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13. BACTERIAL PROFILES IN SEDIMENTS OF THE EASTERN FLANK OF THE JUAN DE FUCA RIDGE, SITES 1026 AND 1027[1]

I.D. Mather[2] and R.J. Parkes[2,3]

ABSTRACT

Bacterial depth profiles were obtained from 45 sediment samples taken from two sites ~100 km east of the crest of the Juan de Fuca Ridge and roughly 80 km east of the nearest extensive basement outcrop using the Acridine orange direct count (AODC) technique. At Site 1026 bacteria were present at all depths. Total bacterial numbers followed the same general depth trend found at other previously analyzed Ocean Drilling Program (ODP) sites. Total counts decreased from near-surface concentrations of 1.47×10^8 to 9.93×10^6 cells cm^{-3} by 8 mbsf. Below this depth, bacterial numbers remained approximately constant to the deepest depth of ~70 mbsf at 1.07×10^7 cells cm^{-3}. Dividing cells were present in all samples and generally paralleled total cell numbers, averaging 12% of the total population. At Site 1027, significant bacterial cells were present in 29 of the 33 samples. Total bacterial numbers were highest at the near surface (1.67×10^8 cells cm^{-3}) but decreased rapidly with depth, reaching 2.16×10^6 cells cm^{-3} in the deepest sample at 565.10 mbsf. The bacterial profile in the top ~300 mbsf followed the same general trend observed at other ODP sites. Between ~374 and ~509 mbsf, bacterial populations were lower than expected. Dividing cells were present in 21 of the 33 samples analyzed and were highest at the near surface (1.97×10^7 cells cm^{-3}), where they represented 12% of the total population. Below this, the numbers paralleled the total count. Dividing cells, however, were absent below 429 mbsf. Two depth zones showed consistently elevated bacterial numbers. The first of these (~166 to ~273 mbsf) was associated with an increase in pore-water ammonia and alkalinity; the second (~527 to ~565 mbsf) was associated with the diffusion of sulfate from the underlying bedrock. As organic carbon concentrations are essentially consistent throughout the bottom 300 m of the hole, the stimulation of bacterial populations in this deeper zone depth is probably caused by the sulfate providing a more efficient electron acceptor at depth. This coincided with removal of CH_4, and, thus, anaerobic methane oxidation, which has previously been associated with deep elevation of bacterial populations at a number of ODP sites. In addition, aspects of the bacterial distributions seemed to indicate the sequential presence of different bacterial temperature groups from the near-surface psychrophilic bacteria to the deeper thermophilic populations.

INTRODUCTION

Extensive research on deep sediments obtained by the Ocean Drilling Program (ODP) has demonstrated the presence of a deep bacterial biosphere in marine sediments to depths of up to 750 m below seafloor (mbsf) (Whelan et al., 1986; Tarafa et al., 1987; Parkes et al., 1990, 1993, 1994; Cragg, 1994; Cragg et al., 1990, 1992, 1996, 1998; Cragg and Parkes, 1994; Cragg and Kemp, 1995; Cragg et al., 1995a; Cragg et al., 1995b; Wellsbury et al., 1997). The presence of bacteria has also been demonstrated in basaltic rocks (Furnes et al., 1996; Giovannoni et al., 1996; Fisk et al., 1998) and Cretaceous shales and sandstones (Krumholz et al., 1997).

Previous studies on the Middle Valley on the northern Juan de Fuca Ridge (Leg 139, Site 858) demonstrated that substantial bacterial populations, including dividing cells, were present in deep sediments within submarine hydrothermal systems, although their depth distributions were limited by the high thermal gradient (1.7°–11°C m^{-1}; Cragg and Parkes, 1994). Sites 1026 and 1027 provided an opportunity to analyze sediments in a similar geological setting, but with a markedly lower thermal gradient (~0.17°C m^{-1}) to clarify the impact of elevated temperature gradients on bacterial depth distributions. These sites also provide "cool" control sites to contrast with bacterial distributions to be subsequently analyzed on Leg 169, which is also in the Juan de Fuca area but subject to high thermal gradients.

MATERIALS AND METHODS

Site Description

The Juan de Fuca Ridge is a seafloor spreading center off the coast of North America (Fig. 1). Sites 1026 and 1027 were situated ~100 km east of the crest of the Juan de Fuca Ridge and roughly 80 km east of the nearest area of extensive basement outcrop. Site 1026 was located over a basement ridge with a sediment thickness of ~250 m. Site 1027 was located about 2.2 km to the east in the adjacent valley with a sediment thickness of ~600 m. Near-surface sediment temperatures were ~2°C, and the extrapolated basement temperatures were very similar at both sites, 61.4°C at Site 1026 and 62.8°C at Site 1027. Thermal gradients were 0.242° and 0.103°C m^{-1} at Sites 1026 and 1027, respectively (Davis, Fisher, Firth, et al., 1997).

Shipboard Handling

A total of 45 1-cm^3 sediment samples were taken from the two sites (Table 1). Samples were removed from the ends of 1.5-m core sections as they were cut on the catwalk. A thin layer of sediment was removed from the cut surface with a sterile scalpel to expose an uncontaminated surface and a sterile (autoclaved) 5-mL plastic syringe, from which the luer end had been removed, was used to take a 1-cm^3 minicore. The sample was sealed with a sterile Suba Seal before being injected directly into a tared serum vial containing 9 mL of filter-sterilized (0.2 µm) 4% formaldehyde in artificial seawater, crimp sealed, and shaken vigorously to disperse the sediment. These preserved samples were returned to the laboratory for analysis.

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[2]Department of Earth Sciences, University of Bristol, Bristol BS8 1RJ, United Kingdom.
[3]Correspondence author: J.Parkes@bris.ac.uk

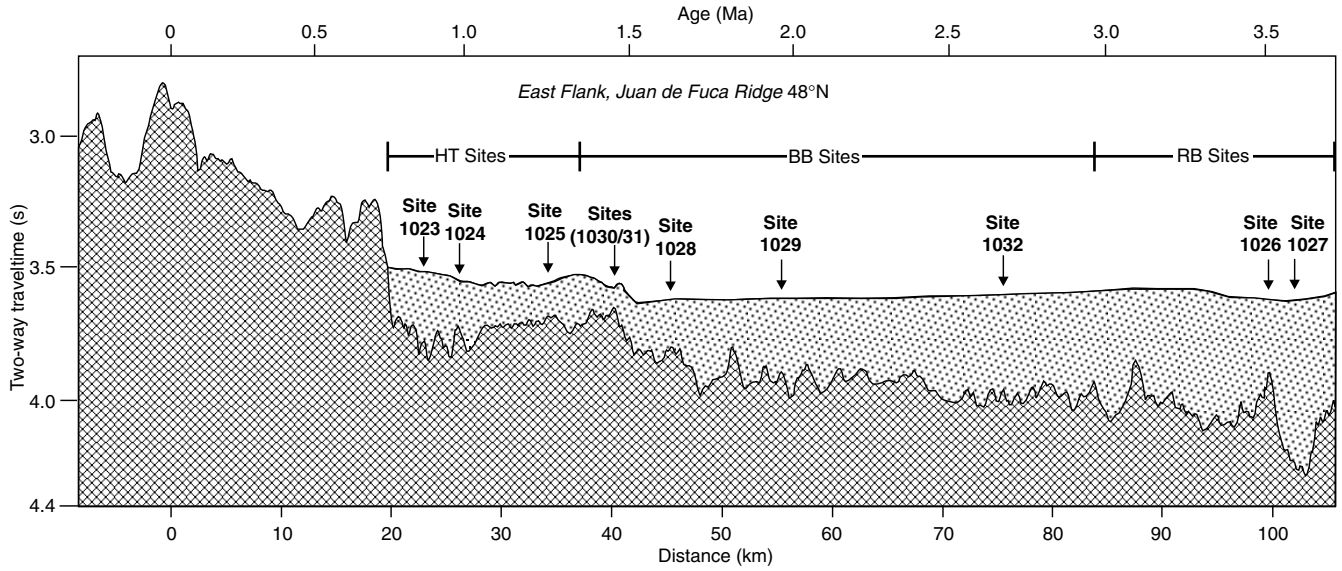

Figure 1. Basement topography and sediment cover of the eastern flank of the Juan de Fuca Ridge showing the location of Sites 1026 and 1027.

Table 1. Location of Holes 1026A and 1027B, water depth, number of samples analyzed, and their maximum sediment depth.

Hole	Position	Water depth (m)	Maximum sample depth (mbsf)	Number of samples
1026A	47°45.757′N, 127°45.552′W	2669.1	69.88	12
1027B	47°45.412′N, 127°43.853′W	2668.3	565.10	33

Laboratory Handling

Direct Microscopic Observations

Acridine orange staining and microscopic quantification were based on the general recommendations of Fry (1988), as outlined in Cragg and Parkes (1994). Preserved samples were vortex mixed, and a 2.5- to 15-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 dm^{-3}. After 3 min, the solution was filtered through a 25-mm, 0.2-µm pore size, Nucleopore black polycarbonate membrane (Costar, High Wycombe, U.K.). The filter was rinsed further with 10 mL of 2% filter-sterilized formaldehyde in artificial seawater and mounted in a small amount 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 $\log_{10}$ 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 Axioscope 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. Green and red fluorescing objects, which resembled bacteria, 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 (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 calculated to be 2.23×10^5 cells cm^{-3}.

RESULTS AND DISCUSSION

Site 1026

Bacteria were present in all the samples from Site 1026 (Fig. 2). Total bacterial numbers followed the same general depth trend of other sites (Cragg et al., 1990, 1992, 1996, 1997, 1998; Cragg and Parkes, 1994; Cragg and Kemp, 1995; Cragg et al., 1995a; Cragg et al., 1995b). Bacterial numbers were initially high at the surface (1.47×10^8 cells cm^{-3}), but decreased rapidly to 9.93×10^6 cells cm^{-3} by 8 mbsf. Below this depth, bacterial numbers remained essentially constant and at ~70 mbsf 1.07×10^7 cells cm^{-3} were present. Dividing cells were also present in all samples, and their depth distributions generally paralleled that of the total count. Numbers of dividing cells, however, were on average only 12% of the total count. Highest numbers were at the near surface (5.19×10^7 cells cm^{-3}) and decreased to 1.47×10^6 cells cm^{-3} at ~70 mbsf, the deepest sample.

Site 1027

Bacterial populations were present in all samples; however, at 374, 413, 470, and 509 mbsf, counts did not significantly exceed blanks (Fig. 3). Total bacterial numbers were high at the surface (1.67×10^8 cells cm^{-3}) and must be adapted to the prevailing low temperatures ~2°C (psychrophilic or psychrotolerant). Direct counts subsequently decreased with depth, with some very low concentrations below ~374 mbsf and, in the deepest sample at 565.1 mbsf, counts were 2.16×10^6 cells cm^{-3}. Dividing cells were present above the detection limit in 21 of the 33 samples. The highest value was at the near surface at 1.97×10^7 cells cm^{-3}, constituting ~12% of the total count. The depth distribution of dividing cells generally paralleled the total count. Below 275 mbsf, dividing cells were only significant in three samples at 316, 354, and 429 mbsf, although below 500 mbsf, numbers of dividing cells did increase, coinciding with an increase in the total bacterial population.

Two regions of Site 1027 were found to have consistently elevated bacterial numbers, both statistically ($P < 0.05$, analysis of variance) and in relation to the general bacterial depth trend at other ODP sites. The first of these was between ~166 and ~273 mbsf with total bacterial numbers maximizing at 1.31×10^6 cells cm^{-3} (223.84 mbsf). This region of elevated bacterial numbers was associated with a subsurface peak in pore-water ammonia (Fig. 4B) and alkalinity (data not shown,

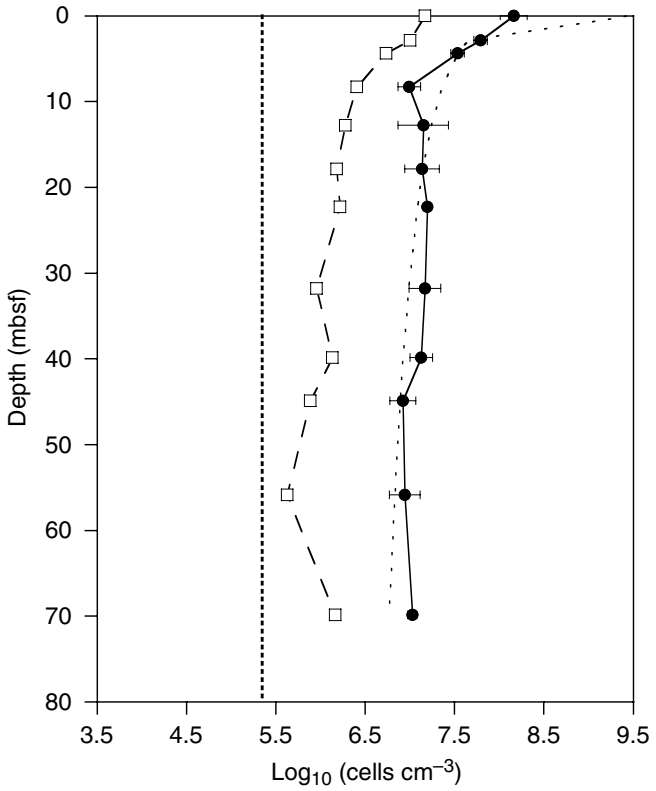

Figure 2. Depth distribution of total bacteria (solid circles) and dividing cells (open squares) to 70 mbsf at Site 1026. The dashed line represents a general regression line of bacterial numbers vs. depth in deep-sea sediments (Parkes et al., 1994). The bold vertical dashed line at $\log_{10}5.3$ is the calculated detection limit. Horizontal bars on the total bacterial data points are 95% confidence limits.

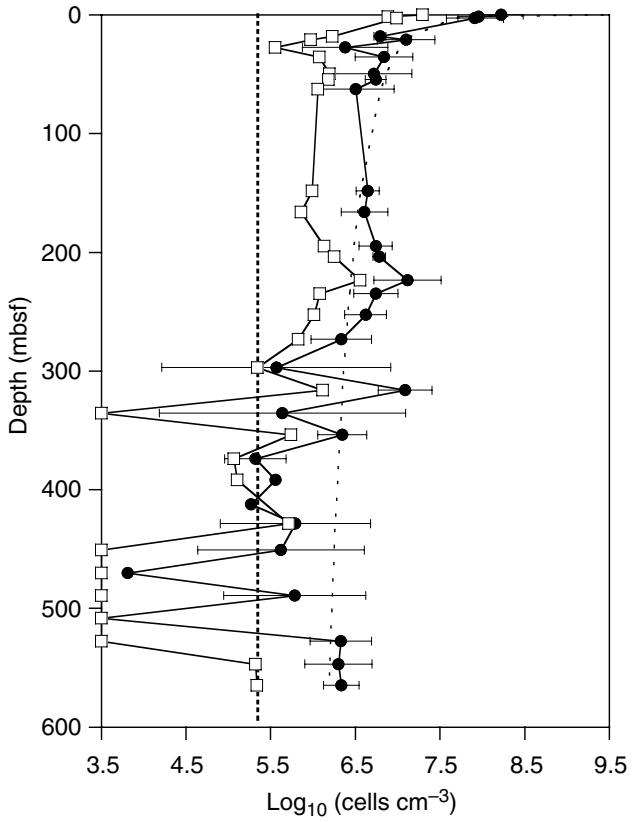

Figure 3. Depth distribution of total bacteria (solid circles) and dividing cells (open squares) to ~565 mbsf at Site 1027. The dashed line represents a general regression line of bacterial numbers vs. depth in deep-sea sediments (Parkes et al., 1994). The bold vertical dashed line at $\log_{10}$ 5.3 is the calculated detection limit. Horizontal bars on the total bacterial data points are 95% confidence limits.

but see Davis, Fisher, Firth et al., 1997). Ammonia and alkalinity formation is usually associated with bacterial degradation of organic matter (Claypool and Kaplan, 1974), and their continued production within the zone confirms the presence of active bacterial populations. The reason for this elevation in bacterial numbers is unclear because although organic carbon concentrations are higher in this zone than at ~100 mbsf, they are similar to those in the remainder of the hole. The second region with elevated bacterial numbers occurs at the bottom of the hole below ~527 mbsf. Bacterial populations in the three samples below this depth (527.84, 547.14, and 565.1 mbsf) are significantly larger ($P < 0.05$, analysis of variance) than in the zone immediately above (374.24–470.24 mbsf, eight samples) which are all very near or below (at 489.44 and 508 mbsf) the detection limit. The presence and increase in dividing cells, below ~520 mbsf, albeit to just at the detection limit, is coincident with the increased total count and together demonstrates that bacteria are active in this region (Fig. 3). During exponential bacterial growth the numbers of dividing cells increase and, hence, the presence of dividing cells should indicate growing and thus active cells. In water samples the percentage of dividing cells provide a reasonable estimate of bacterial growth rates (Newell and Christian, 1981) but for sediments it greatly overestimates growth (Fallon et al., 1983). Despite this, however, dividing cells in sediments do still indicate the presence of growing and thus active cells, particularly when the numbers of dividing cells significantly increase, as occurs below ~520 mbsf at this site, because stimulation of growth in deep sediment bacteria also results in an increase in the number of dividing cells both in the laboratory (Getliff et al., 1992) and in situ (Wellsbury et al., in press), and numbers of dividing cells correlate with an independent measure of growth, [^{3}H]-thymidine incorporation into bacteria DNA (Wellsbury et al., 1996).

The lower than expected bacterial numbers between ~374 and ~509 mbsf is difficult to explain because the total organic carbon concentration was essentially constant (~0.5 wt%) throughout the lower 300 mbsf of the site (Fig. 4). Total bacterial populations at Site 858 on Juan de Fuca Ridge (Leg 139, Cragg and Parkes, 1994) decreased markedly with depth at temperatures around 30°–40°C, and the temperature at 374 mbsf is within this temperature range. Possibly the combined stress of low organic carbon concentration, lack of sulfate as an electron acceptor (Fig. 4), and elevated temperature inhibits a proportion of the total bacterial population, resulting in "low" bacterial numbers in this region. The number of dividing cells as a percentage of the total population in this region, however, is surprisingly high at ~57% compared to the mean at all other depths (~23%), which indicates that a subset of the total population may actually be more adapted to these mesophilic temperatures (growth optimum 20°–45°C).

The increase in bacterial numbers below ~527 mbsf is associated with increasing pore-water sulfate concentrations from the underlying bedrock (Fig. 4; Davis, Fisher, Firth, et al., 1997). This suggests that deep bacterial populations are stimulated by the presence of a more efficient electron acceptor, sulfate, at thermophilic temperatures (~55°–60°C; Fig. 4). The original sea-water sulfate was removed below 200 mbsf during the first elevated zone of bacterial activity, and subsequent bacterial numbers were lower than expected. Coinciding with this deep elevation of bacterial populations was a

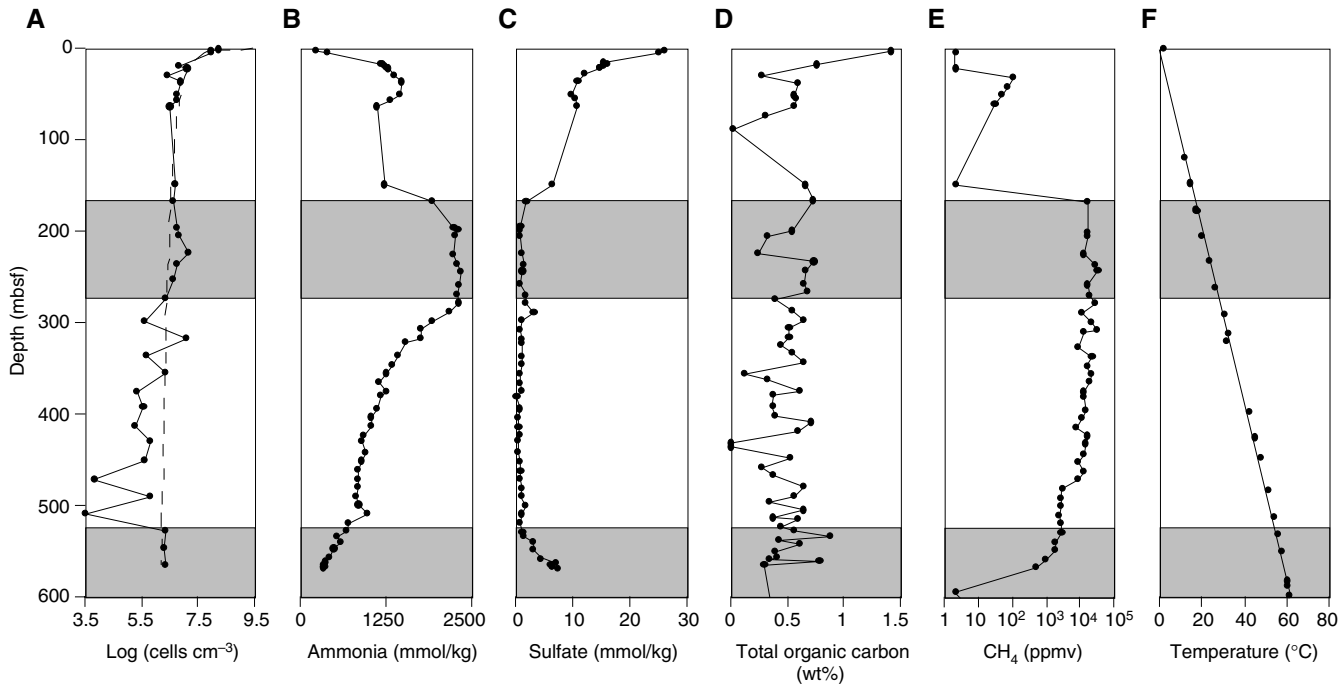

Figure 4. Geochemical and bacterial depth profiles to ~565 mbsf at Site 1027. **A.** Total bacterial count. The dashed line represents a general regression line of bacterial numbers vs. depth in deep-sea sediments (Parkes et al., 1994). **B.** Ammonia. **C.** Sulfate. **D.** Total organic carbon. **E.** Methane. **F.** Temperature. Horizontal lines and shading mark the regions of elevated bacterial populations. Geochemical data from Davis, Fisher, Firth, et al., 1997. Temperature data from Davis and Becker, in press, and Davis, Fisher, Firth, et al., 1997.

marked decrease in the CH_4 gas concentration (Fig. 4; Davis, Fisher, Firth, et al., 1997), suggesting that the sulfate enabled CH_4 to be used as a deep energy source. Although sulfate-reducing bacteria able to grow on methane have not been isolated, there is strong environmental and laboratory evidence for their involvement in anaerobic methane oxidation (e.g., Kosiur and Watford, 1979; Iversen and Jørgensen, 1985; Jørgensen et al., 1990; Hoehler et al., 1994). Stimulation of deep bacterial populations has been observed at a number of previous ODP sites (Cragg et al., 1990; 1992; 1996; Wellsbury et al., 1997), and these are often associated with increases in deep sulfate and methane oxidation.

SUMMARY

Bacterial depth distributions at Site 1026 and the top ~300 mbsf of Site 1027 are consistent with previously analyzed ODP sites. Generally, both the total count and dividing cell count initially decreased rapidly with depth, and then more gradually to the base of the hole. At Site 1027 there were two regions with elevated bacterial populations compared to the surrounding sediment and a zone (~374–509 mbsf) with lower than expected bacterial numbers. The upper region of elevated populations was associated with a peak in ammonia and alkalinity. The second region of increased bacterial numbers occurred at the base of the hole (>520 mbsf). This increase was associated with the diffusion of sulfate from the underlying bedrock and removal of CH_4, suggesting stimulation of deep bacteria by anaerobic methane oxidation, as has been documented at some other ODP sites. In addition, aspects of the bacterial distributions seemed to indicate the sequential presence of different bacterial temperature groups from the near-surface psychrophilic bacteria to the deeper thermophilic populations.

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