Physiological Responses to Stress Conditions and Barophilic Behavior of the Hyperthermophilic Vent Archaeon *Pyrococcus abyssi*

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The physiology of the deep-sea hyperthermophilic, anaerobic vent archaeon *Pyrococcus abyssi*, originating from the Fiji Basin at a depth of 2,000 m, was studied under diverse conditions. The emphasis of these studies lay in the growth and survival of this archaeon under the different conditions present in the natural habitat. Incubation under in situ pressure (20 MPa) and at 40 MPa increased the maximal and minimal growth temperatures by 4°C. In situ pressure enhanced survival at a lethal high temperature (106 to 112°C) relative to that at low pressure (0.3 MPa). The whole-cell protein profile, analyzed by one-dimensional sodium dodecyl sulfate gel electrophoresis, did not change in cultures grown under low or high pressure at optimal and minimal growth temperatures, but several changes were observed at the maximal growth temperature under in situ pressure. The complex lipid pattern of *P. abyssi* grown under in situ and 0.1- to 0.5-MPa pressures at different temperatures was analyzed by thin-layer chromatography. The phospholipids became more complex at a low growth temperature at both pressures but their profiles were not superimposable; fewer differences were observed in the core lipids. The polar lipids were composed of only one phospholipid in cells grown under in situ pressure at high temperatures. Survival in the presence of oxygen and under starvation conditions was examined. Oxygen was toxic to *P. abyssi* at growth range temperature, but the strain survived for several weeks at 4°C. The strain was not affected by starvation in a minimal medium for at least 1 month at 4°C and only minimally affected at 95°C for several days. Cells were more resistant to oxygen in starvation medium. A drastic change in protein profile, depending on incubation time, was observed in cells when starved at growth temperature.

The deep-sea biosphere is influenced by three main parameters which, together, make it particularly extreme: low temperature, low nutrient concentrations, and high hydrostatic pressure (hydrostatic pressure increases by approximately 1 atm per 10-m depth). With the discovery of deep-sea hydrothermal vents, a new, unusual habitat for life was found at the bottom of the oceans which is rich in inorganic and organic energy sources. High biomass and diversity of microorganisms have been identified at these sites (21, 23). In the environs of hydrothermal vents, the temperature, pH, redox state, and nutrient availability change dramatically over spatial scales of centimeters and over time scales measured in seconds, while the hydrostatic pressure remains stable at a given depth.

Over the past decade, anaerobic hyperthermophilic species that grow optimally above 80°C have been isolated from deep-sea vents (for reviews, see works of Deming and Baross [8], Segerer et al. [42], Prieur et al. [37], Baross and Deming [2], and Stetter [44]) and the almost ubiquitous distribution of these microorganisms has been explained by their ability to survive cold oxygenated conditions during dissemination (19). Interestingly, some of the thermophiles have been isolated from areas with temperatures much higher than their maximum growth temperature (10, 18, 49), which suggest that they were transient at the place of capture and not actively growing.

The same could be true for thermophiles isolated from areas with temperatures much below their growth temperature. These observations raise interesting questions about the strategies adopted in the cellular components such as proteins, nucleic acids, and membranes to survive in such dynamic environments. However, there is little known about physiological responses and strategies used under extreme conditions, outside their growth range, and even less in their macromolecular response. One mechanism which may allow them to survive at temperatures above their growth range is the production of heat shock proteins to stabilize, repair, or degrade proteins denatured by thermal stress (16, 34, 46, 47).

Understanding of macromolecular stability in microorganisms able to grow at temperatures exceeding 100°C is progressing, although we are far from having a complete picture as to how they develop their flexible physiology. The archaeon *Pyrococcus furiosus*, originating from a shallow marine vent (11), has been shown to increase the total intracellular organic solutes in response to either increased temperature or salinity (29). The DNA in this species is remarkably stable at high temperatures, and it has been suggested that DNA-binding proteins that protect against hydrolytic damage in hyperthermophiles may be involved with other endogenous protective mechanisms (33). It has also been suggested that the DNA in hyperthermophiles is protected against denaturation by specific mechanisms such as positive supercoiling by reverse gyrase (12) or stabilization by histone-like proteins (41, 43) and that intracellular salt concentration is important for the stability of the DNA primary structure (26).

The effect of pressure on the physiology of hyperthermo-

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philic archaea is essentially unstudied, and little standardization of methodology is available. Nevertheless, the effects of various elevated pressures (hydrostatic and hypobaric) on such microorganisms have been reported (3, 10, 17, 20, 22, 30, 31, 35, 38). Growth was sometimes stimulated by increasing the pressure and the maximal temperature by a few degrees. Moreover, several enzymes purified from hyperthermophilic archaea have shown enhanced thermotolerance under pressure (15, 45). There is evidence for a pressure-induced change in lipid composition in deep-sea psychrophilic barophiles (6, 7), but this has yet to be demonstrated for hyperthermophiles; in addition, experiments involving elevated temperature and pressure in biomass production for analysis are very difficult to conduct.

The present study focused on the growth and survival of the deep-sea hyperthermophilic species *Pyrococcus abyssi* GE5, obtained from a fluid sample having an estimated temperature of 296°C, under experimentally reproduced *in situ* conditions. Our emphases were hydrostatic pressure, high and low temperatures, high and low nutrients, and oxic and anoxic conditions.

MATERIALS AND METHODS

Cultures and media. The test organism used in this study, *P. abyssi* GE5, was originally isolated from hydrothermal vents in the North Fiji Basin at a depth of 2,000 m (10). All cultures used for tests were started from a glycerol stock culture, stored anaerobically at -80°C . Not one culture exceeded more than five generations before experiments. Cells were grown anaerobically as described by Balch and Wolfe (1). Standard YPS medium (10) was used in all cultures except where otherwise stated. Cultures performed in serum flasks or Hungate culture tubes (Bellco, Vineland, N.J.) were cultivated in ventilated ovens (Mettmert). *P. furiosus* DSM 3638 was tested in some pressure experiments.

Starvation conditions. Starvation shifts were initiated when cultures reached the late logarithmic phase of growth in YPS medium at 95°C . Nutrient limitations were achieved by washing (two times) the cells in minimal medium (YPS medium without organic sources and sulfur) under anaerobic conditions in an anaerobic chamber (La Calhène, Vélizy, France). The cells were then resuspended in the starvation medium (30 ml in a 50-ml serum flask), supplemented with sulfur, and incubated at 4, 60, and 95°C . Survival was determined after 3, 6, 12, 24, and 48 h, 1 week, and 1 month, by the three-tube most-probable-number technique (24) in Hungate culture tubes. The final cell density was recorded after 1 to 7 days of incubation at 85°C .

Oxygen tolerance. Oxygen tolerance was tested in normal culture medium and minimal medium. Duplicate cultures, in 50-ml serum bottles, were grown at 95°C in 30 ml of YPS medium. Cultures in the late logarithmic phase of growth were flushed vigorously with air (10 min) until the redox indicator (Reazurin, 0.1 mg/liter) turned pink. Each culture was maintained or progressively cooled to the correspondent test temperature before the air flush. The oxygenic cultures were then incubated at 4, 60, and 95°C , and survival was estimated by the most-probable-number method, after various exposure times, as described above.

Oxygen tolerance in starvation medium was determined under the same conditions as previously described for starvation conditions, except for additional exposure to air for 10 min before incubation at 4, 60, and 95°C .

Pressure experiments. All culturing manipulations preceding pressure experiments were performed anaerobically in an anaerobic chamber. Buffered YPS medium with PIPES [piperazine-*N,N'*-bis(2-ethanesulfonic acid)] buffer (Sigma; 7.0 g liter $^{-1}$ at pH 6.8) was prepared without sulfur in a serum flask. The air was evacuated and replaced by alternatively applying vacuum and N_2 gas (1). Final anaerobiosis was achieved by adding neutral sterile $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ to a final concentration of 0.025% (wt/vol). The medium was then inoculated with 1 to 2% exponential-phase growing cells. Cultivation was performed in sterile gas-tight glass syringes (Ultrafit; Heinke-Sass-Wolf GmbH, Tuttlingen, Germany) with cut pistons. The syringes were sealed with needles (Terumo Eurobe N.V.) inserted in rubber stoppers before the inoculated medium was dispensed in the syringes containing 0.1 g of sulfur. Finally, the pistons were put in place and the gas phase was expelled prior to tightening the seal on each syringe. The syringes were then transferred into the high-pressure and high-temperature incubation system, which was custom-built by Top Industrie S.A. (Industrial zone "Le Plateau de Bière", Dammari-les-Lys, France). The system consists of four stainless steel pressure vessel-incubators which are heated in four vertically positioned ovens (300°C maximal temperature). The temperature and pressure of each vessel can be controlled independently, or the pressure can be equilibrated between the two. Pressure was generated with a hydraulic pump (Top Industrie S.A.), and cold tap water served as the hydraulic fluid. Bourdon gauges (100 MPa) monitored the pressure in each pressure vessel. The maximum working pressure of the system was 60 MPa. Thermocouples (Thermocoax TKA15/501NN), one internal

and one external, were used for each pressure vessel. Each oven was connected to a microprocessor in a temperature regulation unit, whereas the temperature inside each pressure vessel was controlled within $\pm 1^{\circ}\text{C}$. The vessels could be heated from room temperature to 100°C in less than 30 min. Temperature was recorded by a programmed PC computer (IPC Helios) (Fig. 1).

Growth at 0.1 to 20 MPa. Determination of the maximum growth temperature for *P. abyssi* and *P. furiosus* under hydrostatic pressure was performed in triplicate at 20 MPa. Low-pressure controls (0.3 MPa) were run for each temperature tested and in serum bottles at 95°C . Determination of the minimum growth temperature for *P. abyssi* under hydrostatic pressure was performed as described above.

Thermotolerance at 0.1 to 40 MPa. Cultures in the late logarithmic phase (approximately $5 \cdot 10^8$ cells per ml) were used in all experiments. Cells were grown in 0.5-liter serum flasks (250 ml) at 95°C . Each culture was stored at 4°C after the gas phase had been flushed with N_2 to remove the H_2S produced during growth. The syringes (10 ml) were loaded anaerobically, in an anaerobic chamber, with 5 ml of the test culture and without elemental sulfur. At least two syringes were then placed in the hydrostatic pressure vessels and incubated at lethal high temperatures (i.e., temperatures above the maximum growth temperature under atmospheric pressure) under 0.1- to 0.5-, 20-, and 40-MPa pressures. Samples were pressurized to the test pressure before the vessel ovens were heated. Heating started at 22°C , and it took approximately 30 min to achieve test temperatures (105, 106, 107, 108, 109, 110, 111, and 112°C). After various heating periods, the incubations were stopped immediately by cooling the pressurized vessels in a continuous flow of cool water (approximately 2 min cooldown time). The pressure decreased progressively with the temperature.

Thermotolerance was determined by transferring 0.5 ml of heated samples to 4.5 ml of fresh YPS medium and then incubating at 85°C for up to 7 days. Positive growth was scored as survival at a lethal temperature.

Cellular protein extraction and polyacrylamide gel electrophoresis. Whole-cell proteins were extracted from cells as described by Marteinsson et al. (28). Extracts from cultures kept in mineral medium and exposed to 95°C for 0, 12, 18, 24, and 48 h were analyzed on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gradient (5 to 20%) gels (25). Cells exposed to air in minimal and YPS media at 4°C were analyzed as well. As a control, cells without exposure to air or to minimal medium were run in parallel on gels. Protein extraction was performed with cells grown at high and low pressures and at different growth temperatures. Cultures grown at 73, 95, and 103°C under 20 and 0.1 to 0.5 MPa of pressure were stopped at the late exponential phase. Cells were analyzed in the mid-exponential and late exponential growth phases to see if there were any differences in the protein pattern for these phases of cultures. Equal amounts of protein, about 20 μg (protein concentrations were estimated by the Bradford method [5] with the Bio-Rad [Richmond, Calif.] assay kit), from all samples were loaded onto 5 to 20% one-dimensional gradient polyacrylamide gels (5% stacking gels). The following molecular weight standards (with molecular weights in parentheses) were loaded onto each gel: myosin (200,000), *Escherichia coli* β -galactosidase (116,250), rabbit muscle phosphorylase b (97,400), bovine serum albumin (66,200 and 82,000), hen egg white ovalbumin (45,000 and 49,000), bovine carbonic anhydrase (33,300), soybean trypsin inhibitor (28,600), and hen egg white lysozyme (19,400). Coomassie brilliant blue R-250 was used to stain the gels, which were then dried with a gel drying kit (Promega). To confirm visible protein bands, the gels were scanned (Hewlett-Packard Scan Jet 3C) and a densitogram was produced from each lane with the NIH Image 1.54 program.

Lipid analysis. Lipids were extracted from dried cells as described by De Rosa et al. (9) and analyzed by thin-layer chromatography (TLC; with the solvent chloroform-methanol- H_2O [65:25:4, by volume]) in comparison with lipid standards obtained from other archaea (36). The total lipid extract was hydrolyzed in 1 M methanolic HCl to cleave the polar head groups. The core lipids were identified by TLC as described previously by Trincone et al. (48) with the following solvents: (i) *n*-hexane-ethyl acetate (78:22, vol/vol); (ii) *n*-hexane-ethyl acetate (75:15, vol/vol). The lipids were analyzed in cultures grown at 73, 95, and 103°C under 0.1 to 0.5 and 20 MPa (Table 1).

RESULTS

Survival in starvation medium. A good tolerance toward starvation, under anoxic conditions, at both high and low temperatures was observed. The number of viable cells did not change after 1 month in starvation medium at 4°C , and the viability decreased slightly during 1 week at 95°C (Fig. 2A). The viability decreased from $2 \cdot 10^8$ cells/ml to $1 \cdot 10^8$ cells/ml after 48 h and to $3 \cdot 10^7$ cells/ml after 1 week. After 1 month of incubation at 95°C , the number of cultivable cells was reduced to $5 \cdot 10^5$ cells/ml.

Oxygen tolerance. The cells were more oxygen resistant in starvation medium than in YPS culture medium (Fig. 2B). They remained viable for at least 1 month in both media at 4°C , but rapid death was observed at growth range tempera-

FIG. 1. Schematic representation of the high-pressure apparatus "hot bucket" for cultivating microorganisms at high temperature and pressure. One unit of four is shown. Each can be loaded or unloaded separately while the others are kept under constant temperature and pressure conditions. Key: 1, hydraulic pressure generator; 2, Bourdon gauge (100 MPa); 3, water tube (inox); 4, pressure indicator; 5, digital pressure indicator; 6, Bourdon gauge; 7, valve; 8, computerized independent thermoregulators; 9, digital temperature indicator in oven; 10, digital temperature indicator inside the pressure vessel; 11, heating element (oven); 12, bucket for the pressure vessel; 13, O-ring; 14, thermocouple in jacket; 15, stainless steel pressure vessel; 16, the head for the pressure vessel; 17, culture syringe; 18, cut piston; 19, needle embedded in rubber stopper; 20, water; 21, thermocouple for heating unit; 22, computer.

ture. The number of viable cells in YPS culture medium after exposure to oxygen did not change for 48 h at 4°C. After 1 week, about $6 \cdot 10^4$ cells/ml remained cultivable, and $3 \cdot 10^4$ cells/ml remained cultivable after 1 month. The cells remained more oxygen resistant in minimal medium at 4°C, and the titer of viable cells did not change for 1 week, but viable cells decreased to $5 \cdot 10^5$ cells/ml after 1 month. Similar death pat-

terns were obtained for cells in both media at higher temperature. The death rate was higher at 95°C than at 60°C. No cells survived 5 h of exposure to 95°C in either medium, but cells remained cultivable for up to 12 h in minimal medium at 60°C and for up to 8 h in YPS medium.

Growth temperature limits at high pressure. The maximum growth temperature for *P. abyssi* was found to be 106°C and

TABLE 1. Effect of growth temperature and pressure on lipids in *P. abyssi*

Growth conditions	Polar lipids	Core lipids
73°C, 0.1 MPa, in flasks	Three to four phospholipids at R_f 0.1 to 0.15 and at R_f 0.3	Acyclic caldarchaeol
73°C, 20 MPa, in syringes	Two to three phospholipids at R_f 0.1 to 0.16, minor spots between R_f 0.23 and 0.35 and at $R_f > 0.3$	Acyclic caldarchaeol, archaeol (trace amount), unknown minor spots
95°C, 0.1 MPa, in flasks	Phospholipids at R_f 0.09 and at R_f 0.2	Acyclic caldarchaeol, archaeol (trace amount)
95°C, 0.3 to 0.5 MPa, in syringes	Phospholipids at R_f 0.09 and at R_f 0.2	Acyclic caldarchaeol
95°C, 20 MPa, in syringes	Phospholipid at R_f 0.09	Acyclic caldarchaeol, unknown minor spots
103°C, 20 MPa, in syringes	Phospholipid at R_f 0.09	Acyclic caldarchaeol, unknown minor spots

FIG. 2. Survival of *P. abyssi* GE5 under different stress conditions. (A) Number of cultivable cells during exposure in minimal medium at 4°C (■) and 95°C (○); (B) viable cells during exposure to oxygen in YPS culture medium at 4°C (○) and in minimal medium at 4°C (●).

the minimum growth temperature was found to be 71°C with in situ pressure. The maximum growth temperature for *P. furiosus* was 103°C as previously reported (11) and did not change with exposure to 20 MPa of hydrostatic pressure.

Thermotolerance at different pressures. High pressure led to enhanced thermotolerance at a lethal temperature (Fig. 3). The strain was cultivable after 30 min at the maximum temperature of 112°C under 20 and 40 MPa pressure but not at 0.3 MPa. The pressure did not enhance thermotolerance at temperatures higher than 112°C for the onset minimal incubation time of 30 min. The cells did not survive 114 and 115°C for 30 min nor 5 min at 120°C under 20 MPa of pressure. The highest lethal temperature survived by cells for 30 min at low pressure was 111°C. A relatively small difference in survival was found at 110 to 111°C under high and low pressures. We observed a strong significant difference in survival under high and low pressures at 107 to 110°C. The survival time was extended to 3 h for cells under high pressure. The strain was viable for at least 7 h, and up to 16 h, at 105°C under 0.3 MPa of pressure. No significant differences in survival at a lethal temperature under 20 and 40 MPa of pressure was observed (data not shown).

FIG. 3. Time limit to obtain viable cells of *P. abyssi* GE5, examined by subcultivation as described in text, after exposure to different lethal temperatures under pressures of 20 (●) and 0.3 (○) MPa. The time intervals were 30 min, and cultures were scored viable if growth was observed. Each experiment was performed at least twice for each time point to confirm results.

Cellular protein profiles. Comparison of the protein profiles of cells grown at 95°C in the exponential and stationary phases by gel electrophoresis revealed several changes. Many proteins decreased in abundance in the stationary phase of growth, especially one protein band (approximately 74 kDa) which disappeared almost completely (Fig. 4).

The electrophoretic protein profiles of cells exposed to oxygen at 4°C in minimal and normal culture media were not affected for 48 h (data not shown). Protein shifts did occur during exposure to minimal medium for 6, 12, 18, and 24 h at 95°C. The protein band abundance decreased progressively after 6 h of incubation and again after 18 h. Only the major

FIG. 4. Cellular protein profiles of *P. abyssi* grown under different pressures and at different temperatures. Lanes: A and B, GE5 grown in serum bottles at 95°C and in exponential and stationary growth phases, respectively; C to F, GE5 grown in serum bottles (lane C) under atmospheric pressure and in syringes under 0.3 (lane D), 20 (lane E), and 40 (lane F) MPa of hydrostatic pressure; G and H, GE5 grown at 73°C under 0.1 and 20 MPa, respectively; I, GE5 grown at 105°C under 20 MPa. Protein in low abundance and the novel putative protein are marked by arrowheads. The migration of molecular mass standard proteins is indicated in the margin in kilodaltons.

FIG. 5. Cellular protein profile of *P. abyssi* GE5 after exposure to starvation medium at 95°C for 0, 6, 12, 18, and 24 h (lanes A to E, respectively). The two dominating protein bands after 18 h of exposure and the novel putative protein are marked by arrowheads. The migration of molecular mass standard proteins is indicated in the margin in kilodaltons.

protein bands seen in the control (time 0) were well detected with Coomassie blue colorant. Two protein bands (approximately 45 and 47 kDa) were dominant after 18 h, and after 24 h of incubation, a new band appeared with a molecular mass of approximately 150 kDa (Fig. 5). This band was also present after 48 h of incubation (data not shown).

The protein composition of strain GE5 cultivated at 95°C under atmospheric pressure (0.1 MPa in serum bottles and 0.3 MPa in syringes) and in situ pressure did not change (Fig. 4). The profiles were similar for cultures grown at 71 or 73°C under 0.1 MPa and 20 MPa. Comparison of the protein profiles in cells grown at 71, 73, and 95°C under high pressure did not reveal changes. However, cells grown under in situ pressure at 105°C showed significant changes in the protein profile. One protein band (approximately 32 kDa) disappeared almost completely, and one band with a molecular mass of ca. 30 kDa appeared. Proteins with a molecular mass smaller than 50 kDa were poorly expressed in cells (Fig. 4).

Lipid composition. The complex lipid patterns of the strain became more complex with more stress on the cells (Table 1). The samples grown at 95°C, without high pressure, showed the presence of two phospholipids at R_f 0.09 and R_f 0.2, the latter in a trace amount. The spot at R_f 0.2 seems to be the same R_f of the phospholipid present in *P. furiosus*. At 95°C as well as at 103°C under 20 MPa, the spot at R_f 0.09 was present, while that at R_f 0.2 was absent. The profiles of phospholipids produced in cells at 73°C, with high or low pressure, were not all superimposable. Cultures grown at 73°C under 0.3 to 0.5 MPa were phosphorus test positive, with the presence of three to four spots at an R_f between 0.1 and 0.15, while the phospholipid at R_f 0.2 was absent but phospholipid appeared at R_f 0.3. For cultures at 73°C and 20 MPa, two to three phosphorus-positive spots at an R_f between 0.1 and 0.16 were present. Minor phospholipids at an R_f between 0.23 and 0.35 were observed with an abundant spot at an R_f higher than 0.3.

After methanolysis, the lipid of the microorganism grown under standard conditions gave rise to a trace amount of archaeol, a compound with an R_f similar to that of acyclic caldarchaeol, and an additional spot at the R_f of 2+2- or 3+2-cyclized caldarchaeol. When the microorganism was grown at 95°C under low and high pressure, the acyclic caldarchaeol component was present as core lipid with additional unknown minor spots at 20 MPa. The archaeol was not detectable (trace

amount), either due to its absence or because its low amount exceeded the sensitivity of the TLC analysis. At 103°C under 20 MPa pressure, the major core lipid present was the acyclic caldarchaeol, with unknown minor spots. At 73°C under high pressure, the acyclic caldarchaeol was the major spot, with archaeol and unknown spots as minor components. On the contrary, at the same temperature but without pressure, the major spot observable was the caldarchaeol.

DISCUSSION

We have shown that the growth temperature range for *P. abyssi* was increased by 4°C under in situ pressure. The maximum growth temperature was extended from 102 to 106°C, but the strain has been reported to grow at 105°C under in situ pressure and to show dividing cells at 108°C (10). However, growth at 108°C was not observed with the pressure equipment used in this study. The minimum growth temperature was also shifted upward from 67°C at low pressure to 71°C at high pressure. Extension of maximum and optimum growth temperatures in cultures under hydrostatic pressure has been reported for several thermophilic microorganisms (10, 22, 35, 38), but only one paper also reports extension of the minimal growth temperature (35). All of these strains originated in deep-sea hydrothermal vents. Interestingly, the growth temperature of *P. furiosus* (originating in a shallow vent at a depth of 100 m) was not extended at a pressure of 20 MPa as was found for other *Pyrococcus* strains originating in the deep sea. The extension in growth temperature with pressure could possibly be explained by mechanisms involving viscosity in cells. Pressure and heat are parameters which interact with fluidity in cells. The effects of low temperature and high pressure act in an additive manner to cause loss of fluidity (the entropy decreases) and promote the liquid-to-gel transition at minimal growth temperature. On the other hand, high temperatures may counterbalance the gelling effect of pressure (entropy increases with temperature and decreases with pressure), which may lead to stability in the cell and therefore growth.

In this study, we have shown that strategies adopted in the cellular components for shifts in growth range are based on soluble proteins and lipids in membrane. TLC examination of complex lipids of *P. abyssi* grown under different conditions showed that when the pressure and the temperature increased, the polar lipids were composed of only one phospholipid, while when the temperature decreased, many phospholipids were biosynthesized. When the strain was grown under standard conditions, the core lipids found were the same as those previously reported; however, at the TLC level, the archaeol seemed to be a very minor component although it was reported to be about 15% of total lipids by another analysis method (10). Archaeol was not observable in cultures grown at high temperature and pressure. Similar results have been reported for two *Thermococcales* species, ES1 and ES4, in which the ratio of caldarchaeol and archaeol decreased with increasing growth temperature (14). Moreover, some minor spots appeared in cultures of *P. abyssi* GE5 grown under pressure. However, at the TLC level, it is difficult to discriminate between artifacts or degradation compounds and the possible new core lipids.

The shifts in the protein level were less apparent than those in the lipid level. Pressure up to 40 MPa did not produce dramatic changes in the soluble protein composition of *P. abyssi*. This correlates with the observation made for *Pyrococcus* sp. strain ES4 (17). It is quite possible that one-dimensional sodium dodecyl sulfate gradient gels are not capable of detecting differences in protein profile response and that two-

dimensional gels may be more appropriate for such detection. Nevertheless, with this technique, we have already detected differences in the protein composition of a strain originating in a deep-sea site under low- and high-pressure conditions (27). We do observe some changes in the protein profile near the maximum growth temperature under pressure. The maximal growth temperature for the strain is 102°C without pressure, and therefore we did not have a good comparative control of cells grown at 105°C at atmospheric pressure. Nevertheless, the changes in protein profile observed at 105°C were most likely due to high temperature rather than to pressure, whereas such changes were not observed at 71, 73, and 95°C under 20 MPa.

We have shown that pressure is an important parameter for cells to survive at a lethal high temperature. Interestingly, the greatest differences between cells exposed to high and low pressure existed at temperatures up to 110°C but decreased drastically above this limit. This limit is near the maximum growth temperature recorded to date for survival, although it could be higher (4, 8). *P. abyssi* did not survive 120°C under 20 MPa for 5 min, which correlates with findings for the deep-sea strains GB-D and SY (22) but not with findings for the hyperthermophilic strain AL-1 (39). That strain survived 1 h of exposure to 150°C under 20 MPa. However, AL-2 was exposed to the supraoptimal growth temperature of 110°C under pressure before exposure to 150°C. Such an acclimation period may be important for surviving a lethal temperature (16, 17, 47).

We have shown that *P. abyssi* was viable for at least 1 month after exposure to oxygenated medium at 4°C and that the strain seems to be more oxygen resistant in starvation medium than in culture medium. The titer of viable cells did not change for 1 week in the minimal medium, and the number of viable cells was higher, after 1 month, than that of cells kept in culture medium. However, at growth range temperature, the cells did not survive very long but survived longer when kept in starvation medium. Such oxygen resistance suggests again that hyperthermophiles disseminate in cold seawater (13, 19). Moreover, we have shown a relatively high titer of viable cells after a long exposure period. We did not investigate the portion of cells in a dormant state, and it is quite possible that tubes with negative growth contained cells waiting for the right conditions (40). Starvation induces resistance to various environmental stresses in deep-sea psychrophilic marine bacteria (32), which seems to be the case for the archaeon *P. abyssi*. We could not see any changes in the protein composition profile of cells exposed to oxygen for up to 48 h, in both minimal and nutrient-rich media at 4°C, which suggested that other strategies were adopted in the cellular components at low temperatures. However, we observed a drastic change in the protein pattern, with a novel putative (150-kDa) protein, in starved cells at growth temperature. This protein is probably a stress protein which could have a similar role as heat shock proteins.

The influence of growth temperature and pressure on the deep-sea hyperthermophilic archaeon *P. abyssi* is accompanied by changes in the level of phospholipids more than the level of core lipids or proteins. The resistance to a lethal temperature under in situ pressure must be seen as a major factor to survive extreme spatial variability in temperature. Furthermore, the insensitivity toward oxygen under low-temperature and famine conditions clearly shows its resistance to such variability and facilitates its dissemination.

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