

Caldithrix palaeochoryensis sp. nov., a thermophilic, anaerobic, chemo-organotrophic bacterium from a geothermally heated sediment, and emended description of the genus *Caldithrix*

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A novel thermophilic, strictly anaerobic, chemo-organotrophic bacterium, designated MC^T, was isolated from a geothermally heated sediment of a marine hydrothermal system at Palaeochory Bay, Milos, Greece. Cells of strain MC^T were rods of variable length (4–12 µm) and width (0.2–0.3 µm), occurring as single cells or forming large aggregates that were visible as flocs. Strain MC^T grew optimally at pH 7.0 and 60 °C and with 3% (w/v) NaCl. Strain MC^T grew chemo-organoheterotrophically and fermented peptides and di- and polysaccharides in the presence of 0.1 g yeast extract l⁻¹. The DNA G + C content of strain MC^T was 43.3 mol%. Phylogenetic analysis of the 16S rRNA gene sequence placed strain MC^T within the genus *Caldithrix*. However, strain MC^T possessed certain phenotypic features that differentiated it from the type strain of the only species of the genus *Caldithrix* described to date. On the basis of phylogenetic and phenotypic characteristics, it is proposed that strain MC^T represents a novel species, *Caldithrix palaeochoryensis* sp. nov. The type strain is MC^T (=DSM 21940^T =VKM B-2536^T). In addition, an emended description of the genus *Caldithrix* is presented.

The genus *Caldithrix* represents a phylogenetically distinct lineage within the domain *Bacteria* that is comparable to phylum status (Miroshnichenko *et al.*, 2003; Jumas-Bilak *et al.*, 2009). At the time of writing, the genus contains only one species, *Caldithrix abyssi*, the type strain of which was isolated from a sample of a deep-sea hydrothermal chimney on the Mid-Atlantic Ridge (Miroshnichenko *et al.*, 2003). In recent years, an increasing number of 16S rRNA gene sequences have been deposited in public databases from environmental samples that are phylogenetically related to *C. abyssi* LF13^T. The most similar sequences have been retrieved from the Lost City hydrothermal field in the Mid-Atlantic Ocean (GenBank accession no. FJ791936), the Juan de Fuca ridge in the Pacific Ocean (AY280423) and the Kazan mud volcano in the Mediterranean Sea (FJ712503). However, representatives of this phylogenetic group are apparently not restricted to hydrothermal vent fluids, as 16S rRNA gene sequences

sharing about 88 % similarity with *C. abyssi* LF13^T have been retrieved from mangrove soil (GenBank accession no. DQ811936) and a sulfidic cave stream biofilm in Italy (DQ415763). One of the closest phylogenetic relatives to *C. abyssi* LF13^T was detected by denaturing gradient gel electrophoresis of a bacterial community from a shallow hydrothermal vent of Palaeochory Bay (Sievert *et al.*, 2000), located on the south-eastern coast of the Greek island of Milos in the geothermally active area of the South Aegean volcanic arc (Miroshnichenko *et al.*, 2003). This sequence, band ML-5 (GenBank accession no. AF209001), shared 98.2 % 16S rRNA gene sequence similarity to *C. abyssi* LF13^T and represented a dominant fraction of the microbial population in this habitat (Sievert *et al.*, 2000). In this paper, we describe a second species of the genus *Caldithrix*, isolated from a shallow volcanic area of Palaeochory Bay.

The sample site was located 10 m from the shore at about 1 m depth and 0.3–0.5 m in diameter and looked like a round white-yellow spot. The seabed at this location was covered with small pebbles, 0.2–0.5 cm in diameter, and

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain MC^T is FJ999729.

sand. No signs of active venting or bacterial mats were observed. The temperature on the sediment surface at the centre of the site was 40 °C, and there was a steep temperature gradient with depth: 5 cm below the sediment surface, the temperature was 30 °C higher. Samples of sediment with water were taken from 20–30 mm below the surface of the sediment, placed into sterile Falcon tubes and transported to the laboratory at an ambient temperature, where they were stored at 4 °C.

For the enrichment of anaerobic, chemo-organotrophic, thermophilic bacteria, anaerobically prepared BM medium (Miroshnichenko *et al.*, 2003) was supplemented with (l^{-1}) 1 g yeast extract, 1 g acetate, 1 g $Na_2S_2O_3$ and 1 g $NaNO_3$ and the medium was adjusted to pH 7.0 with 6 M HCl. A subsample of the sediment (1 cm^3) was inoculated into 10 ml BM medium in 15 ml Hungate tubes with N_2 as the gas phase and incubated at 55 °C. After 5 days of incubation, the growth of two morphologically diverse micro-organisms was observed: oval motile cells were dominant and thin rods were present as a minor component. Colonies were obtained on BM agar (1.5 %) using the agar-shake method. After 7 days of incubation at 55 °C, the highest dilution showed spherical colonies of two types. Both types of colonies were about 1.5 mm in diameter, but differed in colour, being either white or grey. Individual colonies were transferred into liquid BM medium and, after 3 days of incubation at 55 °C, developed into two morphologically different cultures: oval-shaped cells, designated HM, and rod-shaped cells, designated MC^T . 16S rRNA gene sequence analysis showed that strain HM showed 100 % similarity to *Desulfacinum hydrothermale* MT-96^T, which was isolated previously from Palaeochory Bay (Sievert & Kuever, 1999). Strain MC^T was found to be affiliated to the genus *Caldithrix* and was characterized in detail.

After demonstrating that growth of strain MC^T did not depend on the presence of acetate, nitrate or thiosulfate, we used BM medium supplemented with only 1 g yeast extract l^{-1} for growth experiments or, for growth with di- and polysaccharides, 0.1 g yeast extract l^{-1} . Morphological and physiological studies were performed as described previously (Miroshnichenko *et al.*, 2003). Electron microscopy (Bonch-Osmolovskaya *et al.*, 1990) indicated that cells of strain MC^T were thin rods of variable length (4–12 μm) and width (0.2–0.3 μm) and had one polar flagellum (Fig. 1). Cells of strain MC^T were motile during early exponential growth phase. Formation of spherical empty bodies, which is typical of *C. abyssi*, was also observed for strain MC^T . In liquid culture, cells of strain MC^T aggregated into flocs.

Strain MC^T was an obligate anaerobe that only grew on BM medium when the content of $Na_2S \cdot 9H_2O$ was reduced to 0.5 g l^{-1} . The optimal growth temperature of strain MC^T was 60 °C. No growth was observed above 65 or below 40 °C. The pH range for growth was pH 6.2–7.5 (optimum pH 7.0). Growth was obtained with 0.5–6.5 % (w/v) NaCl

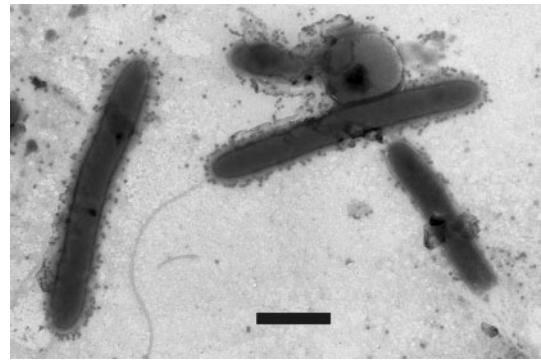


Fig. 1. Electron micrograph of whole cells of strain MC^T at stationary growth phase. Bar, 0.5 μm .

(optimum 3.0 % NaCl). The generation time of strain MC^T was 60 min under optimal conditions.

In the presence of 0.1 g yeast extract l^{-1} , strain MC^T grew chemo-organotrophically by fermentation of variety of proteinaceous substrates and carbohydrates. The following growth substrates were used: yeast extract, beef extract, biotrypticase and peptone (all at 2 g l^{-1}) and maltose, sucrose, glucose, fructose, arabinose, galactose and pyruvate (all at 20 mM). Weak growth of strain MC^T with starch (2 g l^{-1}) and agarose (1 g l^{-1}) was observed. The following substrates were tested but not utilized: fumarate, succinate, trehalose, lactose, pectin, microcrystalline cellulose and xylan. Molecular hydrogen, lactate, acetate, butyrate, ethanol and a trace amount of propanol were produced during growth with yeast extract as a substrate. Strain MC^T was not able to grow by the oxidation of hydrogen using nitrate, sulfur or thiosulfate as electron acceptors. The G+C content of the DNA of strain MC^T , determined as described previously (Miroshnichenko *et al.*, 2003), was 43.3 mol%.

Analysis of 16S rRNA gene sequences was performed according to methods described elsewhere (Miroshnichenko *et al.*, 2008). Briefly, calculations were based on an alignment of almost-complete 16S rRNA gene sequences from the SILVA database (release 95; Pruesse *et al.*, 2007) and included sequences from type strains representing the distantly related phylum *Deferribacteres* for comparison. A maximum-parsimony tree was constructed using programs included in the ARB software package (Ludwig *et al.*, 2004) and a maximum-likelihood tree was constructed with the RaxML program. The phylogenetic analysis (Fig. 2) placed strain MC^T in the genus *Caldithrix* with 96.4 % similarity to the only cultured representative, *C. abyssi* LF13^T. Thus, according to the currently accepted concept of bacterial taxonomy (Rosselló-Mora & Amann, 2001), strain MC^T represents a novel species within the genus *Caldithrix*. Based on 16S rRNA gene similarity, representatives of the genus *Caldithrix* were most closely related to members of the genus *Deferribacter*. However, the similarities were very low (80.7–81.5 %) and do

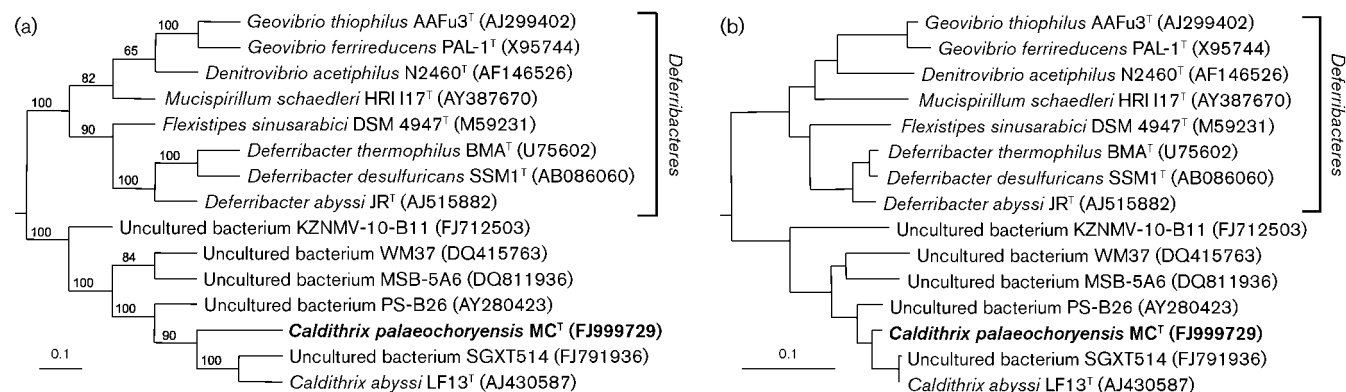


Fig. 2. Maximum-parsimony (a) and maximum-likelihood (b) trees, based on 16S rRNA gene sequences, showing the affiliation of strain MC^T with the genus *Caldithrix*. Percentages at nodes are bootstrap values based on 1000 replications. The sequence of *Escherichia coli* ATCC 11775^T (GenBank accession no. X80725) was used as an outgroup (not shown). Bars, 10% estimated sequence divergence.

not justify the affiliation of the two genera in the same phylogenetic group. In addition, the affiliation of strains of the genus *Caldithrix* with the phylum *Deferribacteres* was not supported by bootstrap values.

Strain MC^T shared some features with *C. abyssi* DSM 13497^T. Indeed, cells of both organisms were thin rods of variable length and often formed spherical protrusion bodies. However, in contrast to *C. abyssi* DSM 13497^T, cells of strain MC^T were motile by use of one polar flagellum. Both strains were moderate thermophiles and obligate anaerobes capable of chemo-organotrophic growth. However, metabolically, *C. abyssi* DSM 13497^T and strain MC^T were distinctly different (Table 1). In contrast to *C. abyssi* DSM 13497^T, which is a facultative chemolithotroph that oxidizes

molecular hydrogen and acetate in the presence of nitrate, strain MC^T showed no signs of the ability to respire anaerobically with these electron donors. With respect to chemo-organotrophic growth, strain MC^T was an obligately fermentative chemo-organotroph that could grow on di- and polysaccharides as well as proteinaceous substrates, in contrast to *C. abyssi* DSM 13497^T, which has a spectrum of organic substrates limited to peptides and pyruvate.

Based on the significant phylogenetic and phenotypic differentiation from *C. abyssi* DSM 13497^T, we propose that strain MC^T should be assigned to a novel species within the genus *Caldithrix*, for which we propose the name *Caldithrix palaeochoryensis* sp. nov.

Emended description of the genus *Caldithrix*

The description of the genus *Caldithrix* is as given by Miroshnichenko *et al.* (2003) with the following amendments. May be able to ferment di- and polysaccharides. The ability to respire anaerobically with molecular hydrogen or acetate as the electron donor and nitrate as the electron acceptor is variable. The DNA G+C content is 42.5–43.3 mol%.

Description of *Caldithrix palaeochoryensis* sp. nov.

Caldithrix palaeochoryensis (pa.lae.o.cho.ry.en'sis. N.L. fem. adj. *palaeochoryensis* pertaining to Palaeochory Bay in Greece, where the type strain was isolated).

Cells are thin motile rods, 0.2–0.3 µm in diameter and 4–12 µm long. Obligate anaerobe. Growth occurs between 40 and 65 °C (optimum 60 °C) and at pH 6.2–7.5 (optimum pH 7.0). Obligate chemo-organotroph and ferments peptides and di- and polysaccharides. The DNA G+C content of the type strain is 43.3 mol%.

Table 1. Comparative characteristics of strain MC^T and *C. abyssi* DSM 13497^T

Data were taken from this study and Miroshnichenko *et al.* (2003). Cells of both strains are rods.

Characteristic	MC ^T	<i>C. abyssi</i> DSM 13497 ^T
Cell size (µm)	0.2–0.3 × 4–12	0.2–0.35 × 4–20
Flagellation	+	–
Lithotrophic growth with H ₂	–	+
Respiration	–	+
Fermentation of carbohydrates	+	–
Fermentation products from yeast extract*	H ₂ , L, A, B, EtOH, PrOH	H ₂ , A, P
DNA G+C content (mol%)	43.3	42.5

*A, Acetate; B, butyrate; EtOH, ethanol; L, lactate; PrOH, propanol; P, propionate.

The type strain, MC^T (=DSM 21940^T =VKM B-2536^T), was isolated from a shallow hydrothermal vent of Palaeochory Bay, Milos, Greece.

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