Caldanaerobacter uzonensis sp. nov., an anaerobic, thermophilic, heterotrophic bacterium isolated from a hot spring

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An anaerobic thermophilic bacterium, strain K67T, was isolated from a terrestrial hot spring of Uzon Caldera, Kamchatka Peninsula. Analysis of the 16S rRNA gene sequence revealed that the novel isolate belongs to the genus Caldanaerobacter, with 95% 16S rRNA gene sequence similarity to Caldanaerobacter subterraneus subsp. subterraneus SEBR 7858T, suggesting that it represents a novel species of the genus Caldanaerobacter. Strain K67T was characterized as an obligate anaerobe, a thermophile (growth at 50–75 °C; optimum 68–70 °C), a neutrophile (growth at pH 6.8–8.0; optimum pH 6.8) and an obligate organotroph (growth by fermentation of various sugars, peptides and polysaccharides). Major fermentation products were acetate, H2 and CO2; ethanol, lactate and l-alanine were formed in smaller amounts. Thiosulfate stimulated growth and was reduced to hydrogen sulfide. Nitrate, sulfate, sulfite and elemental sulfur were not reduced and did not stimulate growth. Thus, according to the strain’s phylogenetic position and phenotypic novelties (lower upper limit of temperature range for growth, the ability to grow on arabinose, the inability to reduce elemental sulfur and the formation of alanine as a minor fermentation product), the novel species Caldanaerobacter uzonensis sp. nov. is proposed, with the type strain K67T (=DSM 18923T =VKM B-2408T).

The thermophilic bacteria currently assigned to the genus Caldanaerobacter (Fardeau et al., 2004) were initially described as Thermoanaerobacter subterraneus (Fardeau et al., 2000), Thermoanaerobacter yonseiensis (Kim et al., 2001) and Thermoanaerobacter tengcongensis (Xue et al., 2001), which were isolated from deep-subsurface thermal habitats, and as Carboxydibrachium pacificum (Sokolova et al., 2001), which was obtained from a deep-sea hydrothermal vent. Described almost simultaneously, these species were not compared with each other and, thus, they were classified either as novel Thermoaeraobacter species or within the novel genus Carboxydibrachium. Subsequently, however, they were found to form a separate phylogenetic branch in the genus Thermoanaerobacter (Subbotina et al., 2003) and so were assigned to a new genus Caldanaerobacter (Fardeau et al., 2004). DNA–DNA hybridization showed that they all belonged to the same species Caldanaerobacter subterraneus; thus, they were reclassified into different subspecies (Fardeau et al., 2004). Here, we report the isolation of a new representative of the genus Caldanaerobacter, strain K67T, from the terrestrial Thermophily hot spring of the Uzon Caldera (54° 49’ N 160° 01’ E) on the Kamchatka Peninsula (Russian Far East).

Strain K67T was obtained from a cyanobacterial mat sample (50–72 °C; pH 6.6–6.8). The isolation procedure was accomplished on the following mineral medium (1): 0.33 g KCl, 0.33 g NH4Cl, 0.33 g KH2PO4, 0.33 g MgCl2, 6H2O, 0.33 g CaCl2, 2H2O, 0.5 g NaHCO3, and 0.5 g Na2S·9H2O. The medium was supplemented with 1 g yeast extract l−1 (Difco) as the growth factor, 0.001 g resazurin l−1 as an indicator of anaerobiosis and solutions (1 ml l−1) of trace elements (Kevbrin & Zavarzin, 1997) and vitamins (Wolin et al., 1963). High-melting-point agarose (1.5%, w/v; MP; Boehringer Mannheim) was added as the growth substrate. Anaerobically prepared 10% (w/v) slurry of the sample (0.5 ml) was placed on top of the agarose block in a Hungate tube. After 3 days of incubation at 55 °C, the upper part of the block became liquid and turbid. Transfer to a semi-liquid medium with 0.5% (w/v) agarose yielded white colonies after 2–4 days of incubation at the same temperature. Isolated colonies were transferred into the medium with 0.2% (w/v) galactose.
Cells of strain K67T were rods with variable morphology. Thin sections of exponential-phase cells of strain K67T were prepared for electron microscopy as described previously (Bonch-Osmolovskaya et al., 1990). After growth in sugar-containing liquid medium, cells were short rods, 0.3–0.5 μm in length (Fig. 1), without spores; motility was never observed. On medium with agarose as the energy and carbon source, cells grew as thin, long rods (12–15 μm), predominantly with round terminal spores swarming the mother cell (Table 1).

Growth of strain K67T was observed at pH 25°C 4.8–8.0, with the optimum at pH 25°C 6.8 (no growth occurred at or below pH 25°C 4.5 and at or above pH 25°C 8.5), at 50–75°C, with the optimum at 68–70°C (no growth at or below 45°C and at or above 80°C) and with 0–2% NaCl (w/v), with the optimum at 0.5%. The following substrates (0.2%, w/v) were utilized by strain K67T as energy and carbon sources: pyruvate, fructose, glucose, galactose, lactose, sucrose, xylose, maltose, arabinose, cellobiose, mannose, trehalose, sorbitol, peptone, dextrin, starch and agarose (Table 1). No growth was observed with Casamino acids, inositol, mannotol, raffinose, rhamnose, ribose and xylitol (all at 0.2%, w/v).

Strain K67T did not grow on anaerobically prepared medium without sodium sulfide. Thiosulfate (0.2%, w/v) was reduced to hydrogen sulfide, stimulating growth in medium supplemented with glucose, fructose or sucrose (all at 0.2%, w/v). Nitrate, sulfate, sulfite (all at 0.2%, w/v) and elemental sulfur (1%, w/v) were not reduced in the course of growth and did not produce stimulating growth effects. Fermentation products (gases and volatile fatty acids) of strain K67T were analyzed using a Crystall-5000.1 gas chromatograph (Chromatech) equipped with a flame-ionization detector and a Superox-FA column (10 mm × 0.53 mm × 1.2 μm; Alltech) with helium as the carrier gas (flow rate 20 ml min⁻¹). Alanine was measured by liquid chromatography (Moore & Stein, 1963), using a B339 automatic analyser (Mikrotechna). According to the method used, the major fermentation end products from growth of strain K67T on 0.2% (w/v) glucose were acetate and H₂/CO₂; lactate, ethanol and L-alanine were also formed, alanine as a minor fermentation end product, with the molar alanine/acetate ratio being 1:10. All representatives of C. subterraneus form these products in equimolar quantities (Fardeau et al., 2000, 2004). The observed minimal doubling time under optimal growth conditions (pH 25°C 6.5, 62°C) was about 1 h.

Genomic DNA of strain K67T was isolated as described by Marmur (1961). The DNA G+C content was determined by melting point analysis (Marmur & Doty, 1962), using Escherichia coli K-12 DNA as a reference, and was 34.2 ± 0.5 mol%. Reference type strains of the C. subterraneus subspecies were obtained from the DSMZ for DNA–DNA hybridizations, which were performed as described previously (Miroshnichenko et al., 1994). Strain K67T showed DNA–DNA relatedness of 21 ± 0.5% with C. subterraneus subsp. subterraneus DSM 13054T and 51 ± 0.5% with C. subterraneus subsp. tengcongensis DSM 15242T.

The 16S rRNA gene was selectively amplified using general bacterial primers and the PCR products were purified from low-melting-point agarose using the Wizard PCR-Prep kit (Promega), according to the manufacturer’s instructions. Sequencing was performed using a Big Dye Terminator version 3.1 sequencing reaction kit with an ABI 3730 DNA automatic sequencer (Applied Biosystems). When the 16S rRNA gene sequence (1417 bp) of strain K67T was aligned with published sequences using BLAST (Altschul et al., 1997), it was found that the sequences most closely related to that from strain K67T were from strains of C. subterraneus subspecies: 16S rRNA gene sequence similarity between strain K67T and C. subterraneus subsp. subterraneus SEBR 7858T was 95.5% and 16S rRNA gene sequence divergence between strain K67T and strains of the other C. subterraneus subspecies was 3.7–4.5%. A phylogenetic tree was constructed with the neighbour-joining method (Saitou & Nei, 1987) provided in MEGA4 software (Tamura et al., 2007). Evolutionary distances were computed using the maximum-composite-likelihood method (Tamura et al., 2004) and bootstrap values (Felsenstein, 1985) were calculated from 1000 replications. Strain K67T clustered with other Caldanaerobacter strains; however, the distance from the nearest strain, C. subterraneus subsp. pacificus JM7, indicated that strain K67T represented a novel species (Fig. 2).

Thus, according to the phylogenetic analysis and the phenotypic differences between strain K67T and the type strains of C. subterraneus subspecies (lower upper limit of temperature range for growth, the ability to grow on arabinose, the production of L-alanine as a minor fermentation product and the inability to reduce elemental sulfur; Table 1), we propose a novel species, Caldanaerobacter uzonensis sp. nov., with strain K67T as the type strain.

**Description of Caldanaerobacter uzonensis sp. nov.**

Caldanaerobacter uzonensis (u.zo.nen’sis. N.L. masc. adj. uzonensis pertaining to the Uzon Caldera, Kamchatka, Far East Russia, from where the type strain was isolated).
Cells are rods of varying size, 0.3–0.5 μm wide and 1.5–3.5 μm long. When grown on agarose, cell length increases to up to 15 μm and spores (1.2–1.6 μm in diameter) are formed. Growth is obligately anaerobic. Thermophilic: grows at 50–75 °C (optimum 68–70 °C). Neutrophilic: grows at pH 4.8–8.0 (optimum pH 6.8). Grows with 0–2 % NaCl (optimum 0.5 %). Obligately organotrophic: grows by fermentation of pyruvate, fructose, glucose, galactose, lactose, maltose and peptone and produced acetate, hydrogen and CO₂. None of the strains used sulfate, sulfite or nitrate as electron acceptors. +, Positive; (+), weakly positive; −, negative; ND, no data available.

### Table 1. Comparative characteristics of *Caldanaerobacter* type strains

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<td>Strains: 1, <em>Caldanaerobacter uzonensis</em> sp. nov. K67T; 2, <em>C. subterraneus</em> subsp. <em>subterraneus</em> DSM 13054T (data from Fardeau et al., 2004); 3, <em>C. subterraneus</em> subsp. <em>tengcongensis</em> ICM 11007T (Xue et al., 2001; Fardeau et al., 2004); 4, <em>C. subterraneus</em> subsp. <em>yonseiensis</em> DSM 13777T (Kim et al., 2001; Fardeau et al., 2004); 5, <em>C. subterraneus</em> subsp. <em>pacificus</em> DSM 12653T (Sokolova et al., 2001; Fardeau et al., 2004). All strains utilized fructose, glucose, galactose, lactose, maltose, peptone and produced acetate, hydrogen and CO₂. None of the strains used sulfate, sulfite or nitrate as electron acceptors. +, Positive; (+), weakly positive; −, negative; ND, no data available.</td>
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reduced and do not produce stimulating growth effects. The DNA G+C content of the type strain is 34.2 mol%.

The type strain is K67T (= DSM 18923T = VKM B-2408T), isolated from Thermophilny Spring, a hot spring of the Uzon Caldera, Kamchatka.

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References


