INTRODUCTION

Recent Ocean Drilling Program (ODP) research on marine sediments (Whelan et al., 1986; Tarafa et al., 1987; Parkes et al., 1990, 1994; Cragg, 1994; Cragg and Parkes, 1994; Cragg and Kemp, 1995; Cragg et al., 1990, 1992, 1995a, 1995b) has confirmed the presence of a deep bacterial biosphere in marine sediments. Previously, this had only been predicted from the extensive amount of indirect geochemical evidence, that is, chemical changes in pore water, gas production, kerogen modification, concretion formation, and isotopic evidence (Krumbein, 1983; Suess and Whiticar, 1989; Kvenvolden and Kastner, 1990; Kastner et al., 1991). The thermal gradient of the Earth’s crust is $\sim 10^3 - 40^\circ$C/km, therefore, in most sediments temperature is unlikely to inhibit bacterial activity until several kilometers below the seafloor as hyperthermophilic bacteria may grow in excess of $110^\circ$C (Huber et al., 1989; Pledger and Baross, 1991; Jørgensen et al., 1992). Deep sediment bacterial populations decrease exponentially with depth (Parkes et al., 1994) and have been shown to be responsive to deep geochemical changes in the sediment, such as brine incursions (Cragg et al., 1990; Parkes et al., 1990) and the presence of methane and hydrogen sulfide gas hydrates (Cragg et al., 1995b; 1996). Additionally, bacterial sulfate reduction and methanogenic activity has been measured in sediments from depths greater than 500 mbsf (Cragg et al., 1992; Getliff et al., 1992; Parkes et al., 1995).

Submarine fans form the largest deep-water sediment bodies on continental margins (Flood, Piper, Klaus, et al., 1995), and the Amazon Fan is one of the largest modern submarine fans containing much of the material eroded from the Amazon drainage basin in the form of muds and silts. The fan has been active during glacial periods with the production of a number of channel and levee systems with sediment deposition rates of $1-25$ m/k.y., but during interglacials sediment has accumulated much more slowly at $<0.1$ m/k.y. These rates compare to $-4$ m/k.y. in the Santa Barbara Basin (Schimmelmann et al., 1990), which is regarded as a particularly high sedimentation rate.

The depositional history of the Amazon Fan is further complicated by the occurrence of very large numbers of turbidites, debris flows, and levee slumps (Damuth and Flood, 1984, 1985). On many occasions, organic-carbon-rich surface sediments have been buried by subsurface sediment particles of relatively low organic carbon concentration, but with pore spaces replenished with sulfate. To our knowledge, this is the first investigation of deep bacterial populations in a submarine fan, a system that is dominated by sediment flow rather than depositional processes.

MATERIALS AND METHODS

Site Description

Sites 934 and 940 are located on the Amazon Fan off northeast Brazil (Fig. 1). Site 934 (5°29.047′N, 47°40.857′W) is located in a cutoff meander bend of the main Amazon Channel of the Amazon Fan (Flood, Piper, Klaus, et al., 1995) in a water depth of 3432 m. Substantial sections recovered at this site are interpreted as overbank spillover from turbidity currents, muddy slump deposits, and sandy debris flows. The current seafloor of the meander is 55 m higher than the main seafloor, resulting from sedimentation in the abandoned meander bend and possibly from incision from the main channel after cutoff. The geothermal gradient at this site was estimated at 35°C/km. Site 940 (5°8.569′N, 47°31.728′W) is located on the flank of the eastern levee of the Amazon Channel in the uppermost middle fan at a present-day water depth of 3195 m (Flood, Piper, Klaus, et al., 1995). This site is located close to two avulsion points that may correlate with rapid growth phases of the levee. A number of slump deposits were recovered at this site. The geothermal gradient here was estimated at 29°C/km. Sedimentation rates across the Amazon Fan...
of 2% filter-sterilized (0.1 µm) formaldehyde in artificial seawater. Acridine orange (50 µL) was added to give a final concentration of 5 mg/dm³. After 3 min the solution was filtered through a 25-µm Nucleopore black polycarbonate membrane (Costair, High Wycombe, United Kingdom) of 0.2-µm pore size. The filter was rinsed with a further 10 mL of 2% filter-sterilized formaldehyde in artificial seawater and mounted in a minimum of paraffin oil under a coverslip. Three replicate filters were prepared from each sample to minimize the variance of the counts (Kirchman et al., 1982). Where 95% confidence limits of the mean count exceeded 0.5 log₁₀ units, further replicate filters were prepared. A minimum of 200 fields of view were counted.

The mounted membrane filters were viewed under incident illumination with a Zeiss Axioskop microscope fitted with a 50-W mercury vapor lamp, a wide-band interference filter set for blue excitation, a 100× (numerical aperture = 1.3) Plan Neofluar objective lens, and 10× eyepieces. Bacteriolytically shaped green and red fluorescing objects were counted. Cells on or off particles were counted separately, and the numbers of those on particles doubled in the final calculations to account for cells hidden from view by particles (Gould, 1977). Dividing cells (those with a clear invagination) and divided cells (pairs of cells of identical morphology) were also counted. The detection limit for bacterial cells was calculated to be 1 × 10³ cells/cm³ (Cragg, 1994).

RESULTS AND DISCUSSION

Site 934

Total bacterial populations are high in the near-surface sediment at 6.05 × 10⁶ cells/cm³ (Fig. 2), and decrease rapidly to 3.46 × 10⁶ cells/cm³ by 108 mbsf (a 175× decrease), with a minimum of 2.1 × 10⁵ cells/cm³ at 99 mbsf. The bacterial population profile for much of the hole generally parallels the general decrease with depth observed for Pacific Ocean sediments (Fig. 2; Parkes et al., 1994). However, between 3 and 69 mbsf, bacterial numbers are consistently and significantly (P < 0.05) greater than expected. Between 10.3 and 69 mbsf, the total bacterial population size is surprisingly constant at 1.95 ± 0.18 × 10⁷ cells/cm³ (mean and 95% confidence limits), apart from a highly significant (P << 0.001) increase (×3.7) in numbers between 16.8 and 26.3 mbsf. This 10.5-m section coincides very closely with a layer rich in plant debris (between 22 and 29 mbsf) and wood fragments (between 14 and 29 mbsf; Fig. 3). There is a second layer of sediment rich in wood fragments between 62 and 68 mbsf. No samples were taken for bacterial counts in this section, however, and thus no bacterial response was observed. Overall, there is no correlation between bacterial numbers and total organic carbon (TOC), although this may reflect the paucity of TOC data in the upper organic-rich layer, and the complete absence of microbiological data in the deeper organic–carbon-rich layer (Fig. 3). Previous work has demonstrated a significant correlation between total bacterial numbers and sediment organic carbon concentrations (Parkes et al., 1993; Cragg and Kemp, 1995; Cragg et al., 1995a). The total bacterial numbers strongly correlate (R = 0.965; N = 16; P << 0.002) with dividing and divided cell numbers (DDC) that represent ~7% of the total population (Fig. 2). This relationship has been commonly observed in Pacific Ocean sites (Parkes et al., 1990; Cragg, 1994; Cragg et al., 1992, 1995a, 1995b; Cragg and Parkes, 1994; Cragg and Kemp, 1995). Between 70 mbsf and the last sample at 108 mbsf, DDC were detected only once at 89 mbsf. Over this same depth range, the total bacterial numbers significantly decreased by a factor of 9 (t = 5.85; d.f. = 6; P < 0.002) when compared to the four data points immediately higher up in the sediment column (Fig. 2). Nevertheless, our complete inability to detect DDC in three out of the four sub-68 mbsf samples was unexpected, although this may be the result of the particularly low organic carbon concentrations over this depth range (mean = 0.36%, assuming that the high value at

Shipboard Handling

A series of 19 × 1-cm³ sediment samples were removed from core sections of Hole 934A, between 0 and 108.3 mbsf, and 32 × 1-cm³ sediment samples at Hole 940A were taken between 0 and 243.5 mbsf. Immediately after a core was cut into 1.5-m sections on the outside catwalk, a thin layer of sediment was removed from the section end using a sterile scalpel to expose an uncontaminated surface. A 1-cm³ sample was then taken with a sterile (autoclaved) 5-mL syringe from which the luer end had been removed. The sample was ejected directly into a tared serum vial containing 9 mL of filter sterilized (0.2 µm) 4% formaldehyde in artificial seawater.

Laboratory Handling

Direct Microscopic Observations

Acridine orange staining and microscopic observations were based on the general recommendations of Fry (1988). Fixed samples were vortex mixed, and a 4- to 10-µL subsample was added to 10 mL of 2% filter-sterilized (0.1 µm) formaldehyde in artificial seawater.

Figure 1. Map of the Amazon Fan showing location of ODP sites in relation to surficial channel systems and debris flows. From Flood et al., (1995); modified from Damuth et al. (1988) and Manley and Flood (1988).

vary from ~1–20 m/k.y. during low sea-level stands to 1 cm/k.y. during periods of high sea level when more riverine-derived sediment is deposited on the continental shelf (Damuth and Kumar, 1975).
108.45 mbsf obtained slightly below the last bacterial count is anomalous). Similar inabilities to detect DDC have been encountered at other sites very low in organic carbon (Cragg, 1994; Cragg and Kemp, 1995). In contrast, above 68 mbsf, where organic carbon concentrations were higher (mean = 0.93%), DDC were observed in all samples.

The interstitial water chemistry was relatively simple at this site. Sulfate concentrations of 23.35 mM at 1.45 mbsf decrease to zero by 7.95 mbsf and remain at less than 0.04 mM (Flood, Piper, Klaus, et al., 1995). Most sulfate reduction should therefore occur in the 0–7.95 mbsf depth range, and this is supported by alkalinity data (Flood, Piper, Klaus, et al., 1995). This depth range also coincides with a higher than expected total bacterial population (Fig. 2).

Methane concentrations were relatively constant with depth (mean = 9460 ppmv between 21.3 and 103.8 mbsf), with a near absence at 1.5 mbsf and a large peak occurring at 11.8 mbsf (55,400 ppmv; Flood, Piper, Klaus, et al., 1995). This may reflect the usual situation of a peak of methane due to local methanogenesis immediately below the zone of sulfate reduction, which ends at ~8 mbsf, or alternatively, this may be a result of the migration of bacterially produced methane from lower sediment layers.

The unexpected general constancy of total bacterial numbers between 10.3 and 69 mbsf is difficult to fully understand, as it implies a degree of sediment homogeneity that does not initially seem to be supported by lithostratigraphic analysis. Any attempt at interpreting this must be speculative as this data is derived from only 12 \( \times 1 \) cm\(^3\) samples taken from a 60-m-deep sediment column; nevertheless, some pertinent observations can be identified. For example, between 30 and 65 mbsf vivianite (hydrated iron phosphate) nodules were recorded as common. This is coincident with an uninterrupted section of uniform bacterial numbers (Fig. 2) and suggests some sediment homogeneity, although this zone covers parts of two different lithostratigraphic units.

Unit III (Fig. 3) consists of a muddy mass flow that has subsequently undergone deformation, plastic folding, injection of sand and mud, and partial mixing (Flood, Piper, Klaus, et al., 1995). Interestingly, there is some uncertainty concerning the exact depth of the base of Unit III (65–70 mbsf). However, if the bacterial numbers can be taken to indicate which unit a particular sediment layer can be at-
tributed to, then the Unit III/IV transition may occur below the last of the “uniform” counts at 68.95 mbsf. Unit II is interpreted as sediment that filled the abandoned meander bend by spillover from turbidity currents flowing down the main Amazon Channel after the meander was cut off (Flood, Piper, Klaus, et al., 1995). Much of Unit II thus might be expected to be bacteriologically relatively homogeneous. This is the case for Subunits IIC and IIH, although superimposed on this is the local increase in populations due to plant debris. Subunit II, however, is not homogeneous as bacterial numbers decrease logarithmically with increasing depth, a trend consistent with near-surface counts in Unit I (Fig. 3). Although Unit IIA does have some inferred mud turbidites, there is a fining-upward trend with hemipelagic laminae above the turbidites, and as this subunit and Unit I are the only units to contain elevated foraminifer concentrations (Flood, Piper, Klaus, et al., 1995), this suggests that a significant component of the sediment in Subunit IIA must be depositional rather than the result of mass flow. This is consistent with the similar trends in bacterial populations within these units and with active sulfate reduction.

Site 940

The total bacterial population is high in the near-surface sediment at 5.62 \( \times 10^5 \) cells/cm\(^3\) (Fig. 4) and is not significantly different (\( t = 0.27; \) d.f. = 4) from that at Site 934 (Fig. 3). Bacterial numbers decrease rapidly to a minimum of 2.69 \( \times 10^5 \) cells/cm\(^3\) at 81.8 mbsf (a 210x decrease). Between ~67 and 214 mbsf, the bacterial profile remains roughly constant at 5 \( \times 10^5 \) cells/cm\(^3\). Below 214 mbsf, however, there is a sustained and significant (\( P < 0.02 \)) increase in total bacterial numbers to a maximum of 2.72 \( \times 10^6 \) cells/cm\(^3\) (a 5x increase). The bacterial population profile for much of the hole generally parallels the decrease with depth observed for Pacific Ocean sediments (Fig. 4; Parkes et al., 1994). Two exceptions to this are between 5.8 and 6.7 mbsf where, with a single exception, are significantly (\( P < 0.05 \)) above the regression line, and between 221 and 243.5 mbsf when, again, all of the data are significantly (\( P < 0.01 \)) above the regression line (Fig. 4). The former group of data approximately coincides with Subunits IIA–IID, with reversion of the bacterial population sizes to predicted levels occurring across Subunit IIE and the uppermost part of Subunit IIIF. The latter group coincides with Subunits III–IIJ (Flood, Piper, Klaus, et al., 1995). Subunits IIA–IID (roughly the Amazon levee system) are characterized by cycles of abundant turbidites (30–60/m) together with moderate bioturbation and color banding. This high frequency of small turbidites combined with bioturbation may well account for the homogeneity of the bacterial counts, particularly the DDC, over this part of the sediment column. The increase in the bacterial population toward the base of this hole will be discussed later.

The total bacterial numbers again strongly correlate (\( R = 0.929; \) N = 32; \( P < 0.002 \)) with DDC numbers that represent ~8.5% of the total population (Fig. 4). After an initially rapid decline in DDC to 11.8 mbsf, the population remains surprisingly constant at ~3.85 \( \times 10^5 \) cells/cm\(^3\) to 54 mbsf, coincident with Subunits IIB–IIID. Thereafter, there is a rapid decline in the DDC, paralleling that in the total count (Fig. 4), to 5.62 \( \times 10^3 \) cells/cm\(^3\) (a 25x decrease) at 81.8 mbsf. Between 81.8 and 214.5 mbsf, bacterial numbers vary considerably around an average of 3.5 \( \times 10^3 \) cells/cm\(^3\), which includes the only occurrence of a “not detectable” DDC result at 182.6 mbsf. Below 214.5 mbsf, which is the start of Subunit III, DDC show a persistent and significant (\( P < 0.02 \)) increase to a peak at 2.22 \( \times 10^4 \) cells/cm\(^3\) at 243.5 mbsf. Overall, for both the total count and DDC data in the lower half of the hole, there is a significant (\( P < 0.01 \) and \( P < 0.002 \), respectively) trend of increase occurring from ~180 mbsf (Fig. 4).

Explaining the changes in the bacterial profile from the geochemical data (Flood, Piper, Klaus, et al., 1995) is particularly difficult for this hole. Organic carbon concentrations are relatively low, and more or less constant throughout, at ~0.81 ± 0.07 wt% (mean ± 95% confidence limits). Interpretation of the inorganic chemistry is complicated by the lack of data between 32.55 and 64.2 mbsf. No data therefore exist for Subunits IID or IIE, and the Subunit IID/IIE boundary at 55.7 mbsf appears to be bacteriologically important (Fig. 4). Consistent with the low organic carbon concentrations at this site (Cragg, 1994; Cragg and Kemp, 1995), pore-water sulfate concentrations do not reach zero until 29.25 mbsf, the greatest depth of sulfate penetration encountered on this leg (Flood, Piper, Klaus, et al., 1995). This depth coincided with the maximum methane concentration of nearly 37,000 ppm at 29.3 mbsf, and suggests that the situation of bacterially mediated sulfate depletion, followed by an increase in methane concentration usually encountered in the top few meters of marine sediments (Jørgensen et al., 1990), may be occurring to much greater depths because of comparatively low rates of sulfate reduction. Bacterial activity, however, does continue below the sulfate/methane transition albeit at reduced levels as indicated by the high, but decreasing, concentrations of alkalinity and phosphate (21.62 mM and 71.8µM at 32.55 mbsf and 6.11 mM and 2.3 µM at 64.2 mbsf, respectively) (Flood, Piper, Klaus, et al., 1995). The lack of any data between these two depths, however, prevents any attempt at correlating the changes in the bacterial profile at ~50–60 mbsf (Fig. 4) with geochemistry.

The significant increase in both total bacterial numbers and DDC below 200 mbsf is surprising. It has been suggested that the high methane concentrations measured over this depth range (an increase from a mean of 9000 to 13,650 ppm), together with relatively high...
sulfate concentrations (1.2–5.3 mM), probably indicate that the sulfate was a sampling artifact (Flood, Piper, Klaus, et al., 1995). It is certainly the case that some cores were subject to considerable drilling disturbance, significantly from 190.8 mbsf and particularly from 236.1 mbsf. Drilling disturbance, however, is recorded for all cores below 103.9 mbsf. Color banding (black N/2), indicative of products of bacterial sulfate reduction, occurs commonly between 181.1 and 219.7 mbsf, and this had been absent since 102 mbsf. This suggests that the measured levels of sulfate could have been real. Additionally, it is difficult to account for such consistent increases in total bacterial and DDC numbers to levels not encountered since 50 mbsf by contamination (Parkes et al., 1990). Similar increases in bacterial numbers at great sediment depth that are associated with increases in sulfate or methane concentrations have been observed at other ODP sites (Cragg et al., 1990, 1995b; Parkes et al., 1990).

**General**

These data represent the first deep sediment samples taken for bacterial analysis from the Atlantic Ocean. Previous work from six ODP legs in the Pacific Ocean has yielded an exponential relationship of bacterial populations with sediment depth (Parkes et al., 1994), and bacterial profiles in Amazon Fan sediments are consistent with this data (Fig. 5). This supports the contention that such bacterial depth relationships are ubiquitous despite widely differing oceanic settings (Parkes et al., 1994).

The topmost parts of Holes 934A and 940A consist of intensely bioturbated foraminifer-nanofossil clay of Holocene age. These layers are small, 0.87 m and 0.24 m in Holes 934A and 940A, respectively, and represent a period of time when the fan has not been active, thus preventing significant terrigenous input to the sediments. In these conditions the near-surface bacterial populations can be directly compared with those from other sites (Table 1). Data from the Amazon Fan occupy a position commensurate with the depth of the overlying water, being greater than near-surface bacterial populations from deeper and less productive sites, not significantly different than those from similar water depths and productivities, and markedly lower than those from much shallower and more productive sites. Correlation analysis produced a highly significant relationship between overlying water depth and sediment near-surface bacterial population size ($R = 0.841; N = 9; P < 0.01$).

**SUMMARY**

This data set represents the first bacterial analysis of deep sediments from the Atlantic Ocean and from a river fan-system. Bacterial populations decline with sediment depth at a rate similar to that previously observed in Pacific Ocean sediments. However, in these sediments, it is apparent that changes in sedimentary input strongly influence both the bacterial profile and sediment geochemistry. Areas of rapid, small turbidites coupled with bioturbation, and areas of massive mud flows or slumps were generally associated with unusually constant depth profiles. Conversely, where such activity was low and sedimentary input was more depositional, the expected gradual decreases in bacterial populations with depth were observed. Overall organic carbon concentrations were low, but where significant amounts of plant material were buried this coincided with highly significant increases in bacterial populations. A strong trend of population increase in both total count and DDC toward the base of Hole 940A was difficult to explain. It was associated with rises in sulfate and methane concentrations, situations previously associated with elevated bacterial populations at depth (Parkes et al., 1990, 1994). Shipboard geochemists, however, consider these to be due to drilling contamination. Nevertheless, the unexpected presence of black color banding, together with the resilience of the direct count technique to contamination, suggests that the bacterial population increase is indeed real and that some sulfate reduction may have occurred at these depths.
Table 1. Comparison of Amazon Fan total bacterial populations in near-surface sediments with those from ODP sites in the Pacific Ocean.

<table>
<thead>
<tr>
<th>Source</th>
<th>Leg</th>
<th>Water depth (m)</th>
<th>Direct count (cells/cm³)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peru Margin</td>
<td>112</td>
<td>150</td>
<td>3.30 × 10^8</td>
</tr>
<tr>
<td>Santa Barbara Basin</td>
<td>146</td>
<td>576</td>
<td>1.27 × 10^8</td>
</tr>
<tr>
<td>Japan Sea</td>
<td>128</td>
<td>900</td>
<td>7.82 × 10^6</td>
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<td>Cascadia Margin (Oregon)</td>
<td>146</td>
<td>1326</td>
<td>6.95 × 10^5</td>
</tr>
<tr>
<td>Cascadia Margin (Vancouver)</td>
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<td>2516</td>
<td>5.32 × 10^4</td>
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<tr>
<td>Lau Basin</td>
<td>135</td>
<td>2692</td>
<td>6.12 × 10^4</td>
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<tr>
<td>Amazon Fan (Site 940)</td>
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<td>3195</td>
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<tr>
<td>Amazon Fan (Site 934)</td>
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<td>3432</td>
<td>6.04 × 10^4</td>
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<tr>
<td>Eastern Equatorial Pacific</td>
<td>138</td>
<td>3761</td>
<td>2.08 × 10^4</td>
</tr>
</tbody>
</table>

Notes: * = calculated bacterial count over top 5 cm of sediment. Depth data from Suess, von Huene, et al. (1990; ODP Leg 128, Site 798); Parson, Hawkins, Allan, et al. (1992; ODP Leg 135, Site 834); Mayer, Piasis, Janecek, et al. (1992; ODP Leg 138, Site 851), Westbrook, Carson, Mungrave, et al. (1994; ODP Leg 146(1), Sites 888/890); and Kennett, Baldauf, et al. (1994 ODP Leg 146(2), Site 893 (Santa Barbara)). Bacterial data from Parkes et al. (1993; Charles Darwin Cruise Leg 38); Cragg et al. (1992; ODP Leg 128); Cragg (1994; ODP Leg 135); Cragg and Kemp (1995; ODP Leg 138); and Cragg et al. (1995a, 1995b; ODP Leg 146 Pts. 1 and 2).

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