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Observations of Barophilic Microbial Activity in Samples of Sediment and Intercepted Particulates from the Demerara Abyssal Plain

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To better understand the ecological significance of pressure effects on bacteria in the abyssobenthic boundary layer, experimental suspensions of sediments and sinking particulates were prepared from samples collected in boxcore and bottom-moored sediment traps at two stations (depth, 4,470 and 4,850 m) in the Demerara abyssal plain off the coast of Brazil. Replicate samples were incubated shipboard at 3°C and at both atmospheric and deep-sea pressures (440 or 480 atm [4.46×10^4 or 4.86×10^4 kPa]) following the addition of [^{14}C]glutamic acid (<10 μg liter^{-1}) or yeast extract (0.025%) and the antibiotic nalidixic acid (0.002%). In seven of the eight samples supplemented with isotope, a barophilic microbial response was detected, i.e., substrate incorporation and respiration were greater under in situ pressure than at 1 atm (101.3 kPa). In the remaining sample, prepared from a sediment trap warmed to 24°C before recovery, pressure was observed to inhibit substrate utilization. Total bacterial counts by epifluorescence microscopy decreased with depth in each sediment core, as did utilization of glutamic acid. Significant percentages of the total bacterial populations in cold sediment trap samples (but not the prewarmed one or any boxcore sample) were abnormally enlarged and orange fluorescing after incubation with yeast extract and nalidixic acid under deep-sea conditions. Results indicated that in the deep sea, barophilic bacteria play a predominant role in the turnover of naturally low levels of glutamic acid, and the potential for intense microbial activity upon nutrient enrichment is more likely to occur in association with recently settled particulates, especially fecal pellets, than in buried sediments.

Recent isolations of barophilic bacteria in pure culture have confirmed that at least some bacteria in the deep sea (depth, >4,000 m) are well adapted to abyssal extremes in hydrostatic pressure (3, 11, 23, 24). However, since most of these isolates have been obtained from specific, relatively nutrient-rich environments, such as the digestive tracts of abyssal invertebrates (2, 4) or their decomposing carcasses (23, 24), the general ecological significance of their occurrence in the deep sea has remained unclear (9). Measurements of the response of natural bacterial populations to substrate enrichment in the relatively nutrient-poor environment of abyssal sediments have shown microbial activity to be affected negatively by in situ pressure (8, 9, 16, 18). The general failure to detect barophilic rates of activity from natural assemblages of bacteria in the deep sea, except in direct association with feeding animals (2, 4), has been attributed to the inability of a small number of barophilic bacteria to compete effectively for nutrients in the presence of an overwhelming majority of nonbarophilic bacteria transported to the deep sea via sinking particulates (9). In fact, the viable microbial component in samples of particulates that have reached the deep ocean floor has never been examined.

In 1979, Rowe and Gardner (15) reported that the organic carbon component of sinking particulates, collected in sediment traps moored near the ocean floor at depths of 2,200 to 3,650 m in the North Atlantic, was three- to fivefold greater than that found in underlying sediments collected in boxcores. Biological utilization of this settling and relatively enriched organic matter was invoked as the most probable explanation for the discrepancy between sediment trap and core analyses. Rowe and Deming (in press) have recently calculated that at least 30% of this utilization can be attributed to bacteria. We hypothesized that indigenous barophilic bacteria, rather than pressure-sensitive, shallow-water intruders, were the predominant participants in this microbial activity.

To test this hypothesis, we joined G. Rowe (Brookhaven National Laboratory, Upton, N.Y.) and M. Sibuet (Centre Oceanologique de Bretagne, Brest, France) aboard the French research vessel *Jean Charcot* in September 1980 to collect boxcore and bottom-moored sediment trap samples at two stations in the Demerara abyssal plain off the north Atlantic coast of Brazil. Responses of natural bacterial assemblages in these samples to [^{14}C]glutamic acid, added in significantly lower concentrations (<10 μg liter^{-1}) than those used in all previous related studies (10, 16, 18), and to an enriching concentration of yeast extract (0.025%) and the antibiotic nalidixic acid were monitored shipboard at 3°C and at both deep-sea and atmospheric pressures. This study, which complements an earlier one on bacteria associated with abyssal holothurians (2) and subsequent studies of abyssal sediments and sinking particulates (Rowe and Deming, in press; J. Deming, in press), provides the first confirmation of barophilic microbial activity in abyssal sediments since the early culturing efforts of ZoBell and Morita (24) and the first indication that such locally adapted bacteria are associated with sinking particulates that have entered abyssal depths of the ocean.

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MATERIALS AND METHODS

Sample collection and preparation. A 0.25-m^2 boxcore was deployed at a depth of 4,470 m at station A (8°09′ N, 49°02′ W) and 4,850 m at station B (10°24′ N, 46°45′ W) in the Demerara abyssal plain. Immediately on retrieval, overlying seawater was drained carefully by siphon, and a subcore (6 by 20 cm) was removed to a refrigerated laboratory (3°C). There the cores were extruded onto sterile foil, cut in half lengthwise, and sampled internally with sterile tools. Samples from the surface (to 1 cm) and at depth intervals of 3, 5, 8, 11, and 15 cm were diluted 1:5 (wet wt/vol) with prefiltered (pore size, <0.2 μm), autoclaved artificial seawater (ASW). Portions of each suspension were filtered and dried to determine water content, while others were fixed in 2% formaldehyde for epifluorescence microscopy. Suspensions from 1-, 5-, and 15-cm depths were used to measure microbial substrate utilization, as described below.

The general design and deployment of sediment traps used in this study have been described in detail by Rowe and Gardner (15). Each trap was rinsed with 70% ethanol and prefiltered (pore size, <0.2 μm) distilled water before deployment. No preservatives were used in the traps, since we were interested in studying the viable microbial component of the intercepted particulates. A trapping period of 9 days was chosen as a compromise among the desire to limit the accumulation of organic matter (which, in the absence of preservative, might stimulate microbial activity), the need to collect an adequate sample size for all parties involved in the sediment trap work, and competing operations of the overall cruise schedule. That the particles were trapped successfully at the desired depth and exposed minimally to warmer surface waters during recovery was indicated by the proper mechanical functioning of the trap, the general absence of surface plankton in the entrapped sample (determined microscopically and by chlorophyll extraction), and a sample temperature measurement of <6°C.

Three sediment trap collections were available for microbiological study. The first was deployed at a depth of 4,270 m at 200 m off the bottom at station A (trap A-200); the second was deployed at a depth of 4,463 m at 7 m off the bottom at the same station (trap A-7); and a third was deployed at a depth of 4,830 m at 20 m off the bottom at station B (trap B-20). Traps A-200 and B-20 were cold on recovery, but the entrapped seawater in trap A-7 had warmed to the sea surface temperature of 24°C as a result of an unavoidable delay of 8 to 10 h in its recovery. Immediately on recovery each trap was secured in a refrigerated laboratory (3°C), where all remaining work was done. Portions (30 ml) of undiluted, well-mixed, particle-laden seawater from traps A-7 and B-20 were fixed in 2% formaldehyde for epifluorescence microscopy. Additional (unfixed) portions were used to measure microbial substrate utilization, as described below.

Particulates in trap A-200 were allowed to settle to the bottom of the trap before being transferred to a sterile petri dish and examined under a dissecting microscope. Eleven intact fecal pellets, similar in their cylindrical, tapered shape and dark-brown color and ranging in length from 100 to 1,400 μm, were located and removed with a sterile Pasteur pipette to 5-ml volumes of ASW, prechilled to 3°C. Individual pellets were washed twice by sequential transfer into separate tubes of fresh, cold ASW. Five intact pellets were fixed in 2% glutaraldehyde, filtered onto Nucleopore filters (diameter, 8 cm; pore size, 0.2 μm), rinsed, dehydrated in an ethanol series, and prepared for scanning electron microscopy by the methods of Paerl and Shimp (13). Four pellets were fixed in 2% formaldehyde for epifluorescence microscopy. One of these was completely disrupted before fixation by using a sterile, hand-operated homogenizer (Wheaton Industries, Millville, N.J.). The two remaining pellets, each measuring 500 by 1,400 μm, were used to measure microbial substrate utilization as described below.

Measurement of microbial substrate utilization. Portions (2 ml) of sediment suspensions from stations A and B and of particle-laden seawater samples from traps A-7 and B-20 were supplemented with [^{14}C]glutamic acid (1.97 μCi μg^{-1}; CEA, France) to a final concentration of 9.4 μg $liter^{-1}$ or 0.10 to 0.14 μg g^{-1} (dry weight) of sediment for core samples. Although natural levels of glutamic acid were not measured in these samples, the concentration of added radiolabeled substrate in the sediments was <10% of that which has been measured in sediment porewaters from similar depths (6; Rowe and Deming, in press).

Incorporation and respiration of [^{14}C]glutamic acid were measured at 12- to 36-h intervals over a 5-day incubation period at 3°C under both in situ pressure (440 or 480 atm [4.46×10^4 or 4.86×10^4 kPa]) and atmospheric pressure (1 atm [101.3 kPa]). Duplicate samples, housed in separate pressure vessels, were used for each time point. Other procedural details have been described previously (2, 16, 18). All measurements of radioactivity (in counts per minute) were corrected against those obtained from control samples and acidified with 0.3 ml of 4 N H_2SO_4 immediately after addition of label. Counting efficiency and quench corrections were determined by use of internal standards. Overall efficiencies, which were low owing to the quenching effects of the sediments and particulates, averaged 45% for core samples and 57% for trap samples.

Portions of the same sediment suspensions and trap samples were also supplemented with an enriching concentration of yeast extract (0.025%) and the antibiotic nalidixic acid (0.002%) by the method of Kogure et al. (12). Duplicate samples were incubated for 3 days at 3°C under in situ and atmospheric pressure before fixation for epifluorescence microscopy. Two fecal pellets, suspended separately in 5-ml volumes of ASW, were also supplemented with yeast extract and nalidixic acid. One was incubated at 3°C and 420 atm (4.25×10^4 kPa), while the other was held at 3°C and 1 atm (101.3 kPa). After 3 days, the entire contents of each tube were fixed for epifluorescence microscopy.

Epifluoresence microscopy. Fixed samples were stored at 3°C aboard ship and during transport to the university laboratory. Each sediment sample required further dilution in ASW ($>10^{-3}$) to minimize particle interference. Approximately 200 bacteria were counted for each sample preparation by the staining and filtration procedures of Hobbie et al. (7). Intact fecal pellets were stained in 0.01% acridine orange for 2 min and transferred with a Pasteur pipette to the surface of a prestained Nuclepore filter (7) for qualitative microscopic examination.

The number of abnormally enlarged, orange-fluorescing bacteria and the total number of bacteria were determined for each sample treated with yeast extract and nalidixic acid. Abnormal enlargement and orange fluorescence were interpreted as evidence for intense metabolic activity (response to enrichment with yeast extract) in the absence of normal cell division (sensitivity to nalidixic acid), as discussed by Kogure et al. (12). Such bacteria were not observed in untreated samples. Total bacterial counts in treated samples were not significantly different from those in duplicate, untreated samples fixed at time zero, indicating that antibi-

otic-resistant division had not occurred during the 3-day incubation period.

RESULTS

Boxcore sediments. Results of incubating sediment suspensions with [^{14}C]glutamic acid at in situ and atmospheric pressures are shown in Fig. 1. In each case, substrate incorporation and respiration were favored by in situ pressure. Respiration accounted for 89 to 94% of total utilization at both pressures tested. No abnormally enlarged, orange-fluorescing bacteria were observed in the sediment samples treated with yeast extract and nalidixic acid, although the counting procedure for sediments would have allowed detection of such bacteria only if they represented ≥0.5% of the total bacterial count.

Depth profiles of bacterial counts in the two sediment cores are presented in Table 1. In general, counts decreased with depth (as did total utilization of [^{14}C]glutamic acid), ranging from a near-surface maximum of 8.29×10^8 bacteria g^{-1} (dry weight) of sediment to a minimum of 1.66×10^7 bacteria g^{-1} at the deepest point examined (15 cm). These counts and profiles do not differ significantly from those obtained more recently by Harvey et al. (5) for sediments at similar depths in the nearby Venezuela Basin.

Trapped particulates. Results of incubating trap samples with [^{14}C]glutamic acid are shown in Fig. 2. Substrate utilization in the cold sample from trap B-20 was barophilic, while activity in the temperature-compromised sample from trap A-7 was barosensitive. In both samples, respiration accounted for 84 to 89% of total substrate utilization (slightly less than in the sediment samples) at both pressures tested. Abnormally enlarged, orange-fluorescing bacteria accounted for 4.0% of the total population in the cold sample from trap B-20, compared with 0.1% in the temperature-compromised

TABLE 1. Depth profiles of bacterial counts[a] in two abyssal sediment cores

Sample	Depth in core (cm)	Bacteria (10^8) (mean ± SD) per g (dry wt) of sediment
Boxcore A (4,470 m)	1	4.65 ± 1.53
	3	8.29 ± 2.20
	5	4.58 ± 1.30
	8	1.05 ± 0.56
	11	0.15 ± 0.11
	15	0.17 ± 0.13
Boxcore B (4,850 m)	1	3.08 ± 0.98
	3	3.10 ± 0.87
	5	2.39 ± 0.61
	8	0.91 ± 0.42
	11	0.74 ± 0.34
	15	0.22 ± 0.19

[a] Determined by acridine orange staining and epifluorescence microscopy.

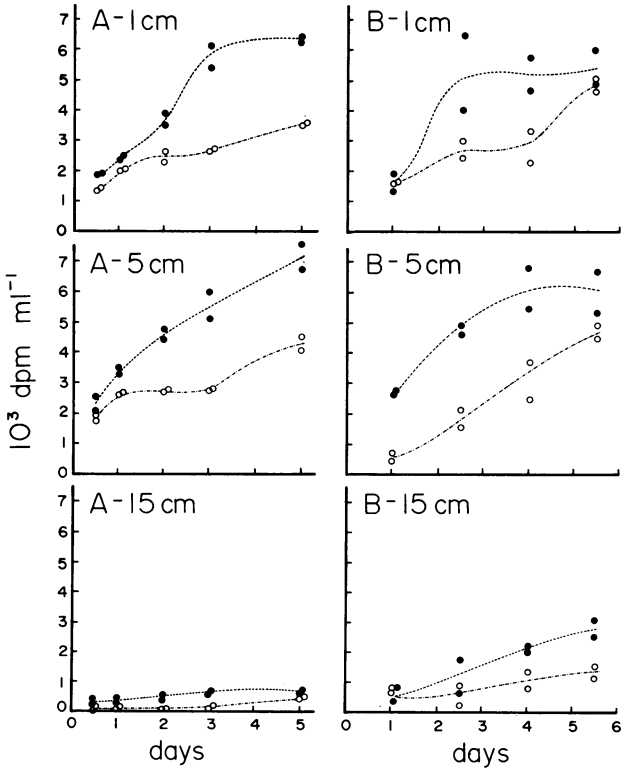

FIG. 1. Total microbial utilization (incorporation plus respiration) of [^{14}C]glutamic acid at 3°C and in situ (●) or atmospheric (○) pressure in sediment suspensions prepared from depths of 1, 5, and 15 cm in boxcores from stations A (depth, 4,470 m) and B (depth, 4,850 m). Respiration accounted for 89 to 94% of total substrate utilization at both pressures.

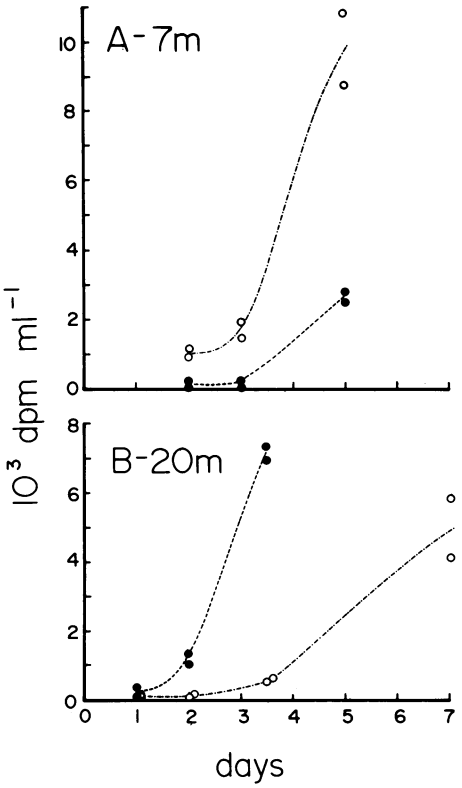

FIG. 2. Total microbial utilization (incorporation plus respiration) of [^{14}C]glutamic acid at 3°C and in situ (●) or atmospheric (○) pressure in seawater suspensions of particulates from temperature-compromised sediment trap sample A-7 (depth, 4,463 m) and cold trap sample B-20 (depth, 4,830 m). Respiration accounted for 84 to 89% of total substrate utilization at both pressures.

sample from trap A-7, after incubation with yeast extract and nalidixic acid under in situ temperature and pressure.

The total bacterial count in trap B-20 was 1.52×10^7 bacteria liter^{-1}, with an average length of <0.5 μm, results consistent with other reports of bacterial number and size in deep-sea water samples (1, 19). In contrast, the temperature-compromised sample from trap A-7 contained 6.38×10^8 bacteria liter^{-1} (42 times the concentration in trap B-20), most of which were significantly larger (1 to 2 μm in length) than those in trap B-20. Less than 20% of the total population appeared to be attached to particulate matter in either trap sample.

Fecal pellets. Scanning electron microscopy of intact and partially disrupted fecal pellets from trap A-200 revealed a variety of organic and inorganic materials, including micrometer-sized cocci, each encased by a peritrophic membrane. No bacteria were observed on the outer surfaces of the pellets.

Examination of additional pellets by epifluorescence microscopy revealed an abundance of small, green-fluorescing bacteria, generally coccoid in shape, with a length of <0.5 μm. Some thin, rod-shaped bacteria, measuring 1.0 to 1.5 μm in length, were also observed. Because the protective membrane covering a fecal pellet is extremely thin and is usually detected only by scanning electron microscopy (J. Turner, personal communication), the extent of external colonization of these particular pellets could not be evaluated. Disrupting the stained pellets by applying pressure to the cover slip revealed numerous bacteria located internally. About 10^4 bacteria were attributed to a single pellet, 200 μm in length, that had been rinsed and homogenized before fixation.

A marked abundance of abnormally enlarged, orange-fluorescing bacteria was observed on a fecal pellet that had been rinsed and suspended in ASW, treated with yeast extract and nalidixic acid, and incubated at 3°C and 420 atm (4.25×10^4 kPa). Of the total number of bacteria observed in the ASW surrounding the pellet, 69% were abnormally enlarged and orange fluorescing. Similar bacteria were not detected on the second pellet, held at atmospheric pressure, but 73% of the bacteria in the surrounding ASW were abnormally enlarged and orange fluorescing. Control samples of ASW, yeast extract, and nalidixic acid incubated under similar conditions were free of bacteria.

DISCUSSION

There are few reports in the literature on the use of dissolved ^{14}C-labeled organic substrates to measure microbial activity in abyssal (depth, >4,000 m) sediment samples at in situ temperature and pressure (8, 9, 16, 18). All have involved additions of substrate at final concentrations of 50 g liter^{-1} or higher. In studies in which deep-sea sediments were examined at both in situ and atmospheric pressures (9, 16, 18), deep-sea pressures were found to inhibit substrate utilization. The adverse effects of pressure on substrate utilization by bacterial isolates from relatively shallow oceanic depths (450 to 2,600 m) are well documented: even a moderate pressure of 200 atm (2.03×10^4 kPa) has been shown to inhibit glutamic acid utilization by as much as 91% (20). Therefore, it was logical to attribute the detection of adverse effects of elevated pressure on similar activity in abyssal sediments to the presence of shallow-water bacteria in sufficient numbers to mask the activities of any indigenous barophiles that might also have been present (8–10).

In marked contrast to these earlier reports, microbial utilization of [^{14}C]glutamic acid in all six of the sediment samples examined in this study was enhanced, rather than inhibited, by in situ pressures. We attribute the consistent detection of these barophilic responses, when barosensitivity has been the rule in the past, to the use of shorter incubation periods and lower (<10 μg liter^{-1}), nonenriching concentrations of added substrate. We interpret these results as evidence that indigenous, barophilic bacteria, rather than shallow-water barosensitive intruders, are functionally predominant in the turnover of naturally low levels of glutamic acid in the abyssal sediment samples we examined. In keeping with this interpretation is the finding that both substrate utilization under in situ pressure (Fig. 1) and the total bacterial count (Table 1) decrease with increasing depth in each core. It also seems clear that most of the bacteria functioning metabolically under elevated pressure in these sediments (whether barophilic or not) were growing slowly, if at all, since (i) substrate incorporation leading to significant bacterial production was minimal (6 to 11% in the sediment samples), even after a 5-day incubation period; and (ii) significant numbers of bacteria responsive to enrichment with yeast extract were not detected in the sediment samples.

Since this study was completed, identical experiments involving the use of equally low or lower concentrations of [^{14}C]glutamic acid have been conducted on sediments from depths of 4,300 and 4,800 m in the Bay of Biscay (Rowe and Deming, in press) and 5,400 m in the Hatteras abyssal plain (J. Deming, unpublished data). In every case, optimal substrate utilization was observed under deep-sea rather than atmospheric pressure, and pure cultures of barophilic bacteria, requiring elevated pressures for the complexity of reactions leading to optimal growth, were isolated readily and at random from each sample (Deming, in press). Thus, the barophilic microbial activity reported in this paper is not a geographic phenomenon unique to sediments of the Demerara abyssal plain. Whether it is a physiological phenomenon unique to the utilization of a single amino acid cannot be assessed definitively until results of additional studies with different substrates or assays for growth of bacterial populations in (rather than pure cultures from) abyssal sediments, are available. However, at present we believe it is important at least to consider the possibility that other microbial processes in abyssal sediments may occur optimally under pressure. If so, then ongoing attempts to measure rates of nitrification (17) and oxygen consumption (14) in abyssal sediments that are decompressed and monitored at atmospheric pressure may be providing underestimates of in situ activity, rather than overestimates, as it has always seemed necessary to assume in the past (17).

Barophilic microbial utilization of [^{14}C]glutamic acid was detected not only in each of the sediment samples examined in this study, but also in a sediment trap sample of sinking particulates that was retrieved while still cold, immediately after the trap had surfaced. Furthermore, and in contrast to results from the sediment core samples, significant percentages of the bacteria in this trap sample and in a fecal pellet sample from another cold trap (4 and 69%, respectively) were observed to be intensely active metabolically under deep-sea conditions, in response to enrichment with yeast extract. The results of both of these experimental approaches to the measurement of microbial activity in sediment trap samples provide support for the hypothesis that sinking particulates reaching abyssal depths are reworked by indigenous, barophilic bacteria. The derivation of some of these barophiles from the digestive tracts of abyssal inver-

tebrates can be inferred from the internal location of bacteria in the fecal pellets we examined.

Neither barophilic utilization of [^{14}C]glutamic acid nor a significant percentage of bacteria responsive to yeast extract under deep-sea conditions was detected in a second trap sample that had been warmed to 24°C at the sea surface during delayed recovery of the trap. The increased size and number of bacteria present in this sample on recovery relative to those observed in the cold-trap sample and in other samples of abyssal seawater (1, 19) suggest that bacterial growth may have occurred in the trap before its recovery. Whether such growth occurred in the trap during deployment, while floating in surface waters, or at all is not clear. However, an 8- to 10-h period of exposure at 24°C would have been sufficient to inactivate deep-sea barophilic bacteria (21) and to stimulate mesophilic, surface water bacteria that may have been present in the trap. Therefore, we must conclude that on recovery, the contents of trap A-7 no longer represented a valid sampling of the bacterial populations originally associated with the particulates and seawater that had been collected at depth and that rapid handling of abyssal samples while still cold is critical to studies of indigenous deep-sea bacteria, as predicted by Yayanos and Dietz (21).

In conclusion, experimental evidence obtained in this study indicated that the recycling of naturally low levels of glutamic acid in cold abyssal sediments is mediated primarily by indigenous, barophilic bacteria rather than by microorganisms that originated in shallow surface waters. The potential for intense microbial activity at abyssal depths in response to nutrient enrichment (0.025% yeast extract in this study) appeared to occur more frequently in association with recently settled particulates, especially fecal pellets, than in older accumulations of sediment.

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