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Dependence of Reproduction Rate on Pressure as a Hallmark of Deep-Sea Bacteria

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Strains of bacteria in axenic culture were isolated from samples of depths between 1,957 and 10,476 m of the Pacific Ocean. All of the bacteria from this range of depths were barophilic. The pressure at which the rate of reproduction was maximal was found to be correlated with the depth of origin of the isolates.

Some deep-sea heterotrophic bacteria grow rapidly and optimally in laboratory culture at the temperatures and pressures of the deep sea (1, 6, 7). Among the recent findings are that barophilic bacteria exist to an extent greater than previously thought (1, 6, 7), that rapidly growing, obligately barophilic bacteria exist in the deepest ocean trench (6), that a barophilic strain from the cold deep is inactivated by temperatures above 10°C at atmospheric pressure (5), and that in two cases the pressure at which the rate of reproduction is maximal at 2°C is less than the pressure at the depth of origin (6, 7). This is part of a continuing study to elucidate the role of these bacteria in nature and to characterize them further. We report here data that show that deep-sea bacteria are ubiquitous in the depth range of 1,957 to 10,476 m and increasingly barophilic with increasing depth of origin.

MATERIALS AND METHODS

Collection of samples. Strain SC2 originated from a 1,957-m depth of the San Clemente Basin located in the Eastern Pacific Ocean. The sample was recovered on 21 August 1980 at latitude 32°53.0′ N, longitude 118°7.0′ W. Strains PE31 and PE35 originated from a depth of 3,584 m just beyond the Patton Escarpment of the eastern Pacific Ocean. The sample was recovered on 5 June 1980 at latitude 31°53.0′ N, longitude 119°54.0′ W. Strain CNPT-3 originated, as previously described (4, 7), from a 5,782-m depth of the central North Pacific Ocean. The sample was recovered at latitude 28°37.4′ N, longitude 155°24.03′ W on 8 June 1978. Isolates MT52 and MT62 were from samples collected on the wall of the Mariana Trench at a depth of 5,672 m. The samples were retrieved on 21 December 1978 at latitude 11°36.2′ N, longitude 142°18.0′ E. Isolate MT41, as previously described (6), was from a sample of a 10,476-m depth taken on 13 December 1978 at latitude 11°20.5′ N, longitude 142°25.8′ E. The temperature and pressure at which a sample was held differed from the conditions prevailing in situ for the time shown in Table 1. After such time, a sample was incubated at the temperature and pressure shown in Table 1.

Note that 1 atm = 1.01325 bars = 0.101325 MPa.

Isolation of bacteria and media. Colonies of bacteria were grown at high pressures with a silica gel pour tube method (7). Isolates were maintained in nutrient silica gels and in marine broth (type 2216; Difco Laboratories, Detroit, Mich.).

Determination of cell concentrations. A Coulter Counter model ZH or ZBI (Coulter Electronics, Hialeah, Fla.) was used as previously described (5) to determine the number of cells in the cultures.

Kinetics of reproduction at high pressures. Cultures growing in marine broth at the temperatures and pressures shown in Table 1 were used to inoculate fresh broth to approximately 10^6 cells per ml. The inoculated medium was dispensed into syringes modified for use at high pressures. The syringes, each containing a glass ball, were placed in pressure vessels and incubated at high pressure. The pressure vessels were mounted on a motor-driven rocking platform, whose motion moved the glass balls in the syringes and mixed the cultures. The pressure in the vessels was checked before the vessels were decompressed and opened for removal of the samples. A special fitting (8) on each vessel allowed the pressure check, opening, closing, and recompression of the vessels to be done quickly. The platform and the vessels on it were submerged in water regulated to 2 ± 0.1°C. As previously reported for bacteria growing with generation times of several hours (6), samples taken from incubations at high pressure with decompression-recompression cycles give growth curves identical to those determined with samples from an apparatus that allows sampling without decompression of the culture. Descriptions of the various high-pressure equipment used are being prepared for publication.

Light microscopy. All cultures were routinely examined with a Zeiss Universal phase-contrast microscope (Zeiss, Oberkochen, West Germany).

RESULTS

The barophilic character of strain SC2 was very slight (Fig. 1A). Strains PE31 and PE35 (Fig. 1B and C) originated from a greater depth than did strain SC2 and exhibited a more pronounced barophilic response than strain SC2 did. As seen in Table 1, the PE strains originated from a 3,584-m depth and the SC strain from a 1,957-m depth. Growth curves for isolates from

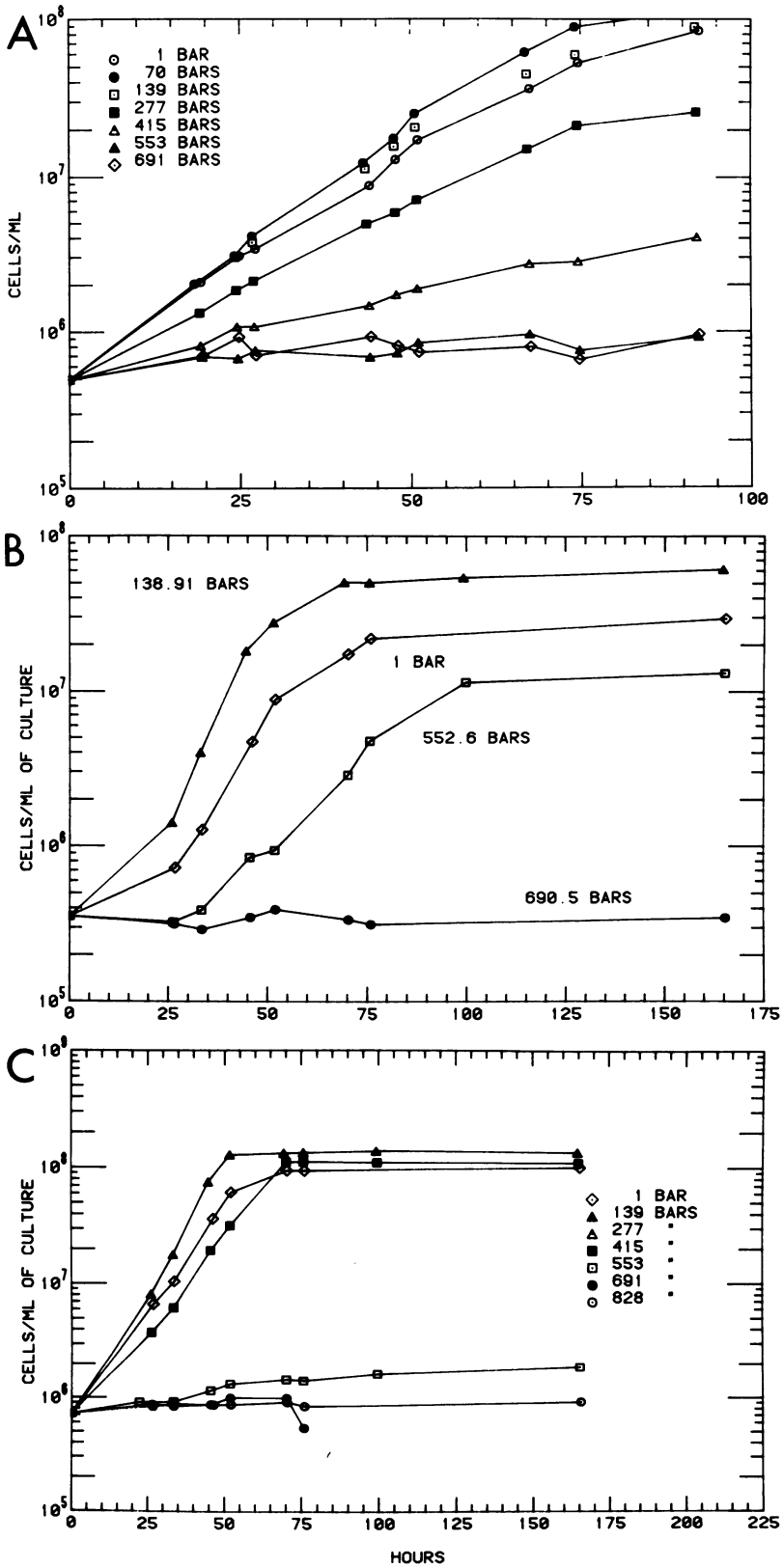

FIG. 1. (A) Growth curves for the bacterial isolate SC2 from a 1,957-m depth were determined at 2°C over a range of pressures. The rate of reproduction is slightly barophilic. Growth curves are also shown for two isolates taken from the Patton Escarpment at a depth of 3,584 m. (B) Isolate PE31; (C) isolate PE35. These isolates are seen to be also slightly barophilic.

TABLE 1. Thermal and pressure history of samples and incubation

Sample[a]	Location	Water depth (m)	Conditions in situ		Retrieval conditions[b]				Incubation conditions	
			Temp (°C)	Pressure (bar)	T_{max} (°C)	Time at T_{max} (h)	P_{min} (bar)	Time at P_{min} (h)	Temp (°C)	Pressure (bar)
Water from a TIFT containing >500 amphipods	San Clemente Basin	1,957	~2.5	201	5	<0.5	1	24	2	242
Water from a TIFT containing 1 amphipod	Near Patton Escarpment	3,584	~2.5	363	3	<1	1	50	2	414
Water around amphipods decaying in a PRAT	Central North Pacific Ocean	5,782	~2.5	586	<10	1.5	559	1.7	2	580
Amphipod from a TIFT	Wall of Mariana Trench	5,672	~2	576	<6	0.5	1	2	2	580
Amphipod from a TIFT	Mariana Trench	10,476	~2	1,062	5.5	0.5	1	2	2	1,035

[a] TIFT, Thermally insulated free-vehicle trap; PRAT, pressure-retaining animal trap that is also thermally insulated.
[b] T_{max}, Maximum temperature; P_{min}, minimum pressure.

an even greater depth, 5,672 m, are shown in Fig. 2. Strains MT52 and MT62 were markedly barophilic and grew over a pressure range greater than 800 bars. Isolates SC2, PE31, and PE35 grew over pressure ranges significantly shorter than 800 bars. It is curious that isolates such as CNPT-3 (7), MT52, and MT62, all originating from depths between 5,600 and 5,900 m, grew over a range of pressures greater than those for isolates from shallower depths, as noted above, or from greater depths (see below). Two sets of data for isolate MT62 are shown in Fig. 2B and C to demonstrate the repeatability among experiments. The generation times for all of the experiments are in Table 2.

Figure 3 shows the pressure dependence of the mean generation time for the bacterial strains originating from depths of 3,584, 5,672, and 10,476 m. The data for strain MT41 are from a previously reported study (6). The pressure dependence of the generation times for strain SC2, which originated from a depth of 1,957 m, is shown in Fig. 4. A proportionality between the value of the mean generation time at the optimal pressure and the depth of origin of the isolates was apparent from the data (Fig. 3). However, strain SC2, from a depth of 1,957 m, grew more slowly (Fig. 4) than did strains from 3,584-m depths (Fig. 3). The data (Fig. 3 and 4) revealed that the breadth of the range of pressure over which an organism grew was also uncorrelated with depth of origin.

All of the bacteria were barophilic in that they grew best or exclusively at pressures greater than atmospheric pressure when held at 2°C. The pressure of the minimum mean generation time at 2°C was chosen as a measure of barophily. This pressure, as revealed in Fig. 3 and 4, was less than the pressure at the depth of origin of the strains. A summary (Fig. 5) of these data and data from similar experiments with additional strains shows a clear relationship between the

TABLE 2. Summary of generation times at 2°C

Pressure (bar)	Generation time (h) for isolate:						
	SC2	PE31	PE35	MT52	MT62	MT62	MT41
1	10.3	7	7.8	22.3	33.1	65	
70	9.1						
139	9.6	5.1	5.8	10.7	9.8	10.9	
277	13.7	5.6	5.9	8.8	8.1	9.4	
346							∞
415	34.1		8.2	8.8	8.0	9.1	
518							52.3
553	∞	12.9	∞	10.5	8.5	10.1	
690		∞		16.2	13.3	13.2	25.2
828				29.1	47.5	23.8	
863							26.8
1,035							33.4

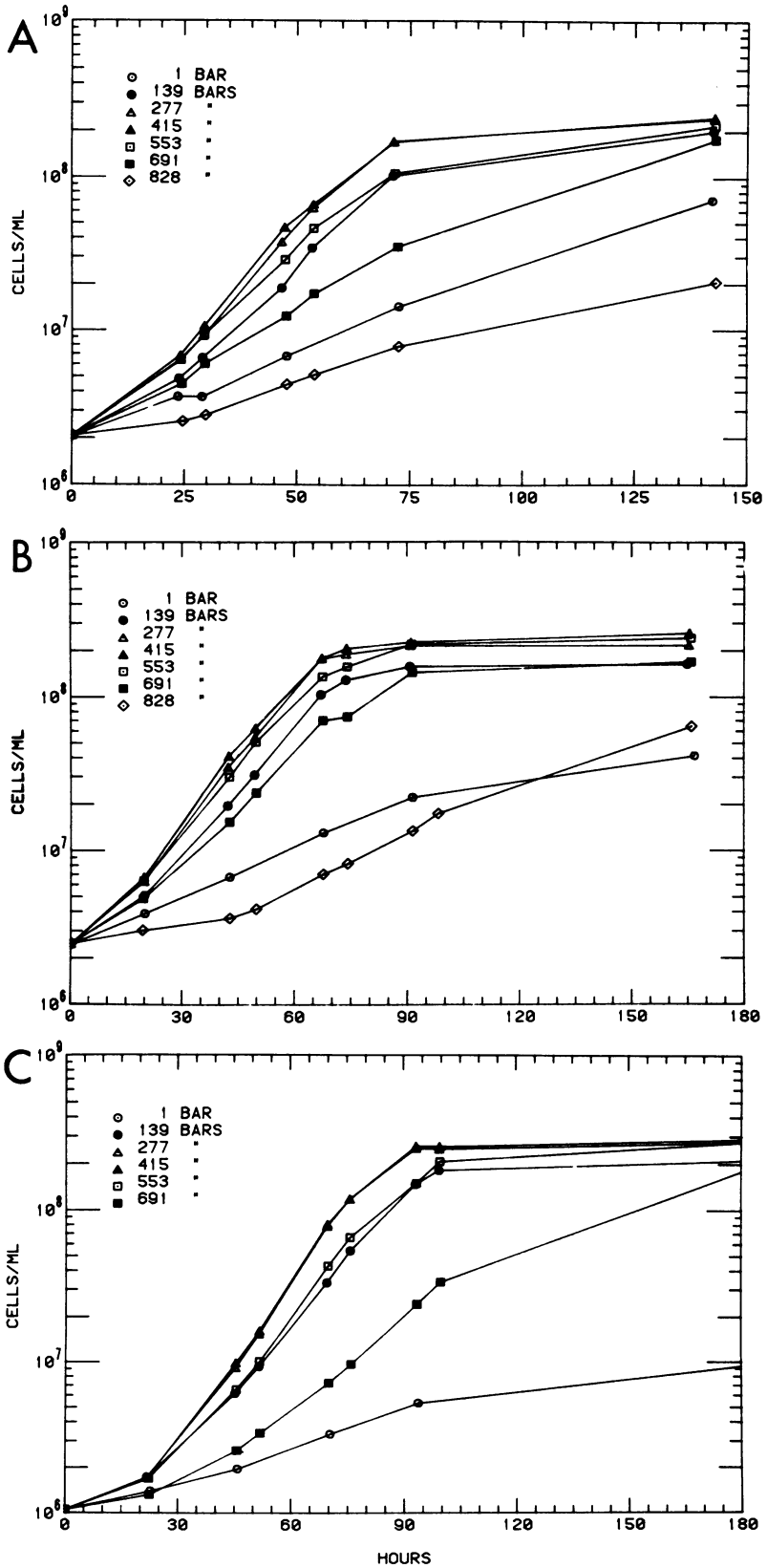

FIG. 2. (A) These growth curves are for isolates MT52, originating from a 5,672-m depth. The rate of reproduction responded in a markedly barophilic fashion. (B and C) Two sets of data are shown for isolate MT62, which originated from a 5,672-m depth. The data were obtained in separate experiments and are shown to illustrate the degree of repeatability that was achieved.

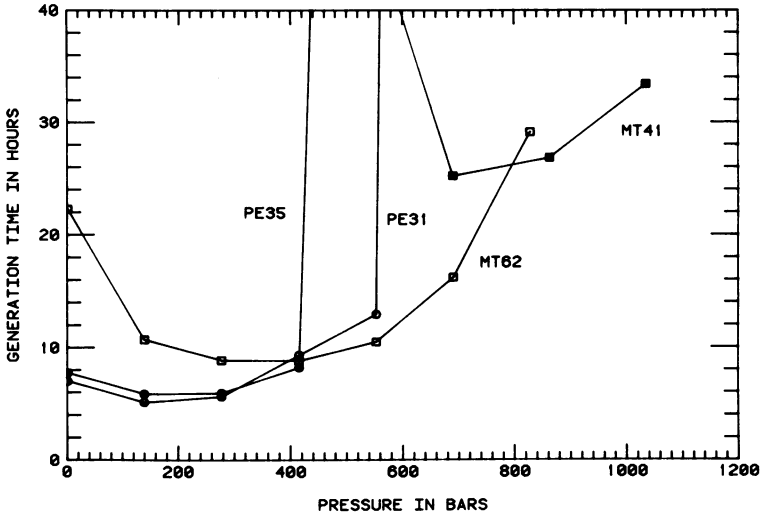

FIG. 3. Pressure dependence of reproduction. All strains were from the Pacific Ocean. PE31 and PE35 were from a 3,584-m depth near the Patton Escarpment; strain MT62 was from a 5,672-m depth on the wall of the Mariana Trench; strain MT41 was from a 10,476-m depth of the Mariana Trench (2).

pressure allowing a maximum rate of reproduction (at 2°C) and the pressure at the depth of origin of each strain. The data were fitted with a least-squares algorithm to a straight line. By extrapolation of this equation, the barophilic characteristic should be absent from isolates obtained from depths of about 1,070 m and less. Furthermore, organisms living at atmospheric pressure (and 2°C) should show optimal growth at a negative pressure. Negative hydrostatic pressures characterize certain metastable states (2, 3).

DISCUSSION

The linear relationship shown in Fig. 5 was interpreted more as a trend than as a rule until more data can be obtained with other isolates. The fact that optimal pressures for deep-sea bacteria were found to be lower than those at their depths of origin was considered to be of fundamental value and significance.

The two dashed lines shown in Fig. 5 thus bound the properties of dozens of deep-sea bacteria (from depths greater than 1,957 m) that we have studied. All of the isolated bacteria were found to be barophilic, that is, yielded points above the lower dashed line; and all of the bacteria showed optimal pressures less than those at their depth of origin, that is, yielded points that were beneath the higher of the two dashed lines.

The fact that the regression line (Fig. 5) extrapolates to a negative hydrostatic pressure (2, 3) might be considered moot since these metastable states are difficult to achieve. Nevertheless, the extrapolated value does seem to reaffirm the well-known nonbarophilic character of bacteria such as *Escherichia coli*.

In summary, these results (i) supported barophily as a ubiquitous characteristic of bacteria from the cold, deep sea and from depths between 1,957 and 10,476 m; (ii) showed that the pressure allowing the maximal reproductive rate at 2°C was always lower than the pressure at the depth of origin; (iii) revealed that the pressure at which the rate of reproduction is maximal may be diagnostic for the depth of origin of a given strain; (iv) gave evidence that bacteria from 5,600- to 5,900-m depths had the broadest range for pressures which permitted growth; (v) showed by extrapolation that bacteria from the shallower depths of deep seas should not be barophilic; (vi) revealed that 5 and 35 h are the lower and upper bounds of the range of genera-

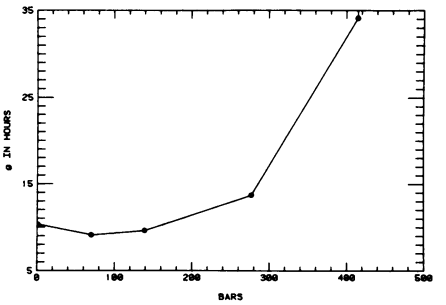

FIG. 4. Generation times of isolate SC2 at various pressures are shown.

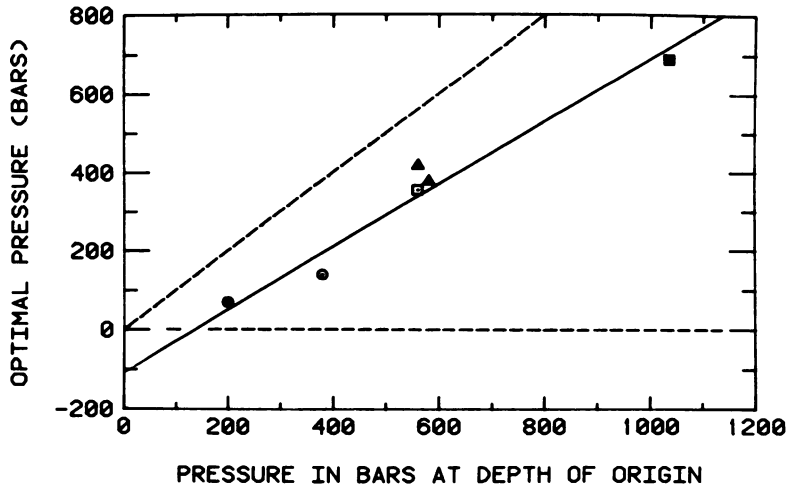

FIG. 5. The data for this plot were derived from Fig. 3 and 4 and from similar data on other bacterial isolates as follows: (●) strain SC2 from a 1,957-m depth in the San Clemente Basin of the Pacific Ocean; (○) strains PE31 and PE35 (Fig. 3) from a 3,584-m depth; strains MT52 (▲) and MT62 (□) (Fig. 3) from a depth of 5,672 m on the wall of the Mariana Trench; (△) strain CNPT-3 from a depth of about 5,782 m in the central North Pacific Ocean (5); and (■) strain MT41 (Fig. 3) (4) from 10,476 m in the Mariana Trench. The upper dashed line is where the pressure of optimal growth would equal the pressure at the depth of origin of the isolates. No experiment yielded points above this line for dozens of isolates studied in the manner described here. Points that lie above the lower dashed line represent barophilic growth.

tion times which these deep-sea bacteria will exhibit at 2°C in nutrient media; and (vii) supported by extrapolation the idea that bacteria from depths shallower than 1,070 m in a 2°C ocean may exhibit maximal growth rates when tested at negative hydrostatic pressures.

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