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The first evidence of anaerobic CO oxidation coupled with H_2 production by a hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent

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Abstract From 24 samples of hydrothermal venting structures collected at the East Pacific Rise (13°N), 13 enrichments of coccoid cells were obtained which grew on CO, producing H_2 and CO_2 at 80°C. A hyperthermophilic archaeon capable of lithotrophic growth on CO coupled with equimolar production of H_2 was isolated. Based on its 16S rRNA sequence analysis, this organism was affiliated with the genus *Thermococcus*. Other strains of *Thermococcales* species (*Pyrococcus furiosus*, *Thermococcus peptonophilus*, *T. profundus*, *T. chitonophagus*, *T. stetteri*, *T. gorgonarius*, *T. litoralis*, and *T. pacificus*) were shown to be unable to grow on CO. Searches in sequence databases failed to reveal deposited sequences of genes related to CO metabolism in *Thermococcales*. Our work provides the first evidence of anaerobic CO oxidation coupled with H_2 production performed by an archaeon as well as the first documented case of lithotrophic growth of a *Thermococcales* representative.

Keywords Anaerobic CO oxidation · Deep-sea hot vents · Hyperthermophilic archaea · *Thermococcus*

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Introduction

Anaerobic, thermophilic, CO-oxidizing, H_2-producing bacteria were found to inhabit diverse volcanic environments (Svetlichny et al. 1991, 1994; Bonch-Osmolovskaya et al. 1999; Sokolova et al. 2001, 2002). These bacteria oxidize CO anaerobically according to the equation $CO + H_2O \rightarrow CO_2 + H_2$ ($\Delta G^{\circ\prime} = -20$ kJ/mol), and recently the term "hydrogenogens" was proposed for this physiological group (Svetlitchny et al. 2001). So far, the extremely thermophilic bacterium *Caldanaerobacter subterraneus* subspecies *pacificus* (formerly *Carboxydobrachium pacificum*) is the only representative of this metabolic group isolated from deep-sea hydrothermal environments (Sokolova et al. 2001; Fardeau et al. 2004). Such habitats are rich in metabolically diverse, anaerobic hyperthermophilic archaea of the kingdoms Euryarchaeota and Crenarchaeota, none of which has been described as a CO oxidizer. Within the kingdom Euryarchaeota, various organotrophic sulfur reducers of the order Thermococcales were isolated from deep-sea hot environments (for references, see Holden et al. 2001). Other organotrophs are sulfate- and/or sulfite-reducing Euryarchaeota of the genus *Archaeglobus* (Burggraf et al. 1990) and sulfur reducers of the genera *Staphylothermus* (Fiala et al. 1986) and *Pyrodictium* (Pley et al. 1991) within the kingdom Crenarchaeota. The lithotrophic hyperthermophilic archaea isolated from deep-sea environments are methanogens of the genera *Methanopyrus* (Kurr et al. 1991), *Methanocaldococcus*, *Methanotorris*, and *Methanothermococcus* (Whitman et al. 2001; Takai et al. 2002), sulfur-reducing *Pyrodictium occultum* (Stetter et al. 1983), *Ignicoccus islandicus* and *I. pacificus* (Huber et al. 2000), and the H_2-oxidizing, facultative aerobe *Pyrolobus fumarii* (Blöhl et al. 1997).

We report here the isolation and primary characterization of a hyperthermophilic archeon capable of lithotrophic growth on CO coupled with production of

H_2. Based on its 16S rRNA sequence analysis, this organism is affiliated with the genus *Thermococcus*.

Materials and methods

Hydrothermal site and sample collection

The hydrothermal vent samples were collected in 1999 during the scientific AMISTAD cruise conducted on the East Pacific Rise with the RV *L'Atalante* and the DSV *Nautile*. The portion of the East Pacific Rise situated at 13°N is about 300 km south of the Orozco fracture zone and 100 km north of a small transform fault located at 11°49′N. Our study focuses on the 13°N vent field lying between 12°48′18″ and 12°50′32″N and between 103°56′39″ and 103°56′80″W. This dome-shaped ridge has a central rift valley varying from 200 m to more than 600 m in width and has a mean depth of about 2,600 m (Hekinian and Fouquet 1985). The volcanic activities at 13°N give rise to a wide range of hydrothermal venting structures, from diffuse vents (with temperature ranging from 5 to 100°C) to black smokers (with temperatures above 350°C). Geographically distant diffuse vents with clear fluid emissions and with a temperature of above 50°C were selected for sample collection (Table 1). Dense populations of *Alvinella pompejana*—a thermotolerant polychaetous annelid worm that builds tubes directly in contact with the sulfides (Cary et al. 1998)—colonized most of the active structures. *Alvinella* populations were usually observed in close association with white microbial mats. Active chimney samples were taken by the hydraulic arm of the submersible and were transferred into enclosed containers to minimize contamination from surrounding seawater during transportation to surface. Once on board, they were immediately transferred into 50-ml glass vials and flooded with a sterile solution of 3% (w/v) Sea Salts (Sigma Chemical, St. Louis, Mo., USA). The vials were then closed tightly with butyl rubber stoppers (Bellco, Vineland, N.J., USA), pressurized with N_2 (100 kPa), reduced with sodium sulfide, and stored at 4°C until return to the laboratory.

Enrichment and isolation of a hyperthermophilic CO-utilizing anaerobe

For the enrichment and isolation of hyperthermophilic CO-utilizing prokaryotes, medium 1 was used (g l^{-1}): NaCl (18), KCl (0.7), $MgSO_4$ (3.9), $CaCl_2$ $2H_2O$ (0.4), NH_4Cl (0.3), Na_2HPO_4 (0.15), Na_2SiO_3 (0.03), $NaHCO_3$ (0.5), cysteine-HCl (0.5), Na_2S $9H_2O$ (0.5), yeast extract (Difco) (0.05), and resazurin (0.002). Trace elements (Kevbrin and Zavarzin 1992) and vitamins (Wolin et al. 1963) were added in an amount of 1 ml l^{-1}. The pH of

Table 1 Origin and types of the 13°N samples used for enrichments

Vent (latitude, longitude)	Type of sample	Sample code	Growth and H_2 production on CO at 80°C[a]
Grandbonum PP52 (12°48.721′N, 103°56.351′W)	Active chimney colonized by *Alvinella*	501	+
Grandbonum PP52 (12°48.721′N, 103°56.351′W)	Inner part of an active chimney colonized by *Alvinella*	502	–
Grandbonum PP52 (12°48.721′N, 103°56.351′W)	Outer part of an active chimney colonized by *Alvinella*	503	+
Genesis PP12 (12°48.666′N, 103°56.429′W)	Outer part of the base of an active chimney	504	–
Genesis PP12 (12°48.666′N, 103°56.429′W)	Outer and inner parts of the base of an active chimney	506	–
Genesis PP12 (12°48.666′N, 103°56.429′W)	Tubes of *Alvinella*	507	+
Elsa PP Hot 14 (12°48.193′N, 103°56.338′W)	Active chimney colonized by *Alvinella*	508	+
La Chaînette PP57 (12°50.342′N, 103°56.903′W)	Outer part of an active chimney	513	–
Elsa PP Hot 14 (12°48.193′N, 103°56.338′W)	Active chimney colonized by *Alvinella*	515	+
Genesis PP12 (12°48.666′N, 103°56.429′W)	Outer part of an active chimney	516	–
Elsa PP Hot 14 (12°48.193′N, 103°56.338′W)	Active chimney colonized by *Alvinella*	517	+
Elsa PP Hot 14 (12°48.193′N, 103°56.338′W)	Outer and inner parts of an active chimney	518	–
Elsa PP Hot 14 (12°48.193′N, 103°56.338′W)	Pyrite sampled from in the inner part of an active chimney	519	–
Genesis PP12 (12°48.666′N, 103°56.429′W)	Outer and inner parts of an active chimney	520	–
Genesis PP12 (12°48.666′N, 103°56.429′W)	Active chimney colonized by *Alvinella*	521	+
La Chaînette PP57 (12°50.342′N, 103°56.903′W)	Outer part of the top of an active chimney	522	+
La Chaînette PP57 (12°50.342′N, 103°56.903′W)	Outer part of the middle of an active chimney	523	+
La Chaînette PP57 (12°50.342′N, 103°56.903′W)	Tubes of *Alvinella*	524	+
Totem (12°48.818′N, 103°56.462′W)	Tubes of *Alvinella*	525	+
Totem (12°48.818′N, 103°56.462′W)	Outer and inner parts of the top of an active chimney	526	–
La Chaînette PP57 (12°50.342′N, 103°56.903′W)	Outer and inner parts of the top of an active chimney	527	–
La Chaînette PP57 (12°50.342′N, 103°56.903′W)	Salt deposits on an active chimney	528	+
La Chaînette PP57 (12°50.342′N, 103°56.903′W)	Outer and inner parts of the top of an active chimney	529	+
Genesis PP12 (12°48.666′N, 103°56.429′W)	Outer and inner parts of the top of an active chimney	530	–

[a] + Microbial growth, CO consumption, and H_2 production; – no growth, CO consumption or H_2 production

the medium was adjusted to 6.8 at room temperature. Anaerobically prepared medium was dispensed into 50-ml flasks under 100% N_2; then, the gas phase (40 ml) was changed to 100% CO. The flasks were inoculated with 1 ml of each hydrothermal sample and incubated at 80°C. A pure culture of a hyperthermophilic microorganism, strain AM4, was obtained by serial tenfold dilutions. Strain AM4 was further cultivated in medium 1 with 0.1 g l^{-1} of yeast extract under a 100% CO atmosphere.

Morphology studies

Cell morphologies of enrichments and pure cultures were examined by phase-contrast and electron microscopy. Light microscopy was carried out using an AU-12 phase contrast microscope with a 90/1.25 oil immersion objective. For electron microscopy, whole cells were negatively stained with 2% phosphotungstic acid. The specimens were examined under a JEM-100 electron microscope (JEOL, Tokyo, Japan).

Metabolic studies

Microbial growth was monitored by direct cell count under an AU-12 light microscope. Quantitative determination of CO, gaseous metabolic products, and short-chain organic acids and alcohols was performed by gas chromatography as described earlier (Sokolova et al. 2001).

Molecular properties

DNA was isolated as described by Marmur (1961). The DNA G+C content was determined by melting-point analysis (Marmur and Doty 1962), using *Escherichia coli* K12 DNA as a reference.

16S rDNA sequence determination, accession number, and phylogenetic analysis

Genomic DNA extraction, PCR-mediated amplification of the 16S rDNA, and sequencing of PCR products were carried out as described by Rainey et al. (1996). Neighbor-joining analysis was done using the algorithm of Felsenstein (1993).

Reference strains

Pyrococcus furiosus DSM 3868^T, *Thermococcus peptonophilus* JCM 9653^T, *T. profundus* DSM 5432^T, *T. chitonophagus* strain E1, *T. chitonophagus* strain 2705, *T. chitonophagus* strain E4, *T. stetteri* strain K15, *T. stetteri* strain 2104, *T. stetteri* strain E3, *T. gorgonarius* DSM 10395^T, *T. litoralis* strain Sh1AM, *T. litoralis* strain 1614, *T. litoralis* strain MW, and *T. pacificus* DSM 10394^T were kindly provided by M.L. Miroshnichenko (Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia). Strains of *Thermococcus* and *Pyrococcus* were cultivated as described earlier (Miroshnichenko et al. 1998; Kostyukova et al. 1999). Their ability to oxidize CO during anaerobic growth was tested on medium 1 with 1 g l^{-1} of yeast extract under a CO (100%) atmosphere.

Database searches

Queries submitted to Entrez at National Center for Biotechnology Information (NCBI) site http://www.ncbi.nlm.nih.gov. combined the generic names *Thermococcus* or *Pyrococcus* with the following term combinations that use to occur in the designations of CO dehydrogenases and CO dehydrogenase/acetyl-CoA synthase enzymes or enzyme complexes: (1) carbon monoxide_dehydrogenase, (2) carbon monoxide_dehydrogenase, (3) acetyl-CoA_synthase, and (4) acetyl-CoA_decarbonylase/synthase.

BlastP and TblastN searches were performed at the NCBI site http://www.ncbi.nlm.nih.gov/BLAST/ and in the genomes of *P. furiosus*, *P. horikoshii*, and *P. abyssi* at the Blast Archaeal Genome Sequence (B.A.G.S.) site http://bix.umbi.umd.edu/genemate/bags.html. The query sequences were those of various subunits of anaerobic CO dehydrogenases and CO dehydrogenase/acetyl-CoA synthase enzymes or enzyme complexes belonging to the four different classes currently recognized (Lindahl 2002): *Methanocaldococcus jannaschii* (*Methanococcus jannaschii*) acetyl-CoA decarbonylase/synthase, subunits α, β, γ, δ, and ϵ (accession number NC_000909); *Methanosarcina thermophila* acetyl-CoA decarbonylase/synthase, subunits α, β, γ, δ, and ϵ (accession number AF173830); *Moorella thermoacetica* (*Clostridium thermoaceticum*) CO dehydrogenase α and β subunits (accession number M62727) and corrinoid/iron-sulfur protein large (accession number AAA23254) and small (accession B46621) subunits; and *Rhodospirillum rubrum* CO dehydrogenase (CooS) (accession number U65510).

Results

Enrichment, isolation, and preliminary characterization of an anaerobic hyperthermophilic CO-oxidizing, H_2-producing archaeon

Twenty-four samples of chimneys and tubes of the polychaete worm *Alvinella pompejana* (Table 1) were used to inoculate flasks containing medium 1 under a CO gas phase. After 3–5 days of incubation at 80°C, 13 samples produced abundant growth of coccoid cells (Table 1). Microbial growth was accompanied by an increase in the gas pressure in the flasks (up to 150 kPa).

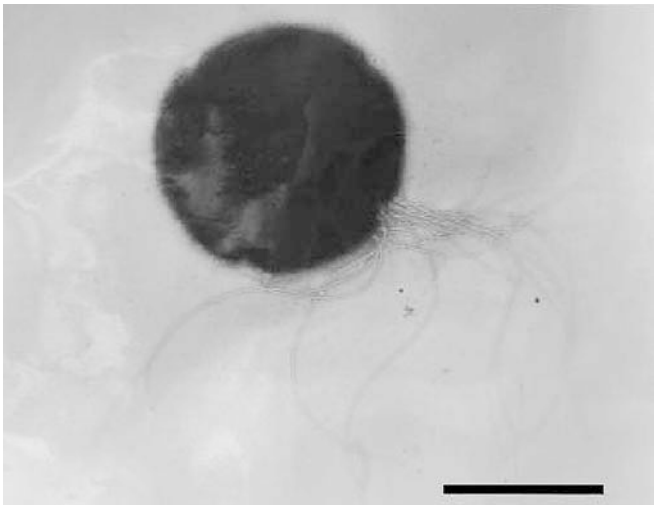

Fig. 1 Electron micrograph of negatively stained cell of strain AM4. *Bar* = 1 μm)

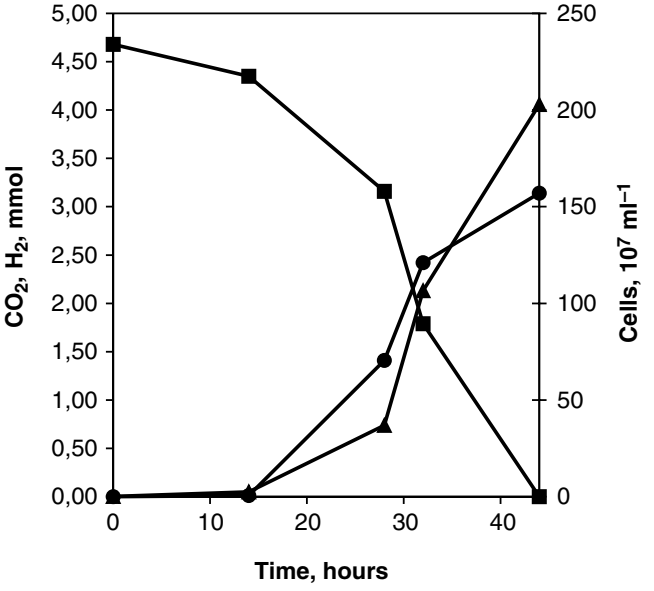

Fig. 2 Growth of strain AM4 at 82°C in medium 1 with 0.1 g l^{-1} of yeast extract (20 ml liquid culture in a 100-ml serum bottle) under a CO atmosphere: cell number (*circles*), CO consumption (*squares*), and H_2 production (*triangles*). The amount of gas is shown as its total amount in the head-space volume

CO content in the gas phase decreased by 50% or more, and a mixture of CO_2 and H_2 appeared. From the enrichment obtained from sample 508, strain AM4 was isolated by serial tenfold dilutions in medium 1 under a 100% CO gas phase.

Cells of strain AM4 were motile cocci, 1–1.5 μm in diameter, with a thick tuft of flagella (Fig. 1). Strain AM4 grew in the temperature range from 45–95°C, with an optimum at 82°C. It grew anaerobically and chemolithotrophically in medium 1 under CO in the gas phase. CO oxidation was coupled with the formation of H_2 and CO_2 in equimolar quantities according to the equation $CO + H_2O \rightarrow CO_2 + H_2$ (Fig. 2). No CH_4 or acetate or any other metabolic product were detected. Yeast extract (0.05 g l^{-1}) was essential for growth. Final cell yield in medium 1 with 0.1 g l^{-1} of yeast extract under 100% CO at pH 6.8 and 82°C was 2.2 10^8 cells ml^{-1}. Strain AM4 did not grow in medium 1 with 0.05, or 0.1, or 1.0 g l^{-1} of yeast extract without CO in the gas phase under N_2 atmosphere. Strain AM4 was able to grow in the same medium supplemented with 10 g l^{-1} of S^0 under N_2 atmosphere with 1 g l^{-1} of yeast extract or soybean extract or peptone.

The G + C content of the DNA of strain AM4 was 55 ± 1 mol%.

Phylogenetic analysis based on 16S rDNA gene sequence comparison indicated a high degree of sequence similarity between strain AM4 and strain 9 N2 (99.9%). Strain 9 N2 has recently been reported in a study on novel groups of hyperthermophilic deep-sea thermococci in the northeastern Pacific Ocean (Holden et al. 2001). These two strains are highly related to some described and as yet undescribed *Thermococcus* species (e.g., *T. gammatolera*ns, 98.9%; *T. peptonophilus*, 98.8%; or *T. stetteri*, 98.7%). The branching pattern of the neighbor-joining tree (Fig. 3) places the two undescribed strains next to the type strains of these species and to *Thermococcus marinus* and *Thermococcus kodakaraensis* (98.8% each). A bootstrap value of 100% supports the relatedness between strains AM4 and 9 N2, while the majority of the other branching points are less stable in the statistical analysis.

Screening of a *Thermococcales* collection for CO oxidizers

Strains *P. furiosus* DSM 3868^T, *T. peptonophilus* JCM 9653^T, *T. profundus* DSM 5432^T, *T. chitonophagus* strain E1, *T. chitonophagus* strain 2705, *T. chitonophagus* strain E4, *T. stetteri* strain K15, *T. stetteri* strain 2104, *T. stetteri* strain E3, *T. gorgonarius* DSM 10395^T, *T. litoralis* strain Sh1AM, *T. litoralis* strain 1614, *T. litoralis* strain MW, and *T. pacificus* DSM 10394^T were tested for their ability to oxidize CO and produce H_2 under the same conditions as strain AM4. After 7 days of incubation in medium 1 with 1 g l^{-1} of yeast extract at 82°C, no CO consumption occurred in any of the reference strains. In the flasks inoculated with strain AM4, CO was completely consumed, and growth to a cell density of 10^9 cells ml^{-1} was observed.

Database searches for enzymes related to CO metabolism in *Thermococcales*

Entrez searches in GenBank revealed several pyrococcal protein sequences that, judging from their names, could bear relation to CO metabolism (acetyl-CoA synthases and acetyl-CoA decarbonylases). However, more

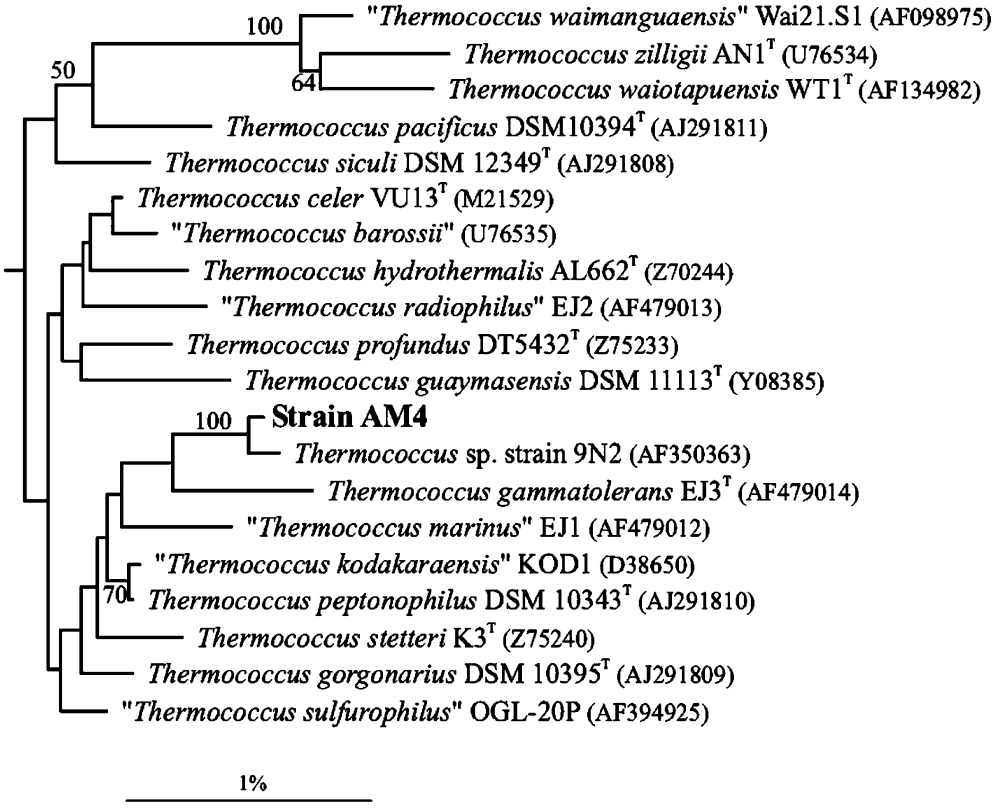

Fig. 3 Relationship of strain AM4 next to type strains of *Thermococcus* species and as yet not validly published species. The dendrogram is based on 16S rRNA gene sequence comparisons, using the neighbor-joining algorithm of Felsenstein (1993). *Numbers* at branching points are bootstrap values (500 resamplings). The *bar* represents 1% sequence divergence as determined by measuring the length of the horizontal lines connecting any two species. The sequence of *Archaeoglobus fulgidus* DSM 4304^T (accession number AE000965) served as a root

thorough inspection of these documents (including BlastP of these sequences as queries) showed that these were enzymes closely related to enzymes fulfilling other functions (e.g., acetyl-CoA synthase in *P. furiosus* was closely related to 3-ketoacyl-CoA thiolases involved in lipid metabolism).

We also performed BlastP searches in GenBank and in the genomes of *P. furiosus*, *P. horikoshii*, and *P. abyssi* at the B.A.G.S. site, using as queries amino acid sequences of various subunits of anaerobic CO dehydrogenases and CO dehydrogenase/acetyl-CoA synthase enzymes or enzyme complexes belonging to the four different classes currently recognized (Lindahl 2002). These searches also failed to reveal any related sequences belonging to *Thermococcales*.

Discussion

CO is a normal component of volcanic exhalations (Symonds et al. 1994). Volcanic exhalations from divergent margin volcanoes contain high concentrations of H_2 and CO: e.g., exhalations of Erta Ale (Ethiopia, December 1971) contained 1.59% of H_2 and 0.79% of CO; of Ardoukoba (Djibouti, November 1978), 1.71% of H_2 and 0.17% of CO; of Surtsey (Iceland, February 1965), 2.27% of H_2 and 0.36% of CO; of Nyiragongo (Zair, 1959), 1.59% of H_2 and 2.72% of CO (Symonds et al. 1994). The main characteristics of East Pacific Rise volcanoes are common to divergent margin volcanoes. CH_4, H_2, and CO were found in superheated waters at 21°N along the East Pacific Rise and at the Rainbow vent site on the mid-Atlantic Ridge (Baross and Deming 1983; Charlou et al. 2002). Thus, CO emanating from deep-sea hydrothermal fluids could serve as a source of C and energy for the growth of lithotrophic microorganisms.

The key enzymes of CO metabolism are CO dehydrogenases, which perform the reaction $CO + H_2O \rightarrow CO_2 + 2e^- + 2H^+$ (Meyer et al. 1993; Svetlitchny et al. 2001; Lindahl 2002). Among anaerobes, CO dehydrogenases are present in microorganisms that form or degrade acetate via the acetyl-CoA pathway found both in the representatives of Archaea and Bacteria domains. Among bacteria, it was found in acetogenic (Drake 1994), sulfate-reducing (see, e.g., Fukui et al. 1999) and some photosynthetic bacteria (Uffen 1983; Ensign 1995); and among archaea, in a variety of methanogens, including species of *Methanosarcina*, *Methanothrix*, and *Methanocaldococcus* (Ferry 1999), and in nonmethanogenic archaea like *Archeoglobus fulgidus* (Möller-Zinkhan and Thauer 1990) and *Ferroglobus placidus* (Vorholt et al. 1997). The number of microorganisms able to grow on CO is smaller. Even fewer microorganisms are capable of hydrogenogenic CO oxidation. Apart from two phototrophs, this capacity was shown for four thermophilic bacteria belonging to the genera *Carboxydothermus* (Svetlichny et al. 1991, 1994), *Caldanaerobacter* (Sokolova et al. 2001; Fardeau et al. 2004), and *Carboxydocella* (Sokolova et al. 2002). Based on 16S rRNA sequence analysis, all of them belong to the low G+C lineage of Gram-

positive bacteria, although they do not form a separate cluster. Among archaea, only the methanogen *Methanothermobacter thermautotrophicus* (Daniels et al. 1977) is known to grow on CO. However, this organism produces CH_4 but not H_2.

Our finding of a CO-utilizing H_2-producing representative of such a well-studied voluminous archaeal group as Thermococcales was rather unexpected. Our Blast searches performed in GenBank and at the B.A.G.S. site failed to reveal any nucleotide or protein sequences belonging to Thermococcales and related to any of the four recognized classes (Lindahl 2002) of anaerobic CO dehydrogenases. This fact, together with our failure to grow several strains of Thermococcales on CO, suggests that the capacity for anaerobic CO utilization is not a previously overlooked property characteristic of these archaea but may have been acquired by a particular *Thermococcus* strain via lateral gene transfer. The latter conclusion is in line with the lack of 16S rRNA sequence clustering of the currently known anaerobic CO oxidizers. It seems possible that the enzymes of anaerobic CO oxidation may be in future found to be housed by other unexpected hosts.

In this work, we present the first evidence of anaerobic CO oxidation coupled with H_2 formation performed by an archaeon. It is also the first example of lithotrophic growth in the order Thermococcales, a group of anaerobic organotrophs.

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References

Baross JA, Deming JW (1983) Growth of "black smoker" bacteria at temperatures of at least 250°C. Nature 303:423–426

Blöhl E, Rachel R, Burggraf S, Hafenbradl D, Jannasch HW, Stetter KO (1997) *Pyrolobus fumarii* gen. and sp. nov., represents a novel group of *Archaea*, extending the upper temperature limit for life to 113°C. Extremophiles 1:14–21

Bonch-Osmolovskaya EA, Miroshnichenko ML, Slobodkin AI, Sokolova TG, Karpov GA, Kostrikina NA, Zavarzina DG, Prokof'eva MI, Rusanov II, Pimenov NV (1999) Biodiversity of anaerobic lithotrophic prokaryotes in terrestrial hot springs of Kamchatka. Microbiology 68:343–351

Burggraf S, Jannasch HW, Nicolaus B, Stetter KO (1990) *Archaeoglobus profundus* sp. nov., represents a new species within the sulphate-reducing archeabacteria. Syst Appl Microbiol 13:24–28

Cary SC, Shank T, Stein J (1998) Worms bask in extreme temperatures. Nature 391:545–546

Charlou JL, Donval JP, Fouquet Y, Jean-Baptiste P, Holm N (2002) Geochemistry of high H_2 and CH_4 vent fluids issuing from ultramafic rocks at the Rainbow vent field (36 degrees 14′N, MAR). Chem Geol 191:345–359

Daniels L, Fuchs G, Thauer RK, Zeikus JG (1977) Carbon monoxide oxidation by methanogenic bacteria. J Bacteriol 132:118–126

Drake HL (1994) Acetogenesis, acetogenic bacetria, and the acetyl-CoA "Wood/Ljungdahl" pathway: past and current perspectives. In: Drake HL (ed) Acetogenesis. Chapman and Hall, New York, pp 1–15

Ensign SA (1995) Reactivity of carbon monoxide dehydrogenase from *Rhodospirillum rubrum* with carbon dioxide, carbonyl sulphide, and carbon disulphide. Biochemistry 34:5372–5381

Fardeau ML, Bonilla Salinas M, L'Haridon S, Jeanthon C, Verhé F, Cayol JL, Patel BKC, Garcia JL, Ollivier B (2004) Isolation from oil reservoirs of novel thermophilic anaerobes phylogenetically related to *Thermoanaerobacter subterraneus*: reassignment of *T. subterraneus*, *Thermoanaerobacter yonseiensis*, *Thermoanaerobacter tengcongensis* and *Carboxydibrachium pacificum* to *Caldanaerobacter subterraneus* gen. nov., sp. nov., comb. nov. as four novel subspecies. Int J Syst Evol Microbiol 54:467–474

Felsenstein J (1993) PHYLIP (PHYlogenetic Inference Package), version 3.5c. Department of Genetics, University of Washington, Seattle

Ferry, JG (1999) Enzymology of one-carbon metabolism in methanogenic pathways. FEMS Microbiol Rev 23:13–38

Fiala G, Stetter KO, Jannasch HW, Langworthy TA, Madon J (1986) *Staphylothermus marinus* sp. nov. represents a novel genus of extremely thermophilic submarine heterotrophic archaebacteria growing up to 98°C. Syst Appl Microbiol 8:106–113

Fukui M, Teske A, Assmus B, Muyzer G, Widdel F (1999) Physiology, phylogenetic relationships, and ecology of filamentous sulfate-reducing bacteria (genus *Desulfonema*). Arch Microbiol 172:193–203

Hekinian R, Fouquet Y (1985) Volcanism and metallogenesis of axial and off-axial structures on the East Pacific Rise near 13°N. Econ Geol 80:221–249

Holden JF, Takai K, Summit M, Bolton S, Zykowski J, Baross JA (2001) Diversity of three novel groups of hyperthermophilic deep-sea *Thermococcus* species from three sites in the northeastern Pacific Ocean. FEMS Microbiol Ecol 36:51–60

Huber H, Burggraf S, Mayer T, Wyschkony I, Rachel R, Stetter KO (2000) *Ignicoccus* gen. nov., a novel genus of hyperthermophilic, chemolithoautotrophic *Archaea*, represented by two new species, *Ignicoccus islandicus* sp. nov. and *Ignicoccus pacificus* sp. nov. Int J Syst Evol Microbiol 50:2093–2100

Kevbrin VV, Zavarzin GA (1992) The influence of sulfur compounds on the growth of halophilic homoacetic bacterium *Acetohalobium arabaticum*. Microbiology (English translation of *Mikrobiologiya*) 61:812–817

Kostyukova AS, Gongadze GM, Polosina YY, Bonch-Osmolovskaya EA, Miroshnichenko ML, Chernyh NA, Obraztsova MV, Svetlichny VA, Messner P, Sleytr UB, L'Haridon S, Jeanthone C, Prieur D (1999) Investigation of structure and antigenic capacities of *Thermococcales* cell envelopes and reclassification of *Caldococcus litoralis* Z-1301 as *Thermococcus litoralis* Z-1301. Extremophiles 3:239–245

Kurr M, Huber R, König H, Jannasch HW, Fricke H, Trincone A, Kristjansson JK, Stetter KO (1991) *Methanopyrus kandleri*, gen. and sp. nov. represents a novel group of hyperthermophilic methanogens, growing at 110°C. Arch Microbiol 156:239–247

Lindahl PA (2002) The Ni-containing carbon monoxide dehydrogenase family: light at the end of the tunnel? Curr Topics Biochem 41:2097–2105

Marmur J (1961) A procedure for the isolation of desoxyribonucleic acid from microorganisms. J Mol Biol 3:208–218

Marmur J, Doty P (1962) Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J Mol Biol 5:109–118

Meyer O, Frunzke K, Morsdorf G (1993) Biochemistry of aerobic utilization of carbon monoxide. In: Murrell JC, Kelly DP (eds) Microbial growth on C_1 compounds. Intercept, Ltd, Andover, Hampshire, UK, pp 433–459

Miroshnichenko ML, Gongadze GM, Rainey FA, Kostyukova AS, Lysenko AM, Chernyh NA, Bonch-Osmolovskaya EA (1998) *Thermococcus gorgonarius* sp. nov. and *Thermococcus pacificus* sp. nov.: heterotrophic extremely thermophilic *Archaea* from New Zealand submarine hot vents. Int J Syst Bacteriol 48:23–29

Möller-Zinkhan D, Thauer RK (1990) Anaerobic lactate oxidation to $3CO_2$ by *Archaeoglobus fulgidus* via the carbon monoxide dehydrogenase pathway: demonstration of the acetyl-CoA carbon-carbon cleavage reaction in cell extracts. Arch Microbiol 153:213–218

Pley U, Schipka J, Gambacorta A, Jannasch HW, Fricke H, Rachel R, Stetter KO (1991) *Pyrodictium abysii* sp. nov. represents a novel heterotrophic marine archaeal hyperthermophile growing at 110°C. Syst Appl Microbiol 14:245–253

Rainey FA, Ward-Rainey N, Kroppenstedt RM, Stackebrandt E (1996) The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of Nocardiopsaceae fam. nov. Int J Syst Bacteriol 46:1088–1092

Sokolova TG, González JM, Kostrikina NA, Chernyh NA, Tourova TP, Kato C, Bonch-Osmolovskaya EA, Robb FT (2001) *Carboxydobrachium pacificum* gen. nov., sp. nov., a new anaerobic, thermophilic, CO-utilizing marine bacterium from Okinawa Trough. Int J Syst Evol Microbiol 51:141–149

Sokolova TG, Kostrikina NA, Chernyh NA, Tourova TP, Bonch-Osmolovskaya EA (2002) *Carboxydocella thermautotrophica* gen. nov., sp. nov., a novel anaerobic CO-utilizing thermophile from a Kamchatkan hot spring. Int J Syst Evol Microbiol 52:1961–1967

Stetter KO, König H, Stackebrandt E (1983) *Pyrodictium*, a new genus of submarine disc-shaped sulfur reducing archaebacteria growing optimally at 105°C. Syst Appl Microbiol 4:535–551

Svetlichny VA, Sokolova TG, Gerhardt M, Ringpfeil M, Kostrikina NA, Zavarzin GA (1991) *Carboxydothermus hydrogenoformans* gen. nov., sp. nov., a CO-utilizing thermophilic anaerobic bacterium from hydrothermal environments of Kunashir Island. Syst Appl Microbiol 14:254–260

Svetlichny VA, Sokolova TG, Kostrikina NA, Lysenko AM (1994) A new thermophilic anaerobic carboxydotrophic bacterium *Carboxydothermus restrictus* sp. nov. Microbiology (English translation of Mikrobiologiya) 63:294–297

Svetlitchny V, Peschel C, Acker G, Meyer O (2001) Two membrane-associated NiFeS-carbon monoxide dehydrogenases from the anaerobic carbon monoxide-utilizing eubacterium *Carboxydothermus hydrogenoformans*. J Bacteriol 183:5134–5144

Symonds RB, Rose WI, Bluth GJS, Gerlach TM (1994) Volcanic gas studies: methods, results and applications. In: Carroland MR, Holloway JR (eds) Volatiles in magma. Mineral Society of America, Washington, pp 1–66

Takai K, Inoue A, Horikoshi K (2002) *Methanothermococcus okinawensis* sp. nov., a thermophilic, methane-producing archaeon isolated from a Western Pacific deep-sea hydrothermal vent system. Int J Syst Evol Microbiol 52:1089–1095

Uffen RL (1983) Metabolism of carbon monoxide by *Rhodopseudomonas gelatinosa*: cell growth and properties of the oxidation system. J Bacteriol 155:956–965

Vorholt JA, Hafenbradl D, Stetter KO, Thauer RK (1997) Pathways of autotrophic CO_2 fixation and of dissimilatory nitrate reduction to N_2O in *Ferroglobus placidus*. Arch Microbiol 167:19–23

Whitman WB, Boone DR, KogaY (2001) Order I. *Methanococcale*s. In: Garrity GM (ed) Bergey's manual of systematic bacteriology, vol 1, 2nd edn. Springer, Berlin Heidelberg New York, p 236

Wolin EA, Wolin MJ, Wolfe RS (1963) Formation of methane by bacterial extracts. J Biol Chem 238:2882–2886