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Obligately barophilic bacterium from the Mariana Trench

(deep sea/high pressure/extreme environment/rate of reproduction)

A. ARISTIDES YAYANOS, ALLAN S. DIETZ, AND R. VAN BOXTEL

Physiological Research Laboratory, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, California 92093

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ABSTRACT **An amphipod (*Hirondellea gigas*) was retrieved with decompression in an insulated trap from an ocean depth of 10,476 m. Bacterial isolates were obtained from the dead and cold animal by using silica gel medium incubated at 1000 bars (1 bar = 10^5 Pa) and 2°C. The isolate designated MT41 was found to be obligately barophilic and did not grow at a pressure close to that of 380 bars found at average depths of the sea. The optimal generation time of about 25 hr was at 2°C and 690 bars. The generation time at 2°C and 1,035 bars, a pressure close to that at the depth of origin, was about 33 hr. Among the conclusions are: (*i*) pressure is an important determinant of zonation along the water column of the sea; (*ii*) some obligately barophilic bacteria survive decompressions; (*iii*) the pressure of optimal growth at 2°C appears to be less than the pressure at the depth of origin and may be diagnostic for the depth of origin; (*iv*) rates of reproduction are slow yet significant and an order of magnitude greater than previously thought; and (*v*) much of deep-sea microbiology may have been done with spurious deep-sea organisms due to warming of samples.**

We recently reported (1, 2) the isolation and partial characterization of a deep-sea bacterium showing barophilic growth in laboratory cultures. Its optimum growth occurs at pressures corresponding to those at ocean depths. This is the first barophilic bacterium to be isolated in nearly 20 years, and it grows much more rapidly than did the previously reported barophile (3). At a deep-sea temperature of 2°C, strain CNPT-3 grows over a range of pressures of nearly 800 bars (1 bar = 10^5 Pa). This range is greater than that of about 500 bars over which many mesophilic (4–6) and psychrophilic (7) bacteria can grow. Accordingly, we would expect to find in the deep trenches of the oceans, where the pressure is 800–1100 bars, bacteria that are either obligately barophilic or barophilic with a tolerance to a range of pressures greater than 800 bars.

We report here on the isolation and partial characterization of an apparently obligately barophilic bacterium from the Mariana Trench at a depth of 10,476 m, where the pressure is in excess of 1,000 bars. This organism is designated MT-41 until a taxonomic study is completed.

MATERIALS AND METHODS

On 13 December, 1978, a trap (a modified 30-liter Niskin bottle) made of polyvinyl chloride, a good thermal insulator, was deployed into the Mariana Trench at 11° 20.5′ N, 142° 25.77′ E (determined by satellite navigation) where the depth was 10,476 m (determined by an echo sounder). The trap was baited with a mackerel and secured to flotage, a submersible radio transmitter, submersible light, ballast, and ballast-releasing devices. Between the sinking and the surfacing of the trap and location system, 23 hr and 49 min elapsed. We estimated that the trap was on the sea floor for 16 hr and 19 min. The trap contained one amphipod, most likely *Hirondellea gigas* (8), which was at 5–6°C and at one atmosphere. The amphipod was not alive as judged by locomotory criteria. It was kept at 0°C, placed in microbiological culture medium (9), and incubated at 1035 bars and 2°C within 2 hr after the recovery of the trap. During 1979, this decomposing animal and the surrounding medium served as the source of inocula for other cultures. These were incubated at 1035 bars, except for intervals of less than 1 hr required to perform inoculations at atmospheric pressure and 0°C.

Colonies of bacteria were grown at 1035 bars by using the silica gel pour tube method as described (9). One of these isolates was designated MT-41 and studied to determine the kinetics of its reproduction as a function of deep-sea pressures at 2°C, a temperature close to that at the bottom of the Mariana Trench (10). A culture of the strain designated MT-41 was grown to between 5×10^7 and 1×10^8 cells per ml at 2–4°C and 1035 bars in type 2216 marine broth (Difco) adjusted to pH 6.8 with HCl and filtered (0.22-μm pore diameter). This culture was decompressed and used to inoculate fresh 2216 medium, pH 6.8, at 2°C to a concentration of 10^6 cells per ml. Portions of this culture were incubated within 1 hr at 2°C and at 1, 173, 346, 518, 690, 863, and 1035 bars. The cultures were confined in plastic syringes, which isolated them from the hydraulic fluid (an ethylene glycol/water mixture) and allowed for removal of samples. The syringes contained glass balls of 1-cm diameter and were put in pressure vessels, which were compressed to the desired pressure and placed on a motor-driven platform in a bath at 2°C. The rocking motion moved the glass balls, thus continuously mixing the cultures. Samples were taken by decompressing a pressure vessel, removing a syringe, and expelling some of the culture from the syringe. The culture remaining in the syringe was repressurized within 1–2 min. The decompressed samples were immediately fixed in an artificial sea water containing 0.05% sodium azide and 0.25% formalin for analyses of cell concentration, size, and shape. The number of cells per ml of culture was determined with a Coulter Counter model B or model ZBI (Coulter). The distribution of cell sizes in cultures was determined with the Coulter Counter ZBI and a multichannel analyzer (Channelizer, made by Coulter). The morphological features of cells were studied with phase microscopy and scanning electron microscopy. The phase-contrast microscope had ×10 oculars, ×63 and ×100 objectives, and tube magnifications of ×1.25, ×1.6, and ×2 (Carl Zeiss, Oberkochen, Federal Republic of Germany). A Hitachi H-500 scanning transmission electron microscope was used in the scanning mode (Hitachi, Tokyo, Japan). The samples were fixed in 1% glutaraldehyde, washed with distilled water, ethanol/water solutions, ethanol, and Freon. The samples were dried in a critical-point drier and then coated with a gold–palladium alloy.

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RESULTS AND DISCUSSION

The question arose if decompressing cultures to take a sample for analysis influenced the nature of the results. Although subtle, there are effects solely attributable to the events of decompression or compression (11) in experiments with *Escherichia coli*. Experiments with strain CNPT-3 and with trench bacteria other than strain MT-41 (unpublished data) show that, for the kind of experiment reported here, there were no discernible effects of the decompression/recompression cycles used to sample the cultures. This was shown with an apparatus (11) that allowed sampling in a gentle fashion without necessitating the decompression of the entire culture and without subjecting the samples to harsh shear forces. Growth curves determined in this manner were indistinguishable from those determined as in this paper.

Growth curves are shown in Fig. 1 *A* and *B* for seven different pressures, all at 2°C. No increase in cell numbers was observed in the cultures at the lowest three pressures. Also, there was no increase in mean cell volume as determined with the Coulter Counter and Channelizer. Thus, even at 346 bars, a pressure found at a sea depth of approximately 3416 m, strain MT-41 did not grow. Growth was observed at 518, 690, 863, and 1035 bars—the highest pressure used and close to that found at a 10,476-m depth of the sea from which MT-41 originated.

The growth curves show that apparently exponential growth ceased when the cultures grew to about 2×10^7 cells per ml. Most of the deep-sea strains we have studied grew exponentially to 2×10^8 to 5×10^8 cells per ml (e.g., strain CNPT-3) (1, 2). A partial explanation for this apparent lower yield is that cells of strain MT-41 formed aggregates in cultures having 2×10^7 to 5×10^7 cells per ml. The aggregates grew into visible floating colonies if the cultures were incubated for more than 3 weeks. The early stages of the formation of aggregates were observed with phase microscopy and with scanning electron microscopy. Fig. 2 shows an aggregate photographed with the scanning electron microscope. The culture was at 863 bars and was sampled at 234 hr after the start of the experiments (0 hr in Fig. 1) and had 3×10^7 particles per ml as determined with the Coulter Counter. Clearly, the Coulter Counter yielded inaccurate values once cellular aggregation occurred. Such aggregates were not observed in cultures having less than 10^7 cells per ml. The morphology of the cellular aggregates (Fig. 2) did not clearly reveal a substance that might be holding the cells juxtaposed, although an indication of extracellular material is evident in the lower right hand portion of the clump.

The mean generation time, g, was calculated from the exponential portions of the curves in Fig. 1. The pressure at which g was determined is shown in Fig. 3 as the depth at which that pressure would occur, thus indicating the depths at which MT-41 could reproduce in a 2°C water column. There is always the possibility that some as yet untested nutrient conditions might allow for growth at shallower depths (lower pressures). If strain

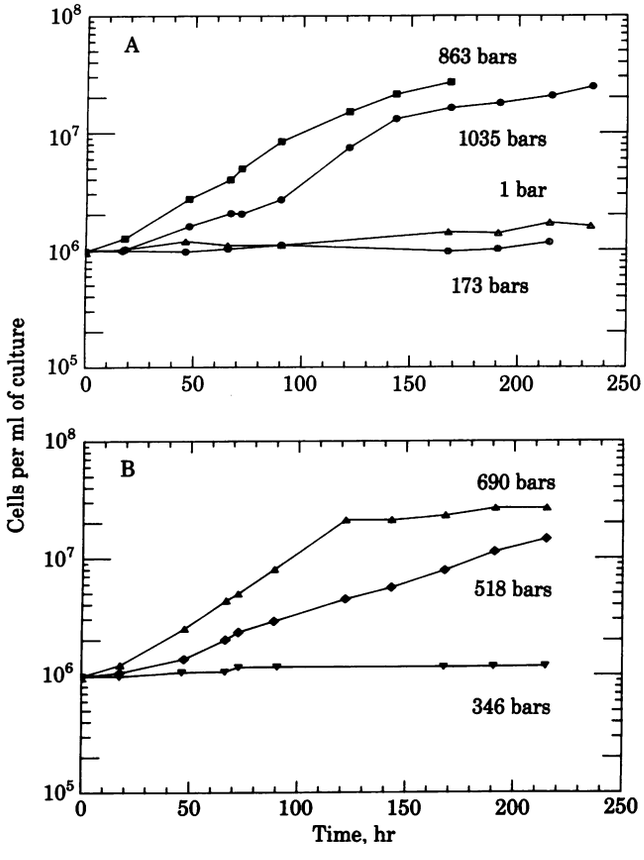

FIG. 1. Reproduction of MT-41 at 2°C. Strain MT-41 was incubated at seven different pressures at 2°C in nutrient medium. Growth was not observed at the three lowest pressures (1, 173, and 346 bars) but was clearly observed at higher pressures and even at 1,035 bars, the highest of the pressures used. (*A*) Data over the full range of pressures: △, 1 bar; ○, 173 bars; ■, 863 bars; ●, 1035 bars. (*B*) Data in the range where pressure change has the maximum effect on growth: ▽, 346 bars; ◆, 518 bars; ▲, 690 bars.

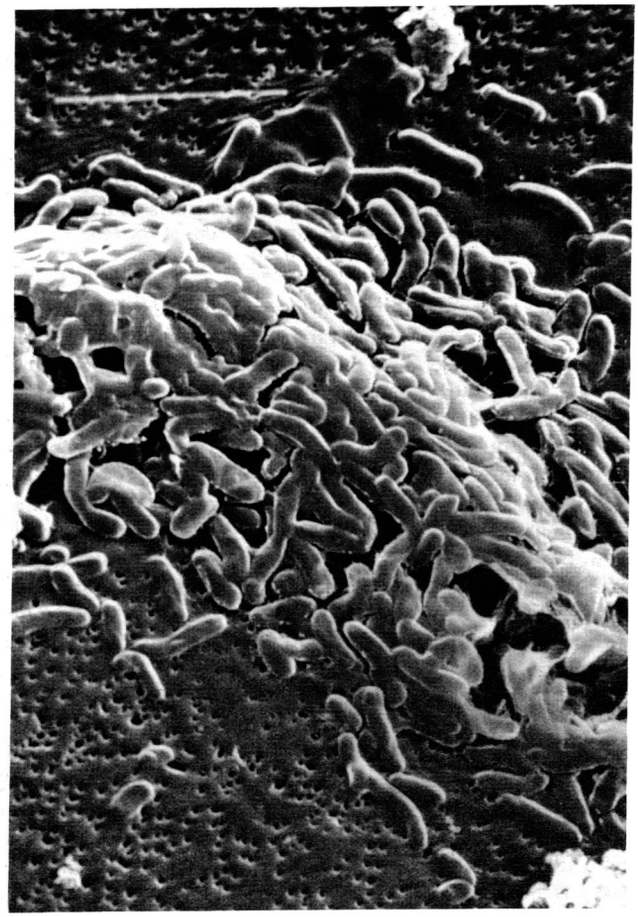

FIG. 2. An aggregate of strain MT-41 was fixed and photographed with the scanning electron micrograph. These large aggregates began to form as cultures reached more than 2×10^7 cells per ml. There is only slight evidence of extracellular material that might be responsible for holding the cells together. The light line in the upper left of the picture is 5 μm long.

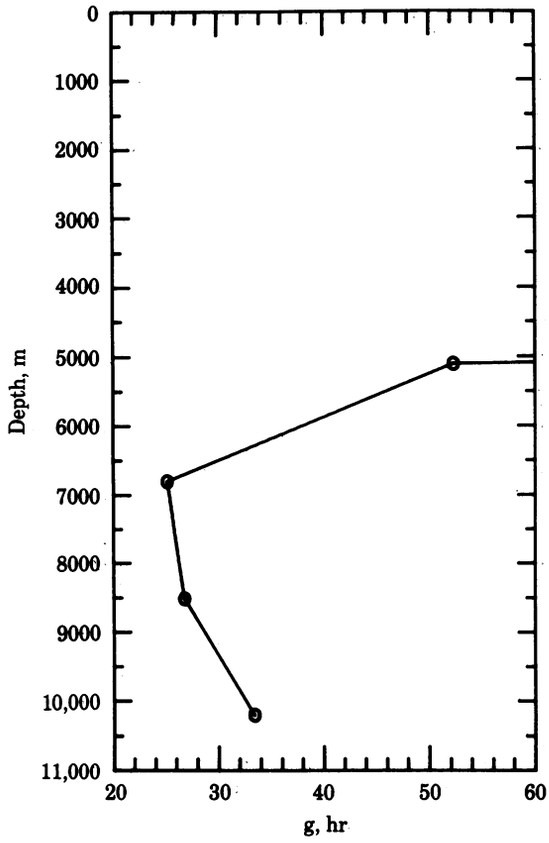

FIG. 3. The values for the generation time, g, of MT-41 were determined from the curves in Fig. 1. The pressure was converted to the depth in the sea at which it would occur. The graph of g versus this depth is shown and illustrates the range of depths in a 2°C ocean over which strain MT-41 could reproduce. The organism is clearly adapted to depths greater than the 3800-m average depth of the sea.

MT-41 will respond to temperature as strain CNPT-3 does (1, 2), then there is no set of naturally occurring temperatures and pressures that would allow for growth at depths shallower than 3416 m. The values of g for strain MT-41 ranged from 25 to 52 hr. The shortest generation time, 25 hr, was about 3 times longer than the 8- to 9-hr value (1, 2) observed with the abyssal plain bacterium, strain CNPT-3, at its optimal pressure. However, strain CNPT-3 does not grow at pressures greater than 850 bars at 2°C. Strain CNPT-3 was isolated (1) from a sample taken thousands of miles from deep trenches. Samples from a 5672-m depth at the rim of the Mariana Trench and only 17.4 miles from the deep portion where strain MT-41 originated yielded bacteria that behaved very similarly to strain CNPT-3 and did not grow above 850 bars.

The above data suggests that there is a barrier at 8500 m that prevents the intrusion of abyssal forms into hadal communities as functional members. Conversely, hadal forms with a slow rate of reproduction that decreases with decreasing pressure cannot successfully compete in the abyssal communities. This data is the first evidence of a physiological nature (12) suggesting genetic adaptation to the deep trenches of the seas. This kind of data on trench organisms is needed to understand to what extent trenches isolated from each other may contain unique communities analagous to those on oceanic islands (13) by offering reproductive isolation. A better understanding of the species composition of trenches, the propagules of these organisms, and the survival of hadal life at abyssal depths is required to assess the effects of the depths of the sill between trenches.

There is no theory for predicting the value of the generation time for bacteria at given conditions of temperature, pressure and nutrient concentrations. Brock (14) tabulated for 11 bacterial strains the temperature at which the generation time (determined in nutrient-rich media) was optimal. He found an intriguing linear relationship between the logarithm of the reciprocal of the optimal mean generation time for each bacterium and the reciprocal of the absolute temperature. If the Brock plot is extrapolated to a generation time of 25 hr (we do not know if this is the optimal value for the barophile MT-41 but use it for the sake of argument), then the temperature of optimal growth is calculated to be −48°C. In this sense barophiles may be viewed as extreme psychrophiles. To what extent is high pressure equivalent to low temperature? Hamann (15) has eloquently discussed how the effects of increasing pressure may be considered from the principle of le Chatelier (which is the basis for most thermodynamic explanations) and from the principle of microscopic ordering. Thus, pressure favors those reactions occurring with a volume decrease and pressure tends to create order at the molecular level. One of the notable exceptions to these principles is water, which freezes at lower temperatures as the pressure is increased and which exhibits a minimum viscosity as the pressure is increased along certain isotherms. In these two examples, increasing pressure produces similar effects to increasing temperature. It is possible that these exceptions do not apply in dealing with aqueous solutions as opposed to pure water; or, that the principles of le Chatelier and of microscopic ordering apply to the nonaqueous components (especially membranes) of living cells that might be important determinants of pressure sensitivity (16). In any event and in the absence of a clear manner [see also Morita (17)] to establish an equivalence between temperature and pressure for complex systems, studies showing the inactivation of deep-sea bacteria (18) by warming to even 10°C support the conclusion from a Brock plot that they are extreme psychrophiles.

The Brock (15) plot shows, furthermore, that there is an intrinsic slowing of life processes as the temperature is lowered. The generation time of the hadal strain MT-41 was slower than that of the abyssal strain CNPT-3. This evidence supports the hypothesis (19–21) that life processes are slowed in the deep sea because of high pressure. However, we note that the extent of the reduction in the rate of biological activities may not be nearly as great as early evidence indicated (19, 20). In addition, the rates of reproduction of both strains CNPT-3 and MT-41 are sufficiently large to ascribe an important role to such bacteria in the biochemical and biogeochemical transformations in the deep sea.

Mean generation times of strain MT-41 of between 25 and 34 hr at temperatures and pressures approximating the natural environmental ones are much more rapid than a 33-day generation time found for *Pseudomonas bathycetes* under similar physical conditions (22). *P. bathycetes* originated from a sediment sample from the Mariana Trench (17). The conclusion should not be drawn that sediment bacteria (perhaps such as *P. bathycetes*) reproduce intrinsically more slowly than animal-associated bacteria (possibly such as MT-41). We have found bacteria from sediments of abyssal plains that reproduced in nutrient-rich media just as rapidly as animal-associated ones did. We are of the opinion that *P. bathycetes*, although originating from a Mariana Trench sample, is not likely part of the indigenous microflora of the Mariana Trench. It is most likely an intruder which entered trench sediments with detritus and remained there in a preserved or low-activity state. This hypothesis is derived in part from the slow generation time that *P. bathycetes* exhibits under low temperatures and high pres-

sures and more so from our studies which suggest that abyssal bacteria and, by extrapolation, trench bacteria will be extremely psychrophilic and barophilic. *P. bathycetes* grows (23) nonbarophilically at atmospheric pressure and at temperatures as great as 25°C.

There has been speculation (5, 21) suggesting that deep-sea bacteria may be sufficiently sensitive to succumb when sampled from the deep sea without retention of the pressure of their native environment. The previous study with the barophilic strain CNPT-3 and the present one with the obligately barophilic strain MT-41, although incomplete, suggest that the barophilic character *per se* does not render a strain sensitive to decompression. The important fact emerging is that deep-sea microorganisms are exquisitely sensitive to increased temperatures as would be encountered in sampling with uninsulated devices and in casual handling in the laboratory. The important conclusion is that much significant (although not all) deep-sea microbiology can be done without elaborate pressure-retaining sampling devices. The ability to handle these organisms in the laboratory is greatly facilitated by their tolerance to decompressions, even if the duration of the decompression must be brief. Finally, we assert that an axiom of deep-sea microbiology is that all cultivations should be conducted at the temperatures and pressures of the natural environment. Only in this way can shallow water microbes be prevented from contaminating and even overtaking the deep-sea microbes in laboratory cultures (24).

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1. Yayanos, A. A., Dietz, A. S. & Van Boxtel, R. (1979) *Science* **205,** 808–810.
2. Yayanos, A. A., Dietz, A. S. & Van Boxtel, R. (1980) *Abstracts, 80th Annual Meeting of The American Society for Microbiology* (American Society for Microbiology, Washington, D.C.), p. 173.
3. ZoBell, C. E. & Morita, R. Y. (1957) *J. Bacteriol.* **73,** 563–568.
4. Yayanos, A. A. & Pollard, E. C. (1969) *Biophys. J.* **9,** 1464–1482.
5. ZoBell, C. E. (1964) in *Recent Researches in the Fields of Hydrosphere, Atmosphere and Nuclear Geochemistry* (Editorial Committee of Sugawara Festival Volume, MARUZEN Co., Tokyo, Japan), pp. 1464–1482.
6. Marquis, R. E. & Matsumura, P. (1978) in *Microbial Life in Extreme Environments,* ed. Kushner, D. J. (Academic, London), pp. 105–157.
7. Baross, J. A., Hanus, F. J. & Morita, R. Y. (1974) in *Effect of the Ocean Environment on Microbial Activities,* eds. Colwell, R. R. & Morita, R. Y. (Univ. Park Press, Baltimore), pp. 180–202.
8. Hessler, R. R., Ingram, C. L., Yayanos, A. A. & Burnett, B. R. (1978) *Deep-Sea Res.* **25,** 1029–1047.
9. Dietz, A. S. & Yayanos, A. A. (1978) *Appl. Environ. Microbiol.* **36,** 966–968.
10. Mantyla, A. W. & Reid, J. L. (1978) *Deep-Sea Res.* **25,** 169–173.
11. Yayanos, A. A. (1975) *Biochim. Biophys. Acta* **392,** 271–275.
12. Belyaev, G. M. (1972) in *Hadal Bottom Fauna of the World Ocean,* ed. Zenkevich, L. A. (Israel Program for Scientific Translations, Jerusalem), pp. 157–158.
13. Dobzhansky, T., Ayala, F. J., Stebbins, G. L. & Valentine, J. W. (1977) *Evolution* (Freeman, San Francisco), pp. 186–187.
14. Brock, T. D. (1967) *Science* **158,** 1012–1019.
15. Hamann, S. D. (1957) *Physico-Chemical Effects of Pressure* (Butterworth, London), pp. 1–6.
16. Gause, E. M., Mendez, V. M. & Rowlands, J. R. (1974) *Spectroscopy Lett.* **7,** 477–490.
17. Morita, R. Y. (1976) in *The Survival of Vegetative Microbes,* eds. Gray, T. R. G. & Postgate, J. R. (Cambridge Univ. Press, Cambridge), pp. 279–298.
18. Yayanos, A. A. & Dietz, A. S. (1980) *Abstracts, 80th Annual Meeting of The American Society for Microbiology* (American Society for Microbiology, Washington, DC), p. 173.
19. Jannasch, H. W., Eimhjellen, K., Wirsen, C. O. & Farmanfarmaian, A. (1971) *Science* **171,** 672–675.
20. Jannasch, H. W. & Wirsen, C. O. (1973) *Science* **180,** 641–643.
21. Jannasch, H. W. & Wirsen, C. O. (1977) *Sci. Am.* **236,** 42–52.
22. Schwarz, J. R. & Colwell, R. R. (1975) *Abstracts, 75th Annual Meeting of the American Society for Microbiology* (American Society for Microbiology, Washington, DC), p. 162.
23. Pope, D. H., Smith, W. P., Swartz, R. W. & Landau, J. V. (1975) *J. Bacteriol.* **121,** 664–669.
24. Yayanos, A. A. (1980) in *Advanced Concepts in Ocean Measurements for Marine Biology,* eds. Diemer, F. P., Vernberg, F. J. & Markes, D. J. (Univ. South Carolina Press, Columbia, SC), pp. 307–318.