Extremophiles, Thermophily section, species description Thermococcus atlanticus sp. nov., a hyperthermophilic Archaeon isolated from a deep-sea hydrothermal vent in the Mid-Atlantic Ridge

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Abstract An extremely thermophilic archaeon, strain MA898, was isolated from a deep-sea hydrothermal vent on the Mid-Atlantic Ridge. This strain is a strictly anaerobic coccus of approximately 0.7–1.2 μm in diameter. Optimal temperature, pH, and NaCl concentration for growth are around 85 °C, pH 7, and 3%, respectively. Strain MA898 grows preferentially in the presence of elemental sulfur, polysulfur, cystine, or L-cysteine. The microorganism requires rich proteinaceous substrates. BHI-S medium supports rapid growth, with a final concentration of more than 1.2 · 10⁹ cells ml⁻¹, but strain MA898 exhibits poor growth on 2216S medium (yeast/peptone) and poor growth on starch. Growth is inhibited by rifampicin and chloramphenicol at a concentration of 100 μg/ml. The DNA G+C content is 50 mol%. Sequencing of the 16S rRNA gene indicates that strain MA898 belongs to the Thermococcus genus, and from DNA/DNA hybridization data it is proposed as a new species: Thermococcus atlanticus. The deposition numbers are CIP-107420T and DSM15226.

Keywords Hydrothermal · Thermophiles · Thermococcus · Mid-Atlantic Ridge · Proteolysis

Introduction

The Thermococcales family is widely distributed in thermal environments (Takai and Sako 1999). It is now represented by three genera, Pyrococcus (Fiala and Stetter 1986), Thermococcus (Zillig et al. 1983), and the newly described Paleococcus (Takai et al. 2000). These organisms are all obligate heterotrophs growing preferentially on proteinaceous substrates, although some of them are also able to degrade carbohydrate substrates such as starch (Godfroy et al. 1997), chitin (Huber et al. 1995), and xylan (Ronimus et al. 1997). Because of these properties, they have been intensively studied for biotechnological purposes (Ciaramella et al. 1995). Their thermophily and their easy cultivation procedures make them good candidates for the development of molecular biology tools such as DNA polymerases (Cambon-Bonavita et al. 2000; Perler et al. 1996; Takagi et al. 1997) or conjugative plasmids (Charbonnier et al. 1992; Noll and Vargas 1997).

The normal approach for isolation of new species requiring purification and identification often reveals isolates that belong to known species. For this reason, we used qualitative dot-blott hybridization as a tool prior to purification, using known Thermococcales species as controls. This approach led to the isolation of a new hyperthermophilic, heterotrophic archaeal species from chimney wall fragment. The description of this new species is presented in this paper.

Materials and methods

Reference strains

Thermococcus aggregans (DSM 10597T), Thermococcus stetteri (DSM 5262), Thermococcus celer (DSM 2476T), Thermococcus litrantis (DSM5474T), Pyrococcus woesi (DSM3773T), and Thermotoga maritima (DSM 3109T) were obtained from the Deutsche Sammlung von Mikroorganismen, (Braunschweig-Stöckheim, Germany). Thermococcus barophilus (CNCM I-1946T) was obtained from the CNCM (Institut Pasteur, Paris, France). Thermococcus profundus (DT5432T) was provided by Tetsuo Kobayashi (RIKEN, Wako, Japan). Pyrococcus abyssi strain ST549 (CNMC I 1318), Pyrococcus glycovorans (CNMC I-2120T), Thermococcus hydrothermalis (CNMC I-1319T), Thermococcus fumicolans (CIP 104690T), and Thermococcus sp strain ST554 were isolated in our laboratory. Thermococcus sp. strains GE3, GE8, and GE20 were provided by Gael Erauso (IUEM, Brest, France) (Marteinsson et al. 1995).
Culture conditions

BHI-S medium consists (per liter) of brain-heart infusion (Difco), 9 g; NaCl, 23 g (instead of sea salt from Sigma, to prevent precipitations); PIPES, 6.05 g; sulfur, 10 g; and resazurin, 1 mg. 2216-S medium (Belkin and Jannasch 1985) consists (per liter) of peptone (Difco), 2 g; yeast extract (Difco), 0.5 g; sea salt, 30 g; PIPES, 6.05 g; sulfur, 10 g; and resazurin, 1 mg. 20AA-S medium consists (per liter) of sea salt, 30 g; PIPES, 6.05 g; sulfur, 10 g; resazurin, 1 mg; 10 ml mineral solution (Balch et al. 1979); 10 ml vitamin solution (Balch et al. 1979); and a solution of 20 amino acids each at 0.1 g/l. A stock solution containing each amino acid (from Sigma) at a concentration of 1 g/l was sterilized separately by filtration (Nalgene filterware, 0.22 μm). Unless otherwise indicated, the pH was adjusted to 7.0 with 5M-NaOH. The medium was sterilized by steaming twice at 100 °C for 30 min on two successive days, transferred into an anaerobic chamber containing N2/H2/CO2 (90:5:5), reduced by addition of sodium sulfide to a final concentration of 0.5 g/l, and distributed in Hungate tubes or in 50-ml serum vials with butyl rubber stoppers. Unless otherwise indicated, cultures were incubated at atmospheric pressure under the anaerobic chamber gas mixture at 80 °C.

Sampling

Sampling of hydrothermal chimneys was conducted by the submersible Alvin during the American-French MAR’93 cruise in June 1993 at the hydrothermal TAG (26°N) and Snake Pit (23°N) sites on the Mid-Atlantic Ridge. Whenever possible, samples were brought to the surface in insulated boxes. On board, chimney fragments were aseptically subsampled according to their mineral zonation. Each subsample was crushed in 40 ml sterile seawater in fragments were aseptically subsampled according to their mineral zonation. Each subsample was crushed in 40 ml sterile seawater in an anaerobic chamber and stored at room temperature in serum vials under anaerobic conditions (N2 headspace and 0.5 g/l sodium sulfide). The bacterium Thermotoga maritima was also investigated.

Enrichment cultures and purification

Enrichment cultures were performed on a BHI-S medium into 100-ml serum vials containing 80 ml of the medium. Each sample was used as inoculum at three different dilutions (1/1, 1/10, and 1/100). Cultures were performed in an oven at 80 and 95 °C. Positive cultures were identified by turbidity and microscopic observation. Strains were purified by streaking on BHI-S medium, solidified with Gelrite (Scott Laboratories, Long Island, USA), and incubated in anaerobic jars at 80 °C (Errasou et al. 1995). Purified isolates were stored at 4 °C and could serve as inoculum for at least 1 year.

Determination of cell numbers

Cell numbers in liquid cultures were determined by direct visual counting of cells using a Thoma chamber with an Olympus BH-2 microscope. Optical density (600 nm) was also measured with a spectrophotometer (Spectronic 301 instrument, Milton Roy, Rochester, USA) for determination of optimum growth conditions, and the correlation between turbidity and cell number was determined.

Determination of growth parameters

For optimal temperature determination, cells were grown in Hungate tubes containing 6 ml of BHI-S medium. The headspace was 100 kPa of N2/CO2/H2 (90:5:5). Cultures were incubated in aluminum heating blocks (Bioblock, Illkirch, France) and monitored with temperature probes placed in control tubes. For determination of growth rates at different pH values, BHI-S medium was modified using the following buffers (Sigma, St. Louis, USA) each at 20 mM: pH 3 and 4 — no buffer; pH 5 (MES), buffer; pH 6 — PIPES buffer; pH 7 — HEPES buffer; and pH 8 and 9 — AMPSO buffer. After addition of sodium sulfide, the pH was adjusted with 1M-HCl. To determine the salt requirement, BHI-S medium was prepared with different dilutions of NaCl (0, 15.6, 23, 30.6, and 46 g/l). The effects of pH and salinity were determined at 80 °C. Three replicates were performed simultaneously at each temperature, pH, or salinity.

Determination of growth requirements

The ability of the isolate to consume different carbon sources was tested in medium where the yeast extract and peptone of the 2216-S medium were replaced by 0.4 g/l ammonium chloride, 10 ml mineral solution (Balch et al. 1979), 10 ml vitamin solution (Balch et al. 1979), and a variety of carbon sources. Tests were performed in tubes containing no nitrogen or carbon substrates to serve as controls for growth on substrates carried over in the inoculum. Most of the carbon sources (peptone, yeast, tryptone, meat, maltose, glucose, cellobiose, xylose, lactose, fructose, saccharose, malt, casein, casamino acids, xylan, and dextrane) were tested at 5 g/l. Starch, cellulose, and chitin were tested at 10 g/l. Formate, acetate, propionate, pyruvate, succinate, and lactate were tested at 2 g/l, and ethanol, glycerol, and methanol were tested at 5 ml/l. Growth on cystine (10 g/l), HCl-cysteine (20 mM), and polysulfide (10 mM) (Blumentals et al. 1990) was also investigated.

Influence of the gas headspace

To test the influence of the gas composition of the headspace, cells were cultured on BHI-S media with various headspace gases: N2, N2/CO2 (80:20), H2/CO2 (80:20), and N2/H2/CO2 (90:5:5). Pro- duction of H2 and CO2 was measured using a gas chromatograph (Shimadzu). To test the growth under aerobic conditions, BHI-S medium was prepared under ambient air, without being reduced by addition of sodium sulfide.

Antibiotic sensitivity

Sensitivity to vancomycin, penicillin, kanamycin, streptomycin, rifampicin, and chloramphenicol at final concentrations of 100 μg/ml was tested under the standard growth conditions (BHI-S, pH 7, 80 °C). The bacterium Thermotoga maritima was used as control to establish the effectiveness of the antibiotic at 80 °C.

Scanning electron microscopy

Cells were harvested at the end of log phase. For scanning electron microscopy, cells were fixed with formalin 10% (v/v) for 1 h, and then harvested by centrifugation for 20 min at 2,000 × g. Pellets were resuspended in 23 g/l NaCl, displayed on 0.22 μm filters (Nucleopore), and dried overnight at room temperature. Samples were then coated with gold (SCD640, Balzers) and examined with a scanning electron microscope XL 30 LaB6 (Philips).

Analysis of amino acids, organic acids, and aromatic acids

Each sample was centrifuged 30 min at 8,000 g to remove the cells. Half of the supernatant was transferred to an ultrafree-CL-PLGC