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Pyrococcus CH1, an obligate piezophilic hyperthermophile: extending the upper pressure-temperature limits for life

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Abstract:

A novel hydrothermal site was discovered in March 2007, on the mid-Atlantic ridge during the cruise 'Serpentine'. At a depth of 4100 m, the site 'Ashadze' is the deepest vent field known so far. Smoker samples were collected with the ROV 'Victor 6000' and processed in the laboratory for the enrichment of anaerobic heterotrophic microorganisms under high-temperature and high-hydrostatic pressure conditions. Strain CH1 was successfully isolated and assigned to the genus *Pyrococcus*, within the Euryarchaeota lineage within the Archaea domain. This organism grows within a temperature range of 80 to 108 °C and a pressure range of 20 to 120 MPa, with optima for 98 °C and 52 MPa respectively. *Pyrococcus* CH1 represents the first obligate piezophilic hyperthermophilic microorganism known so far. Comparisons of growth yields obtained under high-temperature/high-pressure conditions for relative organisms isolated from various depths, showed clear relationships between depth at origin and responses to hydrostatic pressure.

Keywords: Archaea, high hydrostatic pressure, deep-sea hydrothermal vent, extremophile, Thermococcales

Introduction

Among psychrophilic piezophilic prokaryotes isolated from deep oceans, few are obligate piezophiles (Bartlett, 2002). Since the discovery of black smokers in 1979, many thermophilic prokaryotes have been isolated from deep-sea hydrothermal vents, but only *Thermococcus barophilus* and *Marinitoga piezophila* (Marteinsson *et al.*, 1999; Alain *et al.*, 2002) are piezophiles, although not obligate. Here, we report the isolation of the first obligate piezo-hyperthermophilic archaeon (*Pyrococcus* CH1) from the deepest vent field explored so far.

Materials and methods

Active black smoker sulphide samples brought up to the surface in a biobox were immediately transferred in an anaerobic chamber, inoculated into sterile syringes filled with reduced artificial seawater, transferred into high-pressure vessels and then kept at 40 MPa and 4 °C.

In the laboratory, subsamples of chimney CH1 were depressurized on ice and inoculated under strict anaerobic conditions (Balch and Wolfe, 1976) into syringes loaded with modified MJYP medium supplemented with 20 mM $NaNO_3$ and 5 mM $FeSO_4$ (Takai *et al.*, 2000). These syringes were then placed into a high-pressure/high-temperature incubation system, custom-built by Top-Industrie (Vaux le Pénil Cedex, France), pressurized to 42 MPa (*in situ* pressure) and heated to 95 °C until the culture became turbid (Marteinsson *et al.*, 1997). When a significant growth was observed, the dilution-to-extinction technique was employed to obtain a pure culture. All experiments in this study were conducted in duplicate.

Pressure assays (0.1–130 MPa) were carried out at 95 °C. Under optimal pressure (52 MPa), growth yield was monitored for temperatures ranging from 60 to 110 °C. To determine optimal salt and pH requirements, MJYP medium was prepared with different concentrations of NaCl (0–6% salinity) and different pH buffers (pH 5–10).

Growth requirements and substrate utilization were tested in the MJYP mineral base supplemented with 002% (w/v) yeast extract, various electron acceptors and carbon and nitrogen sources.

Cells were counted in a Thoma chamber (depth, 0.02 mm) using a light microscope (Model BH2; Olympus, Japan).

For comparison and determination of the pressure range allowing growth of CH1 strain and five other *Thermococcales*, Thermococcales rich medium that had the following composition (per litre distilled water) was used: 3.3 g Pipes disodium salt, 23 g NaCl, 5 g $MgCl_2.6H_2O$, 0.7 g KCl, 0.5 g $(NH_4)_2SO_4$, 1 ml KH_2PO_4 5%, 1 ml K_2HPO_4 5%, 1 ml $CaCl_2.2H_2O$ 2%, 0.05 g NaBr, 0.01 g $SrCl_2.6H_2O$, 1 ml Na_2WO_4 10 mM, 1 ml $FeCl_3$ 25 mM, 1 g yeast extract, 4 g tryptone and 1 mg resazurin. The medium was adjusted to pH 6.8, autoclaved and then reduced with 0.5 g sodium sulphide before use.

The 16S rRNA gene was amplified by PCR using the primers Arch 8F and 1492R (Delong, 1992) and the PCR product was sequenced (1424 bp, EU682399) by Genome-Express Company (France). 16S rRNA gene sequences were aligned and compared with GenBank database using the BLAST network service (http://www.ncbi.nlm.nih.gov/blast). Phylogeny was inferred as described by Cambon-Bonavita *et al*., 2003.

Results and discussion

In March 2007, during the 'Serpentine' cruise on board RV 'Pourquoi Pas?', the hydrothermal field Ashadze 1, located on the mid-Atlantic Ridge (12°58. 4′ N-44°51.8′W) was explored for the first time with the ROV 'Victor 6000'. At 4000–4100 m depth, it is the deepest active hydrothermal field known so far (Fouquet *et al*., 2007). Hydrothermal end member fluids (370 °C) are controlled by seawater–peridotite interactions and show low pH (3.5), low silica and H_2S contents, but are particularly enriched in H_2, CO_2, CH_4 and abiogenic hydrocarbons (Charlou *et al*., 2007).

Anaerobic enrichment cultures inoculated with smoker sulphide samples were carried out at 95 °C under a pressure of 42 MPa (Marteinsson *et al*., 1997). After 2 days of incubation, growth was observed and strain CH1 was purified. CH1 cells are highly motile irregular cocci with a diameter of about 1–1.5 µm. Growth was observed only under strictly anaerobic conditions, within a temperature range of 80–108 °C and a pressure range of 20–120 MPa, with optima at 98 °C and 52 MPa. No growth was observed after 48 h of incubation under atmospheric pressure at various temperatures ranging from 60 to 110 °C. Thus CH1 strain should be considered as an obligate piezophile. pH and salt concentrations allowing growth were in the range 6.0–9.5 (optimum 7.5–8) and 2.5–5.5% NaCl (optimum 3.5%) respectively. Under these optimal conditions, generation time was about 50 min. CH1 is a chemoorganotrophic organism growing on

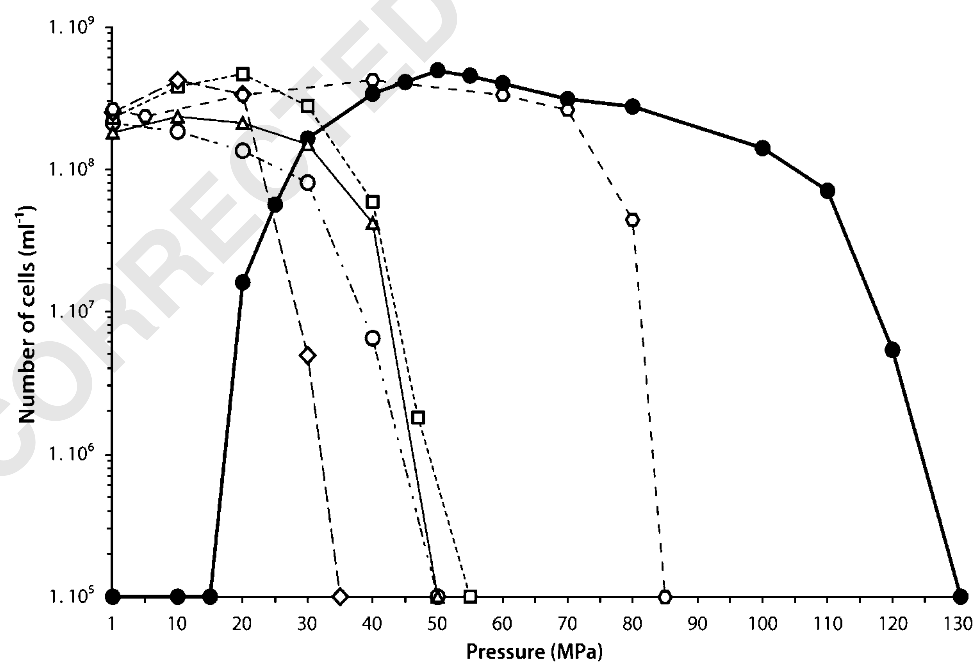

Figure 1 Growth yields recorded for Thermococcales originated ~~growth~~ from different depths (see below) as a function of hydrostatic pressure:–◇–P.*furious* (DSM 3638) (shallow vent),–○– P.*horikoshi* (JCM 9974,DSM 12428) (1400 m),–□–*P.abyssi* (CNCM I-1302) (2200 m),–△–*P.glycovorans* (CNCM I-2120) (2650 m), -○- *T.baraphilus* (DSM 11836) (3550 m) and —●— *Pyrococcus* CH1(4100 m). Except for *T.baraphilus* (85°C) cells of the other strains were grown at 95°C.

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complex carbon sources such as peptone, tryptone, yeast extract or beef extract. Poor growth was recorded on casamino acids, and no growth on carbohydrates, alcohols or single amino acids. With appropriate carbon sources, growth was stimulated by elemental sulphur.

Among the *Thermococcales* order, 16S rRNA gene sequences analyses are valuable to separate the three genera: *Paleococcus*, *Thermococcus* and *Pyrococcus* but not to discriminate species among a genus due to very high sequence conservation (above 97% which is the limit of species description (Stackebrandt and Goebel, 1994; Stackebrandt *et al*., 2002)). According to our phylogenetic analyses, *Pyrococcus* CH1 share more than 97% of 16S rRNA gene sequence similarity with *Pyrococcus furiosus*. The G + C content of the genomic DNA of strain CH1 was 49.0 ± 0.5 mol%.

The growth yields of CH1 and 5 *Thermococcales* strains isolated from samples collected at different depths were determined for increasing hydrostatic pressures: *P. furiosus* (shallow vent) (Fiala and Stetter, 1986), *P. horikoshii* (1400 m) (González *et al*., 1998), *P. abyssi* (2200 m) (Erauso *et al*., 1993), *P. glycovorans* (2650 m) (Barbier *et al*., 1999) were grown at 95 °C and *T. barophilus* (3550 m) (Marteinsson *et al*., 1999) at 85 °C (Figure 1). After 48 h incubation in Thermococcales rich medium maximum growth yields were observed from 1 to 20 MPa for *P. furiosus*, and from 1 to 30 MPa for *P. horikoshii*, *P. abyssi* and *P. glycovorans*, whereas no growth was observed beyond 35, 50 or 55 ~~Mpa~~, respectively. *T. barophilus* and CH1, both isolated under high-pressure and high temperature conditions, grew well up to 80–120 MPa, respectively. Noticeably, CH1 was unable to grow for pressures below 15 MPa and represents the first obligate piezo-hyperthermophilic organism. By combining the physiological properties of hyperthermophiles and strict piezophiles, CH1 extends the pressure/temperature limits for life.

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