**Methanocaldococcus indicus** sp. nov., a novel hyperthermophilic methanogen isolated from the Central Indian Ridge

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An autotrophic, hyperthermophilic methanogen, strain SL43T, was isolated from a deep-sea hydrothermal chimney sample collected on the Central Indian Ridge at a depth of 2420 m. The coccoid, surface-layer-carrying, Gram-negative-staining cells were heavily flagellated and exhibited a slight tumbling motility. The temperature range for growth at pH 6–5 was 50–86 °C, with optimum growth at 85 °C. The optimum pH for growth was 6–6 and the optimum NaCl concentration for growth was 30 g l⁻¹. The novel isolate used H₂ and CO₂ as the only substrates for growth and produced methane. Selenium and yeast extract stimulated growth significantly. In the presence of CO₂ and H₂, the organism reduced elemental sulfur to hydrogen sulfide. Growth was inhibited by chloramphenicol and rifampicin, but not by ampicillin, kanamycin, penicillin or streptomycin. The G+C content of the genomic DNA was 30–7 mol%. On the basis of 16S rRNA gene sequence analysis, this organism was most closely related to *Methanocaldococcus infernus* ME (3–2 % distance). Its phylogenetic distinctiveness was confirmed by RFLP analysis of the 16S rDNA, a reliable tool for differentiating hyperthermophilic methanococci. On the basis of phylogenetic and physiological characteristics, it is proposed that strain SL43T (=DSM 15027T = JCM 11886T) be designated as the type strain of a novel species, *Methanocaldococcus indicus* sp. nov.

On the basis of differences in 16S rRNA gene sequences and the diversity of temperature growth ranges, the strictly anaerobic, methane-producing members of the order *Methanococcales* have recently been separated into two families, the mesophilic and thermophilic members of the *Methanococcaeae* (optimal temperature ≤70 °C) and the hyperthermophilic members of the *Methanocaldococcaseae* (Whitman et al., 2001). The family *Methanocaldococcaseae* includes two new genera, *Methanocaldococcus* and *Methanotorris* (Whitman & Jeanthon, 2002). Members of these genera possess less than 93 % 16S rRNA gene sequence similarity and have been exclusively isolated from deep-sea hydrothermal vents. Within the genus *Methanocaldococcus*, the 16S rRNA sequence similarity between species is greater than 95 %.

To date, *Methanocaldococcus* species have been isolated from actively venting sulfide deposits at deep-sea hydrothermal vents at the East Pacific Rise (13°N and 21°N) (Jones et al., 1983; Jeanthon et al., 1999a) and the Mid-Atlantic Ridge (14°45’N and 23°N) (Jeanthon et al., 1998, 1999b) and from hydrothermally heated sediments from Guaymas Basin in the Gulf of California (Zhao et al., 1988; Jones et al., 1989; Jeanthon et al., 1999b).

Because of the paucity of readily identifiable phenotypic characteristics, taxonomic distinction between *Methanocaldococcus* species on this basis is difficult. Reliable comparison of strains can be made by RFLP analysis of the 16S rDNA (Jeanthon et al., 1999b). This method was used to reveal a novel species isolated from sulfide deposits of a deep-sea hydrothermal vent on the Central Indian Ridge. In this paper, we describe the isolation and characterization of this novel organism.

The novel strain was isolated from chimney samples...
strains were observed at pH 5–7, with optimum growth at around pH 6–7. Growth occurred in NaCl concentrations ranging from 15 to 50 g l\(^{-1}\), with optimum growth at 30 g l\(^{-1}\). No growth was observed at 10 or 60 g l\(^{-1}\). Growth in NaCl concentrations ranging from 15 to 50 g l\(^{-1}\), with optimum growth at 30 g l\(^{-1}\). No growth was observed at 10 or 60 g NaCl l\(^{-1}\). Under the optimal conditions for growth, the doubling time of the novel organism was 25–30 min.

Strain SL43\(^{T}\) is a strictly anaerobic, autotrophic organism. Growth was prevented in the presence of low levels of oxygen, and H\(_2\) and CO\(_2\) served as the only substrates for...
growth. No growth was observed on acetate (2 g l\(^{-1}\)), formate (5 g l\(^{-1}\)), methanol (0.5 %, v/v), monomethylamine (2 g l\(^{-1}\)) or yeast extract (2 g l\(^{-1}\)) with a N\(_2\)/CO\(_2\) (80:20; 200 kPa) or H\(_2\) (100 %; 200 kPa) headspace. In the presence of H\(_2\) and CO\(_2\), methane production paralleled growth (Jeannot et al., 1998). Ammonium (10 mM) was the preferred nitrogen source, but significant growth also occurred in the presence of yeast extract, tryptone, urea, glutamate (all at 2 g l\(^{-1}\)) and nitrate (10 mM). When supplemented individually, selenate, tungstate and yeast extract stimulated the growth rate. When sulfur (5 g l\(^{-1}\)) was added to the sulfate-free medium in the presence of CO\(_2\) and H\(_2\), growth occurred and H\(_2\)S was produced. No dissimilatory reduction of cystine (5 g l\(^{-1}\)), sulfate or thiosulfate (at 20 mM) was observed.

Sensitivity to antibiotics supplemented at 25, 50, 75, 100 and 200 μg ml\(^{-1}\) was tested in the culture medium at 80 °C. Strain SL43\(^{T}\) was resistant to ampicillin, penicillin, streptomycin and kanamycin (200 μg ml\(^{-1}\)) and was sensitive to chloramphenicol (75 μg ml\(^{-1}\)) and rifampicin (25 μg ml\(^{-1}\)).

DNA was isolated after disruption of cells using a French pressure cell (Thermo Spectronic) and purified by hydroxyapatite chromatography (Cashion et al., 1977). The DNA was hydrolysed with P1 nuclease and the nucleotides were dephosphorylated with bovine alkaline phosphatase (Mesbah et al., 1989). The G+C content of the DNA of strain SL43\(^{T}\) was 30.7 mol%, as determined by the HPLC method (Tamaoka & Komagata, 1994), and is in the range reported for other members of the genus Methanocaldococcus (Whitman et al., 2001).

A total of 1409 nucleotides of the 16S rRNA gene were sequenced as described previously (Götz et al., 2002). Distance and maximum-likelihood trees (De Soete, 1983; Olsen et al., 1994) (1354 nucleotides were used) placed strain SL43\(^{T}\) as a relative of Methanocaldococcus infernus ME\(^{T}\) (3.2 % distance), Methanocaldococcus jannaschii JAL-1\(^{T}\) (4.1 % distance), Methanocaldococcus vulcanius M7\(^{T}\) and Methanocaldococcus fervens AG86\(^{T}\) (5.3 % distance) (Fig. 2). However, the level of 16S rDNA sequence similarity between strain SL43\(^{T}\) and these organisms is lower than the limit (97 %) used to define distinct species at the DNA level without the requirement for DNA–DNA reassociation tests (Stackebrandt & Goebel, 1994). This significant phylogenetic distinctiveness was confirmed by RFLP analysis of the 16S rDNA (Jeannot et al., 1999b), a reliable and useful method for distinguishing species of Methanocaldococcus from each other (Whitman et al., 2001).

Although the novel isolate shared a number of phenotypic features with its relatives, obvious differences exist (Table 1). Strain SL43\(^{T}\) is distinct from other Methanocaldococcus species, and particularly from Methanocaldococcus infernus, its closest phylogenetic relative, in its maximum pH and temperature for growth and in the numbers of regions of insertion of flagella into the cell body. This distinctiveness was confirmed by comparing whole-cell protein patterns of strain SL43\(^{T}\) with those of type strains of described

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**Table 1. Differentiating characteristics of Methanocaldococcus species**

<table>
<thead>
<tr>
<th>Taxa</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
<td>Temperature for growth (°C):</td>
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<tr>
<td>Range</td>
<td>50–86</td>
<td>55–91</td>
<td>50–91</td>
<td>49–89</td>
<td>48–92</td>
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<tr>
<td>Optimum</td>
<td>85</td>
<td>85</td>
<td>85</td>
<td>80</td>
<td>85</td>
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<tr>
<td>pH range for growth</td>
<td>5–7</td>
<td>5–7</td>
<td>5–7</td>
<td>5–7</td>
<td>5–7</td>
</tr>
<tr>
<td>Flagellation apparatus</td>
<td>One extended insertion site</td>
<td>Three tufts of flagella</td>
<td>Two bundles of flagella</td>
<td>Three tufts of flagella</td>
<td>ND</td>
</tr>
<tr>
<td>Resistance to rifampicin*</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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*Concentration tested was 50 μg ml\(^{-1}\); +, resistant; –, sensitive.
Methanocaldococcus and Methanotorris species (Fig. 3). We propose that strain SL43^T represents a novel Methanocaldococcus species, named Methanocaldococcus indicus sp. nov.

Description of Methanocaldococcus indicus sp. nov.

Methanocaldococcus indicus (in’di.cus. L. masc. adj. indicus of India, referring to the Indian Ocean, where the type strain was isolated).

Cells exhibit a tumbling motility by means of numerous flagella, predominantly inserted at one specific region of the cell body. They are cocci (1–3 μm in diameter), covered with a hexagonal S-layer lattice, and occur singly and in pairs. Cells lyse rapidly in SDS (0–01 %) and in distilled water. Pale-yellow, round colonies about 1 mm in diameter form on Phytigel plates. Growth occurs at between 50 and 86 °C, with an optimum at 85 °C, between pH 5·5 and 6·7, with the optimum at around pH 6·5, and between 15 and 50 g NaCl l^{-1}, with an optimum of 30 g l^{-1}. Obligately anaerobic. Chemolithotrophic. Uses H_2 and CO_2 as energy and carbon sources to produce methane. Growth is stimulated by selenate, tungstate and yeast extract. Sulfur and carbon sources to produce methane. Growth is stimulated by selenate, tungstate and yeast extract. Sulfur and carbon sources to produce methane. Growth is stimulated by selenate, tungstate and yeast extract. Sulfur and carbon sources to produce methane. Growth is stimulated by selenate, tungstate and yeast extract. Sulfur and carbon sources to produce methane. Growth is stimulated by selenate, tungstate and yeast extract. Sulfur and carbon sources to produce methane. Growth is stimulated by selenate, tungstate and yeast extract. Sulfur and carbon sources to produce methane. Growth is stimulated by selenate, tungstate and yeast extract. Sulfur and carbon sources to produce methane.

The type strain, strain SL43^T (=DSM 15027^T = JCM 11886^T), was obtained from a deep-sea hydrothermal vent chimney in the Kairei vent field on the Central Indian Ridge.

Acknowledgements

The work performed in Plouzane was supported by a grant from PRIR (Programme de Recherche d’Intérêt Régional, Conseil Régional de Bretagne) and INTAS (International Association for the Promotion of Co-operation with Scientists from the New Independent States of the Former Soviet Union) (grant no. 99-1250). The work performed at Portland was supported, in part, by grants NSF-OCE9712358 and NSF-OCE0083134. The work performed in Wien was supported by the Austrian Science Fund (project P13840). We thank Harald Mayer and Andrea Scheberl for excellent technical assistance.

References


