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Bacterial growth in deep-sea sediment trap and boxcore samples

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ABSTRACT: This study was to determine whether heterotrophic bacteria associated with deep-sea particulates are adapted more to the moderate temperatures and pressures of surface waters or to the extremes of the deep sea, and how such microorganisms respond to substrate enrichment. Samples of sinking particulates, fecal pellets, and deposited sediments were collected in bottom-moored sediment traps and boxcores at station depths of 1850, 4120, and 4715 m in the North Atlantic. Homogenized seawater suspensions of samples were incubated for 2 to 7 d under both shallow and deep-sea temperatures and pressures, with and without substrate enrichment (yeast extract or chitin). Increases in total bacterial number or percent dividing cells were measured by epifluorescence microscopy. Probable origins of bacteria in a given sample were evaluated according to temperature and pressure regimes affording bacterial growth. With a few exceptions, results indicated a predominance of shallow water bacteria in sediment-trap, but not boxcore, samples and an increasingly significant fraction of deep-sea bacteria, adapted to low temperature and elevated pressure, in fecal pellet samples trapped at increasing depth. Under deep-sea conditions, bacterial doubling times in sediment core samples were weeks or months, regardless of substrate enrichment, while in some trap samples were days (>1.5) without enrichment, and hours (7.4 to 14) with enrichment. Doubling times of barophilic bacteria, isolated in pure culture from the deeper trap and core samples, ranged from 6 to 13 h under *in situ* temperature and pressure. These findings suggest that particulate organic matter, prior to its burial in abyssal sediments, is altered by indigenous, deep-sea bacteria, some of which are capable of rapid activity at low temperature and elevated pressure.

INTRODUCTION

Recent investigations of particulate matter settling through the oceanic water column have revealed subsurface areas of intense microbial activity that is mediated by indigenous bacteria adapted to *in situ* conditions, and not by surface-derived microorganisms that have settled with the particulates (Fellows et al. 1981, Fuhrman & Azam 1983, Gowing & Silver 1983, Karl et al. 1984, Silver et al. 1984). These findings have extended to about 2000 m, the depth at which *in situ* microbial activities are known to contribute significantly to the recycling of surface productivity and the nutritional support of aphotic pelagic ecosystems. To what greater ocean depths such microbial activity may occur is not known.

Most of the available data from the abyssopelagic environment, which is based on seawater samples and not collections of sinking particulates, indicate that microbial activities are reduced by orders of magnitude relative to shallower environments, and that elevated pressures act to keep rates low (Jannasch & Taylor 1984). Thus, it has appeared that the activities of surface-derived bacteria, which are known to be inhibited by deep-sea temperatures and pressures (Wirsen & Jannasch 1975, Jannasch & Taylor 1984), dominate over any locally-adapted microorganisms that may occur at great depth. Otherwise, pressure-enhanced or barophilic microbial activities would have been measured regularly in deep-sea samples. Instead, such activity has been detected only in invertebrate guts (Schwarz et al. 1976, Deming et al. 1981, Deming & Colwell 1982), with some preliminary evidence that barophilic activity may also occur in the abyssopelagic environment in sediments and in association with fecal pellets (Deming & Colwell unpubl.).

To understand more fully the role of bacteria in altering the organic carbon that fuels the deep-sea benthos (Rowe & Gardner 1979, Rowe & Deming in press), it is important to know if intense areas of *in situ*

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microbial activity, similar to those detected above 2000 m, also occur in association with particulate matter descending into the benthic boundary layer. To this end, sinking particulates and deposited sediments were collected in sediment traps and cores at station depths of 1850 (similar to the maximum oceanic depth already studied by others), 4120, and 4715 m in the North Atlantic. Conclusions about the origin of bacteria in these samples and their potential for rapid activity *in situ* are based on relative rates of bacterial growth, as measured in homogenized seawater suspensions incubated under shallow water and deep-sea temperatures and pressures, with and without enrichment with yeast extract or chitin.

MATERIALS AND METHODS

Sample collection. Samples were collected aboard the French Research Vessel 'Jean Charcot' at station depths of 4120 m (47°35' N, 9°41' W) and 4715 m (46°31' N, 10°27' W) in the Bay of Biscay during the Biogas expedition of September 1981. Additional samples were collected aboard NOAA Ship 'Mt. Mitchell' at a station depth of 1850 m (72°26' N, 38°57' W) off the US East Coast during the NEMP cruise of September 1983. At the 4120 m station, sinking particulates were collected in a 40 l cylindrical sediment trap (described by Rowe & Gardner 1979), moored 200 m above the sea floor. At the 4715 m station, a larger cone-shaped trap (described by Sibuet et al. 1984) was deployed 20 m above the seafloor. Both traps were open at depth for 8 d and sealed upon command prior to ascent and recovery. The temperature of the entrapped seawater upon recovery was about 6 °C in the first trap and 2.8 °C in the second. For a shallow water comparison, a Rowe trap was floated for 8 h at a depth of 20 m below sea surface at the 4120 m station. Water temperature upon recovery was 15.5 °C. At the 1850 m station, particulates were collected in a Rowe trap moored 10 m above the seafloor for 3 d with a recovery temperature of 13.8 °C. Surface seawater at that site measured 25 °C.

At the 4120 and 4715 m stations, cold (6.5 and 5.5 °C) sediment samples were retrieved using a 0.25 m^2 boxcore. After careful siphoning to remove overlying seawater, surface sediment was sampled to a depth of 1 cm using a sterile spatula. At the 1850 m station, equally cold sediment was obtained by gravity corer and subsampled aseptically at depths of 1, 5, and 15 cm after core extrusion.

Sample treatment and analysis. All trap and core samples were kept at < 3 °C (in a refrigerated laboratory or using ice baths), immediately after recovery and during the following procedures. Particulates from each trap sample were examined under a dissecting microscope to locate and remove individual fecal pellets, ranging in length from 100 to 1000 µm, using a sterile Pasteur pipet. Each pellet was rinsed twice by sequential transfer into sterile seawater (SSW; 0.2 µm filtered, autoclaved seawater collected by Niskin bottle from a depth of 4700 m) before being pooled in 1 tube and homogenized, using a hand-operated tissue homogenizer. Fecal pellet suspensions, prepared from the subsurface trap and bottom-moored traps at the 1850, 4120, and 4715 m stations, contained 2, 24, 28, and 24 pellets, respectively. An undiluted, homogenized seawater suspension of the complete particle collection from the 1850 m station was also prepared. Sediment samples were diluted 1:5 (wet wt:vol) in SSW from the appropriate station and mixed by vortex.

Portions of each of the 10 sample suspensions prepared for this study were fixed immediately in 2 % formaldehyde for later determination of total bacterial number ml^{-1} by acridine orange staining and epifluorescence microscopy, using the methods of Hobbie et al. (1977) as applied to sediment samples by Deming & Colwell (1982). Bacterial concentrations in sediment samples were normalized to g^{-1} dry wt sediment, after determining percent water content from subsamples dried at 60 °C for 18 h. The total number of bacteria g^{-1} dry wt fecal pellet was calculated, using Honjo's estimate, from a study of several hundred deep-sea fecal pellets, that the dry weight of a pellet in the same size class as those used in this study is 3.2 µg (Honjo 1978).

Half of the remaining (unfixed) volume of each sample suspension from the 4120 and 4715 m stations was enriched with 0.2 µm filtered, autoclaved yeast extract (YE) to a final concentration of 0.025 %. Replicate portions of the enriched and unenriched samples were then loaded into sterile plastic syringes and incubated for 2 d at 22 °C and 1 atm, simulating approximate sea surface conditions; at 3 °C and 400, 420, or 470 atm in pressure vessels, simulating *in situ* conditions at depth of collection; and at 3 °C and 1 atm to differentiate pressure from temperature effects. Subsamples were fixed in 2 % formaldehyde before and after incubation for later analysis by epifluorescence microscopy.

Sample suspensions from the 1850 m station were split into 5 aliquots, 4 of which were supplemented with YE to a final concentration of 0.001, 0.01, or 0.1 %, or with a solution of chitin, purified from squid pens and added to a final concentration of about 3 % (wet wt:vol). Replicate portions of each enriched and unenriched aliquot were then incubated under 3 sets of conditions, similar to those described above except that room (and sea surface) temperature was warmer (25 °C) and simulated *in situ* pressure was lower (180 atm). Subsamples were fixed for microscopy at 0, 2, and 5 d for sediment trap samples and at 0, 3, and 7 d for core sediments. Separate pressure vessels were used for each incubation period.

The total number and the number of dividing bacteria ml^{-1} were determined by epifluorescence microscopy for each fixed subsample from these incubation experiments. Standard error (SE) averaged 66 % for total counts of $<10^6$ bacteria ml^{-1}, and 16 % for those $>10^6$ bacteria ml^{-1}. Counting procedures allowed detection of dividing cells if they were present in numbers > 0.5 % of the total count. For 2 d end-point experiments in which the total bacterial number increased significantly (>2 SE) above starting level, bacterial doubling time was calculated assuming a linear increase during incubation. For 5 and 7 d time-course experiments, doubling times were calculated using linear regression analysis if stationary phase had not been reached by the end of the experiment and the correlation coefficient (r) was >0.800 (r averaged 0.962). If growth was rapid and maximum yield attained by the end of the first incubation interval, the experiment was treated as an end-point experiment, again assuming a linear increase. Doubling times determined in this manner may be conservative, since the possibility of an initial lag period followed by more rapid growth cannot be taken into account. The longer time-course experiments would have revealed a lag period of 2 or 3 d, but no such lag was evident in experiments where growth occurred. If no significant growth (<2 SE) was detected in either end-point or time-course experiments, but the end percentage of dividing cells had increased above starting level, the net gain was interpreted as an index of unusually slow or incipient bacterial growth.

Pure culture work. Replicate 0.1 ml aliquots of suspensions prepared from trap and boxcore samples from the 4120 and 4715 m stations were inoculated into each of 4 silica gel pour tubes, prepared according to Dietz & Yayanos (1978). Inoculated tubes were sealed with parafilm and incubated in pressure vessels at 3 °C and 410 or 470 atm for 3 wk. Upon decompression, colony-forming units were selected at random and purified, using a purification scheme that involved 2 complete serial transfers from gel to 2216 broth (Difco) to new gel, using a 10 d culturing period at 2 °C and the appropriate *in situ* pressure after each transfer into gel or broth. (One colony was obtained from the subsurface trap sample, but it failed to grow at 2 °C and 400 atm upon the first transfer.) Growth rates at 2 °C and hydrostatic pressures of 1 to 816 atm were determined for 6 of the purified bacterial stains (designated BBS2 and 3 for 4120 m surface sediment isolates, BBDT1 and 2 for 3920 m fecal pellet isolates, and BBP5 and 6 for 4695 m fecal pellet isolates) using methods described by Deming et al. (1984). Also included in this pure culture work were 2 bacterial isolates (BBG3 and 4) obtained from the hindgut contents of an abyssal holothurian (*Psychropotes* sp.) collected at the 4120 m station and dissected according to techniques described by Deming & Colwell (1982).

RESULTS

Bacterial concentrations in fecal pellet samples, collected near the seafloor, and in all sediment samples are listed in Table 1. The total number of bacteria g^{-1} in fecal pellets was 9 to 72 times greater than that measured in underlying surface sediments. Bacterial concentrations in sediments decreased linearly with depth in the sediment core ($r = 0.950$) and with station depth ($r = 0.998$). The concentration of bacteria in fecal pellets from the 20 m subsurface trap at the 4120 m station (3.07×10^{10} bacteria g^{-1} dry weight) was comparable to that determined for the deep-sea collections of fecal pellets (Table 1). The undiluted, homogenized seawater suspension of the complete particle collection from the 1850 m station contained 1.44×10^8 bacteria l^{-1}, a concentration about 20 times higher than that reported in seawater from similar depths (Carlucci & Williams 1978, Williams et al. 1980).

Results of growth studies using seawater suspen-

Table 1. Total number of bacteria g^{-1} in fecal pellet and sediment samples. Bacteria in sample suspensions were enumerated by acridine orange staining and epifluorescence microscopy and normalized to g^{-1} dry wt sediment. For fecal pellet samples, bacteria g^{-1} = total number bacteria in homogenized suspension ÷ number pellets ÷ 3.2×10^{-6} g, the dry wt of a 200 µm pellet as determined by Honjo (1978)

Sample	Depth above (+) or below (−) seafloor	Station depth 1850 m	4120 m	4715 m
Fecal pellets	+200 m		3.06×10^{10}	
Fecal pellets	+ 10 or 20 m	8.59×10^9		1.71×10^{10}
Sediment	− 1 cm	9.41×10^8	4.26×10^8	3.34×10^8
Sediment	− 5 cm	4.24×10^8		
Sediment	− 15 cm	2.50×10^7		

Table 2. Doubling time of total bacterial number in seawater suspensions of homogenized fecal pellets and surface sediments from near-surface water and station depths of 4120 and 4715 m

Sample	Collection depth (m)	Incubation conditions		Bacterial doubling time (h)[a]	
		Temperature (°C)	Pressure (atm)	no additions	0.025 % YE added
Fecal pellets	20	22	1	18	3.8
		3	1	NG	NG
		3	400	NG	NG
Fecal pellets	3920 (200 m above seafloor)	22	1	9.0	6.1
		3	1	NG (1 %)	13
		3	400	NG	NG (17 %)
Fecal pellets	4695 (20 m above seafloor)	22	1	NG (1 %)	7.4
		3	1	NG	12
		3	470	NG (4 %)	13
Sediment	4120 (1 cm below surface)	22	1	NG	NG
		3	1	NG	NG
		3	420	NG	NG
Sediment	4715 (1 cm below surface)	22	1	NG	12
		3	1	NG	NG
		3	470	NG	NG

[a] Based on a 2 d endpoint experiment in which total bacterial number, determined by epifluorescence microscopy, increased significantly above starting level
YE = yeast extract
NG = no growth; no significant increase (+2 SE) in bacterial number above starting level. For NG experiments in which percentage of dividing cells increased above starting level, net gain in percentage is noted parenthetically

Table 3. Doubling time of total bacterial number in seawater suspensions of homogenized fecal pellets, other particulates, and sediments from a station depth of 1850 m

Sample	Collection depth (m)	Incubation conditions		Bacterial doubling time (h)[a]				
		Temperature (°C)	Pressure (atm)	No additions	0.001 % YE added	0.01 % YE added	0.1 % YE added	3 % chitin added
Fecal pellets	1840 (10 m above sea floor)	25	1	12	4.7	4.0	4.0	5.1
		3	1	NG	NG	NG (4 %)	NG (1 %)	NG
		3	180	NG (1 %)	NG (14 %)	NG (6 %)	NG (4 %)	37
Sinking particles	1840 (10 m above sea floor)	25	1	11	8.6	5.2	4.6	5.7
		3	1	NG (4 %)	46	NG	NG	53
		3	180	36	14	7.7	8.5	7.4
Sediment	1850 (1 cm below surface)	25	1	–[b]	–	–	–	–
		3	1	NG (1 %)	NG (1 %)	–	NG (2 %)	NG (2 %)
		3	180	NG	NG (1 %)	–	NG (3 %)	190
Sediment	1850 (5 cm below surface)	25	1	–	–	–	–	–
		3	1	NG	NG (< 1 %)	–	NG	NG
		3	180	NG	NG (< 1 %)	–	NG	NG
Sediment	1850 (15 cm below surface)	25	1	–	–	–	–	–
		3	1	NG (1 %)	NG (2 %)	–	NG (1 %)	NG
		3	180	NG (2 %)	NG	–	NG (1 %)	NG (1 %)

[a] Based on 5 to 7 d time-course experiments in which total bacterial number, determined by epifluorescence microscopy, increased significantly above starting level
[b] Experiment not done
YE = yeast extract
NG = no growth; no significant increase (+ 2 SE) in bacterial number above starting level. For NG experiments in which percentage dividing cells increased above starting level, net gain in percentage is noted parenthetically

sions of homogenized fecal pellets and surface sediments from the 4120 and 4715 m stations are shown in Table 2. Results of similar experiments on samples from the 1850 m station are given in Table 3. With only naturally-available substrates present, no significant bacterial growth was detected in any sediment sample under any of the incubation conditions tested. Increasing the total incubation period from 2 d (Table 2) to 7 d (Table 3) resulted in the occasional observation of small net gains in the percentage of dividing cells under deep-sea temperature and pressure. Enrichment with YE induced growth in 1 sediment sample under shallow water conditions (Table 2), but had no significant effect at low temperature or elevated pressure for any of the sediment samples tested (Table 2 and 3). Enrichment with chitin, however, induced a barophilic growth response at 3 °C from bacteria in the 1850 m surface sediment sample (Table 3).

Results obtained with samples of fecal pellets from bottom-moored sediment traps (depths of 1840, 3920, and 4695 m) differed from those obtained with sediment core samples. Under shallow water conditions, significant bacterial growth occurred without substrate enrichment in the 2 samples of shallower origin. Additions of YE or chitin increased these growth rates and stimulated growth in the third and deeper sample. At the same pressure (1 atm) but lower temperature (3 °C), growth was not detected unless the sample was enriched with YE (Table 2). When these fecal pellet samples were pressurized, as well as chilled, substrate additions induced a bacterial growth rate or net gain in percent dividing cells that was equivalent to or greater than that measured at 1 atm and 3 °C (Table 2 & 3). Similar results were obtained for the sample containing a representative portion of all particulates intercepted by the 1840 m sediment trap, with 2 exceptions: under deep-sea temperature and pressure, significant growth occurred without substrate enrichment; and, with additions of YE or chitin, 'deep-sea' doubling times (7.4 to 14 h) were markedly faster than those measured at 3 °C and atmospheric pressure (46 h to no growth) and almost as rapid as those measured at 25 °C and 1 atm (4.6 to 8.6 h).

The most rapid doubling time in this study (3.8 h) was recorded for bacteria in the suspension of fecal pellets from near-surface waters when enriched with YE and incubated at 22 °C and 1 atm. No significant growth or net gain in percent dividing cells was measured in this sample when chilled or chilled and pressurized.

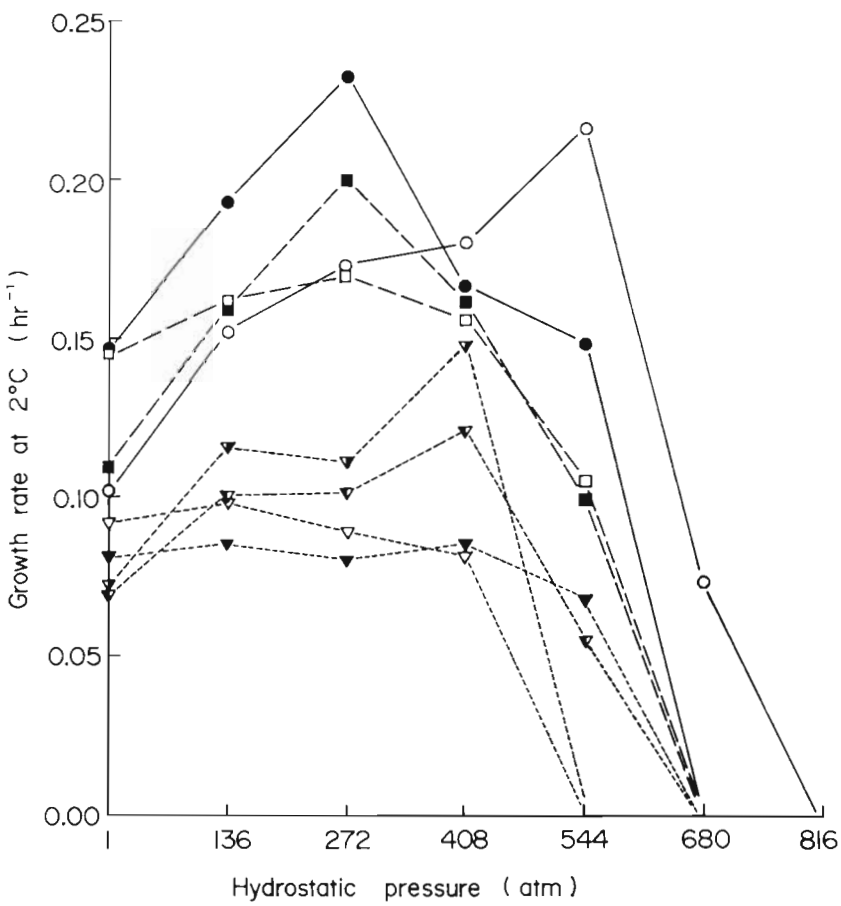

Fig. 1. Barophilic growth characteristics of bacterial strains BBS2 (□) and 3 (■), BBDT1 (▽) and 2 (▽), BBP5 (▼) and 6 (▽), and BBG3 (●) and 4 (○), isolated in pure culture from sediment trap (___), box-core (– –), and holothurian gut (——) samples from station depths of 4120 and 4715 m in the Bay of Biscay

Growth rates measured at 2 °C for 8 strains of bacteria, isolated in pure culture from deep-sea samples collected at the 4120 and 4715 m stations, are plotted in Fig. 1 as a function of increasing hydrostatic pressure. All strains were barophilic or highly barotolerant, growing 1.1 to 2.2 times faster at elevated pressures than at atmospheric pressure, as similarly reported for other strains of deep-sea, barophilic bacteria (Jannasch et al. 1982, Yayanos et al. 1982, Deming et al. 1984). The most rapid doubling times (4.3 and 4.5 h) and the strongest barophilic responses were demonstrated by the gut isolates (Strains BBG3 and 4).

DISCUSSION

Although recent progress in the development of microbiological methods has resulted in estimates of the *in situ* doubling times of natural bacterial assemblages in relatively accessible marine environments (Hagstrom et al. 1979, Karl 1979, Fuhrman & Azam 1980, Ducklow et al. 1982), bacterial growth rates have not been measured *in situ* at depths greater than 2000 m. *In situ* microbiological studies in the deep sea have focused instead on measurements of substrate utilization rates, using ^{14}C-organic compounds and pressure-retaining water samplers or remote-controlled sediment tripods (see review by Jannasch & Taylor 1984). Results of these studies indicated that total utilization rates were 10 to 1000 times slower than rates measured under shallow water conditions and that only about 10 % of any substrate utilized actually contributed to bacterial production or growth. By inference, bacterial growth rates in the deep sea must be quite slow.

Carlucci & Williams (1978) estimated *in situ* doubling times of bacteria in the deep sea (145 h at 1500 m; 210 h at 5550 m) on the basis of pure culture studies in which pelagic marine bacteria were isolated at atmospheric pressure and later returned to simulated *in situ* conditions of temperature, pressure, and substrate availability (unamended seawater). A similar study using bacteria selectively isolated under *in situ* conditions has not been attempted. Yayanos et al. (1982) reported growth rates for bacteria isolated under *in situ* temperatures and pressures, but the microorganisms were enriched from a specialized source (trapped, decomposing abyssal amphipods) and cultured in nutrient-rich broth. This study represents a first attempt to measure simulated *in situ* growth rates for natural bacterial assemblages associated with sinking particulates (primarily fecal pellets) and sediments in the deep sea.

The resulting rates must be viewed with caution for several reasons. Many were based on end-point experiments in which lag periods or rapid spurts of growth are not recognized. Experimental design would have selected for aerobic, heterotrophic bacteria in a population which, left undisturbed *in situ*, might have been more complex and heterogeneous in community structure and activity. Growth rates might have been influenced artificially, in unknown ways, by the necessity to homogenize the samples and, in the case of sediments, dilute them. On the other hand, potential 'bottle effects' (which can never be discounted completely) leading to overestimates of bacterial growth were minimal in these experiments, since unusually slow or no growth was measured in many of the samples, even after substrate enrichment and incubation for 7 d. Furthermore, the unavoidable but brief period of decompression to which all samples were exposed during retrieval and handling did not prevent detection of significant populations of barophilic bacteria, since doubling times in some samples under *in situ* pressure were equivalent to or more rapid than those measured at atmospheric pressure, all other conditions being equal. Until better estimates can be obtained *in situ*, the bacterial doubling times reported here may be of some use in evaluating microbial activities and developing models of the abyssal benthic boundary layer (Rowe & Gardner 1979, Rowe & Deming in press). The rates calculated for a given sample under variable conditions are of immediate use on a comparative basis in assessing the origins of bacteria present in the deep sea, and their potential response to substrate enrichment.

From this perspective, some of the results of this study support current views on bacteria in the deep sea, as recently reviewed by Jannasch & Taylor (1984), and some do not. Experimental data from the subsurface trap sample were in keeping with the view that surface-derived bacteria, settling through the water column with the flux of particulate matter, are severely limited in their activities at first by low temperature and at increasing depths by the combined extremes of low temperature and elevated pressure. Although reduced availability of utilizable energy sources may also limit surface-derived bacteria at great depth in the sea (Novitsky & Morita 1978), results of experiments with such bacteria in this study indicate that restrictions imposed on their growth by deep-sea temperature and pressure cannot be overcome by the introduction of fresh energy sources in the form of yeast extract. It was not surprising that no bacteria were isolated in pure culture from the near-surface trap sample when deep-sea incubation conditions of 3 °C and 410 atm and an enriched culturing medium were used.

Nor was it surprising that the slowest growth rates in this study were measured for bacterial populations in the deep-sea sediment samples incubated under *in situ* conditions. Growth in these samples was sufficiently

slow to preclude detection of significant increases in the total bacterial number, even after enrichment with yeast extract and an extended incubation period of 7 d. Thus, the *in situ* doubling times of bacteria in deep-sea sediments must measure in weeks or months, in keeping with earlier views developed on the basis of *in situ* measurements of substrate utilization rate in similar sediments (Jannasch 1979).

That enrichment of deep-sea sediment samples with yeast extract failed to stimulate more rapid bacterial growth under deep-sea temperature and pressure is reminiscent of the behavior of surface-derived bacteria in the deep sea. However, other results are inconsistent with the interpretation that most of the bacteria in these sediment samples originated in shallow water: (1) barophilic bacteria, capable of doubling every 6 h in nutrient broth under *in situ* temperature and pressure, were isolated readily from the sediment samples; and (2) enrichment with chitin, rather than yeast extract, induced measurable bacterial growth under *in situ* temperature and pressure (doubling time of 190 h) in a sediment sample from 1850 m, when no growth was detected at atmospheric pressure, all other conditions being equal. No general conclusions can be drawn from a single chitin-enrichment experiment and no other precedent exists in the literature for bacteria in relatively shallow sediment (1850 m) requiring *in situ* pressure for optimal growth; nevertheless, the detection of this barophilic growth response argues strongly for the predominant role of locally-adapted bacteria in sediment processes, as has already been proposed for particle-associated processes in the water column at a similar depth (Karl et al. 1984, Silver et al. 1984). Clearly, detection of this response may also be linked specifically to the remineralization of chitin, a refractory substance commonly available in the deep sea and a substrate of choice for future experiments.

Ultimately, it is the results of experiments with samples of deep-sea fecal pellets and sinking particulates, rather than with surface-water pellets or bottom sediments, that do not fit conventional views of limited microbial biomass and activity in the deep sea. A comparison of bacterial concentrations in the various samples examined (Table 1) reveals that fecal pellets, and probably sinking particulates in general, represent an enriched source of bacteria in the benthic environment. Note that simulated *in situ* growth in the undiluted seawater suspension of particulates trapped for 2.6 d at 1840 m (doubling time of 36 h) was too slow to account for the observed bacterial concentration (1.44×10^8 bacteria l^{-1}) being 20 times higher than expected in seawater at that depth. These particulate sources of bacteria, regardless of their *in situ* activities, probably serve as a quality food supply for benthic animals (Honjo 1978, Morita 1979, Rowe & Gardner 1979, Khripounoff & Sibuet 1980). The fecal pellets, in particular, may have already passed through or originated from the digestive systems of pelagic animals and planktonic forms (Honjo 1978), some of which are known to concentrate and feed at surprisingly high rates in the benthic boundary layer (Wishner & Meise-Munns 1984). An abyssal gut origin for the fecal pellets collected at depth in this study is supported by the isolation of barophilic bacteria from carefully rinsed pellets. It is interesting to note, however, that bacteria isolated directly from an invertebrate gut still show a stronger barophilic response (as also observed by Jannasch & Wirsen 1984) and grow more rapidly than those isolated from the pelagic environment, all other conditions being equal (Fig. 1).

Evidence that many of the bacteria associated with particulates reaching the ocean floor were indigenous to the deep sea, and not surface-derived, came from experimental data for each of the samples prepared from deep trap collections, as well as from the pure culture work. Exemplary data was obtained with a chitin-enriched suspension of sinking particulates intercepted at a depth of 1840 m, 10 m above seafloor. In this sample, the bacterial doubling time was rapid at 25 °C and 1 atm (5.1 h), considerably slower at 3 °C and 1 atm (53 h), but almost as rapid again under deep-sea temperature and pressure (7.4 h) as under shallow-water conditions. These results can be interpreted as evidence that many surface-water bacteria do reach bottom depths via particulate matter, but that at depth they are slowed in their activities primarily by low temperature. Remarkably rapid growth under *in situ* temperature and pressure, following sample enrichment with chitin, suggests the presence of a separate population of indigenous deep-sea bacteria as the dominant agents in the remineralization of organic carbon, even when highly refractory, at ocean depth.

This study is part of an ongoing research program in the deep sea that, along with the work of many others, may eventually confirm or refine the various interpretations and conclusions drawn here. However, it seems reasonable on the basis of the current data set to propose that particulate organic matter arriving at the ocean floor provides focal points for intense microbial activity in the deep sea, not unlike those already detected by others at mid-oceanic depths.

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