Caloramator australicus sp. nov., a thermophilic, anaerobic bacterium from the Great Artesian Basin of Australia

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A strictly anaerobic, thermophilic bacterium, designated strain RC3T, was isolated from microbial mats colonizing thermal waters of a run-off channel formed by free-flowing waters from a bore well (registered no. 17263) of the Great Artesian Basin, Australia. The slightly curved rods (2.5–4.2×0.8–1.0 μm) of strain RC3T stained Gram-positive and grew optimally in tryptone-yeast extract-glucose medium at 60 °C (range 45–70 °C) and pH 7 (range pH 5–9). Strain RC3T grew poorly on yeast extract (0.2 %) but did not grow on tryptone (0.2 %) as a sole carbon source; yeast extract was required for growth on other energy sources, which included glucose, fructose, galactose, xylose, maltose, sucrose, raffinose, mannose, cellobiose, cellulose, starch, amylopectin, xylan, peptone, amyl media (Research Achievement), threonine and pyruvate but did not include arabinose, ribose, lactose, CM-cellulose, myo-inositol, mannitol, chitin, casein, formate, acetate, succinate, propionate, lactate, benzoate, glycerol, ethanol, Casamino acids, arginine, alanine, serine, glycine, glutamine, leucine, isoleucine, methionine or asparagine. The end products of glucose fermentation were ethanol and acetate. In the presence of 0.2 % yeast extract, iron(III), manganese(IV) and elemental sulfur were reduced but not sulfate, sulfite, thiosulfate, nitrate or nitrite. Iron(III) was also reduced in the presence of peptone, tryptone, amyl media, threonine and glycerol but not chitin, xylan, pectin, starch, pyruvate, acetate, benzoate, lactate, propionate, succinate, inositol, ethanol, mannitol, arginine, glutamine or serine. Strain RC3T was not able to utilize molecular hydrogen and/or carbon dioxide in the presence or absence of iron(III). In the presence of iron(III) and glycerol, increased concentrations of Fe(II) corresponded to increased cell numbers, demonstrating that strain RC3T was able to conserve energy to support growth from the reduction of Fe(III) to Fe(II). Chloramphenicol, streptomycin, tetracycline, penicillin and ampicillin and NaCl concentrations greater than 2 % inhibited growth. The G+C content of the DNA was 34±1 mol% as determined by the thermal denaturation (Tm) method. 16S rRNA gene sequence analysis indicated that strain RC3T was affiliated to Caloramator fervidus (95.8 % similarity to the type strain) and to other Caloramator species (average similarity of 91.6 %) within the phylum Firmicutes. On the basis of phylogenetic and phenotypic characteristics, it is proposed that strain RC3T should be classified in the genus Caloramator as a representative of a novel species, Caloramator australicus sp. nov. The type strain is RC3T (=JCM 1508T =KCTC 5601T).

The Great Artesian Basin (GAB) is the world’s largest subsurface aquifer, underlying approximately one-fifth of the subarid regions of the Australian continent and covering an area of over 1.7×10⁶ km², with a water-storage capacity of 8.7×10¹² m³ (Habermahl, 1980). The GAB provides a vital water resource for rural semi-arid communities and also contains the largest onshore oil and gas reserves in Australia. The GAB is composed of alternating layers of water-bearing permeable sandstone and non-water-bearing impermeable shale. These geological formations have an immense influence on the chemical composition of GAB groundwaters, which can be bicarbonate-, chloride-, sulfate- or iron-rich. The depth of the aquifer is estimated to be 3000 m and the underground water flow from the recharge areas at the edge of the basin to the discharge areas in central Australia as mound springs is estimated to be 1–5 m year⁻¹. The water is heated by the Earth’s magma due to its depth, and the age of the water is calculated to be over 2 million years. Not only do more than 5000 free-
flowing bores, with source temperatures ranging between 100 and 30 °C, depending on bore depth, provide an important water resource to the outback communities, but the GAB is also a conducive environment for the growth of a wide diversity of microbial life. Distinct thriving macroscopic microbial mat communities can be seen colonizing specific temperatures along the temperature gradient of run-off channels formed by the free-flowing bores. A range of thermophilic and mesophilic microorganisms have been characterized from the GAB waters and include sulfate-reducers (Love et al., 1993; Redburn & Patel, 1994), carbohydrate-fermenters (Andrews & Patel, 1996), strict aerobes (Kanso & Patel, 2003; Spanevello et al., 2002) and dissimilatory iron(III)-reducing microorganisms (DIRM) (Kanso et al., 2002). DIRM are known to have a significant impact on the geochemistry of deep subsurface aquifers through their cycling of metals and organic matter, and thereby influence water quality and taste (Lovley, 1997). Our finding of DIRM (Kanso et al., 2002) in the GAB environment has led to the concern that their colonization of, and growth on, the metal casing of bores could lead to corrosion, causing significant water leakage and therefore economic loss and hardship to communities that rely on aquifer waters.

In this paper, we describe a novel enrichment strategy performed under thermophilic conditions for the enrichment and identification of DIRM. For this, we have used Biolog MicroPlates amended with Fe(III). The Biolog system was originally developed as a diagnostic tool to identify bacterial isolates based on sole-carbon-source utilization. The Biolog MicroPlate consists of 95 different substrates each contained in a separate well (and a control containing no substrate), to which a minimal growth medium and inoculum are added. The wells also contain the redox dye tetrazolium violet, which turns purple in the presence of electron transfer-conferring substrate utilization. The use of Biolog MicroPlates amended with soluble Fe(III) and trace amounts of yeast extract allows the simultaneous enrichment of Fe(III) on 95 different substrates. In this report, we describe a novel strictly anaerobic, thermophilic DIRM that inhabits a microbial mat of a GAB bore run-off channel, designated strain RC3\textsuperscript{T}. Strain RC3\textsuperscript{T} represents a novel species of the genus Caloramator.

The microbial mat samples used in this study were collected from the run-off channel of the New Lorne bore (registered bore no. 17263) situated near Blackall, some 1000 km north-west of Brisbane, Queensland (24° 54’ 48” S 145° 08’ 18” E). The bore, drilled in 1966, is 1613 m deep, and the temperature of the free-flowing water at the bore head was 88 °C, with a pH of 8.5 and a flow rate of 7.6 l s\textsuperscript{-1} at the time of sampling. The iron concentration of the free-flowing water was 0.01 mg l\textsuperscript{-1}. Red-coloured microbial mats that thrived at 66 °C were collected in sterile glass vessels and the vessels were filled to the brim with water from the run-off channel, capped, transported to the laboratory and stored at 4 °C until use. The microbial mats have been described by Spanevello et al. (2002).

Enrichment was performed by inoculating wells of Biolog GN2 MicroPlates with 100 μl red mat suspension that had been prepared by mixing 0.2 ml mat sample in 10 ml inoculating fluid (IF) containing ammonium ferric citrate (0.1%) and yeast extract (0.001%) followed by incubation at 70 °C for 24 h in humidified, sealed plastic containers. To determine the influence of Fe(III), the enrichment was replicated but in IF lacking ammonium ferric citrate. All wells with a purple colour due to the reduction of tetrazolium dye were regarded as positive (Bochner, 1989). Wells containing the substrates D-psicose, L-rhamnose and myo-inositol were considered to be of immediate interest, as these were positive in Fe(III)-amended plates but not in plates prepared with IF lacking Fe(III). Samples (60 μl) from each of these three wells were inoculated in anaerobic Pl medium with the addition of 2 g yeast extract l\textsuperscript{-1} followed by incubation at 60 °C for 3 days. PL medium contained (per l deionized water) 1 g NH\textsubscript{4}Cl, 0.6 g K\textsubscript{2}HPO\textsubscript{4}, 0.3 g KH\textsubscript{2}PO\textsubscript{4}, 0.1 g MgCl\textsubscript{2} 6H\textsubscript{2}O, 0.1 g CaCl\textsubscript{2} 2H\textsubscript{2}O, 1.0 g NaCl, 1 ml vitamin solution (Wolin et al., 1963), 1 ml trace-element solution (Zeikus et al., 1979), 12.0 g HEPES, 0.2 g yeast extract and 2 g ammonium ferric citrate (brown). The pH of the medium was adjusted to 7.2 with 1 M NaOH and 1 M HCl and the medium was heated for 10 min in an autoclave at a pressure of 1–1.5 kg cm\textsuperscript{-2}, cooled under a stream of N\textsubscript{2} and then dispensed into Hungate tubes or serum bottles under oxygen-free N\textsubscript{2} and the medium was sterilized for 30 min at a pressure of 1–1.5 kg cm\textsuperscript{-2}. Initial attempts to subculture from the L-rhamnose and myo-inositol wells failed, and hence further pure culture isolation was carried out from the D-psicose well. For this, the subculture was serially diluted and then incubated under the same conditions and the tube containing the lowest serial dilution was selected; the procedure was repeated at least three times before a strain designated RC3\textsuperscript{T} was isolated and regarded as a pure culture. Pure cultures were stored at −20 °C in glycerol–PL medium (50:50).

Phase-contrast microscopy and electron microscopy (Kanso & Patel, 2003) showed that cells of strain RC3\textsuperscript{T} were slightly curved, peritrichously flagellated rods (2.5–4.2 × 0.8–1.0 μm) which occur singly, in pairs and rarely in chains and exhibit a ‘roll and tumble’ motility. Spores were not observed under various growth conditions tested, though cells are transferable after heating for 30 min at 95 °C, indicating heat resistance. Cells of strain RC3\textsuperscript{T} stained Gram-positive and electron micrographs of thin sections revealed a thick peptidoglycan layer, characteristic of a Gram-positive cell-wall ultrastructure (Fig. 1).

Growth of strain RC3\textsuperscript{T} was tested in anaerobic tryptone-glucose (TYEG) medium as described by Patel et al. (1985a, b), which consisted of low-phosphate-buffered salts (LPBS) amended with 0.2 % each of tryptone, yeast extract and glucose, at temperatures ranging from 26 to 75 °C and pH 4.5–9.5. Growth was estimated from turbidity at 580 nm by inserting Hungate tubes directly into a modified cuvette holder of a Novaspec LKB
spectrophotometer (Pharmacia-Biotech). Strain RC3$^T$ grew optimally in TYEG medium at 60 °C (growth range 45–70 °C) and pH 7 (growth range pH 5–9) with a generation time of 0.53 h. Unless indicated otherwise, all subsequent growth experiments were conducted under these optimal conditions.

Strain RC3$^T$ was tested for its ability to use 0.2 % tryptone (TYEG minus YE), 0.2 % yeast extract (TYEG minus TG), 0.2 % each of tryptone and yeast extract (TYEG minus G) and 0.2 % glucose (TYEG minus TYE) as sole carbon sources. No growth was observed on tryptone or glucose but growth was noted on yeast extract with or without added tryptone. However, this growth was 25–30 % lower than that obtained in TYEG medium. In addition, glucose was fermented in the presence of yeast extract only (YE medium) and not tryptone (TG medium), suggesting that components in yeast extract but not tryptone were required for glucose fermentation. No growth of strain RC3$^T$ was observed when yeast extract concentrations in YEG medium were lowered to 0.01 %, and approximately 20 % less growth was observed with 0.1 % yeast extract compared with growth in 0.2 % yeast extract. This indicates that components in yeast extract were required for glucose fermentation in a dose-dependent manner.

Substrate-utilization tests were performed in 0.2 % YE medium (TYEG minus TG). Soluble substrates were added to the sterile medium 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 into the tubes and sterilized. Turbidity was measured at hourly intervals at 580 nm until stationary phase was reached. Growth was taken as positive if the turbidity of the culture in YE medium containing substrates was higher than that in YE medium that lacked the substrates. Strain RC3$^T$ grew on glucose, fructose, galactose, xyllose, maltose, sucrose, raffinose, mannose, cellobiose, cellulose, starch, amylpectin, xylan and peptone but not on arabinose, ribose, lactose, CM-cellulose, myo-inositol, mannotol, chitin or casein. Amino acids utilized by strain RC3$^T$ include amyl media (a casein peptone pancreatic digest; Research Achievement) and threonine, but not Casamino acids, arginine, alanine, serine, glycine, glutamine, leucine, isoleucine, methionine or aspartate. Strain RC3$^T$ utilized pyruvate but not formate, acetate, succinate, propionate, lactate or benzoate or alcohols (glycerol and ethanol).

End products from glucose fermentation in TYEG medium were determined by GC as described previously (Chrisotomos et al., 1996) with the exception that the oven and injector temperatures were 200 and 215 °C, respectively. End products from glucose fermentation were ethanol and acetate.

To determine the substrates used by strain RC3$^T$ with reduction of Fe(III), substrates were added to PL medium from sterile, anaerobic stock solutions and cultures were incubated for 8 days at 60 °C. Fe(III) reduction was inferred when transformation of the reddish-brown colour of the Fe(III) to a dark precipitate [Fe(II)] and clearing of the medium were observed. Fe(III) reduction was confirmed by measuring Fe(II) accumulation using the ferrozine method (Sørensen, 1982). A sample (0.1 ml) was mixed with 3 ml ferrozine reagent and, after 1 min, the absorbance at 562 nm was determined. A culture containing PL medium lacking a substrate was used as a negative control and a culture containing PL medium amended with 2 g yeast extract l$^{-1}$ was used as a positive control. At elevated temperatures, Fe(III) reduction can occur spontaneously in the presence of carbohydrates under sterile conditions (Zavarzina et al., 2002) and, hence, carbohydrates were not tested (with the exception of rhamnose). Fe(III) was reduced in the presence of yeast extract, tryptone, peptone, amyl media, threonine and glycerol but not rhamnose, chitin, xylan, pectin, starch, pyruvate, acetate, benzoate, lactate, propionate, succinate, inositol, ethanol, mannotol, arginine, glutamine or serine.

As Fe(III) was reduced in the presence of glycerol, 60 ml serum bottles containing 35 ml PL medium amended with glycerol (0.2 %) were used to investigate Fe(III) reduction linked to cell growth. Fe(III) reduction was monitored by measuring Fe(II) concentrations as described above and cell numbers were determined by the most-probable-number technique (Greenberg et al., 1992). A culture lacking Fe(III) and a culture lacking the inoculum were used as negative controls. Strain RC3$^T$ grew and reduced Fe(III) in PL medium with glycerol (0.2 %) and, following an initial lag period, the onset of Fe(II) production corresponded to the most rapid increase in cell numbers; Fe(II) concentrations did not increase once cells had reached death phase (Fig. 2). Fe(II) concentrations did not

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**Fig. 1.** Transmission electron micrograph of a thin section of strain RC3$^T$, revealing a typical Gram-positive cell-wall ultrastructure. CM, Cytoplasmic membrane; P, peptidoglycan; S, septum. Bar, 50 nm.
increase in the control lacking strain RC3^T and no growth was observed in the control lacking Fe(III). Thus, RC3^T was able to conserve energy to support growth from the reduction of Fe(III) to Fe(II) and therefore can be regarded as a facultative DIRM.

The potential for molecular hydrogen or carbon dioxide to serve as an electron donor for strain RC3^T in the presence or absence of Fe(III) was studied in 60 ml serum bottles containing 10 ml PL medium with H_2 (100, 10 and 5 %, v/v)/CO_2 (balance) or CO_2 (100 %) as the gas phase and 10 ml LPBS medium amended with yeast extract (0.02 %) with CO_2/H_2 (80:20) or CO_2 as the gas phase, respectively. Strain RC3^T was not able to reduce Fe(III) in the presence of CO_2 or H_2/CO_2 (100, 10 and 5 %, v/v) or grow with CO_2 or CO_2/H_2 (80:20).

Electron acceptor utilization tests for 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 determined in YE medium lacking the reductant Na_2S. The reduction of sulfate, sulfite, elemental sulfur and thiosulfate was tested using the method of Ramamoorthy et al. (2006). Fe(NH_4)_2(SO_4)_2 (0.5 %) was added to culture tubes, which were scored positive for the reduction of oxidized sulfur compounds if a black FeS precipitate was formed. The reduction of nitrate and nitrite to dinitrogen was determined by using Durham tubes. Cultures were scored positive if the amount of gas collected in the Durham tube was greater than that found in control tubes lacking nitrate or nitrite. Reduction of nitrate to nitrite was determined using nitrite test kits as described by the manufacturer (Aqua One). The reduction of nitrate to ammonium was subsequently tested in the same solution with the addition of 1 mg zinc powder (Merck). The strain was scored positive for the reduction of nitrate to ammonium if no colour change occurred or scored negative for nitrate reduction if a pink colour change occurred. The reduction of nitrite to ammonium was tested using an ammonia test kit (Aqua One). Electron acceptor utilization tests for amorphous Fe(III) oxyhydroxide (Lovley & Phillips, 1986) and MnO_2 (Lovley & Phillips, 1988) were done in PL medium amended with 2 g yeast extract 1^{-1} in which ammonium ferric citrate was replaced by 2 g of the electron acceptor. Fe(III) reduction was determined as described above and Mn(IV) reduction was inferred by a clearing of the medium and was further confirmed using the leuco crystal violet manganese oxide detection technique (Spratt et al., 1994). The MnO_2 concentration was determined by mixing 0.2 ml sample with 4 ml leuco crystal violet reagent, which was diluted, and the absorbance at 562 nm was determined. Strain RC3^T was able to reduce amorphous Fe(III) oxyhydroxide, Mn(IV) and elemental sulfur, but not sulfate, sulfite, thiosulfate, nitrate or nitrite.

Strain RC3^T was unable to grow under aerobic conditions in medium D (Brock & Freeze, 1969) or in aerobic YE medium.

Antibiotic sensitivity was determined by adding antibiotics from filter-sterilized stock solutions to sterilized TYEG medium to final concentrations of 10 and 100 μg ml^{-1}. Growth inhibition of each antibiotic was calculated from cultures grown in antibiotic-free TYEG medium. Strain RC3^T did not grow in the presence of 10 μg ampicillin or tetracycline ml^{-1} or 100 μg chloramphenicol, streptomycin or penicillin ml^{-1}. Strain RC3^T grew poorly in the presence of 500 μg sodium azide ml^{-1} (43 % inhibition). Strain RC3^T was able to grow without NaCl and also grew well with 1 % NaCl, but did not grow with 2 % NaCl.

To determine the G+C content of strain RC3^T, DNA extraction and genome amplification was performed using the Amersham Biosciences TempliPhi Amplification kit using a modification of the conditions and protocols described by the manufacturer, in which a longer incubation period (4–4.5 min at 95 °C) was used to release the bacterial chromosomal DNA for amplification. An aliquot of 0.4 μl concentrated exponential-phase cells, washed three times in distilled water, was used for each TempliPhi reaction. The quality of the DNA was assessed by agarose gel electrophoresis, ethidium bromide staining and visualization under a UVP GDAS 1200 Gel Documentation Analysis System. The G+C content of the genomic DNA was determined by the thermal denaturation method (Marmur & Doty, 1962) using a CINTRA20 spectrophotometer (GBC Scientific Equipment) as described by Spanevello et al. (2002) and was calculated to be 34 ± 1 mol% (mean ± SD from three determinations).

Inorganic Fe(III) present in PL medium interferes with genomic DNA extraction and PCR (Kashefi et al., 2003). Thioglycollic acid pre-treatment was used to reduce and precipitate Fe(III) prior to genomic DNA extraction of cultures grown in PL medium. For this, 40 μl thioglycollic acid was added to 35 ml exponential-phase PL medium-grown cultures; the mixture was mixed by inversion and
allowed to settle for 5 min and the supernatant was centrifuged at 8000 r.p.m. at 4 °C for 15 min (Sigma 4K15). The pellet was collected and resuspended in 7.5 ml TE buffer and then 10 μl thioglycollic acid was added and the solution mixed by gentle inversion. The mixture was allowed to settle, the supernatant was centrifuged as described above and the cell pellet was washed in 3.5 ml TE buffer to remove traces of thioglycollic acid; the pellet was finally resuspended in 460 μl TE buffer. Genomic DNA was prepared using a modification of the method of Marmur (1961) in which achromopeptidase (final concentration 0.3 mg ml⁻¹) was used with lysozyme to improve cell lysis. The spooled DNA was dissolved in 10 mM Tris/ HCl (pH 8.0) and the quality was assessed by agarose gel electrophoresis, ethidium bromide staining and visualization under a UVP GDAS 1200 Gel Documentation Analysis System. Aliquots (2 μl) of the DNA was used as a template for amplification of the 16S rRNA gene (Andrews & Patel, 1996). Sequencing was performed essentially as described previously (Andrews & Patel, 1996). The partial sequences that were generated were assembled using BioEdit v. 5.0.1 (Hall, 1999) and the consensus sequence of 1472 nucleotides was corrected manually for errors. The most closely related sequences in GenBank (version 152) and the Ribosomal Database Project II (release 9.37) identified using BLAST (Altschul et al., 2005) were extracted and aligned and the alignment was adjusted manually according to the 16S rRNA secondary structure using BioEdit. Nucleotide ambiguities were omitted and evolutionary distances were calculated by using the Jukes and Cantor option (Jukes & Cantor, 1969) and the consensus sequence of 1472 nucleotides was corrected manually for errors. The most closely related sequences in GenBank (version 152) and the Ribosomal Database Project II (release 9.37) identified using BLAST (Altschul et al., 1997) and the Sequence Match program (Cole et al., 2005) were extracted and aligned and the alignment was adjusted manually according to the 16S rRNA secondary structure using BioEdit. Nucleotide ambiguities were omitted and evolutionary distances were calculated by using the Jukes and Cantor option (Jukes & Cantor, 1969) in TREECON (Van de Peer et al., 1997).

Phylogenetic trees were constructed from evolutionary distances using the neighbour-joining method (Saitou & Nei, 1987). Tree topology was re-examined by the bootstrap method (1000) of resampling (Felsenstein, 1985). 16S rRNA gene sequence analysis indicates that strain RC3T is related to members of the genus Caloramator, most closely to Caloramator fervidus (95.8% similarity to the type strain) (Fig. 3).

Fig. 3. Dendrogram showing the phylogenetic position of strain RC3T with members of the genus Caloramator. Clostridium butyricum ATCC 19398T was used as an outgroup. GenBank accession numbers are given in parentheses. Bootstrap values >98% are shown. Bar, 2 changes per 100 nucleotide positions.

In this paper, we have demonstrated that IF amended with soluble Fe(III), trace amounts of yeast extract and minimal environmental sample inoculum can be used successfully for the enrichment of novel thermophiles, including DIRM, in the Biolog system. However, the Biolog system is only qualitative, and not quantitative, as it measures not growth but metabolic activities. In addition, a homogeneous sample is required, and this is difficult to achieve with environmental samples. It is therefore not entirely surprising to note that the two substrates, glycerol and threonine, that are substrates in the Biolog GN2 MicroPlate were negative for our enrichments, yet were subsequently found to be positive with the isolate. Increasing the environmental sample inoculum correspondingly increases the numbers of wells that are positive. However, this complicates studies, especially when one is interested only in strains that are present in small numbers, as we have been, and we therefore used a small environmental inoculum. In our study, our initial attempts to subculture from the L-rhamnose and myo-inositol wells failed; however, successful subcultures into TYE medium were obtained in a repeat experiment after 4 h incubation of the Biolog MicroPlate instead of 24 h, suggesting that the Biolog system is subject to variability, perhaps due to cell lysis/inactivation after prolonged incubation at high temperatures. Pure cultures of these subcultures were obtained by repeat serial dilutions, as described above, and 16S rRNA gene sequencing revealed identical sequences to strain RC3T, so no further characterization was performed. As strain RC3T does not utilize myo-inositol or L-rhamnose in the presence or absence of Fe(III), we hypothesize that strain RC3T may be able to metabolize these substrates but not grow by their utilization, or perhaps another organism was metabolizing D-psicose, L-rhamnose and myo-inositol, and strain RC3T was utilizing its end product for growth.

Strain RC3T was able to grow quickly, conserving energy for growth from dissimilatory Fe(III) reduction or from the fermentation of numerous energy substrates, with a temperature optimum and pH range consistent with the conditions of the red microbial mats existing in the bore from which it was isolated. Thus, it is likely that strain RC3T is largely involved in the cycling of Fe(III) and organic matter within the microbial mats and the GAB environment.

Strain RC3T shares common characteristics with all members of the genus Caloramator, which include strict anaerobic growth, thermophily, Gram-positive cell-wall ultrastructure, carbohydrate fermentation (in particular glucose, fructose, maltose, galactose and sucrose) and growth with yeast extract as the sole carbon source. However, there are a number of traits that differentiate strain RC3T from its closest relative, C. fervidus, which include differences in temperature optimum and range, G+C content, doubling time and Gram-staining behaviour and the ability to utilize serine and tryptone (Patel et al., 1987). Furthermore, cells of C. fervidus exhibit sluggish motility, while cells of strain RC3T exhibit roll and tumble.

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motility. Based on the phenotypic differences and the phylogenetic distance that separates strain RC3\(^T\) from other members of the genus Caloramator, we propose that strain RC3\(^T\) should be placed in a novel species of the genus, Caloramator australicus sp. nov.

**Description of Caloramator australicus sp. nov.**

Caloramator australicus (aus.tra’li.cus. N.L. masc. adj. australicus pertaining to Australia, from where the type strain was isolated).

Cells are strictly anaerobic, slightly curved rods, 2.5–4.2 x 0.8–1.0 μm. Gram reaction is positive. Cells possess peritrichous flagella and exhibit roll and tumble motility. No spores are observed. Cells are heat resistant (95 °C for 30 min). Growth occurs between 45 and 70 °C (optimum temperature 60 °C) and at pH 6–9 (optimum pH 7.0). Grows on yeast extract but not tryptone as the sole carbon source. Yeast extract but not tryptone is required for growth. Utilizes glucose, fructose, galactose, xylose, maltose, sucrose, raffinose, mannose, cellobiose, cellulose, starch, amylopectin, xylan, peptone, tryptone, amyl, media, threonine and pyruvate but not arabinose, ribose, lactose, CM-cellobiose, myo-inositol, mannitol, chitin, casein, Casamino acids, arginine, alanine, serine, glycine, glutamine, leucine, isoleucine, methionine, aspartate, formate, acetate, succinate, propionate, lactate, benzoate, glycerol, ethanol, H\(_2\) or CO\(_2\). Fe(III), Mn(IV) and elemental sulfur serve as electron acceptors but not sulfate, sulfite, thiosulfate, nitrate or nitrite. Fe(III), Mn(IV) and elemental sulfur are reduced in the presence of yeast extract but not tryptone as the sole carbon source. Reduced growth in the presence of sodium azide. The G+C content of the genomic DNA of the type strain is 34 ± 1 mol%.

The type strain, RC3\(^T\) (=KCTC 5601\(^T\) =JCM 1508\(^T\)), was isolated from a microbial mat sample from the outflow of a Great Artesian Basin bore (the New Lorne bore) in Queensland, Australia.

**References**


