Zahn, R., Comas, M.C., and Klaus, A. (Eds.), 1999
Proceedings of the Ocean Drilling Program, Scientific Results, Vol. 161

34. BACTERIAL PROFILES IN DEEP SEDIMENTS OF THE ALBORAN SEA, WESTERN MEDITERRANEAN, SITES 976–978[1]

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ABSTRACT

Sediments from three sites in the Alboran Sea, western Mediterranean were investigated for the presence of bacteria in deep sediments. A series of 54×1–cm^3 sediment samples were removed from core sections of Hole 976B between 26.9 and 646.6 mbsf (16 samples), Hole 977A between 0 and 525.8 mbsf (29 samples), and Hole 978A between 346 and 644.6 mbsf (9 samples) for direct microscopic determination of bacteria. Sites 977 and 978 were only 24 km apart, contained similar sedimentary sequences and interstitial water profiles, and covered complimentary depth ranges to provide samples from the surface to 644 mbsf. Therefore, these two sites were treated as one site. Significant bacterial populations were present in the deepest samples of both cores at Sites 977/978 (644 mbsf) and 976 (647 mbsf), (3.5×10^6 cells/cm^3 and 1.6×10^6, respectively). These bacterial depth distributions correspond well with previous data from Atlantic and Pacific Ocean sites, and they also extend considerably the previous deepest marine sediment samples that have been analyzed for bacteria (514 mbsf), thus extending the depth of the bacterial biosphere in this environment. Because the rate of decrease in bacterial populations does not change in these deep samples, it is likely that bacteria are present even deeper than 650 mbsf in marine sediments. At Site 977/978 a deep brine incursion supplies sulfate below 400 mbsf, and absence of methane in this zone suggests active bacterial anaerobic methane oxidation. Previously a brine incursion in the Peru Margin has been shown to stimulate bacterial activity and to increase bacterial viability at depths of 80 mbsf and ~1 Ma. A similar effect may be occurring at Site 977/978. This effect would explain why the bacterial profile does not decrease with depth in the deeper layers and demonstrates the viability of bacteria in ancient deposits (6 Ma).

INTRODUCTION

A deep bacterial biosphere in the marine environment has now been confirmed by an extensive amount of work on sediment cores primarily from the Ocean Drilling Program (ODP) (Whelan et al., 1986; Tarafa et al., 1987; Parkes et al., 1990, 1993, 1994, 1995; Cragg, 1994; Cragg et al., 1990, 1992, 1995a, 1995b, 1996, 1997, 1998; Cragg and Parkes, 1994, Cragg and Kemp, 1995). In addition, bacteria have been shown to be present in basaltic basement rocks (Furnes et al., 1996; Giovannoni et al., 1996) and Cretaceous shales and sandstones (Krumholz et al., 1997). In marine sediments the depth profile of bacteria is remarkably consistent across different oceans, with population sizes of $\sim 9 \times 10^8$ cells/cm^3 at the near surface decreasing exponentially to $\sim 1.5 \times 10^6$ cells/cm^3 at 500 mbsf. At the near surface, bacteria act as a filter on sedimented organic material and are crucially involved in the degradation and selective preservation of organic matter in marine sediments. They are, therefore, intimately involved in biogeochemical cycling (Jørgensen, 1983; Novitsky and Karl, 1986; Jørgensen et al., 1990; Parkes et al., 1993). At much greater depths geochemical evidence has indicated that bacterial populations can remain active (Krumbein, 1983) and more recently low levels of bacterial activity have been demonstrated to 500 mbsf in the Japan Sea (Cragg et al., 1992; Getliff et al., 1992; Parkes et al., 1994). Most recently, work in the eastern Mediterranean on sediment cores containing organic-rich (up to 30% total organic carbon [TOC]) sapropels has provided evidence of continued bacterial activity and actual population growth in sediments up to 4.7 Ma (Cragg et al., 1998).

The Alboran Sea is situated in the mouth of the Mediterranean and Atlantic inflow generates gyre circulation, which stimulates small-scale upwelling of deep, nutrient-rich waters to the surface layers, locally increasing productivity (Comas, Zahn, Klaus, et al., 1996). The Alboran Basin contains some of the greatest accumulations of Neogene and Quaternary deposits of the western Mediterranean, with sediments up to 7 km thick. Our previous work in the Japan Sea (Cragg et al., 1992) has demonstrated bacterial populations down to 514 mbsf, the deepest samples analyzed; however, there were only two sub-400 mbsf samples. The aim of this work was to obtain deep sediment samples to confirm the presence of bacteria below 400 mbsf and, potentially, to extend the depth of the bacterial biosphere in marine sediments.

MATERIALS AND METHODS

Site Description

Sites 976–978 are located in the Alboran Sea Basin of the western Mediterranean (between 2 and 4.5°W and ~36°N) on a convergent plate boundary between Africa and Eurasia (Comas, Zahn, Klaus, et al., 1996). The Alboran Sea is ~400 km long and 200 km wide with a maximum water depth of 2000 m. Overlying water depths for the three sites were 1108 m (Hole 976B), 1984 m (Hole 977A), and 1929 m (Hole 978A). Surface-sediment temperature was ~12.5°C and the geothermal gradient is estimated at 72°C/km. Sedimentation rates vary considerably throughout the sediment column (33–275 m/Ma). Site 976 has an average of ~152 m/Ma. Despite rapid sedimentation rates, approximate age at 670 mbsf is 12.2 Ma, a result of a significant hiatus in the sediment column. Conversely, Site 977 has a much slower average sedimentation rate (75 m/Ma), but has an estimated age of ~5 Ma at 540 mbsf. The average sedimentation rate for Site 978 is 126 m/Ma and at 645 mbsf sediment age is ~6.7 Ma (Comas, Zahn, Klaus, et al., 1996).

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Shipboard Handling

A series of 54×1 cm^3 sediment samples were removed from core sections of Hole 976B between 26.9 and 646.6 mbsf (16 samples), Hole 977A between 0 and 525.8 mbsf (29 samples), and Hole 978A between 346 and 644.6 mbsf (9 samples). 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 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 2.5 to 10 µL subsample 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/L. After 3 min, the solution was filtered through a 25-mm Nucleopore black polycarbonate membrane (Costair, High Wycombe, U.K.) of 0.2-µm pore size. The filter was rinsed with a further 10 mL of 2% filter-sterilized formaldehyde in artificial sea water and mounted in a minimum of paraffin oil under a cover slip. 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 $\log_{10}$ units, further replicate filters were prepared. A minimum of 200 fields of view, or 400 cells, 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 (450 – 490 nm; Zeiss, Oberkochen, Germany), a 100× (numerical aperture = 1.3) Plan Neofluar objective lens, and 10× eyepieces. Bacterially shaped green and red fluorescing objects were counted. Cells that were 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 (Goulder, 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 estimated as 5×10^4 cells/cm^3.

RESULTS AND DISCUSSION

Hole 976B

Bacterial populations were detected at all depths sampled (Fig. 1). The uppermost sample was at 27 mbsf with 2.8×10^7 cells/cm^3, which gradually decreased to 1.6×10^6 cells/cm^3 at 646.6 mbsf.

Dividing and divided cells (DDC) were present in all but two samples (Fig. 1) at 352.5 and 522 mbsf. The reason for these two "not-detected" data is unclear as the associated total counts are not significantly lower than adjacent total count data**.** Their occurrence prevented correlation analysis between total bacterial numbers and DDC data, as they had indeterminable values between zero and the detection limit (5×10^4 cells/cm^3). DDC represent ~7.2% of the total count.

Total organic carbon is generally below 1% throughout this hole, averaging 0.5% (Fig. 2) with a maximum of 1.58%. The trend of TOC decrease is linear with depth ($R = 0.656$; $N = 139$; $P << 0.002$). Notable exceptions in this data are the few points that have unusually high TOC. These were all related to organic-rich layers, of which 28 examples were identified in Unit I (0–362 mbsf). These layers also had unusual magnetic susceptibilities and amorphous organic components in addition to the terrigenous organic plant remains more generally distributed throughout the rest of the core (Comas, Zahn, Klaus, et al., 1996). Unfortunately, no microbiological samples were obtained from these layers. Additionally, between ~335 mbsf and 502 – 536 mbsf (no precise depth can be given due to lack of TOC data in this zone [Fig. 2]), TOC concentrations are unusually and significantly lower than expected ($\chi^2 = 13.5$; d.f. = 1; $P < 0.0005$). This depth range broadly coincides with lithostratigraphic Unit II (361–518 mbsf), which is characterized by low and disturbed recovery with high sand concentrations (Comas, Zahn, Klaus, et al., 1996). Such sediments might be expected to have low bacterial populations as a result of the low TOC concentrations. Although the total bacterial populations in this zone are not low and fit with the general decreasing trend in bacterial populations with increasing depth, it is interesting that the two occurrences of zero DDC (see above) occur within this low TOC band, even though they do not fall within the precise boundaries for Unit II.

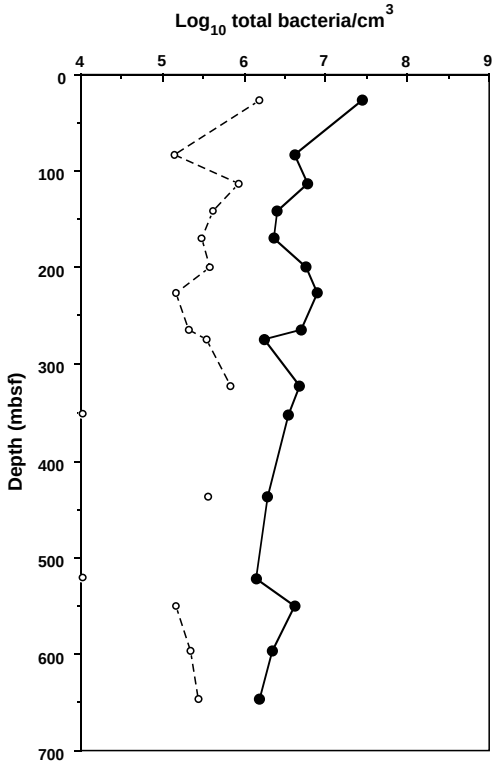

Figure 1. Depth distribution of total bacteria (solid circles) and dividing/divided cells (open circles) at Hole 976B using the Acridine Orange Direct Count (AODC) technique. Open circles on the vertical axis unconnected with other data are dividing/divided cell counts of zero. Average variance for sample enumerations in this data set was 0.019 Log_{10} units. This produced average 95% confidence limits of 0.297 Log_{10} units (range 0.081 – 0.427).

The increase in salinity with depth from 37 g/L at the near surface to 76 g/L at 646 mbsf, in addition to increases in other conservative elements, is indicative of a deep-seated brine source (Comas, Zahn, Klaus, et al., 1996). However, as there are no deep Messinian evaporitic salts locally, a lateral flow from elsewhere along the basement-sediment contact has been suggested (Comas, Zahn, Klaus, et al., 1996). Additionally, as sulfate concentrations do not increase with depth (Fig. 2), this suggests that it is halite rather than gypsum dissolution providing the brine. Sulfate is completely depleted by 19.95 mbsf (Comas, Zahn, Klaus, et al., 1996) and shows values close to zero throughout the rest of the core (Fig. 2). Initial sulfate depletion is probably due to anaerobic sulfate-reducing bacteria utilizing the la-

bile near-surface organic matter (Parkes et al., 1993). Methane concentrations increase below the depth of sulfate depletion and high C_1/C_2 values support a biogenic origin for the methane. The highest concentration of methane, 66,555 ppmv, occurred at 98 mbsf (Fig. 2). Below about 300 mbsf, there is little evidence of significant methanogenic activity despite low or negligible concentrations of sulfate. This may be a reflection of the low concentrations of TOC, which average only 0.27% between 300 mbsf and the bottom of the hole, and that organic matter at this depth may be rather resistant to bacterial degradation.

Holes 977A and 978A

Sites 977 and 978 were 24 km apart and were both cored to investigate similar sedimentary sequences. Hole 978A was drilled from only 178 mbsf, but went ~100 m deeper than Hole 977A. Microbiological samples were obtained principally in the lower section of Hole 978 for the purpose of extending the data obtained from Hole 977A. Interstitial water chemical data suggest that these two holes are very similar, and, hence, bacterial data from the two holes have been combined.

Bacterial populations were detected at all depths sampled (Fig. 3). At the near surface, numbers were high at 1.01×10^9 cells/cm^3 and rapidly decreased to 7.9×10^6 cells/cm^3 by 13.6 mbsf. From 13.6 to 85 mbsf, bacterial numbers were broadly constant at 1.7×10^7 cells/cm^3. Between 85 and 105 mbsf, there was a substantial decrease in bacterial numbers. Below this, surprisingly, bacterial numbers remained more or less constant to the base of the core at 644.6 mbsf (5.3×10^6, mean; $3.8 - 6.8 \times 10^6$ cells/cm^3, 95% confidence interval). The largest population below the near-surface few meters of 2.8×10^7 cells/cm^3 at 84.5 mbsf was coincident with one of the 38 organic-rich layers (TOC > 1%) that were identified between 0 and 300 mbsf (Comas, Zahn, Klaus, et al., 1996). Unfortunately, this was the only sample associated with an organic-rich layer.

DDC were present in all samples (Fig. 3). DDC and total count were significantly correlated (R = 0.910; N = 38; $P << 0.002$) with DDC representing ~8.7% of the total count. The strong relationship between total count and DDC has been regularly observed in deep marine sediments at other ODP sites (Cragg et al., 1990, 1992; Parkes et al., 1990; Cragg, 1994; Cragg and Kemp, 1995).

TOC is generally below 1% throughout this hole, averaging 0.55% (Fig. 4) with a maximum of 2.47% at 177.7 mbsf. The decrease in TOC is linear with depth (R = 0.390; N = 398; $P << 0.002$). The amount of data scatter above 300 mbsf is markedly greater than that below 300 mbsf; however, of all ten TOC data with >1.5% organic carbon, eight were associated with the organic-rich layers (Comas, Zahn, Klaus, et al., 1996). The rate of decrease in TOC was similar to that observed in Hole 976B (0.07%/100 m compared to 0.1%/100 m); thus, between 500 mbsf and the end of the core, TOC concentrations at the sites are comparable at 0.34% (976B = 0.27%). Average bacterial population sizes are also similar below 500 mbsf at 7.0×10^6 (Hole 976A) and 2.2×10^6 (Holes 977A/78A) cells/cm^3.

Salinity increases with depth (Comas, Zahn, Klaus, et al., 1996), although, unlike the situation in Hole 976B, this does not start to occur until ~400 mbsf. Below 510 mbsf, data is available from Hole 978A only and a continued increase from 46 g/L to 79 g/L by 692 mbsf was measured. It has been suggested that the brine source is lateral flow of evaporitic brines from another section of the basin (Comas, Zahn, Klaus, et al., 1996), and the sulfate concentration increase at the base of the hole (Fig. 4) indicates that the brine originates from gypsum dissolution.

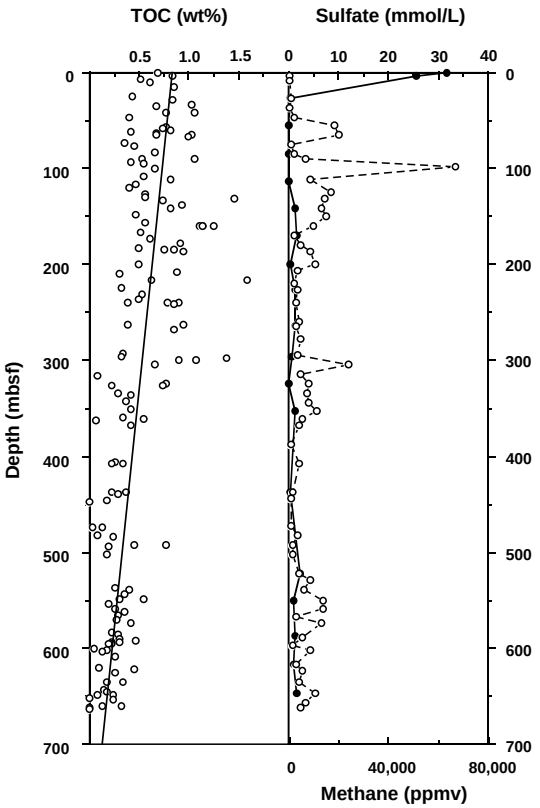

Figure 2. Depth distribution of total organic carbon (TOC; open circles), pore-water sulfate (solid circles), and methane (open circles) in Hole 976B. (Graph redrawn from Comas, Zahn, Klaus, et al., 1996.)

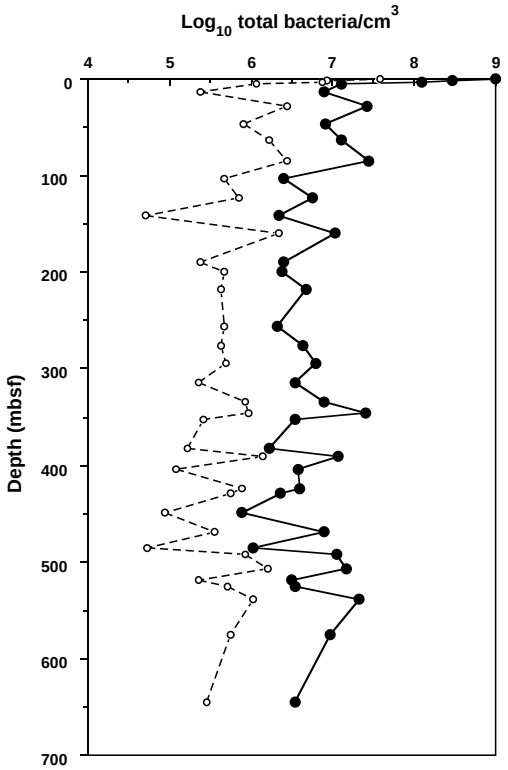

Figure 3. Depth distribution of total bacteria (solid circles) and dividing/divided cells (open circles) at Hole 977A/978A using the Acridine Orange Direct Count (AODC) technique. Average variance for sample enumerations in this data set was 0.023 Log_{10} units. This produced average 95% confidence limits of 0.270 Log_{10} units (range 0.034–0.494).

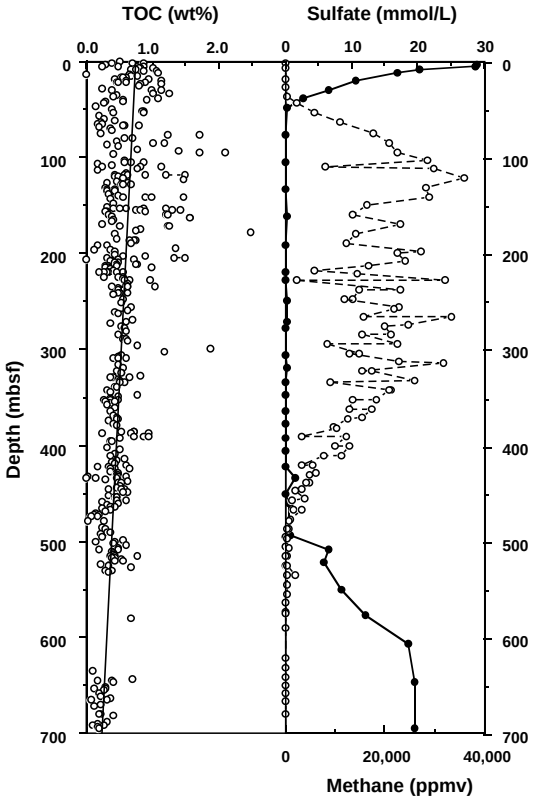

Figure 4. Depth distribution of TOC (open circles), pore-water sulfate (solid circles), and methane (open circles) in Hole 977A/978A. (Graph redrawn from Comas, Zahn, Klaus, et al., 1996.)

Sulfate concentrations decrease rapidly over the upper 50 m from 28.9 mM to zero (Fig. 4). Rapid bacterial degradation of organic matter over this depth range is supported by increases in alkalinity (1.45 mM to 15 mM at 27.45 mbsf) and phosphate (17.9 µM to 56.2 µM at 6.9 mbsf). Ammonium increases rapidly (0.25 mM to 1.4 mM at 9.9 mbsf) and then continues to increase to 3.94 mM at 218 mbsf as a result of methanogenic bacterial activity. Sulfate concentrations increase again from ~450 mbsf and appear to stabilize at 19 mM from 600 mbsf.

Methane concentrations again show a substantial increase starting within the base of sulfate depletion (Fig. 4). The maximum concentration was 35,700 ppmv at 122 mbsf, and between ~80 and 420 mbsf methane concentrations averaged 20,000 ppmv. High C_1/C_2 ratios indicate that this methane is biogenic (Comas, Zahn, Klaus, et al., 1996). This is supported by the low methane concentrations when sulfate occurs at depth (Fig. 4), as sulfate-reducing bacteria can outcompete methanogens for common growth substrates (Schonheit et al.,1982; Kristjansson et al., 1982; Claypool and Kvenvolden, 1983). The geochemical data thus indicates rapid bacterial activity in the top 50 m and then lower, but continued, activity throughout the core, and the bacterial distributions are consistent with this interpretation (Fig. 3).

Bacterial profiles below about 100 mbsf at Site 977/978 are surprisingly constant with little or no decrease with depth. This suggests continued low bacterial activity at depth, which is consistent with the presence of high concentrations of biogenic methane. This methane is removed deeper in the core when sulfate concentrations increase, probably a result of anaerobic methane oxidation utilizing the sulfate (Iversen and Jørgensen, 1985; Suess and Whiticar, 1989; Hoehler et al., 1994), thus providing energy for the deeper bacterial population. Direct evidence for this has been obtained for ODP sites on Leg 146, using ^{14}C-CH_4 radiotracer activity measurements. Significant stimulation in anaerobic methane oxidation occurred at depth, and this was associated with a significant (10×) increase in the total bacterial population at a discrete gas hydrate zone (Cragg et al.,1996).

Brine incursion at Site 681 on the Peru Margin has been shown to result in a stimulation in bacterial activity and an increase in the viable bacterial population at depth of 80 mbsf and in sediments 1 Ma (Parkes et al., 1990). The brine incursion at Site 977/978 may have a similar effect resulting in bacterial populations not decreasing with depth; however, these deposits are much deeper and older than the Peru Margin (6 Ma and almost 650 mbsf). These results support the growing evidence demonstrating the viability of bacterial populations in ancient sediments.

General

The bacterial population profiles with depth at both sites are typical of what would be expected in sediments with depth-related, bacterially-mediated changes in pore-water chemistry and methane gas. The bacterial population profiles obtained from these data were plotted with a general bacterial depth profile derived from Pacific and Atlantic Ocean ODP sites (Parkes et al., 1994; Cragg et al., 1997) and since augmented (Cragg et al., 1998) given by the expression:

$$\text{Log}_{10} \text{ bacterial numbers} = 7.95 - 0.64 \text{ Log}_{10} \text{ depth (m)}$$

$$(R = 0.691;\ N = 585;\ P << 0.002).$$

There is exceptionally close agreement between data from Holes 976 – 978 and the general bacterial depth trend (Fig. 5). These bacterial data confirm that significant bacterial populations are present below 400 mbsf in marine sediments and extend the presence of bacterial populations to 646 mbsf, 133 m deeper than previous observations (514 mbsf; Parkes et al., 1994).

SUMMARY

Direct microscopic analysis of bacterial populations at two sites on Leg 161 has demonstrated the presence of significant bacterial populations at depth (mean bacterial population between 400 mbsf, and the deepest samples analyzed 644 and 647 mbsf in Sites 977/978 and 976, respectively, were 3.5×10^6 cells/cm^3 and 1.62×10^6 respectively). This extends by 133 m the depth of the bacterial biosphere in marine sediments, as the previous deepest results were samples from 514 mbsf from the Japan Sea (Cragg et al., 1992). These bacterial depth distributions correspond well with previous data from Atlantic and Pacific Ocean sites (Cragg et al., 1997, 1998), and, as the rate of decrease in bacterial populations does not change in the deep samples, it is likely that bacteria are present even deeper than 650 mbsf in marine sediments.

Sites 977/978 show classical zonation of near-surface sulfate reduction followed by deeper methanogenesis. An incursion of high concentrations of sulfate sub-400 mbsf is coincident with a decrease in biogenic methane concentrations to zero, suggesting that active bacterial sulfate reduction is probably utilizing the methane at depths below 400 mbsf. This deep bacterial activity may explain why bacterial populations at this site do not decrease in the deeper depths and confirms the viability of bacteria in ancient deposits (6 Ma).

ACKNOWLEDGMENTS

We would like to thank ODP for allowing us access to samples from Leg 161 and shipboard personnel who assisted in taking sam-

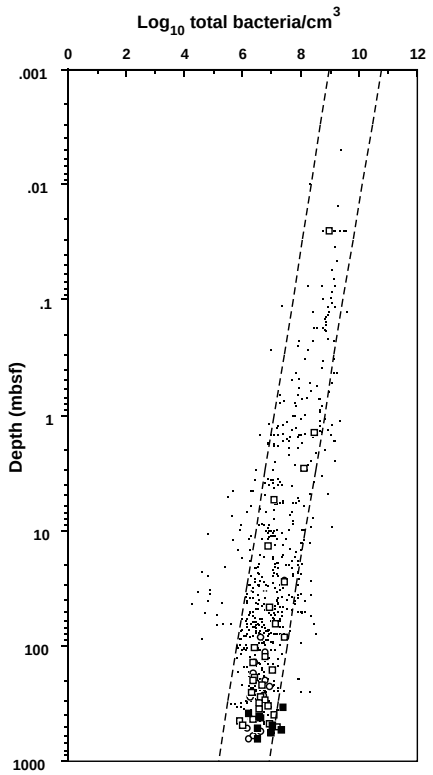

Figure 5. Depth distribution of total bacteria from western Mediterranean Holes 976B (open circles), 977A (open squares), and 978A (solid squares) compared to the distributions of bacterial populations (n = 585) with depth at 16 other sites from eight ODP legs (dots). Heavy slanting lines represent the 95% prediction limits for the regression line given by Log_{10} bacterial numbers = 7.95 – 0.64 × Log_{10} depth (m) (Cragg et al., 1997).

ples. We acknowledge the support and facilities provided by Bristol University and the Natural Environment Research Council U.K. (NERC) who funded this work.

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Date of initial receipt: 9 May 1997
Date of acceptance: 19 January 1998
Ms 161SR-267