828\(^T\) = KCTC 5735\(^T\)), was isolated from a water sample from the outflow of a GAB bore (the Mitchell Bore) in Queensland, Australia. The genomic DNA G+C content of the type strain is 38.4 ± 0.9 mol%.

### References


