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2. Bacterial Profiles in a Sulfide Mound (Site 1035) and an Area of Active Fluid Venting (Site 1036) in Hot Hydrothermal Sediments from Middle Valley (Northeast Pacific)[1]

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ABSTRACT

Sediment samples (1 cm^3 each) were obtained from two sites (Ocean Drilling Program [ODP] Sites 1035 and 1036) in the Middle Valley of the northern Juan de Fuca Ridge for direct microscopic determination of bacterial depth distributions in a region influenced by hydrothermal activity. These data were compared to data gathered during Leg 139, Site 858, at the same location. Site 1035 was cored to 170 meters below seafloor (mbsf), and significant numbers of bacterial cells were detected in most samples with 4×10^5 cells/cm^3 at the base of the hole. Dividing and divided cells were only found above 64 mbsf. The temperature at the base of the hole was estimated at ~113°C—the current estimated upper temperature limit for bacteria. When the data were divided according to growth-temperature ranges of bacteria (mesophile = 10°–45°C, thermophile = 45°–80°C, and hyperthermophile = >80°C), the bacterial profile clearly displayed three bands of bacterial populations. Only populations in the upper mesophilic section of the hole agreed with a general population profile obtained from many other ODP legs. At higher temperatures bacterial populations were markedly lower than this general profile.

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Samples from Holes 1036A, 1036B, and 1036C all showed reduced populations when compared to the general profile. The deepest reliable enumeration was at 30 mbsf in Hole 1036B with 5×10^5 cells/cm^3. Bacteriological sampling from Hole 1036C stopped before very high temperatures were encountered in the borehole; however, at Holes 1036A and 1034B, samples from temperatures apparently >200°C were obtained. Bacterial populations decreased rapidly from the surface and became nondetectable at ~110°C, but, at ~155°–185°C, intact cells were observed. This was similar to data from Site 858, where intact bacterial cells were also detected in this temperature range. Analysis of geochemical data suggested, however, that the reasons for the presence of these cells may be different. For Site 858, bacterial cells could be explained by a constrained lateral flow of entrained seawater carrying cells from shallower sediments down into the hot sediments containing hydrothermal fluids. This was not the case at Site 1036, where rapid seawater recharge occurred throughout the depths where bacteria were detected, which may have distorted, and significantly reduced, the assumed temperature profile. These populations appear to exist in a thermophilic/hyperthermophilic environment at the edge of hydrothermal sediment layers. There was some chemical evidence of in situ bacterial activity and they may be utilizing the products of hydrothermal alteration (e.g., methane) rising up from deeper sediment layers.

INTRODUCTION

Previous research on deep marine sediments obtained from the Ocean Drilling Program (ODP) has conclusively demonstrated the presence of a deep bacterial biosphere to at least 750 meters below seafloor (mbsf) (Whelan et al., 1985; 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, 1996, 1997, 1998; Wellsbury et al., 1997). Additionally, indirect chemical evidence (e.g., chemical changes in pore water and isotopic evidence) corroborates the evidence that microbial activity is continuous to considerable sediment depths. In the majority of sediments, high temperatures do not impose a limit on bacterial activity because the thermal gradient of the Earth's crust is ~10°–40°C/km and bacteria can grow at temperatures of 113°C (Huber et al., 1989; Pledger and Baross, 1991; Stetter et al., 1990; Blochl et al., 1997). Nevertheless, in areas of high geothermal activity such as mid-ocean ridges and subduction zones, where there may be diffuse or focused hydrothermal fluid flow, temperatures in excess of 350°C can occur. This exceeds the growth temperature of even the most hyperthermophilic bacteria ("hyperthermophile" is defined as bacteria able to grow above 80°C) and hence limits bacterial distributions (Karl, 1985; Karl et al., 1988; Stetter et al., 1990).

Venting chimneys, vent fluids, the surrounding water, and adjacent shallow sediments of hydrothermal systems have been microbiologically investigated and a number of extreme thermophiles and hyperthermophiles isolated (Baross and Deming, 1985; Jannasch and Mottl, 1985; Deming and Baross, 1986; Fiala and Stetter, 1986; Jannasch et al., 1988; Karl et al., 1988; Huber et al., 1989, 1990; Stetter et al., 1990; Straube et al., 1990), including samples from the Juan de Fuca Ridge (Pledger and Baross, 1989, 1991; Reysenbach and Deming, 1991). However, deeper sediment layers have been little studied. One investigation at the Juan de Fuca Ridge (Cragg and Parkes, 1994) showed, with two

exceptions, no bacterial cells below ~16 mbsf, or at >76°C, in four holes at Site 858. The two exceptions were in two "hot" holes (Holes 858B and 858D), where a single occurrence of significant bacterial populations (>5 × 10^6 cells/cm^3) was measured in each hole at ~17 mbsf at an estimated in situ temperature of 165°–170°C, well above the current documented upper survival temperature for bacteria. Contamination during drilling was not a likely explanation for the presence of these bacteria, and Leg 169 represented an opportunity to revisit Site 858 and re-examine bacterial distributions in these deep, high-temperature hydrothermal sediments.

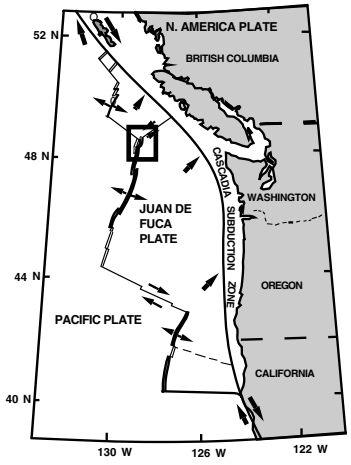
MATERIALS AND METHODS

Site Descriptions

Sites 1035 and 1036 are in Middle Valley on the northern Juan de Fuca Ridge off the northwest coast of North America (Fig. F1). Hole 1035A is 75 m west of the center of the Bent Hill Massive Sulfide deposit. The mound is ~300 m in diameter, extensively weathered to iron oxyhydroxides, and partially buried by sediment (Fouquet, Zierenberg, Miller, et al., 1998). The mineralogical composition and primary sulfide textures (Goodfellow and Peter, 1994) suggest that this deposit was formed by fluids venting at 350°–400°C at or near the seafloor. The current temperature gradient is ~1.24°C/m.

Site 1036 is situated on the Dead Dog active hydrothermal mound, an area previously cored as Site 858. Hole 1036A was cored ~9 m west, Hole 1036B ~37 m northwest, and Hole 1036C ~71 m northwest of an active vent discharging water at 268°C (Fouquet, Zierenberg, Miller, et al., 1998). The thermal gradients at this site are estimated at 12°C/m, 6°C/m, and 3°C/m, respectively (Fouquet, Zierenberg, Miller, et al., 1998).

F1. Location map of the Juan de Fuca plate, p. 13.



Shipboard Handling

A total of 59 1-cm^3 sediment samples were taken: Hole 1035A, 25 samples from the surface to 168.2 mbsf; Hole 1036A, 10 samples from the surface to 26.5 mbsf; Hole 1036B, 16 samples from 1.5 to 46.1 mbsf; and Hole 1036C, 8 samples from the surface to 31.5 mbsf. Samples were removed from the cut 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 ejected 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 plug.

Laboratory Handling

Direct microscopic observation of bacteria by Acridine orange staining was based on the general recommendations of Fry (1988). Between 5 and 50 µL of formaldehyde-preserved subsample was stained with Acridine orange (50 µL of 1 g/L solution) in 10 mL of filter-sterilized (0.1-µm pore size) 2% formaldehyde for 3 min and then filtered through a polycarbonate (0.2-µm pore size) membrane (Costar, High

Wycombe, United Kingdom). The membrane was then rinsed with 10 mL of 2% filter-sterilized formaldehyde and mounted in a minimum of paraffin oil under a coverslip.

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. Three replicate filters were prepared from each sample to minimize count variance (Kirchman et al., 1982). Where replicate $\log_{10}$ counts differed by more than 0.5 log units, an additional replicate filter was prepared. A minimum of 200 fields of view, and up to 400 fields of view, was counted when a total of <20 cells was encountered during enumeration of a single membrane filter. The number of bacterial cells counted on particles was doubled to account for cells hidden from view (Goulder, 1977). Dividing cells were defined as co-joined cells of identical morphology with an invagination between the two cells. Divided cells were defined as two adjacent cells of identical morphology with a space visible between the cells. Where zero bacterial cells were encountered on a membrane, then the results from all membranes of that particular sample were combined for calculation. In some instances this meant population estimations were based on the observation of >1200 fields of view. A consequence of this approach, however, was that population estimations were very low and variable, meaning that statistical variance could not be calculated. Thus, these data, although reported, all fall below the significance level and are regarded as unreliable. Reagent and membrane filter blanks were regularly counted, and bacterial numbers were calculated after subtraction of the appropriate blanks. The detection limit was estimated at 7×10^4 cells/cm^3. This particularly low limit was a result of the use of large volumes of sample on each membrane (to 50 µL).

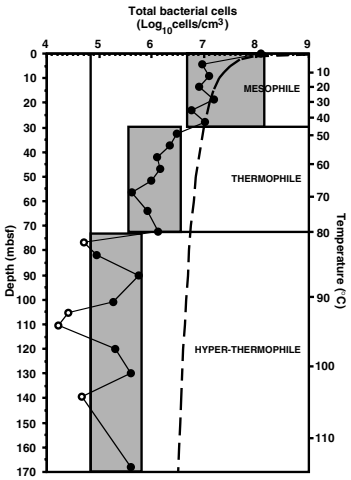
RESULTS AND DISCUSSION

Site 1035

Bacteria were detected in all samples examined (Fig. F2), although in four samples the results were considered unreliable. The greatest population was at the near surface with 1.22×10^8 cells/cm^3. Thereafter, numbers of bacteria declined rapidly; the smallest reliable population was found at 81.7 mbsf, with 9×10^4 cells/cm^3 representing only 0.07% of the near-surface population. Dividing and divided cells (DDC) were detected only in the upper sections of Hole 1035A above 64 mbsf (data not shown). The overall average percentage DDC was 7.6%, although where DDC were detected in every membrane count (<28 mbsf) they represented 9.1% of the total numbers; however, where they were observed intermittently (28–64 mbsf), DDC represented only 6.1% of the total bacterial numbers.

Above 28 mbsf, the total bacterial profile was consistent with the general regression line obtained from a number of ODP sites worldwide: Log_{10} numbers of bacteria = 7.98 – 0.57 × Log_{10} depth (m), ($R^2 = 0.561$, $N = 829$). However, below 28 mbsf the profile significantly deviated below predicted levels. In situ temperatures have been estimated by extrapolation below the last temperature measurement at 55 mbsf, which was 69.4°C (Fig. F2). Despite the dangers of extrapolation, the measured temperatures produced a very tight depth relationship, and the extrap-

F2. Depth and temperature distribution of total bacterial cells at Hole 1035A, p. 14.



olated temperature and confidence limits for the deepest sample (170 mbsf) was estimated at 115°C ± 8°C.

Bacteria are classified into broad groups based on their temperature growth range and optimum growth temperature. Mesophiles grow above 10° to ~45°C, thermophiles from ~40° to 80°C, and the more recently encountered hyperthermophiles above 80°C (Brock et al., 1997). Interestingly, when the bacterial profile of Hole 1035A was divided according to these temperature boundaries, it clearly separated into three distinct zones (Fig. F2). The upper mesophile zone had a mean bacterial population of 1.40×10^7. This decreased to 1.24×10^6 in the thermophile zone (~91% reduction) and to 2.6×10^5 in the hyperthermophile zone (a further ~80% reduction). The sequential reduction in bacterial numbers was statistically significant in both cases ($t = 5.537$, $N = 15$, $P < 0.0005$; and $t = 4.555$, $N = 14$, $P < 0.0005$, respectively, where t was obtained from a Student's t-test and P = probability). The decrease in cell numbers with temperature is consistent with the fact that thermophiles and hyperthermophiles represent consecutively smaller and smaller subsets of the original bacterial population at mesophilic temperatures. Additionally, despite presumably being adapted to their in situ temperature, they are inhabiting an increasingly hostile environment as bioavailable carbon concentrations decrease rapidly with depth; thus, an enforced reduction in activity would be reflected in a lower total biomass. The DDC percentage also split their distribution around the mesophile/thermophile boundary at 28 mbsf by being consistently present at ~9.1% above 28 mbsf and only intermittently present at ~6.1% below 28 mbsf.

Clearly, this interpretation of the data is not the only one possible; however, temperature does appear to be the dominant variable at this hole. Apart from 9 m of clastic sulfides at the top of Hole 1035A and a 2-m interval of massive sulfide at 55 mbsf, the lithostratigraphy was essentially uniform to ~170 mbsf with hemipelagic and turbiditic sediments containing anhydrite veins. Induration increased with depth (Fouquet, Zierenberg, Miller, et al., 1998). Additionally, a silicified layer at ~170 mbsf was interpreted as an impermeable barrier to hydrothermal fluids. Pore-fluid chemistry above this depth was relatively constant with no indication of significant lateral flow and was consistent with seawater subjected to gradually increasing hydrothermal alteration with depth, with no indication of the presence of vent fluids (Fouquet, Zierenberg, Miller, et al., 1998).

Temperature would therefore seem to be the controlling factor for bacterial distribution at this site, as analysis of interstitial water (IW) and solid phase chemistry provided no consistent correlation with bacterial populations that could account for the significant stepwise reductions in bacterial numbers at ~30 and ~72 mbsf (data not shown). Alkalinity gradually increased from 2.45 mM at the near surface to 4.12 mM at 24.5 mbsf and then declined to 0.92 mM at 56 mbsf before rapidly increasing to >9 mM by ~100 mbsf. Similarly, NH_4 increased from 0 µM at the near surface to 610 µM at 31 mbsf before declining to ~100 µM at 54 mbsf and then gradually increasing to >1400 µM by ~170 mbsf. Interstitial water sulfate concentrations required correction for anhydrite dissolution (Fouquet, Zierenberg, Miller, et al., 1998), but sulfate was more or less constant from the near surface to 31 mbsf at 27–28 mM. Thereafter, it gradually decreased to ~4 mM by ~110 mbsf. However, because sulfate concentrations >3 mM are generally not limiting to sulfate-reducing bacteria (Capone and Kiene, 1988), it seems unlikely that the reduced sulfate concentration would limit them above ~110

mbsf. Even if it did, they would be replaced by other terminal oxidizers such as methanogens. In addition, sulfate-reducing bacteria normally represent only a small proportion of the total bacterial population (Cragg et al., 1990, 1992); thus, it is unlikely that low sulfate concentrations could be responsible for the decrease in total bacterial numbers around 100 mbsf and below. A cause for decreases in bacterial numbers can be depletion of buried, bioavailable carbon. At this site, however, the total organic carbon concentration (TOC) was always low. With the single exception of the near surface, all measurements were below 0.65%; with the further exception of two other points in the upper 24 mbsf, the remainder of the measurements were all less than 0.4%. The average concentration below 24 mbsf was relatively constant at 0.25%–0.35% with very little variability, and below 112 mbsf TOC concentrations dropped to an average of only 0.1% to 170 mbsf.

Site 1036

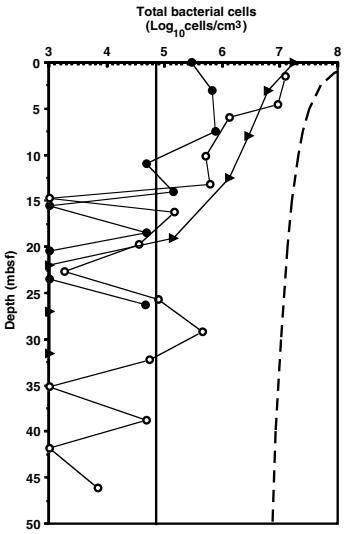
Bacterial populations were, generally, present only in the upper 20 m at this site (Fig. F3). At the sediment surface, all populations were significantly lower than expected, with both Holes 1036B and 1036C having ~1.4 × 10^7 cells/cm^3 and Hole 1036A having much reduced numbers at 2.8 × 10^5 cells/cm^3, some 2% of the population seen at the other two holes. These reduced populations may be because of their proximity to the active vent. Sediments near vents tend to suffer significant fallout of metal/mineral particles, producing an environment that can be severely depleted in organic carbon and high in heavy metals and, hence, toxic to bacteria (Juniper and Tebo, 1995). Additionally, the effect of seawater being pulled across the seafloor for entrainment into the base of the plume leaves these areas sediment and organic matter starved (Winn et al., 1995). The particularly low numbers in Hole 1036A may be caused by high bottom-water temperature because this hole was only 9 m from the active vent, although this cannot be confirmed because no temperature measurements were made at the seafloor.

Dividing cells were only present in the cooler, upper sections of these holes and represented an overall average of ~11.5% of the total count (data not shown); however, because of low total numbers of bacteria, these data were very variable and probably represent an overestimate. Dividing cells were not detected below 7.5 mbsf, 13.2 mbsf, and the surface in Holes 1036A through 1036C, respectively.

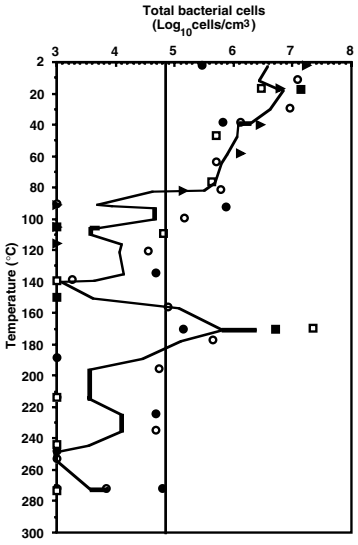
The temperature increase with depth differed widely for each hole at this site, related to the respective distance from the active vent. Thus, these bacterial profiles were plotted against temperature to give a clearer indication of the effects of increasing temperature at depth (Fig. F4). This approach produced a much tighter data distribution from the assumed 2°C sediment surface to ~110°C. Bacterial profiles from Holes 858B and 858D taken during Leg 139 some 5 yr previously also fitted into this temperature distribution and enhanced the data set (Fig. F4).

Between ~155° and 185°C, significant bacterial populations were detected in four of the five holes (Holes 1036A, 1036B, 858B, and 858D), but not in Hole 1036C (Fig. F4). The latter was because Hole 1036C was not sufficiently deep to reach these high temperatures. The presence of bacterial cells at such high temperatures was unexpected because the generally accepted temperature maximum for bacteria is ~113°C (Blochl et al., 1997). Nevertheless, finding cells at these temperatures in cores from all four holes in the same hydrothermal location, taken as two

F3. Depth distribution of total bacterial cells at Holes 1036A, 1036B, and 1036C, p. 15.



F4. Temperature distribution (a function of depth) of total bacterial cells at Sites 1036 and 858, p. 16.



samples 5 yr apart, strongly suggests that these are real data and a consistent feature rather than artifacts.

The possibility that these bacteria were an effect of the coring procedure has to be addressed. It is unlikely that their presence was the result of contamination from sediment from above, because at both sites positive samples were obtained below sediment layers where no cells were detected (Figs. **F3, F4;** Cragg and Parkes, 1994). Elevated bacterial populations might, however, still be introduced into deeper layers if the sediment becomes harder and the coring procedure changes to one prone to contamination from drilling fluids. No change in coring occurred in three of the holes, and all three samples that contained deep, high-temperature populations were taken within sequences drilled by the advanced hydraulic piston corer (APC), which recovers sediment ahead of the coring bit. Only in Hole 1036B did a coring changeover occur from the APC to the extended core barrel (XCB), which recovers sediment closer to the coring bit than the APC. The second of two significant high-temperature bacterial enumerations from Hole 1036B occurred at this changeover (Fig. **F3**).

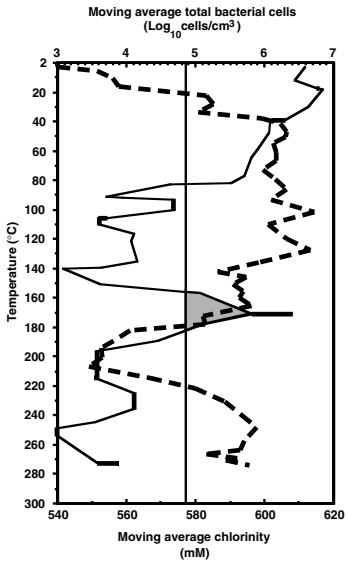
The production of microspheres of protein and nucleic acids at high temperatures (White, 1984; Yanagawa and Kojima, 1985) also seems unlikely as an explanation because this cannot account for the presence of dividing cells in two of the four sets of samples (Holes 858B and 858D) or for the rapid disappearance of such cell-like structures as temperature further increased with sediment depth. The absence of dividing cells in the samples from Holes 1036A and 1036B was not unexpected because total bacterial populations were significantly lower than in Holes 858B and 858D and because the average DDC, where present, was only 11.5% of the total population, which would have been undetectable.

The presence of bacteria at these depths and temperatures does not demonstrate that they were living under these conditions, as they may have been transported from cooler regions into the sediments where they were found by lateral flow, or draw down of entrained seawater.

Both Holes 858B and 858D are influenced by lateral fluid flow below a constraining hydrothermally cemented layer acting as a cap (Davis, Mottl, Fisher, et al., 1992). The effect of seawater recharge into these sediments should be visible through reductions in IW chlorinity. Chlorinity data for Holes 858B and 858D were combined and plotted as a moving average against temperature for comparison with the total bacteria profile (Fig. **F5**). Clearly, there is evidence of seawater ingress between 165° and 245°C with chlorinity concentrations the same as those of bottom seawater (~550 mM) between ~190 and 210°C, although the effect of moving average calculation will mask what is a wider band than 20°C. It is interesting that cells were detected in the upper portion of this seawater zone where there is a mix of hydrothermal fluid and seawater. It seems probable that bacteria in this low-chloride zone are directly derived from seawater and/or swept in from cooler sediments along the seawater's flow path. Although most bacteria would be lysed as a result of high temperature, some bacteria may have survived as intact cells at the lowest temperatures of this zone (155°–185°C; Fig. **F5** [shaded area]). Alternatively, it is interesting to speculate that bacteria in this zone may occupy a unique maximum high-temperature habitat provided by mixing of seawater and hydrothermal fluid.

Bacterial profiles from Holes 1036A and 1036B show the presence of intact cells within the same high-temperature zone as for Holes 858B and 858D. Interstitial water chemical data at Hole 1036A showed a distribution of conservative ion species above 20 mbsf (<240°C) similar to

F5. Temperature distribution of combined total bacterial cells from Holes 1036A, 1036B, 858B, and 858D, p. 17.

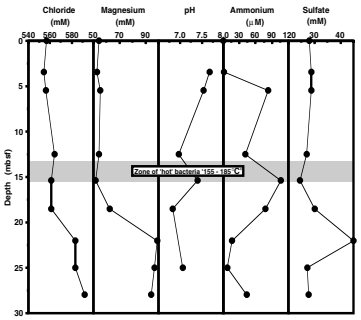


that of seawater, suggesting that these sediments were rapidly recharged with seawater (Fig. F6). Between 20 and 30 mbsf IW chemistry was consistent with intense lateral flow of hydrothermally altered seawater above a capping layer separating these fluids from the hydrothermal fluids below 33 mbsf. At Hole 1036B, pore fluids were essentially entrained seawater to 40 mbsf (<240°C; Fouquet, Zierenberg, Miller, et al., 1998), with significant hydrothermal alteration of fluids occurring from ~30 mbsf. Thus, in Holes 1036A and 1036B all of the data were obtained only from sediments with heated seawater pore fluids, and there is no zone of seawater recharge below a zone of hydrothermal, high chlorinity fluids (such as that found at Site 858) to explain the sudden reoccurrence of cells at ~170°C and ~15 mbsf in Hole 1036A and ~30 mbsf in Hole 1036B. Indeed, if bacterial cells were present at these depths it might be expected that they should occur at all depths to the maximum depth at which they were detected rather than occur to ~100°C and then, after an absence, again at ~170°C.

Temperature was not actually measured at Site 1036, and the gradients were estimated from the first occurrence of anhydrite, which undergoes dissolution at temperatures less than ~120°C (Fouquet, Zierenberg, Miller, et al., 1998), which was ~9.5 mbsf in Hole 1036A. At 15 mbsf, where "high-temperature" bacterial cells were observed, there were increases in pH and ammonium and a decrease in sulfate concentration (Fig. F6), all usually indicative of bacterial heterotrophic and sulfate-reduction activity. However, at these temperatures, production of ammonium by hydrothermal alteration of organic matter may be more likely. There was also an increase in thermogenic methane concentration (to 500 ppmv) at 18.5 mbsf, which could be a potential carbon source for bacteria as TOC concentrations were only 0.11% below 18.5 mbsf (Fouquet, Zierenberg, Miller, et al., 1998).

As the bacterial profile for Hole 1036A was totally within a high-flow seawater recharge zone and the temperature profile was based on the estimated depth of a single isotherm, it is quite possible that the temperature profile was nonlinear. Rapid inflow of cold seawater could maintain temperatures well below the ~170°C at 15 mbsf, low enough for continued bacterial existence. In this situation, a thermophilic or hyperthermophilic bacterial population could inhabit the edges of a low–organic carbon, high-temperature zone being continually recharged with sulfate from both above and below and by methane from below. This would be consistent with the presence of bacterial cells and changes in pore-fluid chemistry suggesting bacterial activity at 15 mbsf (Fig. F6) and compatible with data from Holes 858B and 858D, where lateral flow of entrained seawater is linked with the presence of bacteria at an apparent temperature of ~170°C.

F6. Interstitial water geochemistry at Hole 1036A, p. 18.



SUMMARY

Significant bacterial populations were detected to ~170 mbsf in Hole 1035A and to a maximum of 30 mbsf in three holes at Site 1036. The distribution of bacteria at Site 1035 suggested that the profile demonstrated mesophilic, thermophilic, and hyperthermophilic preferred growth temperature ranges. At Site 1036, the distributions of bacteria were similar to those observed previously at Leg 139 Site 858 (the same location 5 yr previously that had a small number of samples containing bacterial cells at an unexpectedly high 155°–185°C). For Leg 139 Site 858, analysis of the data indicated that the presence of cells in, appar-

ently, very hot sediments of Site 858 may be linked to seawater ingress beneath geothermal fluids and lateral flow at depth below constraining hydrothermally altered sediments. Conversely, at Site 1036, hydrothermal fluids were not evident until well below the depth of the last detectable bacterial cell, and the entire upper zones were rapidly recharged with seawater. Here, it is possible that the thermal gradient may not be linear and that these deep bacteria may be alive in situ, living in cooler than predicted pore fluids, on the edge of the hydrothermal fluids, and possibly benefiting from hydrothermal alteration of sediments below them.

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Figure F1. Location map of the Juan de Fuca plate. The area containing Sites 1035 and 1036 is enclosed by the rectangle (from Westbrook, Carson, Musgrave, et al., 1994).

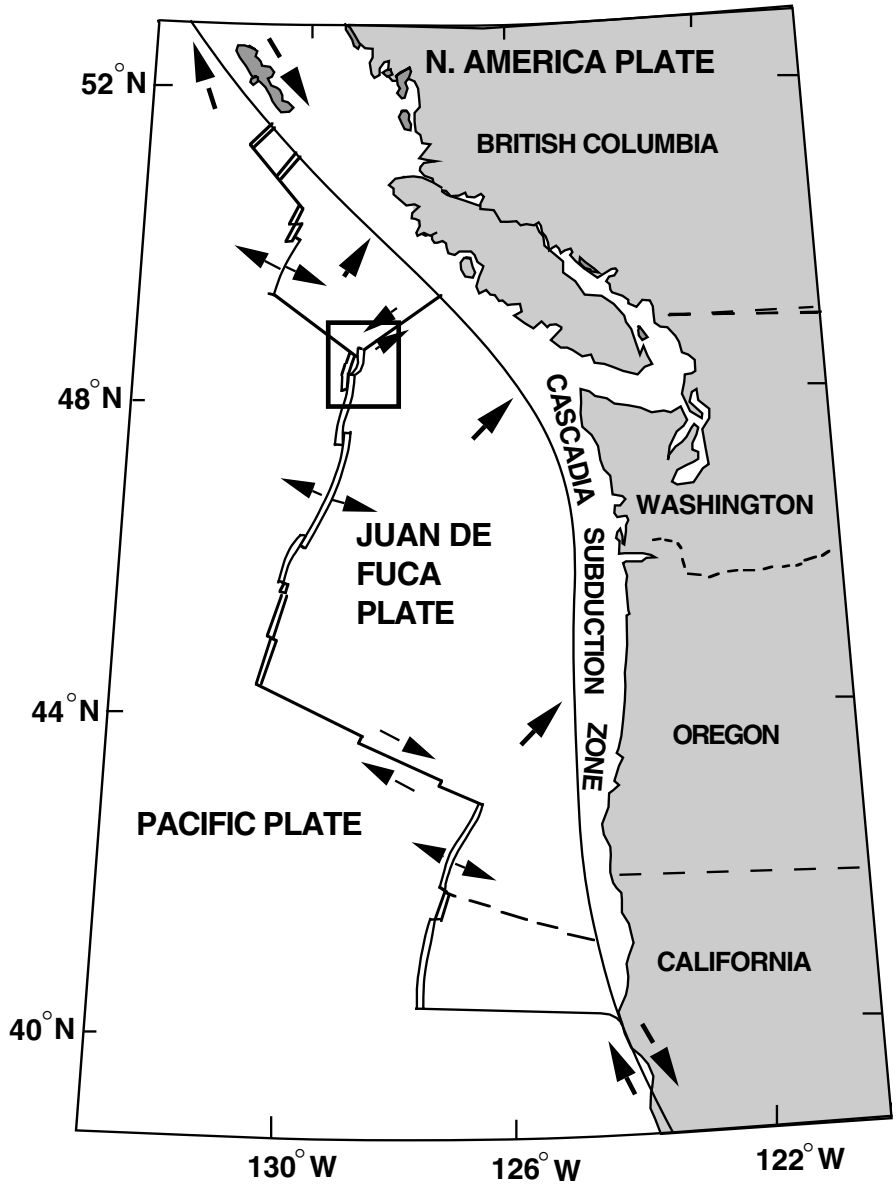

Figure F2. Depth and temperature distribution of total bacterial cells at Hole 1035A. Vertical line = the significance limit, and open circles = unreliable bacterial enumerations. Dashed line = the general ODP regression line for bacterial population numbers against depth expressed by Log_{10} bacterial numbers = 7.98 – 057 × Log_{10} depth (m) (R^2 = 0.561, N = 829). The three shaded areas putatively identify three temperature-limited groups of bacteria: mesophiles (~10°–45°C), thermophiles (40°–80°C), and hyperthermophiles (>80°C). Temperatures >70°C were obtained by extrapolation.

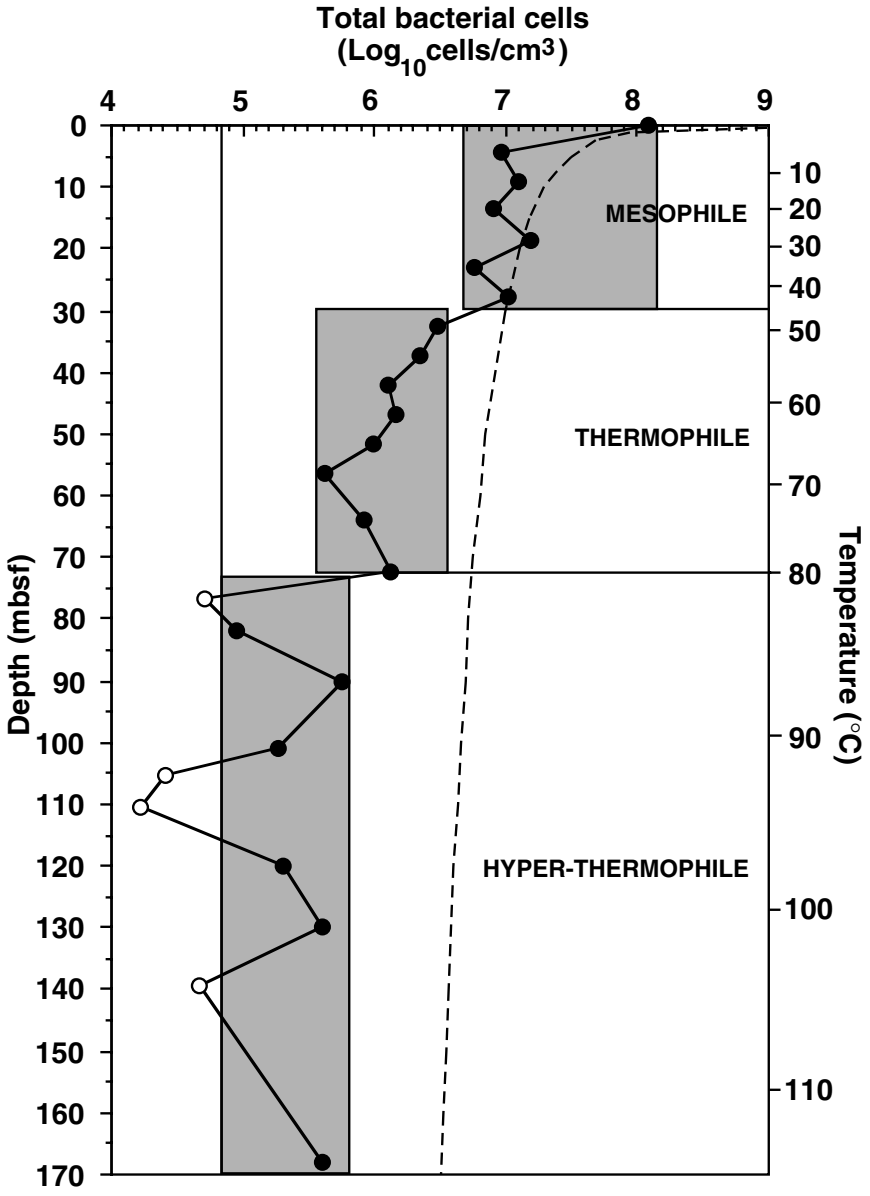

Figure F3. Depth distribution of total bacterial cells at Holes 1036A (solid circles), 1036B (open circles), and 1036C (solid triangles). Vertical line = the significance limit and all data to the left of this line are unreliable. Dashed line = the general ODP regression line for bacterial population numbers with depth expressed by: Log_{10} bacterial numbers = 7.98 – 057 × Log_{10} depth (m) (R^2 = 0.561, N = 829). Symbols on the vertical axis indicate no bacteria detected.

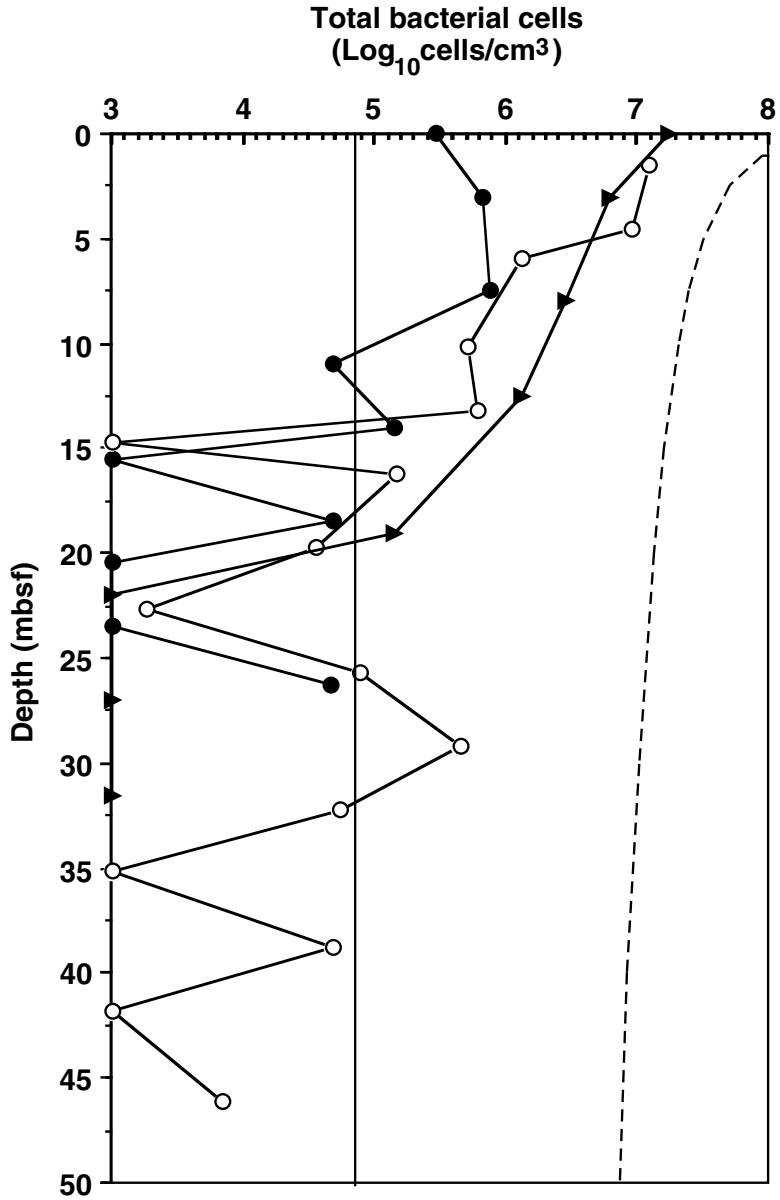

Figure F4. Temperature distribution (a function of depth) of total bacterial cells at Holes 1036A (solid circles), 1036B (open circles), 1036C (solid triangles), 858B (solid squares), and 858D (open squares). Heavy line = the moving average calculated from all data shown. Vertical line = the significance limit and all data to the left of this line are unreliable. Symbols on the y axis = no bacteria detected.

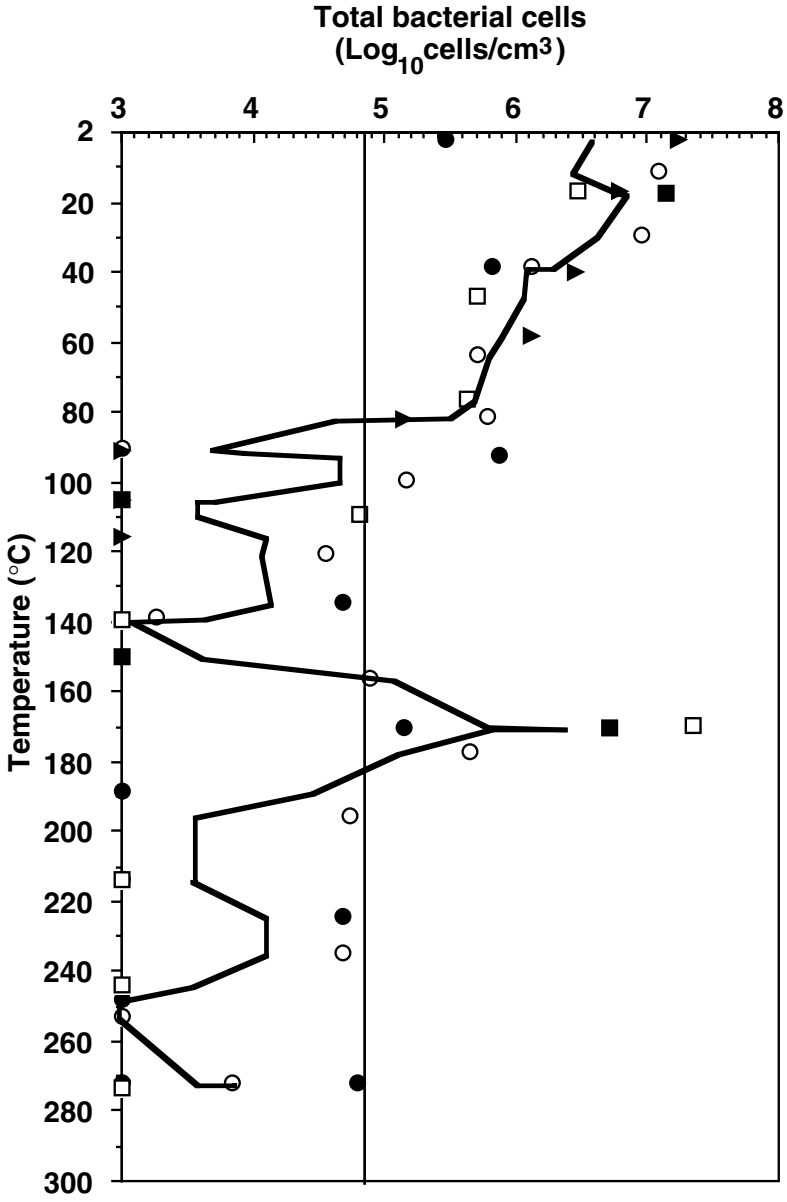

Figure F5. Temperature distribution of combined total bacterial cells from Holes 1036A, 1036B, 858B, and 858D expressed as a moving average (solid line) and interstitial water chlorinity (Holes 858B and 858D only), also expressed as a moving average (dashed line). Shaded zone = the presence of significant numbers of bacterial cells at very high temperature and rapidly decreasing chlorinity. Vertical line = the significance limit, and all microbiological data to the left of this line are unreliable.

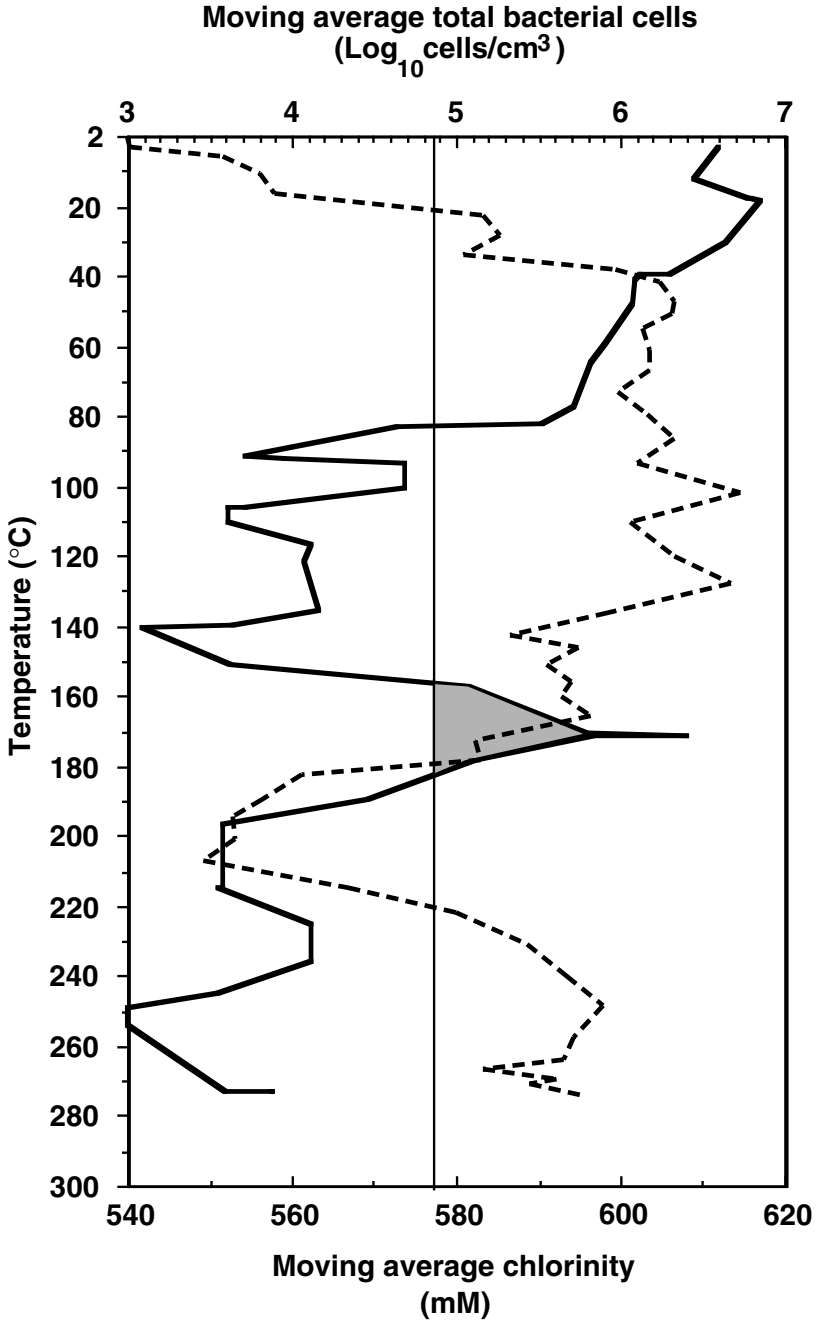

Figure F6. Interstitial water geochemistry at Hole 1036A.