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# 37. LIVING BACTERIA IN ANTARCTIC SEDIMENTS FROM ODP LEG 119[1]

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**ABSTRACT**

A feasibility study was made of the shipboard determination of living microbial cell densities in deep-sea sediments using fluorescence microscopy and the DAPI staining technique. In sediments down to 38 m below seafloor the number of living bacterial cells per milliliter of sediment ranged from near 0 to near $10^6$, which is well within the range of microbial densities reported from aquatic and sedimentary environments elsewhere. We suggest that this method be used on future ODP drilling cruises, where recovery of carbon-enriched ancient sediments is expected, to verify the long-term diagenetic influence of living microbes in black shale sequences.

## INTRODUCTION

Results from studies of the diagenetic alteration of sediments, particularly those enriched in organic carbon, strongly indicate that microbial breakdown of organic matter after burial is an important and widespread process (e.g., Irwin et al., 1977; Berner, 1981; Thierstein and Roth, in press). The possibility of encountering organic carbon-rich sediments of Mesozoic age along the continental margin of Antarctica bore the potential of verifying the presence of surviving microbes in ancient marine sediments at considerable burial depths. As an exploratory study we therefore prepared for an investigation of major changes in bacterial cell densities using a standard fluorochrome staining technique for active DNA (Coleman, 1980; Porter and Feig, 1980). Unfortunately, no organic carbon-rich sediments of Mesozoic age were recovered on Ocean Drilling Program (ODP) Leg 119. Nevertheless, for documentation purposes we report here the results of our study of the relatively young sediment samples.

## METHODS AND TECHNIQUES

### Sampling

Water and sediment samples were taken with a disposable, sterile 1-mL syringe immediately after the cutting of the core liner on the catwalk next to the drill floor. A few sediment samples from within the cores were taken in a similar way upon splitting the cores into two halves, which was usually done about 2–3 hr after arrival of the cores on deck.

Samples were drawn after we pushed the tip of the syringe about 0.5–1 cm below the accessible sediment surface to minimize contamination derived from the core liner or outside air. Between 0.1 and 0.5 mL of the sample was then transferred to a sterile 2-mL polyethylene container with a screw cap. The microbes were preserved by adding 0.2 mL formaldehyde solution (37%–40%). To disperse bacteria from clay surfaces, 0.2 mL of 1% hexa-meta-phosphate (Calgon) solution was added. The sample container was then filled with filtered (0.2-$\mu$m pore size), sterilized water to a total volume of 2 mL. After this preparation, the samples were stored for up to 2 days in the refrigerator at 4°C before staining was done. Storage at that point should be possible without alteration for at least 2 weeks (Porter and Feig, 1980).

### Preparation of the Filter System

Nucleopore polycarbonate filters (Hobbi et al., 1977) with a pore size of 0.2 $\mu$m and a diameter of 13 mm were dyed overnight in a irgalanblack-acetic acid solution (2 g/L of 2% acetic acid). The filters were then washed with sterile water. The stained filters were mounted wet onto an in-line filter holder (Millipore-Swinnex 13) with a diameter of 13 mm and then closed. The filter holder gaskets had been stored sterile in concentrated methyl-alcohol. Handling of the filters was done with sterilized tweezers.

### Staining

A stock solution of 1 mg DAPI (4′,6-Diamidino-2-phenylindol-dihydrochlorid monohydrate, puriss.) per milliliter $H_2O$ was prepared, which can be stored refrigerated for months. From this stock a dilute solution with a concentration of 10 $\mu$g per milliliter $H_2O$ was prepared, which is preservable for 2 weeks. After we added 0.2 mL of this solution to the sample, the vial was closed again, the sediment homogenized by shaking, and then left for 10 min at room temperature in the dark. Immediately afterward, 1 mL of the supernatant of the sample was siphoned off with a sterile syringe and pressed through the previously prepared irgalanblack polycarbonate filter. Subsequently the filter was washed once or twice with sterile $H_2O$ and mounted wet on a microscope glass slide by adding a drop of $H_2O$ on the filter and sealing the cover glass with nail polish. These slides can be stored refrigerated for 24 weeks without any observable alteration (Porter and Feig, 1980).

### Microscopy

The DNA-DAPI compound fluoresces bright blue (main emission around 390 nm) when stimulated by fluorescent light at 365 nm and with a filter cut off of 430 nm (Porter and Feig, 1980). We mounted a Zeiss fluorescence attachment consisting of a condenser (Zeiss model 466300), reflector housing (466301), filters (487701) with housing (467259), collector 100 (467274), and an illumination system (468032, 392642, and 381619) to the shipboard Zeiss Standard WL microscope. On the water-mounted slides the bacteria appeared as small (1–2 $\mu$m) blue dots or sticks, individually or as chains and agglomerates. The number of individual cells was determined (using a Plan-Neofluar 63× phase objective) in 10 subsequent fields of view, which represented an area of 0.491 mm$^2$ (i.e., 1/270 of the total filter area). This choice was a compromise between time available for preparation and study of samples on board and statistical reliability of results. By making a ratio of the counted bacterial cells multiplied by 270 with the volume of the sediment or

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water sample filtered, the number of bacterial cells per milliliter of sediment or water was determined. Based on the two duplicate preparations and counts (samples 20 and 21, samples 22 and 23, Table 1) the reproducibility of the derived cell densities is ±11.5% and ±9.3%, respectively, and can be expected to be better in samples with higher cell densities.

## RESULTS

A total of 31 bacterial counts was done during the cruise (Table 1). The number of bacterial cells in a milliliter of sediment or core water was variable and ranged from less than 270 (none found) to nearly $10^6$. Water from various faucets in the paleontology lab of *JOIDES Resolution* (samples 28–30 in Table 1) contained from 11,000 to 170,000 cells/mL. No bacteria were found in a blank sample (sample 31) of doubly distilled water from the shipboard chemistry laboratory, from which water was used for all preparations.

**Table 1. Microbial cell densities of Leg 119 samples.**

| No. | Sample (core, section, interval in cm) | Depth (mbsf) | Cells ($\times 10^3$/mL) |
|---|---|---|---|
| | 119-736A- | | |
| 1 | 1H (water) | 0.01 | 4 |
| 2 | 1H-1, 1 | 0.01 | 1 |
| 3 | 1H-1, 40 | 0.40 | 24 |
| 4 | 1H-7, 2 | 9.00 | 0 |
| 5 | 3H-2, 33 | 20.33 | 0 |
| 6 | 4H-2, 126 | 30.76 | 10 |
| 7 | 5H-3, 102 | 37.02 | 400 |
| 8 | 5H-4, 19 | 37.69 | 50 |
| | 119-738A- | | |
| 9 | 1H (water) | 0.01 | 0 |
| 10 | 1H-1, 1 | 0.01 | 1 |
| | 119-739A- | | |
| 11 | 1H-1, 1 | 0.01 | 4 |
| 12 | 1H-CC | 4.60 | 16 |
| 13 | 2H (water) | 4.61 | 980 |
| | 119-739B- | | |
| 14 | 1H-CC | 9.00 | 330 |
| 15 | 2X-CC | 3.01 | 11 |
| | 119-739C- | | |
| 16 | 4R-CC | 28.70 | 0 |
| | 119-740A- | | |
| 17 | 1R (water) | 0.01 | 51 |
| 18 | 1R-1, 1 | 0.01 | 90 |
| 19 | 2R-CC | 13.70 | 390 |
| 20 | 3R-2, 35 | 16.55 | 62 |
| 21 | 3R-2, 35 | 16.55 | 73 |
| 22 | 3R-3, 55 | 18.25 | 14 |
| 23 | 3R-3, 55 | 18.25 | 16 |
| | 119-742A- | | |
| 24 | 1R (water) | 0.01 | 12 |
| 25 | 1R-1, 1 | 0.01 | 14 |
| | 119-744A- | | |
| 26 | 1H (water) | 0.01 | 39 |
| 27 | 1H-1, 1 | 0.01 | 3 |
| | Water samples measured in the shipboard laboratory and plotted in Figure 1 at 10 m above seafloor: | | |
| 28 | Paleontology lab drill water | | 130 |
| 29 | Paleontology lab salt water | | 170 |
| 30 | Paleontology lab distilled water | | 11 |
| 31 | Chemistry laboratory distilled water | | 0 |

The range of cell concentrations in our sediment samples is highly variable and within the range of concentrations found, for instance, in groundwater aquifers (e.g., Nehrkorn, 1988) or in coastal waters (e.g., Cavari and Colwell, 1988). In the water and surface sediment samples 17 and 18 from the coastal Site 740 (at a water depth of 808 m) the cell concentrations are about two orders of magnitude lower than those observed in the nearby meromictic Ace and Deep Lakes of the Vestfold Hills near David Station (Hand, 1980). On the other hand, cell concentrations in our Leg 119 sediment samples are commonly considerably higher than those found in marine sediments of the Gulf of California and the Arabian and Caspian seas (Belyaev et al., 1980).

There is no clearly discernible depth trend in the measured microbial concentrations, although a general decrease from the sediment/water interface downcore is observed (Fig. 1). A possible exception to this trend is in Sample 119-736A-5-3, 102 cm, which is from an interval with slightly elevated dissolved $SiO_2$ in the interstitial waters and above-average organic carbon contents (Barron, Larsen, et al., 1989). It is possible that the abundance of diatom frustules leads to increased porosity, which when combined with the elevated organic carbon contents provides an optimum environment for continued diagenetic microbial activity (R. Cranston, pers. comm., 1989). Unfortunately, only very rarely were there porosity measurements, or carbonate and organic carbon determinations, or interstitial water chemistry measurements available from the same levels as the bacterial samples. No evaluation of any correlation between bacterial cell densities and either of those physical or chemical parameters was therefore possible in the available sample set.

Samples taken from inside the cores (samples 3 and 5–8 in Table 1) within a few hours after the cores arrived on deck are not systematically enriched in bacteria compared to samples collected on deck, suggesting that storage and splitting did not lead to contamination.

Most of the cells encountered were of coccoid form, with minor numbers of rod-shaped cells and rare occurrences of chained cells.

We conclude that such living bacterial cell density determinations can be carried out with a reasonable effort by paleontologists during future ODP drilling cruises where recovery of deeply buried organic carbon-enriched sediments is expected.

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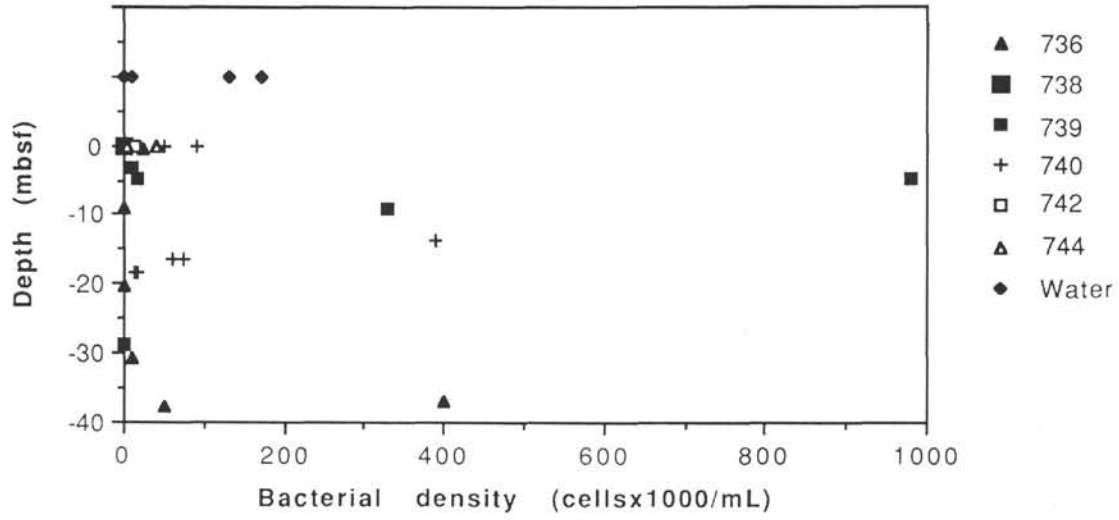


Figure 1. Densities of living bacterial cells determined in sediment and water samples at varying levels in the drill holes of ODP Leg 119. Microbial cell concentrations of various water samples are plotted at 10 m above the seafloor (0). Key to leg symbols is at right.