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3. Bacterial Populations in Deepwater Low-Sedimentation-Rate Marine Sediments and Evidence for Subsurface Bacterial Manganese Reduction (ODP Site 1149, Izu-Bonin Trench)[1]

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ABSTRACT

Bacterial distributions were determined at Site 1149 in the Izu-Bonin Trench, which is a deepwater (5818 m) low-sedimentation-rate area. This was the first Ocean Drilling Program (ODP) leg where contamination checks were conducted for microbiology. These demonstrated that the inner portion of cores, where the microbiological samples were taken, were free from any potential sampling contamination. Bacterial populations were present in all samples (deepest at 171.2 meters below seafloor [mbsf]). The highest numbers were near the surface (1.4 mbsf; 7.2×10^6 cells/cm^3), but these declined rapidly within the upper 10 mbsf. Below this, numbers decreased at a more gradual rate to 7.2×10^5 cells/cm^3 at 172 mbsf, a 10-fold reduction. This two-stage bacterial depth distribution has been observed at several other ODP sites (e.g., Amazon Fan and Santa Barbara Basin). Bacterial depth distributions at this site were well below those predicted by the general equation for global deep-sediment bacteria and predominantly below the lower 95% prediction limits. These low bacterial populations were thought to reflect the low sedimentation rates and low input of bioavailable organic matter that is characteristic for deepwater sites. Consistent with this trend is that there was only limited removal of pore water sulfate and, thus, bacterial sulfate reduction. Most of this removal was in the top ~5

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mbsf, coinciding with the highest bacterial populations, the presence of small amounts of methane, and an increase in pore water manganese and ammonia. In the deeper sediments, however, there was still indirect evidence of continuing low bacterial activity, with increases in pore water ammonia, soluble manganese, bioavailable acetate, and decreasing sulfate. Interestingly, manganese reduction, sulfate reduction, and a limited amount of methanogenesis seemed to be occurring simultaneously at depth in this low–organic matter site, and the coincident detection of bacterial populations provides further support for the universal presence of a deep biosphere in marine sediments.

INTRODUCTION

The presence of a deep bacterial biosphere in marine sediments has been established by extensive research on sediments primarily obtained from the Ocean Drilling Program (ODP) (Parkes et al., 2000). In addition, bacteria have been shown to be present in basaltic basement rocks (Furnes et al., 1996; Giovannoni et al., 1996; McKinley and Stevens, 2000) and Cretaceous shales and sandstones (Krumholz et al., 1997). Bacteria appear to be ubiquitous in marine sediments, and depth profiles of bacteria are remarkably consistent across different oceans. Population sizes range from ~109 cells/cm^3 at the near surface, decreasing exponentially to ~106 cells/cm^3 at the mean ocean sediment depth of 500 meters below seafloor (mbsf). Near the sediment surface, bacteria act as a filter on the burial of sedimented organic material and in the subsurface they continue degradation, recycling, and selective preservation of organic matter, albeit at much lower rates. These subseafloor bacterial populations have been shown to be stimulated by variations in their in situ environment (i.e., influxes of electron acceptors—a brine intrusion replenishing sulfate at the Peru margin [Cragg et al., 1992b]; the presence of high concentrations of organic carbon—sapropels containing up to 30 wt% organic carbon in the Mediterranean [Cragg et al., 1998]; the presence of free methane gas around hydrates—below the bottom-simulating reflector at Cascadia margin [Cragg et al., 1996] and the Blake Ridge [Wellsbury et al., 2000]; and the presence of thermogenic methane in Japan Sea sediments [Cragg et al., 1992a]). Conversely, where organic carbon concentrations are low or electron acceptors are depleted, bacterial populations tend to be considerably reduced (e.g., the Lau Basin [Cragg, 1994]; the eastern equatorial Pacific [Cragg and Kemp, 1995]; the sedimented flanks of the Juan de Fuca Ridge [Mather and Parkes, 2000], and in the relatively deepwater low–organic carbon sediments of the Woodlark Basin [Wellsbury et al., 2002]).

ODP Site 1149 in the western equatorial Pacific is a deepwater area (5818 m). The bulk of the material deposited as sediments onto the seafloor has consisted of carbonate-free clays with mixtures of volcanic ash accounting for 35%–50% of the sediment to 118 mbsf. Below this, dark brown pelagic clay, devoid of calcareous and siliceous microfossils, dominates to 179.1 mbsf (Shipboard Scientific Party, 2000). This site represents the greatest water depth sampled to date for microbiological studies. At these depths, only very low amounts of bioavailable organic carbon may reach the sediments, which could limit the depth distribution of sediment bacterial populations. Core samples were obtained from this site in order to determine the presence, concentration, and

depth distribution of sediment bacteria in such an extreme and challenging environment.

MATERIALS AND METHODS

Shipboard Handling

Sediment samples were obtained from 23 core sections between 1.4 and 171.2 mbsf of Hole 1149A. 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-cm^3 sample was then taken with a sterile (autoclaved) 5-mL syringe from which the luer end had been removed. The sample was aseptically ejected directly into a serum vial containing 9 mL of filter-sterilized (0.2 µm) 4% formaldehyde in artificial seawater for preservation and storage. This was the first ODP leg where contamination checks were conducted for microbiology, and these checks demonstrated that the inner portion of cores, where the microbiological samples were taken, were free from potential sampling contamination (Smith et al., 2000).

Laboratory Handling

Direct Microscopic Observations

Acridine orange staining and microscopic observations were based on the general recommendations of Fry (1988) with minor modifications. Fixed samples were vortex mixed and a subsample of between 15 and 40 µL was added to 10 mL 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/dm^3. After 3 min the solution was filtered through a 25-mm Nucleopore black polycarbonate membrane (Osmonics Inc. of Minnetonka, Minnesota) of 0.2-µm pore size. The filter was rinsed further with 10 mL of 2% filter-sterilized formaldehyde and mounted in a minimum amount of paraffin oil under a coverslip.

Mounted membrane filters were viewed under incident illumination with a Zeiss Axioskop microscope fitted with a 50-W mercury vapor lamp, a wideband interference set for blue excitation, a 100× (numerical aperture = 1.3) Plan Neofluar objective lens, and 10× eyepieces. Green fluorescing bacterial cells were counted. Cells attached or unattached to particles were counted separately, and the numbers of those attached to particles were doubled in the final calculations to account for cells hidden from view by particles (Goulder, 1977).

We prepared three replicate membranes for each sample; however, where calculated 95% confidence limits of the mean population size were greater than ±0.5 $\log_{10}$ units, additional membranes were prepared and enumerated until the confidence limits were reduced below ±0.5 $\log_{10}$ units. All 23 samples were satisfactorily enumerated with three membranes, and the mean confidence limit for all 23 samples was ±0.33 $\log_{10}$ units.

Interstitial Water and Pore Space Chemistry

The concentrations of ammonium, sulfate, and methane, plus the measurement of pH, were obtained directly from the database gener-

ated during Leg 185 (Shipboard Scientific Party, 2000). The methods are described in detail therein. Dissolved manganese and acetate concentrations were subsequently obtained from shore-based laboratory analysis, and methods are described below.

Dissolved Manganese

Dissolved Mn was measured by inductively coupled plasma–atomic emission spectrometry (ICP-AES) at Boston University, following the general analytical procedures described by Murray et al. (2000), using a Jobin-Yvon 170C ICP spectrometer. Calibration standards were constructed using Mn-spiked seawater (of Scituate, Massachusetts) to provide an appropriate matrix match. Replicate analyses of separate natural samples indicated that the data were precise to 2% of the measured values. Accuracy was checked by comparison to a Mn-spiked aliquot of International Association for the Physical Sciences of the Oceans standard seawater.

Acetate

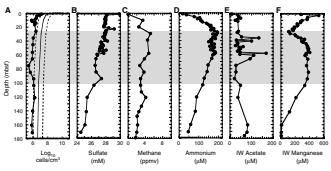
Pore water acetate concentrations were measured at Bristol University following the enzymatic method of King (1991), which measures bioavailable acetate, using high-performance liquid chromatography (Dionex [UK] Ltd.) separation. Samples were injected onto an analytical column (Supelco LC-18-T, 25 cm × 4.6 mm) with a mobile phase of 0.1-M KH_2PO_4 (pH 6.0) at 30°C at 0.8 mL/min (Wellsbury and Parkes, 1995). Detection was by UV/VIS at 254 nm and quantification by peak-area integration (Spectra-Physics, United Kingdom).

RESULTS AND DISCUSSION

Bacterial cells were present in all samples, although absolute numbers were low (Fig. F1). The highest cell concentrations of 7.2×10^6 cells/cm³ were detected near the surface (1.4 mbsf), then populations declined rapidly over the upper 10 m, followed by a much slower rate of decrease for the rest of the core, to 7.2×10^5 cells/cm³ at 172 mbsf. The smallest population of 2.1×10^5 cells/cm³ occurred at 75 mbsf. When compared to the general equation of Parkes et al. (2000) that describes depth distributions for global deep-sediment bacterial populations, it is clear that the population profile at Site 1149 is well below the predicted distribution, and in fact, bacterial numbers are also below the lower prediction limits of this equation (Fig. F1). Whereas low bacterial populations have been observed at other ODP sites such as the eastern equatorial Pacific (EEQ) (Cragg and Kemp, 1995), this is the first time that such persistently low counts have occurred.

In sediments, the substrate for bacterial growth and maintenance is normally organic material input from the euphotic zone. At extreme water depth such as that found at Site 1149, by the time sinking organic matter has reached the sediment surface, much of the labile material has already been mineralized, resulting in a lower, more recalcitrant, organic carbon supply (Jørgensen, 1983). This effectively restricts bacterial population size and growth rates. It is unfortunate that total organic carbon measurements were not made at Site 1149 to directly confirm this.

F1. Depth profiles for total bacteria and interstitial water components, p. 11.



Another factor controlling concentrations of buried organic matter, and therefore bacterial population size, is the sedimentation rate. Deposited organic detritus that remains at the sediment surface for a longer period of time will generally be more efficiently mineralized under oxic and suboxic conditions by aerobic heterotrophic and fermentative bacteria, thereby reducing the amount of organic matter buried (Hartnett et al., 1998). At Site 1149, the average sedimentation rates were particularly low at ~18 m/m.y., however, this average is misleading as, particularly in the upper layers, these rates were boosted by volcanic ash layers. A more realistic average is 7–13 m/m.y. (Shipboard Scientific Party, 2000). This compares to sites with high-organic and high-sedimentation rates, such as the Peru margin (80 m/m.y) and to EEQ sites with low organic, low sedimentation rates (35 m/m.y.) (Suess, von Heune, et al., 1988; Mayer, Pisias, Janacek, et al., 1992). It is interesting to note that in the upper 80 m of sediment at the EEQ site (Site 851) sedimentation rates were very much lower at ~20 m/m.y. (Mayer, Pisias, Janacek, et al., 1992). This coincided with particularly low bacterial populations compared to the distribution predicted by Parkes et al. (2000), with many data that also fall below the lower prediction limit (Cragg and Kemp, 1995). Hence, the low sedimentation rates at Site 1149 probably contributed to the consistently low bacterial populations.

With the presumed low levels of recalcitrant organic carbon, rates of bacterial activity would also be expected to be low. The sulfate profile in the uppermost 5 m at this site, where the sulfate concentration falls from 30 to 28 mM, is the most rapid rate of sulfate removal, and hence sulfate reduction, at this site. Below 5 mbsf, much lower rates of sulfate reduction occur, based on the smaller decrease in IW sulfate with depth (Fig. **F1**). Between 5 and 170 mbsf, sulfate removal is relatively small, decreasing from 28 to 24.5 mM, and even at 407 mbsf, the sulfate concentration remains over 19 mM (Shipboard Scientific Party, 2000). The removal of only about one-third of the sulfate in the pore fluids of sediments deposited for some 130 m.y. (Shipboard Scientific Party, 2000) provides strong circumstantial evidence that the sediment column is relatively depleted in bioavailable organic matter. High concentrations of sulfate throughout the sediment column would also explain the apparently low activity of bacterial methanogenesis in these sediments (Fig. **F1**), where maximum methane concentrations reach only 5 ppmv (Shipboard Scientific Party, 2000). It is surprising that any methane is present in this core, as in most marine sediments sulfate concentrations >3 mM inhibit methanogenesis (Capone and Klein, 1988). Additionally, methane can be used as a substrate for sulfate reduction (Hinrichs et al., 1999; Boetius et al., 2000; Nauhaus et al., 2002). However, a similar coexistence of sulfate and methane has been observed at other low–organic carbon ODP sites (e.g., EEQ) (Cragg and Kemp, 1995). The reason for this is unclear.

Consistent with low levels of bacterial sulfate reduction and methanogenesis, the geochemical evidence indicates that other bacterial activities occur at low levels. Ammonium concentrations increase from ~20 µM near the surface to a broad maximum of ~175 µM between 20 and 70 mbsf (Fig. **F1**), indicating low levels of organic matter degradation. The subsequent slow decrease in ammonium between 70 and 170 mbsf may well reflect uptake during clay diagenesis rather than declining levels of bacterial activity with increasing depth (Shipboard Scientific Party, 2000). Between ~35 and 70 mbsf, bioavailable acetate shows local increases from a background of 30 µM to a maximum of 170 µM at 57

mbsf (Fig. F1). These data suggest there may be localized elevated rates of bacterial acetogenesis. Such elevated acetate concentrations are unexpectedly high compared to other low–organic carbon sediments. In the sediments of the Southern Ocean (Leg 177), acetate concentrations were in the range of 0–15 µM (Wellsbury et al., 2001), although many localized spikes (to 110 µM) were present in sediments containing diatom-rich lamellae. Additionally, in the western Woodlark Basin (Leg 180), acetate concentrations were again in the range of 1–20 µM (Wellsbury et al., 2002).

Site 1149 has received considerable inputs of hydrothermal plume material in the past; thus, there are significant metalliferous components within the sediments (Shipboard Scientific Party, 2000). The two most microbiologically important metals for sediment bacteria are iron and manganese (Lovley and Chappelle, 1995). Whereas there was no evidence for iron reduction, as dissolved iron concentrations were always below detection (Shipboard Scientific Party, 2000), there was clear evidence for manganese reduction with significant increases of reduced manganese in the interstitial water (Fig. F1). The maximum Mn(II) concentration was 528 µM at 2.9 mbsf. Below this, concentrations declined to a minimum of 110 µM at 26 mbsf. However, below 26 mbsf reduced manganese concentrations exhibited a broad increase to ~100 mbsf, with a local maximum value of 403 µM at 56 mbsf. This broad subsurface dissolved manganese profile corresponds with high ammonium and acetate concentrations.

Despite the presumed low levels of recalcitrant organic matter at this site, there is clear geochemical evidence for bacterial activity at all depths with maximum activity restricted to the top ~5 m and much lower activity below. This is consistent with the high, but rapidly decreasing, bacterial populations in the top ~10 mbsf, and smaller populations below, that decrease more slowly. This type of distribution has been observed previously at several ODP sites (e.g., Amazon Fan and Santa Barbara Basin) (Cragg et al., 1996, 1995) and probably reflects the removal of the most degradable organic matter fractions followed by very slow utilization of recalcitrant organic matter. It seems improbable that any organic matter would be degradable after millions of years of burial, but Cretaceous marine sediments have been shown to support bacterial activity, including acetate formation and sulfate reduction (Krumholz et al., 1997). At Site 1149, deep manganese reduction is also occurring (Fig. F1). Manganese reduction is energetically more favorable than sulfate reduction (Madigan et al., 2000), and hence it is surprising that both soluble manganese is produced and sulfate is removed in the top ~10 m. However, this might just be an artifact of the limited depth resolution of the samples (first sample taken at 1.4 mbsf). Nevertheless, there is clear evidence of simultaneous manganese and sulfate reduction in deeper layers. This situation may reflect heterogeneous conditions in the sediment, the use of noncompetitive substrates by the different metabolic types, or cooperative metabolism between the different bacterial types. Additionally, the possibility that manganese reduction is occurring through the chemical oxidation of sulfide must be considered (Schippers and Jørgensen, 2001, 2002). It is difficult to demonstrate that this is not occurring, as the maximum increase in Mn(II) between 25 and 100 mbsf (~300 µM) would be difficult to detect as equivalent replenished sulfate; 300 µM would represent only an ~1% increase in the sulfate concentration; and sulfate reduction is simultaneously occurring. Hydrogen sulfide concentrations were not measured in Hole 1149A, although H_2S is unlikely to be present in any significant

quantity as these sediments are only slightly suboxic throughout (Shipboard Scientific Party, 2000). However, the continued gradual decrease in sulfate concentration over this depth range, the increase in ammonium, and the local increases in bioavailable acetate in low-temperature sediments all indicate that bacterial activity, albeit at low levels, is occurring (Fig. F1). Given that bioavailable acetate is present and that manganese reduction by acetate metabolism is considerably more favorable energetically than sulfate reduction, it seems probable that a significant amount, if not all, of manganese reduction will be by way of bacterial activity. Alternatively, if some of the manganese reduction is due to a biological reduction via H_2S, this suggests greater rates of bacterial sulfate reduction than indicated by sulfate removal. Whatever the explanation, there is clear geochemical evidence for bacterial activity in the deep subsurface of this deepwater site, which is the deepest so far analyzed for sediment microbiology.

SUMMARY

Site 1149 is a deepwater site characterized by very low sedimentation rates and probably low organic matter concentrations. The lack of geochemical evidence for high levels of sulfate reduction supports the view that organic carbon arriving on the seafloor is of insufficient quantity or quality to fuel rapid sulfate depletion by bacterial sulfate reduction. This is in agreement with the consistently low bacterial populations. However, other geochemical evidence suggests some limited bacterial turnover of carbon is occurring throughout the sediment column, with increases in ammonium and the production of bioavailable acetate. These changes in the geochemical profiles occur with increases in reduced manganese in the interstitial water, demonstrating that active bacterial manganese reduction may be an important process, to considerable depths, at this site. It is interesting that manganese and sulfate reduction and limited methanogenesis seem to be occurring together at this low–organic matter site. This is in stark contrast to the predicted sequential separation of these bacterial activities on thermodynamic grounds, which are often observed in shallow-water sediments (Jørgensen, 1983). These bacterial activities together can provide energy to support the small deep bacterial population detected by direct microscopy at Site 1149.

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Figure F1. Depth profiles. **A.** Total bacteria at Site 1149 using the acridine orange direct count technique. Solid sloping line = the regression line of best fit derived from 16 ODP legs of deep bacterial profiles, dashed lines = the 95% prediction limits (Parkes et al., 1994) and interstitial water (IW) geochemistry. **B.** Sulfate. **C.** Methane. **D.** Ammonium. **E.** Acetate. **F.** Reduced manganese. The shaded area highlights the broad peak in bacterial manganese reduction activity between 26 and 100 mbsf.