The influence of nutrition on the physiology of piezophilic bacteria

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ABSTRACT

The adaptation known as piezophily is a hallmark of the true bacterial inhabitants of the cold deep sea below a threshold depth of about 2,000m. Because of advection of water masses and particle transport, there is mixing of bacterial populations of different depths. There is also a variability in nutrient supply. An understanding of bacterial processes in the oceans thus requires studies of how bacteria respond to changes in temperature, pressure, nutrition and community structure. A first step towards understanding this complexity was to employ pressure and temperature as coordinate variables in experiments. This led to the definition of piezophily. The next step is to add the variable of nutrition. The pressure response of cells of isolate PE36 was found to be a function of the carbon compound they were utilizing. The piezopsychrophile PE36 was grown in a minimal salts medium with glycerol, glucose, glutamate or citrate as a carbon source. Growth on each of these carbon sources also resulted in a distinct membrane protein profile (results to be published elsewhere) that was also a function of the pressure during growth.

Introduction

The classification of a microorganism with respect to its growth as a function of temperature is based on the temperature range wherein its maximum growth rate occurs. Microorganisms are accordingly psychrophilic, mesophilic, thermophilic and hyperthermophilic. This classification arose from studies of organisms inhabiting mostly atmospheric pressure habitats. The concept of barophily was proposed by ZoBell and Johnson [17] to categorize bacteria "whose growth or metabolism is favored by pressure." Through the study of deep-sea bacteria in pure culture, this concept of barophily has been found ambiguous and inadequate for describing them [15, 16]. For example, some microorganisms, which have their pressure-temperature maximum growth rate at atmospheric pressure, grow better at a high pressure along some isotherms. Are these barophiles? Also, other bacteria have their maximum growth rate at a high pressure but not at the temperature of their environment. Are these barophiles?

The concept of piezophily [15, 16] arose in the study of bacteria from deep seas having three different temperatures: 2°C, 9.8°C, and 13.5°C. Bacteria from these diverse habitats are adapted not to a particular pressure or temperature, but to a condition defined by both of these environmental parameters. Microorganisms grow over a *(P,T)*-domain, not simply over a *T* or *P* range. The *T* range of growth is a function of the *P* at which it is determined and vice versa. The maximum growth rate in the *(P,T)*-domain can be visualized with *PTk*

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diagrams which are plots of experimental values of *k* versus *P* and *T*. So far, it appears that the growth rate of a particular bacterial isolate has a single maximum growth rate, k_{max} at (P_{kmax},T_{kmax}) on a *PTk*-diagram. Piezophiles are bacteria or archaea with a $P_{kmax} > 0$ and the piezophiles of the cold deep sea can be placed into two broad classes: [1] piezopsychrophiles ($P_{kmax} > 0.1$ *MPa*) and [2] hyperpiezopsychrophiles ($P_{kmax} > 50$ *MPa*). A *PTk*-diagram for the deep-sea piezopsychrophile PE36, which was used for the studies reported below, is shown in Figure 1. Piezomesophiles, but not piezopsychrophiles, have been found in the deep 13.5°C Mediterranean Sea [15]. There are not yet clear examples, however, of piezothermophiles and hyperpiezothermophiles.

Several other environmental parameters in addition to temperature and pressure affect the function of bacteria in the oceans. For example, at any depth in the sea there is an admixture of bacteria originating from other depths, hydrothermal habitats, fluvial transport, aeolian transport, sewage inputs, industrial wastes, and other sources. The mix of allochthonous and autochthonous bacteria complicates the interpretation of measurements of bacterial transformation rates in the sea. Another set of environmental parameters defines bacterial nutrition that modifies bacterial activity both in studies of natural populations of bacteria and in research with pure cultures. Here we report studies on bacterial isolate PE36 that show both growth rate and its pressure dependence are greatly affected by the carbon source in the culture medium.

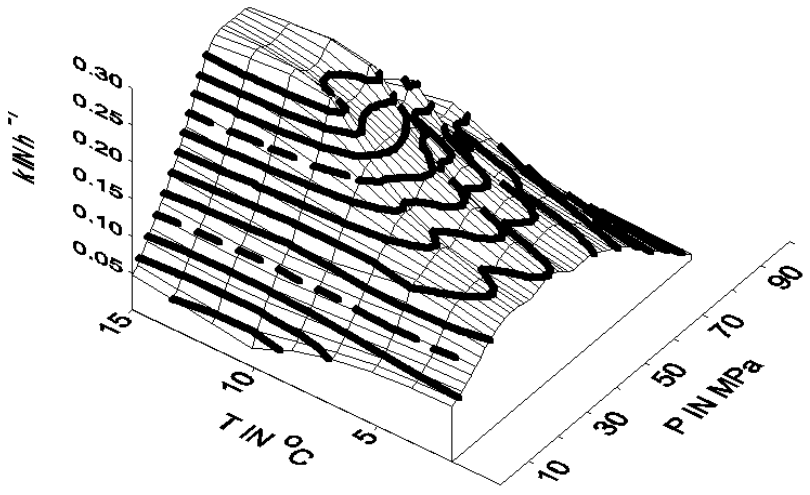

Fig. 1. A *PTk*-diagram showing the growth rate of the piezopsychrophile PE36, genus *Moritella*. Exponential growth rate constants, *k*, were determined for cells growing in type 2216 marine broth (Difco) at approximately 30 combinations of temperature, *T*, and pressure, *P*. The plot shown was generated with SURFER (Golden Software, Golden, CO).

Methods

The piezopsychrophile PE36, a gram negative rod with a single polar flagellum and assigned to the genus *Moritella* [3], has several essential characteristics for the investigation of

nutritional effects. First, PE36 grows on a minimal salts medium supplemented with any one of several carbon sources. Second, PE36 can be plated for a colony forming ability assay. Whether cells are incubated on plates or in pour tubes, it is crucial to avoid exposing the plating medium to radiation from fluorescent lights. Isolate PE36 is highly sensitive to the direct [11] and indirect (unpublished) effects of UV radiation. Cells plated at atmospheric pressure do not grow well nor, yet found, with a high plating efficiency. Cells "plated" in pour tubes incubated at high pressure, however, exhibit a plating efficiency of over 94%. Finally, PE36 regulates its membrane fatty acid composition as a function of *T* and *P* [4].

The high-pressure methods have been previously described [16]. Two pressures at 4°C were employed in the study reported here. PE36 was grown in a minimal sea salts medium in heat sealed plastic bags with glycerol, glucose, glutamate or citrate as a carbon source.

Results and Conclusions

The results in Fig. 2 show that the growth rate of isolate PE36 in minimal salts medium at 4°C and either at 0.1 or 40 MPa was a function of the carbon source. The fastest growth occurred in type 2216 medium and the slowest in minimal medium supplemented with citrate. The exponential growth rate constant, *k*, of cells in minimal salts medium at 40 MPa (close to the

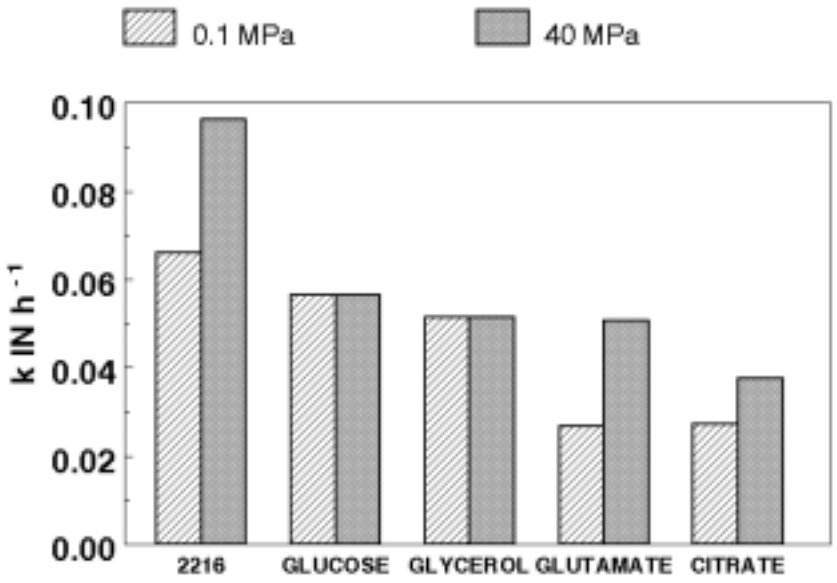

Fig. 2. Shows the growth rate constant, *k*, for bacterial isolate PE36 grown in five different media at two pressures and 4°C. Type 2216 (Difco) is a complex growth medium. The other cultures were grown in minimal salts medium plus 5 g/l of the indicated compound.

habitat pressure of isolate PE36) were in the order $k_{glucose} > k_{glycerol} > k_{glutamate} > k_{citrate}$. Similar relative growth rates occur in cultures of *E. coli* [7] and *Serratia marcescens* [10] grown at 37°C at atmospheric pressure.

The growth rate of isolate PE36 in type 2216 medium and in minimal salts medium with either citrate or glutamate as the sole carbon source was increased by pressure as seen in Figure 2. Under these conditions, isolate PE36 is barophilic (has a pressure-facilitated

growth rate). Other effects of pressure on the growth rate were evident. First, at atmospheric pressure $k_{glutamate} \approx k_{citrate}$ whereas at 40 MPa $k_{glutamate} > k_{citrate}$. Second, cells grown on glycerol divided at almost the same rate either at 0.1 MPa or at 40 MPa. The identical result was observed with cells grown on glucose. These experiments were done close to the habitat temperature of PE36. As can be seen in Figure 1, the effect of pressure on growth rate in complex medium is most pronounced at temperatures close to 13°C. We have not yet determined the growth rate of isolate PE36 at 13°C in defined media. We hypothesize that all growth rates will be pressure-facilitated at 13°C.

It has been known for a long time that glycerol enters many gram negative cells via diffusion through outer membrane porins into the periplasmic space. It then enters the cytoplasm via a process of facilitated diffusion which involves the protein GlpF in the cytoplasmic membrane [1, 9, 14]. GlpF interacts with glycerol kinase (GlpK) and somehow facilitates the capture of intracellular glycerol. We hypothesized that the piezopsychrophile PE36 will not exhibit a pressure-facilitated growth response when grown at low glycerol concentrations. The reasoning was that since diffusion would be the limiting growth factor at low glycerol concentrations, then growth would be affected only slightly by pressure change because diffusion itself in aqueous systems is affected only slightly over the 100 MPa pressure range found in the sea. As seen in Figure 2, even cells in mM levels of glycerol grew at nearly the same rate at 0.1 and 40 MPa. Experiments at glycerol concentrations as low as 1 μM gave the same result shown in Figure 2 (Yayanos and Chastain, unpublished). A glycerol concentration of 1 μM was not, however, growth limiting. Since bacterial isolate PE36 grows much more slowly than does *Escherichia coli*, the concentration of glycerol needed to limit the growth of PE36 may be much lower than the μM levels which limit the growth rate of *E. coli*.

Bacterial isolate PE36 has the phosphoenolpyruvate:glucose phosphotransferase system (PTS) for the uptake of glucose [5, 13]. Some of the PTS components in two piezophiles are pressure-stimulated and others pressure-inhibited [5]. In three strains of marine bacteria, there is no correlation between the degree of pressure adaptation (ability to grow at high pressure in complex medium) and the nature of the pressure response of methyl α-glucoside (α-MG) phosphorylation or of α-MG glucose-6-phosphate transphosphorylation. In cells of PE36, the rates of both of these reactions were reduced by pressure. In spite of these two pressure-inhibited PTS components, the uptake of α-MG by PE36 cells is greater at pressures up to nearly 80 MPa than at 0.1 Mpa [5]. α-MG is phosphorylated and transported into cells by the PTS but not metabolized. Thus the result in Figure 2 showing that the growth of PE36 on glucose in minimal medium was at a rate nearly the same at 0.1 and 40 MPa was surprising.

Profiles of membrane proteins were determined with PAGE in each of the experiments shown in Figure 2. Membrane protein composition was affected by carbon source and by pressure in all cases, even when pressure did not affect the growth rate (Yayanos and Chastain, unpublished). This and the results in this paper lead us to conclude that bacterial adaptation to pressure and temperature needs to be examined from the perspective of system dynamics rather than solely from a consideration of the pressure sensitivity of individual system components.

Finally, a problem in marine ecology is the interpretation both of the uptake rates of radiolabeled compounds by communities of bacteria and of the pressure dependence of

those rates. It is well known that the uptake of one amino acid can be repressed by the presence of another [6, 8, 12]. For example, L-phenylalanine at 1 nM or 0.165 μg/l completely inhibits the uptake of DL-tryptophan [2]. Thus, the amendment of natural seawater with a single radiolabeled compound without knowledge of background levels of other compounds may give misleading results. Very few studies of marine bacteria have been done to determine the presence of repression in uptake experiments [12]. The results in Figure 2 imply that we need to find out more about the variety of substrate transport systems in marine bacteria and, in particular, the response of these systems to pressure-temperature changes. For example, if glycerol uptake is widespread among marine bacteria and if it is as insensitive to pressure change as it is in isolate PE36, then the use of radiolabeled glycerol may be advantageous for the assessment of certain activities in natural bacterial populations in the deep sea.

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