Carboxydocella manganica sp. nov., a thermophilic, dissimilatory Mn(IV)- and Fe(III)-reducing bacterium from a Kamchatka hot spring

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Abbreviations: AQDS, 9,10-anthraquinone 2,6-disulfonate; CFA, cellular fatty acid.

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A supplementary table is available with the online version of this paper.

Metal-reducing micro-organisms play important roles in the cycling of carbon and metals in various anaerobic ecosystems, including thermal environments. Dissimilatory Fe(III)- and Mn(IV)-reducing micro-organisms have been found in a variety of thermal biotopes, including continental hot springs, a deep terrestrial subsurface, submarine petroleum reservoirs, deep-sea hydrothermal vents and anthropogenic systems (Lovley et al., 2004; Slobodkin, 2005). Dissimilatory manganese reduction by thermophilic prokaryotes is much less well studied than iron reduction. Of 43 described species of Fe(III)-reducing thermophiles belonging to different phylogenetic groups of the Bacteria and Archaea, the capacity for Mn(IV) reduction has been reported for six species (Slobodkin, 2005). Only two micro-organisms, Bacillus infernus (Boone et al., 1995) and Deferrribacter thermophilus (Greene et al., 1997), were first isolated using Mn(IV) as an electron acceptor. In this article, we report the isolation and characterization of a novel anaerobic, thermophilic, Mn(IV)- and Fe(III)-reducing micro-organism from a hydrothermal spring on the Kamchatka peninsula.

Strain SLM 61T was isolated from a sample of sediment collected from a hot spring at the ‘Trostnikovy’ field, Uzon Caldera, Kamchatka, Russia. The conditions at the sampling site were 60 °C, pH 6.2, Eh ~400 mV. Samples were taken anaerobically in tightly stoppered bottles and transported to the laboratory. Enrichment cultures were initiated by inoculation of 10% (w/v) of the sample into anaerobically prepared, bicarbonate-buffered, sterile (135 °C, 1 h) liquid medium with lactate (14 mM) as an electron donor and manganese(IV) oxide (25 mmol l⁻¹) as an electron acceptor. Medium composition and preparation techniques were described previously (Slobodkin et al., 1999). MnO₂

A thermophilic, anaerobic, dissimilatory Mn(IV)- and Fe(III)-reducing bacterium (strain SLM 61T) was isolated from a terrestrial hot spring on the Kamchatka peninsula. The cells were straight rods, 0.5–0.6 μm in diameter and 1.0–6.0 μm long, and exhibited tumbling motility by means of peritrichous flagellation. The strain grew at 26–70 °C, with an optimum at 58–60 °C, and at pH 5.5–8.0, with an optimum at pH 6.5. Growth of SLM 61T was observed at 0–2.0 % (w/v) NaCl, with an optimum at 0.5 % (w/v). The generation time under optimal growth conditions was 40 min. Strain SLM 61T grew and reduced Mn(IV), Fe(III) or nitrate with a number of organic acids and complex proteinaceous compounds as electron donors. It was capable of chemolithoautotrophic growth using molecular hydrogen as an electron donor, Fe(III) but not Mn(IV) or nitrate as an electron acceptor and CO₂ as a carbon source. It also was able to ferment pyruvate, yeast extract, glucose, fructose, sucrose and maltose. The G+C content of DNA of strain SLM 61T was 50.9 mol%. 16S rRNA gene sequence analysis revealed that the closest relative of the isolated organism was Carboxydocella thermautotrophica 41T (96.9 % similarity). On the basis of its physiological properties and phylogenetic analyses, the isolate is considered to represent a novel species, for which the name Carboxydocella manganica sp. nov. is proposed. The type strain is SLM 61T (=DSM 23132T =VKM B-2609T). C. manganica is the first described representative of the genus Carboxydocella that possesses the ability to reduce metals and does not utilize CO.
sequences in GenBank was carried out using the CLUSTAL W Alignment with a representative set of other 16S rRNA gene sequences in GenBank (Benson et al., 1999) using BLAST (Altschul et al., 1997) to identify the closest relatives. Alignment with a representative set of other 16S rRNA gene sequences in GenBank was carried out using the CLUSTAL W program provided by the phylogenetic analysis package MEGA version 4 (Tamura et al., 2007) and then adjusted manually. Pairwise similarity values were calculated by means of EzTaxon (Chun et al., 2007). A phylogenetic dendrogram was reconstructed by the neighbour-joining method (Saitou & Nei, 1987) with the maximum composite likelihood model (Tamura et al., 2004) using MEGA version 4. Carboxydocella thermotrophica DSM 12356T and C. sporoproducens DSM 16521T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) and used as reference strains.

Cells of strain SLM 61T were straight rods, 0.5–0.6 μm in diameter and 1.0–6.0 μm long. The cells occurred singly or in pairs and were motile by means of peritrichous flagellation. Spores were not observed. Ultrathin sectioning of strain SLM 61T revealed the presence of a distinct electron-dense material typical of the peptidoglycan layer of a Gram-positive-type cell wall.

The temperature range for growth of strain SLM 61T was 26–70 °C, with an optimum at 58–60 °C. No growth was detected at or above 71 °C or at 25 °C or below after incubation for 3 weeks. The pH range for growth was 5.5–8.0, with an optimum at pH 6.5. No growth was noted at or below pH 5.0 or at or above pH 8.5. Growth of strain SLM 61T was observed at 0–2.0 % (w/v) NaCl with an optimum at 0.5 % (w/v); no growth was evident at or above 2.5 % (w/v) NaCl. Yeast extract was not required for growth, but addition of 0.20 g yeast extract l–1 increased final cell yield 10– to 15-fold. Potential electron acceptors were tested with sodium lactate (14 mM) as an energy source in the presence of 0.20 g yeast extract l–1. Manganese(IV) oxide (25 mmol MnO2 l–1), poorly crystalline iron(III) oxide (ferrihydrite) [90 mmol Fe(III) l–1], iron(III) citrate (10 mM) and potassium nitrate (10 mM) were used as electron acceptors for growth of strain SLM 61T. Sulfate (14 mM), elemental sulfur (10 g l–1), thiosulfate, 9,10-anthraquinone 2,6-disulfonate (AQDS), fumarate (20 mM each) and oxygen (3 or 20 %, v/v, in the gas phase) were not reduced and did not support growth. During Mn(IV) reduction, black insoluble MnO2 turned to a light-brown precipitate containing 5–7 mM Mn(II). Ferrihydrite was reduced to a black magnetic precipitate with an Fe(II) content of 25–30 mM. No changes in colour or volume of precipitate were detected in uninoculated controls with Mn(IV) and Fe(III) incubated under the same conditions. Nitrate was reduced to ammonium. Lactate was oxidized incompletely to acetate. Strain SLM 61T was able to grow and reduce Fe(III), Mn(IV) and nitrate with lactate, pyruvate (14 mM each), formate, butyrate, succinate (20 mM each), yeast extract, peptone, tryptone (2.5 g l–1 each) and H2/CO2 (80/20 v/v) as electron donors. Strain SLM 61T could grow chemolithoautotrophically in the absence of yeast extract, using molecular hydrogen as an electron donor and ferrihydrite [but not Mn(IV) or nitrate] as an electron acceptor and bicarbonate/ CO2 as a carbon source. It also was able to grow without electron acceptors by fermentation of pyruvate, glucose, fructose, succrose, malonate (15 mM each) and yeast extract (2.5 g l–1). The main product of glucose fermentation (15 mM initial concentration) was acetate (28–30 mM),

(birnessite) was prepared by O2 (air) oxidation of MnCl2 in alkali medium (Feng et al., 2004). A pure culture was obtained from an enrichment positive for Mn(IV) reduction by serial dilutions in the same medium followed by the selection of well-separated colonies that had developed in anaerobic agar blocks (1.5 % agar in growth medium). Since MnO2 is a black, insoluble compound, it is inconvenient for colonies to be obtained in solidified medium, and it was replaced by KNO3. Light-brown, irregular-shaped colonies, 0.5–1.0 mm in diameter, appeared after 3 days of incubation at 60 °C. Individual colonies were checked for manganese-reducing ability by transferring them to liquid medium with MnO2 and, for positive cultures, the process of obtaining colonies was repeated two more times. Finally, a single colony that had developed in the highest agar block dilution (10–6) and possessed Mn(IV)-reducing ability was designated strain SLM 61T and used for further studies.

Physiological studies on substrate and electron acceptor utilization, temperature, pH and salinity ranges for growth, light and electron microscopy, analytical techniques, DNA extraction and determination of G+C content were performed as described previously (Slobodkin et al., 1999). Manganese (Mn2+) contents were determined by the formaldoxime method (Goto et al., 1962). Ammonium and nitrite were determined by HPLC with a conductivity detector (Aquilon C1P column, 4 mM HNO3, for ammonium and Aquilon A1.2 column, 3.5 mM carbonate buffer, for nitrite). Cellulose fatty acid (CFA) profiles were determined by GC-MS as methyl ester derivatives prepared from 5 mg dry cell material (Sasser, 1990). CFA content was determined as percentages of total ion current peak area. The 16S rRNA gene was selectively amplified from genomic DNA by PCR using primers 11F and 1492R (Lane, 1991). The PCR was carried out in 50 μl reaction mixture containing 50 ng DNA template, 5 pmol (each) primers, 12.5 nmol (each) dNTPs and 3 U Taq DNA polymerase (Fermentas) in Taq DNA polymerase reaction buffer (Fermentas). Temperature cycling was done by using the following program: first cycle of 9 min at 94 °C, 1 min at 55 °C and 2 min at 72 °C, followed by 30 amplification cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C. A final extension step was carried out at 72 °C for 7 min. PCR products were purified using the Wizard PCR Preps kit (Promega) as recommended by the manufacturer. The 16S rRNA gene was sequenced in both directions with the use of Big Dye Terminator version 3.1 (Applied Biosystems), as described in the manufacturer’s instructions, using an ABI PRISM 3730 sequencer (Applied Biosystems). Sequences were assembled and checked for accuracy manually using the alignment editor BioEdit version 5.0.9 (Hall, 1999). The full-length 16S rRNA gene sequence was compared with other sequences in GenBank (Benson et al., 1999) using BLAST (Altschul et al., 1997) to identify the closest relatives. Alignment with a representative set of other 16S rRNA gene sequences in GenBank was carried out using the CLUSTAL W program provided by the phylogenetic analysis package MEGA version 4 (Tamura et al., 2007) and then adjusted manually.
with trace amounts (<1.0 mM) of ethanol, formate, lactate and succinate; no molecular hydrogen was produced. The generation time under optimal growth conditions on pyruvate was 40 min. Isolate SLM 61T did not utilize acetate (10 mM), propionate, fumarate, maleinate, methanol, ethanol or glycerol (20 mM each) with iron(III) citrate (5 mM), manganese(IV) oxide (25 mmol l⁻¹) or potassium nitrate (10 mM) as electron acceptor. No growth was observed on peptone or tryptone (2.5 g l⁻¹) without an electron acceptor. Xylose, l-arabinose, lactose, galactose, cellobiose (15 mM each), starch (2.5 g l⁻¹) and citrate (10 mM) were not utilized with or without an electron acceptor. No growth or electron acceptor reduction was observed during cultivation on carbon monoxide (5, 15, 45 or 100% CO in gas phase) with ferrihydrite [90 mmol Fe(III) l⁻¹], manganese(IV) oxide (25 mmol l⁻¹) or KNO₃ (10 mM) or without an acceptor added. Penicillin, ampicillin, streptomycin, novobiocin, kanamycin and neomycin (10 mM) or without an acceptor added. Penicillin, ampicillin, streptomycin, novobiocin, kanamycin and neomycin (10 mM) or without an acceptor added. Penicillin, ampicillin, streptomycin, novobiocin, kanamycin and neomycin (10 mM) or without an acceptor added. Penicillin, ampicillin, streptomycin, novobiocin, kanamycin and neomycin (10 mM) or without an acceptor added. Penicillin, ampicillin, streptomycin, novobiocin, kanamycin and neomycin (10 mM) or without an acceptor added.

The G+C content of the genomic DNA of strain SLM 61T was 50.9 mol% (Tm). A comparison of 1522 nt of the 16S rRNA gene sequence of strain SLM 61T with those available in the GenBank database showed that strain SLM 61T belonged to the genus Carboxydocella (Fig. 1). The 16S rRNA gene sequence of the new isolate had the highest pairwise similarity to that of C. thermautotrophica DSM 12326T (96.9%). The level of 16S rRNA gene sequence similarity to C. sporoproducens KarT was 96.7%. Trees reconstructed by using the maximum-likelihood and maximum-parsimony algorithms displayed the same topology (not shown). Transversion analysis (Woese et al., 1991) did not affect the phylogenetic position of the novel strain.

The new isolate described in this report represents a thermophilic micro-organism capable of reduction of Mn(IV) and Fe(III) and, phylogenetically, it is closest to members of the genus Carboxydocella. At the time of writing, the genus Carboxydocella consists of two species with validly published names, C. thermautotrophica Sokolova et al. 2002 (type species) and C. sporoproducens Slepova et al. 2006. The novel isolate shares many phenotypic features with them. All members of the genus Carboxydocella display motile, rod-shaped cells and grow under anaerobic conditions, have very similar ranges of growth temperature and pH and were isolated from hot springs in Kamchatka. However, strain SLM 61T displays a number of significant differences (Table 1). The most significant distinction of strain SLM 61T from the other representatives of the genus Carboxydocella is its inability to grow on carbon monoxide, not only at 100% CO in the gas phase but also at lower concentrations. It was reported previously that nitrate inhibits growth of C. sporoproducens. In our study, we found that C. thermautotrophica DSM 12326T could also not grow and reduce nitrate with lactate or CO as an electron donor. Unlike other members of the genus Carboxydocella, strain SLM 61T is able to use nitrate as an electron acceptor. It utilizes a wider spectrum of substrates, including hydrogen, mono- and disaccharides, organic acids and complex proteinaceous compounds. We performed additional studies on sugar utilization by C. thermautotrophica DSM 12326T in the presence of yeast extract in the culture medium used for cultivation of strain SLM 61T and confirmed its inability to consume carbohydrates.

Strain SLM 61T was enriched on Mn(IV) as electron acceptor and confirmed its inability to consume carbohydrates. Strain SLM 61T was enriched on Mn(IV) as electron acceptor and confirmed its inability to consume carbohydrates. Strain SLM 61T was enriched on Mn(IV) as electron acceptor and confirmed its inability to consume carbohydrates. Strain SLM 61T was enriched on Mn(IV) as electron acceptor and confirmed its inability to consume carbohydrates. Strain SLM 61T was enriched on Mn(IV) as electron acceptor and confirmed its inability to consume carbohydrates. Strain SLM 61T was enriched on Mn(IV) as electron acceptor and confirmed its inability to consume carbohydrates.

Descriptive features of strain SLM 61T. The strain represents a novel species, Carboxydocella manganica sp. nov.

Carboxydocella manganica (man.ga’ni.ca. N.L. fem. adj. manganica pertaining to manganese).

Cells are straight, motile rods, 0.5–0.6 μm in diameter and 1.0–6.0 μm long. Spores are not observed. The cell wall is of the Gram-positive type. Cells form light-brown,
irregular-shaped colonies (0.5–1.0 mm in diameter) in anaerobic agar blocks. The temperature range for growth is 26–70 °C, with an optimum at 58–60 °C. The pH range for growth is 5.5–8.0, with an optimum at pH 6.5. Growth occurs at 0–2.0 % (w/v) NaCl, with an optimum at 0.5 % (w/v). Anaerobic. Reduces insoluble oxides of Mn(IV) and Fe(III). Utilizes peptone, tryptone, yeast extract, molecular hydrogen, formate, butyrate, lactate, pyruvate and succinate with Mn(IV), Fe(III) or nitrate as an electron acceptor. Lactate is oxidized incompletely to acetate. Capable of chemolithoautotrophic growth using molecular hydrogen as an electron donor, Fe(III) as an electron acceptor and CO₂ as a carbon source. Ferments pyruvate, yeast extract, glucose, fructose, maltose and succrose. The main product of glucose fermentation is acetate. Does not utilize acetate, glucose, fructose, sucrose and maltose. The main product of growth is 5.5–8.0, with an optimum at pH 6.5. Growth occurs at 0–2.0 % (w/v) NaCl, with an optimum at 0.5 % (w/v). Anaerobic. Reduces insoluble oxides of Mn(IV) and Fe(III). Utilizes peptone, tryptone, yeast extract, molecular hydrogen, formate, butyrate, lactate, pyruvate and succinate with Mn(IV), Fe(III) or nitrate as an electron acceptor. Lactate is oxidized incompletely to acetate. Capable of chemolithoautotrophic growth using molecular hydrogen as an electron donor, Fe(III) as an electron acceptor and CO₂ as a carbon source. Ferments pyruvate, yeast extract, glucose, fructose, maltose and succrose. The main product of glucose fermentation is acetate. Does not utilize acetate, propionate, fumarate, maleinate, methanol, ethanol, glycerol, xylene, L-arabinose, lactose, galactose, cellobose, starch or citrate, with or without electron acceptor. Sulfate, elemental sulfur, thiosulfate, AQDS, fumarate and oxygen are not reduced and do not support growth. No growth on carbon monoxide with or without electron acceptor. Penicillin, ampicillin, streptomycin, novobiocin, kanamycin and neomycin inhibit growth at 100 μl ml⁻¹. The major fatty acid is C₁₆:0. The G+C content of DNA of the type strain is 50.9 mol% (Tₘ).

The type strain, SLM 61ᵀ (=DSM 23132ᵀ =VKM B-2609ᵀ), was isolated from a terrestrial hot spring at Kamchatka, Russia.

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References


