Thermococcus hydrothermalis sp. nov., a New Hyperthermophilic Archaeon Isolated from a Deep-Sea Hydrothermal Vent

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An extremely thermophilic archaeon, strain AL662^T, was isolated from a deep-sea hydrothermal vent located on the East Pacific Rise at a latitude of 21°N. This strain is a strictly anaerobic coccus, and its cells range from 0.8 to 2 µm in diameter. The optimum temperature, pH, and Sea Salt concentration for growth are 85°C, 6, and 20 to 40 g/liter, respectively. Strain AL662^T grows preferentially on proteolysis products, on a mixture of 20 amino acids, and on maltose in the presence of elemental sulfur. The membrane lipids consist of di- and tetraether glycerol lipids. The DNA G+C content is 58 mol%. Sequencing of the 16S rRNA gene showed that strain AL662^T belongs to the genus *Thermococcus*. On the basis of hybridization results, we propose that this strain should be placed in a new species, *Thermococcus hydrothermalis*.

Since the discovery of hyperthermophilic archaea, strains belonging to the order *Thermococcales* have been isolated from both terrestrial and marine hot environments and described. Species belonging to the genus *Thermococcus* have been isolated from various deep-sea and shallow marine hydrothermal areas and described. All of the strains have quite similar physiological characteristics and can be divided into the following two groups on the basis of their G+C contents: (i) a group of strains with high G+C contents (50 to 58 mol%), including strains belonging to two species from shallow marine environments, *Thermococcus celer* (34) and *Thermococcus stetteri* (20), and three species from deep-sea environments, *Thermococcus profundus* (14), "*Thermococcus peptonophilus*" (8), and *Thermococcus fumicolans* (7); and (ii) a group of strains with low G+C contents (38 to 47 mol%), including strains belonging to two species from deep-sea environments, "*Thermococcus chitonophagus*" (11) and "*Thermococcus alcaliphilus*" (13), and an organism from a shallow marine environment, "*Thermococcus litoralis*" (22). All of these species are able to use proteinaceous carbon substrates in the absence or presence of sulfur. Some of these strains are able to grow in the absence of elemental sulfur, but sulfur greatly enhances growth. In this paper, we describe a new hyperthermophilic heterotrophic archaeal species that was isolated from a deep-sea hydrothermal vent on the East Pacific Rise at a latitude of 21°N. This organism was selected on the basis of the results of a preliminary screening for alcohol dehydrogenase (ADH) activity in 71 hyperthermophilic heterotrophic strains that were isolated at 80 and 95°C from various deep-sea hydrothermal vent areas (15).

MATERIALS AND METHODS

Reference strains. *Thermococcus celer* DSM 2476^T, "*Thermococcus litoralis*" DSM 5474^T, *Thermococcus stetteri* DSM 5262^T, *Sulfolobus acidocaldarius* DSM 639^T, and *Thermotoga maritima* DSM 3109^T were obtained from the Deutsche Sammlung von Mikroorganismen, Braunschweig-Stöckheim, Germany. Other strains were directly obtained from researchers. *Thermococcus profundus*

DT5432^T was provided by Tetsuo Kobayashi (RIKEN, Wako, Japan). *Thermococcus fumicolans* CIP104680^T was isolated in our laboratory.

Growth conditions. 2216-S medium (2) contains (per liter) 2 g of peptone, 0.5 g of yeast extract, 30 g of Sea Salt (Sigma Chemical Co., St. Louis, Mo.), 6.05 g of PIPES [piperazine-*N,N'*-bis(ethanesulfonic acid)] buffer (Sigma), 10 g of sulfur, and 1 mg of resazurin. BHI-S medium contains (per liter) 9 g of brain heart infusion, 23 g of NaCl (instead of Sea Salt, to prevent formation of a mineral precipitate), 6.05 g of PIPES buffer, 10 g of sulfur, and 0.5 mg of resazurin. 20AA-S medium contains (per liter) 30 g of Sea Salt, 6.05 g of PIPES buffer, 10 g of sulfur, 1 mg of resazurin, 10 ml of a mineral solution (1), and 10 ml of a vitamin solution (1) and each of the 20 classical amino acids at a concentration of 0.2 mM. A stock solution containing each of the amino acids at a concentration of 2 mM was sterilized separately by filtration (pore size, 0.22 µm; Nalgene filterware). Unless indicated otherwise, the pH was adjusted to 5.5 with 5 N NaOH. In each case, the medium was sterilized by steaming it at 100°C for 30 min on 2 successive days, transferred into an anaerobic chamber containing N₂, H₂, and CO₂ (90:5:5), reduced by adding sodium sulfide to a final concentration of 0.5 g/liter, and then distributed into Hungate tubes or into 50-ml serum vials with butyl rubber stoppers. Unless indicated otherwise, cultures were grown on 2216-S medium at pH 5.5 and incubated at atmospheric pressure under the anaerobic chamber gas mixture at 80°C.

Enrichment cultures and purification. After samples (rock suspensions) were collected, they were stored at room temperature in serum vials filled with sterile seawater under anaerobic conditions (N₂ headspace gas and 0.5 g of sodium sulfide per liter). Enrichment cultures were grown on 2216-S medium that was incubated at 80°C for 2 or 3 days. Cultures were purified by streaking samples onto 2216-S medium that was solidified with Gelrite (Scott Laboratories, Long Island, N.Y.) and incubated in anaerobic jars at 80°C (6). Purified isolates were stored at 4°C and could be used as inocula for at least 1 year. For long-term storage, pure cultures were stored anaerobically at -70°C in the presence of 5% (wt/vol) dimethyl sulfoxide (Sigma) as a cryoprotectant.

Determination of cell number. The numbers of cells were determined by direct cell counting by using a Thoma chamber (depth, 0.02 mm). When cells were grown on medium containing high particle concentrations (e.g., on culture medium supplemented with starch or on medium at temperatures above 100°C), the numbers of cells were determined by epifluorescence (10). Samples were diluted in sterile water containing Sea Salt (30 g/liter), formalin (2.5%), and acridine orange (0.01%), filtered with black polycarbonate membrane filters (pore size, 0.2 µm; Nuclepore), and observed with an Olympus model BH-2 microscope.

Determination of growth parameters. To determine the optimum temperature, cells were grown in Hungate tubes containing 6 ml of 2216-S medium. The headspace gas was N₂-CO₂-H₂ (90:5:5) at a pressure of 100 kPa for temperatures under 100°C and at a pressure of 200 kPa for temperatures of 100°C or more. Temperatures were maintained with aluminum heating blocks (Bioblock, Illkirch, France) and were monitored with temperature probes placed in control tubes. To determine growth rates at different pHs, 2216-S medium was modified by using the following buffers (Sigma), each at a concentration of 1 g/liter: for pH 2.5, 3.5, and 4.5, no buffer; for pH 5.5, MES (morpholineethanesulfonic acid) buffer; for pH 6.5, PIPES buffer; for pH 7.5, HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer; and for pH 8.5 and 9.5, AMPSO [3-(1,1-dimethyl-2-hydroxyethyl)amino)-2-hydroxypropanesulfonic acid] buffer. After sodium sulfide was added, the pH was adjusted with 1 M HCl. To determine the salt requirement, 2216-S medium was prepared with different dilutions of Sea

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Salt. The effects of pH and salinity were determined at 80°C. Three replicates were studied simultaneously at each temperature, pH, or salinity.

Determination of growth requirements. The ability of isolate AL662^T to utilize various carbon sources was tested in media in which the yeast extract and peptone of 2216-S medium were replaced by 0.4 g of ammonium chloride per liter, 10 ml of a mineral solution (1) per liter, 10 ml of a vitamin solution (1) per liter, and carbon sources. Tests were performed by using tubes containing no nitrogen and no carbon substrates as controls for growth on substrates carried over in the inoculum. Most of the carbon sources were tested at a concentration of 5 g/liter; the exceptions were starch, which was tested at a concentration of 10 g/liter, and ethanol, which was tested at a concentration of 5 ml/liter. Growth on BHI-S medium and growth on 20AA-S medium were also tested. In addition, autotrophic growth with H₂-CO₂ (80:20) headspace gas as the sole carbon source was tested. To determine the ability of the isolate to grow in the absence of elemental sulfur, cells were cultured on 2216-S medium from which sulfur compounds had been omitted. In this experiment, the headspace gases were N₂, N₂-CO₂ (80:20), and H₂-CO₂ (80:20) at a pressure of 100 kPa, and the medium was reduced by adding titanium nitrilotriacetate (21) at a concentration of 200 µM. Growth on cystine (10 g/liter) and growth on polysulfide (10 mM) (4) were also investigated.

Antibiotic susceptibility. Susceptibility to the antibiotics vancomycin, penicillin, kanamycin, streptomycin, rifampin, and chloramphenicol, each at a final concentration of 100 µg/ml, was determined under the standard growth conditions. *Thermotoga maritima* was used as a control to establish the effectiveness of antibiotics at 80°C.

Electron microscopy. For transmission electron microscopy, cells were harvested at the end of the log phase. The pellet was resuspended and fixed for 1 h at room temperature in 0.4 M cacodylate buffer (pH 7.4)–5.5% NaCl–8% glutaraldehyde (2:1:1) and then postfixed for 1 h at 4°C in 0.4 M cacodylate buffer (pH 7.4)–10% NaCl–2% OsO₄ (1:1:2). After dehydration with increasing ethanol concentrations, cells were embedded in Spurr's resin, thin sectioned, contrasted with 1% (wt/vol) uranyl acetate and 1% (wt/vol) lead citrate (Bio-Rad S.A., Ivry, France), and examined with a model EM201 transmission microscope (Philips, Eindhoven, The Netherlands).

Lipid analysis. (i) **Lipid extraction.** Lipids were extracted from lyophilized cells (50 to 100 mg) by using a modified Bligh-Dyer method (3, 33). The extracted lipids were fractionated into neutral lipids, glycolipids, and polar lipids by silicic acid column chromatography by using chloroform, acetone, and methanol, respectively. The methanol fraction containing polar ether lipids was dried under a vacuum and stored under N₂ until further analysis. The lipid-extracted residue was hydrolyzed in 1 N HCl (5 h at 100°C). After cooling, the solution was transferred to a separatory funnel with washes of dichloromethane. Two phases were allowed to separate, and the organic phase containing the bound ether lipids was collected for further analysis.

(ii) **Glycerol ether analysis.** The polar phase of the Bligh-Dyer extraction preparation was subjected to strong acid hydrolysis, and the resulting core ether lipids were digested with 55% HI for 18 h at 100°C. The resulting alkyl iodides were extracted with hexane and successively washed with Na₂S₂O₃ and Na₂CO₃ solutions (12, 16, 23). After extraction with hexane, the iodide derivatives were analyzed by gas chromatography with an instrument equipped with an electron capture detector by using 1,2-di-*o*-hexadecyl-rac-glycerol as the internal standard. Authentic glycerol diethers and diglycerol tetraethers were purchased from Sigma or were isolated from *S. acidocaldarius*.

Organic acid production. The isolate was grown on BHI-S medium and on 20AA-S medium at 80°C for 15 h. Organic acids were analyzed by gas chromatography and gas chromatography-mass spectrometry as *tert*-butyl dimethylsilyl derivatives (27).

DNA base composition. Cells were cultured in 4.5 liters of 2216-S medium at 80°C and harvested at the end of the exponential phase of growth. After centrifugation, the cell pellet was suspended in 5 ml of lysis buffer (100 mM Tris-HCl, 100 mM NaCl, 0.50 mM EDTA; pH 8), 1% (wt/vol) Sarkosyl was added, and then 1% (wt/vol) sodium dodecyl sulfate and proteinase K (final concentration, 0.4 mg/liter) were added for cell lysis. After 3 h of incubation at 40°C, three phenol-chloroform-isoamyl alcohol (24:24:1) extractions and one chloroform extraction were performed. The DNA was precipitated by adding 2 volumes of ethanol at –20°C. The DNA pellet was resuspended in TE buffer (10 mM Tris-HCl, 2 mM EDTA; pH 7.4) and treated with RNase (5 µg/liter) for 1 h at 60°C. The DNA base composition was determined by the thermal denaturation method (19).

16S rRNA sequence analysis. The *rrs* gene was amplified by PCR (28) as described previously (5), except that archaeon-specific primers were used. The primer sequences were 5'-TCC GGT TGA TCC TGC CGG AC-3' (*Escherichia coli* 16S rRNA gene positions 2 to 21) and 5'-CTT TCG GTC GCC CCT ACT-3' (*Thermococcus celer* 23S rRNA gene positions 257 to 234). As described in the SureClone ligation kit (Pharmacia Biotech, Orsay, France) protocol, the purified PCR product was prepared for ligation by using the 3'-5' exonuclease activity of the Klenow fragment to remove single-base 3' overhangs on the fragments. The PCR fragments were concomitantly phosphorylated with T4 polynucleotide kinase. After phenol-chloroform-isoamyl alcohol (24:24:1) extraction and Micro-Spin column (Pharmacia) purification, the PCR product was ligated to a *Sma*I-digested and calf intestine alkaline phosphatase-dephosphorylated BlueScript SK+ vector (Stratagene cloning system; Stratagene, La Jolla, Calif.). *E. coli* TG2

competent cells were then transformed with a portion of the reaction mixture. Recombinant colonies were selected by α -complementation (32), and the size of the cloned plasmid was determined by agarose electrophoresis. After this, the plasmid was purified by using a Plasmid Midi kit (Qiagen, Hilden, Germany). The DNA sequence was determined on double-stranded DNA by the dideoxy chain termination method (30) and with the Applied Biosystems automatic DNA analysis system; this analysis was performed by workers at the Euro Sequence Gene Service, Montigny le Bretonneux, France. A total of 1,975 bp was sequenced. The sequence was then aligned with a representative collection of 16S rRNA sequences. The alignment was performed with 1,450 bp. A multiple alignment, a phylogenetic tree, and levels of similarity were obtained by using MegAlign of the DNASTAR package (Promega, Madison, Wis.), the Clustal (version V) method (9) with weighted residue, and the neighbor-joining method (29).

Quantitative DNA-DNA hybridization. The DNAs of the reference strains of *Thermococcus celer*, *Thermococcus litoralis*, and *Thermococcus fumicolans* were first used as labeled probes in a single hybridization experiment with strain AL662^T, and strain AL662^T DNA was then used as the labeled probe in experiments with *Thermococcus stetteri* and *Thermococcus profundus*. Up to 20 liters of culture was needed to get enough DNA for the experiment. A 4- to 5-µg portion of each reference strain DNA was labeled by incorporation of both [³H]dATP and [³H]dGTP by using a Megaprime kit (Amersham, Amersham Place, United Kingdom). The nuclease S1 method for quantitative DNA-DNA hybridization was carried out as described by Popoff and Coynault (25) by using DE81 filters (Whatman International, Maidstone, United Kingdom) and a β matic IV scintillation counter (Kontron Instruments, Montigny le Bretonneux, France).

Nucleotide sequence accession number. The nucleotide sequence of the 16S rRNA of strain AL662^T has been deposited in the GeneBank database under accession no. Z70244.

RESULTS

Collection of samples. Chimney walls covered with Alvinne-lids tubes were sampled by using the manned submersible "Alvin" during the American cruise MVT'90 in 1990 on the East Pacific Rise at a latitude of 21°N (26).

Enrichment and purification. After 2 or 3 days, increases in turbidity and microscopic observation indicated that growth had occurred. Purification was performed by plating samples onto solid sulfur-containing medium under anaerobic conditions (26).

Morphology. Phase-contrast microscopy revealed that isolate AL662^T cells were coccoid and occurred singly, in pairs, or in small aggregates (Fig. 1). The cell diameter ranged from 0.8 to 2 µm. The cells appeared to be motile, and polar flagella were observed by phase-contrast microscopy after specific coloration with Spot Test flagellum stain (Difco Laboratories, Detroit, Mich.). The cells most likely divided by constriction (data not shown). A two-layer cell envelope was observed with membrane fragments (Fig. 2).

Antibiotic susceptibility. Isolate AL662^T was resistant to vancomycin, penicillin, kanamycin, streptomycin, rifampin, and chloramphenicol, each at a concentration of 100 µg/ml.

Lipid analysis. The polar lipid fraction of strain AL662^T contained primarily glycerol ether lipids, and diphytanyl glycerol diether (C₂₀) was the predominant lipid and accounted for 80% of the total lipid fraction. In contrast, the tetraethers predominated (accounting for 75% of the total lipids) in the lipids extracted by the Bligh-Dyer method. No cyclopropane rings were observed either in the organic fraction or in the residual phase. The latter phase also contained a significant amount of unidentified compounds; complete identification of these unusual lipids is under way (8a).

Determination of growth parameters. Isolate AL662^T grew at 55 to 100°C, and the optimum temperature for growth was 80 to 90°C (Fig. 3a). At the optimal temperature the generation time was around 90 min; at 100°C it was around 5 h. Growth was observed at pH 3.5 to 9.5, and the optimum pH was between 5.5 and 6.5 (Fig. 3b). Growth was observed at salt concentrations ranging from 20 to 80 g/liter, and the optimum salt concentration was between 30 and 40 g/liter (correspond-

FIG. 1. Phase-contrast micrographs of strain AL662^T. Bar = 10 μ m.

ing to 19.5 to 26 g of NaCl per liter); no growth was detected at salt concentrations of 5, 10, and 100 g/liter (Fig. 3c).

Determination of growth requirements. 2216-S media supported rapid growth of isolate AL662^T. Yeast extract-peptone could be replaced by meat extract and brain heart infusion (Table 1). Significant growth was also observed on yeast extract, malt extract, peptone, and the 20 amino acids tested. Slow growth was also observed on casein and Casamino Acids. Maltose supported rapid growth of isolate AL662^T. Growth on cellobiose and very low levels of growth on pyruvate were observed after 24 h of culture. None of the other sugars, alcohols, carboxylic acids, individual amino acids, or polysaccharides tested supported growth of isolate AL662^T. H₂-CO₂ (80:20) headspace gas did not support autotrophic growth and did not modify the growth of AL662^T on BHI-S medium (Table 1). Slow growth occurred in the absence of elemental sulfur, and growth was rapidly inhibited by 80% H₂ (Table 1). Cystine and polysulfide supported rapid growth of AL662^T (Table 1). When elemental sulfur was present in the culture medium, growth of AL662^T was accompanied by the production of large amounts of hydrogen sulfide (data not shown).

Organic acid production. In BHI-S and 20AA-S media, acetate (6.53 and 4.7 mM, respectively), phenyl acetate (3.06 and 0.83 mM), lactate (1.17 and 1.75 mM), and 2-methylpropionate (2.25 and 0.53 mM) were the major fermentation products; propanoate (0.65 and 0.79 mM), indoleacetate (0.26 and 0.37 mM), 3-methylthiopropionate (0.87 and 0.36 mM), and *p*-hydroxy-phenylacetate (0.77 and 0.2 mM) were also present.

DNA base composition. The G+C content of the DNA of isolate AL662^T is 58 mol%.

16S rRNA sequence analysis. The sequence of the 16S rRNA-encoding gene of strain AL662^T was determined and aligned with the other available 16S rRNA sequences of strains belonging to the *Thermococcales*. A comparison of the 16S rRNA sequences in which a phylogenetic tree was constructed

(Fig. 4) revealed that strain AL662^T belongs to the order *Thermococcales*. The levels of similarity between the 16S rRNA of AL662^T and the 16S rRNAs of other *Thermococcus* species are as follows: *Thermococcus celer*, 99.1%; "*Thermococcus litoralis*," 94.5%; *Thermococcus profundus*, 98.7%; *Thermococcus stetteri*, 98.5%; "*Thermococcus peptonophilus*," 97.2%; and *Thermococcus fumicolans*, 93.9%. The results of this analysis clearly indicate that AL662^T belongs to the genus *Thermococcus*.

DNA-DNA hybridizations. Considering the phylogenetic tree based on 16S rRNA gene sequences and considering the high levels of similarity (more than 98%) (31) between the AL662^T 16S rRNA gene sequence and the *Thermococcus celer*, *Thermococcus profundus*, and *Thermococcus stetteri* 16S rRNA gene sequences, quantitative DNA-DNA hybridization experiments were performed. When strain AL662^T was used as the labeled probe, the levels of DNA reassociation were as follows: *Thermococcus stetteri*, 5%; and *Thermococcus profundus*, 6%. When reference strains were used as the labeled probes, the levels of DNA reassociation were as follows: *Thermococcus celer*, 26%; "*Thermococcus litoralis*," 8%; and *Thermococcus fumicolans*, 27%. These data confirm that strain AL662^T is a new species of the genus *Thermococcus*.

DISCUSSION

The novel marine hyperthermophilic strain AL662^T belongs to the archaeal domain on the basis of its resistance to antibiotics, the presence of di- and tetraether lipids, and its 16S rRNA sequence. The G+C content and the 16S rRNA sequence indicate that strain AL662^T belongs to the genus *Thermococcus*. The morphological and physiological characteristics of strain AL662^T are consistent with the morphological and physiological characteristics of this genus.

FIG. 2. Transmission electron micrograph of strain AL662^T. Bar = 1 μ m.

FIG. 3. Temperature, pH, and Sea Salt concentration optima for growth of isolate AL662^T on 2216-S medium. (a) Specific growth rate as a function of temperature (in the presence of 30 g of Sea Salt per liter at pH 7.5). (b) Specific growth rate as a function of pH (in the presence of 30 g of Sea Salt per liter at 80°C). (c) Specific growth rate as a function of salinity (at 80°C and pH 7.5). Growth rates were calculated by performing a linear regression analysis along the logarithmic parts of the growth curves. If enough data were available, information about the precision of the growth rate is given (regression coefficient $\pm$ 2 standard errors).

Strain AL662^T differs from "*Thermococcus litoralis*," "*Thermococcus alcaliphilus*," and "*Thermococcus chitonophagus*" in its DNA G+C content; from *Thermococcus stetteri*, *Thermococcus profundus*, strain ES1 (24), and "*Thermococcus alcaliphilus*" in its temperature range (it grows at temperatures up to 100°C); from "*Thermococcus litoralis*", *Thermococcus profundus*, "*Thermococcus alcaliphilus*", and *Thermococcus fomicolans* in its pH optimum; and from "*Thermococcus litoralis*", *Thermococcus stetteri*, *Thermococcus profundus*, "*Thermococcus peptonophilus*", and *Thermococcus fomicolans* in its resistance to rifampicin. Although the physiological features of strain AL662^T resemble those of *Thermococcus celer*, strain AL662^T is more tolerant to low salt concentrations. *Thermococcus celer* lyses at salt concentrations below 35 g/liter (34). Strain AL662^T grows optimally in the presence of proteinaceous substrates and also on a mixture of 20 amino acids as sole carbon and energy sources, as previously described for other strains belonging to the order *Thermococcales* (7). Significant growth on maltose and slow growth on cellobiose, characteristics which have been observed previously for *Thermococcus fomicolans*, were observed, and a small amount of proteinaceous substrate is needed (7). Like the growth of most other *Thermococcus* species, the growth of strain AL662^T is not strictly dependent upon elemental sulfur. Elemental sulfur

TABLE 1. Growth requirements of strain AL662^T

Compound(s)	Growth after ^a :		
	8 h	15 h	24 h
Carbon sources			
Yeast extract-peptone (2216-S medium)	+++	+++	ND
Meat extract	++	+++	ND
Yeast extract	+	++	+++
Malt extract	++	+++	+++
Peptone	++	+++	+++
Brain heart infusion (BHI-S medium)	+++	+++	ND
Casein	+	++	++
Casamino Acids	ND	—	+
Glucose	—	—	—
Sucrose	—	—	—
Maltose	+++	ND	+++
Cellobiose	+	ND	++
Starch	—	—	—
Ethanol	—	—	—
Mannitol	—	—	—
Acetate	—	—	—
Pyruvate	—	+	+
20 Amino acids (20AA-S medium)	+	++	++
None (under H ₂ -CO ₂)	—	—	—
BHI-S medium (under H ₂ -CO ₂)	+++	+++	ND
Electron acceptors			
Sulfur	+++	ND	+++
None (under N ₂)	++	ND	++
None (under N ₂ -CO ₂ , 80:20)	++	ND	++
None (under H ₂ -CO ₂ , 80:20)	—	ND	—
Cystine	++	+++	ND
Polysulfide	++	ND	ND

^a +++, $>2 \times 10^8$ cells per ml of culture (final concentration); ++, 5×10^7 to 10^8 cells per ml of culture (final concentration); +, 2×10^7 to 10^7 cells per ml of culture (final concentration); —, no growth ($<10^7$ cells per ml of culture); ND, not determined.

greatly enhances the growth of strain AL662^T, but it can be replaced by cystine or polysulfide (however, growth is less efficient).

Strain AL662^T possesses an NADP-dependent ADH activity

FIG. 4. Phylogenetic relationships based on 16S rRNA sequences. Most of 16S rRNA sequences were obtained from the GenBank and EMBL databases. The exceptions were the sequences of *Thermococcus litoralis*, *Thermococcus profundus*, *Thermococcus stetteri*, and *Thermococcus fomicolans*, which were determined in our laboratory. Alignment was performed with sequences from representatives of *Pyrococcus* and *Pyrodicticum* species. Only part of the resulting tree is presented. The units for the scale are number of substitution events inferred per 100 bp. The accession numbers of the sequences of the organisms used in this analysis are as follows: *Thermococcus celer*, M21529 I; "*Thermococcus peptonophilus*", D37982; *Pyrococcus abyssi*, L19921; *Pyrococcus shinkai*, D45214; *Pyrococcus furiosus*, U20163; *Pyrodicticum occultum*, M21087; "*Thermococcus litoralis*", Z70251; *Thermococcus profundus*, Z75233; *Thermococcus stetteri*, Z75240; and *Thermococcus fomicolans*, Z70250.

that oxidizes alcohol and reduces aldehydes (15). Purification and characterization of the enzyme are under way. Within the genus *Thermococcus*, "*Thermococcus litoralis*" and *Thermococcus* sp. strain ES1 have an NADP-specific ADH activity (17, 18), and it has been proposed that the physiological reaction of the enzyme might be aldehyde reduction. Ma and coworkers (17) have shown with *Thermococcus* sp. strain ES1 that the level of ADH activity increases when the amount of elemental sulfur is limited, and they have proposed that when sulfur is limiting, ADH reduces compounds to alcohols, the aldehydes that are generated during fermentation of amino acids, to remove excess reductant. Thus, the presence of ADH activity in strain AL662^T, which is able to grow on amino acids, is not surprising.

On the basis of its morphological and physiological characteristics, its 16S rRNA sequences, and DNA-DNA hybridization data, new isolate AL662^T represents a new species of the genus *Thermococcus*. This species is named *Thermococcus hydrothermalis* because it was isolated from a hydrothermal area.

Description of *Thermococcus hydrothermalis* sp. nov. *Thermococcus hydrothermalis* (hy.dro.ther.mal'is. N.L. adj. *hydrothermalis*, pertaining to a hydrothermal vent). Cells are cocci (diameter, 0.8 to 2 µm) that are motile by means of polar flagella. Cell division occurs by constriction. Obligately anaerobic. Grows optimally in the presence of 30 to 40 g of Sea Salt per liter and at a pH around 6. Growth occurs at 55 to 100°C, and the optimum temperature is around 85°C. Obligately chemorganotrophic. Grows preferentially on proteolysis products, a mixture of amino acids, and maltose. Sulfur is not necessary for growth but greatly enhances growth. The results of 16S rRNA sequence comparisons place *Thermococcus hydrothermalis* in the *Thermococcales*. Type strain AL662 (= CNCMI1319 [Collection Nationale de Cultures de Microorganismes, Institut Pasteur, Paris, France]) was isolated from an active chimney wall fragment recovered from a hydrothermal site on the East Pacific Rise at a latitude of 21°N.

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