In situ morphologies of deep-sea and sediment bacteria

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Deep-sea and sediment bacteria at the bottom of an approximately 1200-m water column were sampled by means of pressure vessels attached to a remote underwater manipulator. Cells were immediately fixed in situ with glutaraldehyde, and after processing in the laboratory their morphologies were observed with the scanning electron microscope. Most bacteria were coccolid and less than 0.4 μm in diameter or width. Few filamentous bacteria were observed. Bacteria were in aggregates or free-living. It is concluded that morphologies of deep-sea bacteria collected and fixed at the hydrostatic pressure of their environment are, in general, similar to the observed morphologies of deep-sea bacteria determined at 1 atm pressure after collection and decompression during ascent through the water column.


Des bactéries des fonds marins et du sédiment à la base d'une colonne d'eau d'approximative-ment 1200 m de profond ont été échantillonnées au moyen de conteneurs résistant à la pression attachés à un manipulateur submergé télécommandé. Les cellules ont été immédiatement fixées in situ avec le glutaraldéhyde, et après manipulation au laboratoire, leurs morphologies ont été observées par balayage au microscope électronique. La plupart des bactéries étaient coccoïdes ou en forme de bitouillons et moins que 0.4 μm en diamètre ou en épaisseur. Quelques bactéries filamentueuses ont été observées. Les bactéries étaient en agrégats ou à l'état libre. Il a été conclu...
The morphologies of deep-sea bacteria under natural conditions have not been studied. The reason for the lack of such studies has been one of logistics; methodology has not been sophisticated enough to collect and maintain bacteria under conditions similar to their environment. Several pressure samplers have been described (1, 2), but to date none has been used in morphological studies of deep-sea bacteria in situ. Earlier work (see review 11) which described the morphologies of marine bacteria at elevated pressures relied on direct microscopic observations of bacteria that were collected at depths of less than 1 to greater than 12 000 m in a range of pressures of 1 to over 1200 atmospheres (atm) and then exposed to 1 atm for culturing and maintenance. The effects of decreasing pressure during the time the bacteria are brought to the sea surface (in some instances up to 3 or more hours) are not known.

Compression has produced marked morphological changes in bacteria. Laboratory studies (3, 11, 12, 13) have shown that a number of bacteria, including freshwater types such as Escherichia coli and Bacillus subtilis, form long (to over 40 µm) filaments or chains of cells when subjected to increased hydrostatic pressures.

A major problem in the laboratory study of pressure effects on deep-sea bacteria is the initial and subsequent periodic compression and decompression. While it has been concluded by ZoBell (11) from a number of studies that compression and decompression, each for a duration of about 15 s, do not affect the morphologies of bacteria, the effects of the initial, considerably longer, decompression remained unknown.

This communication describes the morphologies of deep-sea bacteria which were sampled and fixed in situ. Barokams or pressure vessels (3, 10) of about 300-ml volumes were scrupulously cleaned with detergent and rinsed many times with double-glass-distilled water to minimize possible bacterial contamination. From our experience, this treatment would reduce bacterial levels to the point where those remaining would be diluted out during processing for the scanning electron microscope (SEM). Twelve millitres of 50% glutaraldehyde was added to each, giving a concentration of glutaraldehyde of 2% of the total volume of the barokam. The barokam was assembled and air-evacuated with suction drawn from a laboratory vacuum line and applied to the end of a stainless steel tube (30 x 0.3 i.d. cm) which was connected to a needle-valve unit. The needle valve was then closed and the barokam kept in ice until sampling.

The sites of sampling were on the ocean floor under about 1200 m of water in the San Diego Trough. During October and November of 1973, a remote underwater manipulator (RUM) was regularly lowered from a secured floating platform, referred to as Oceanographic Research Buoy (ORB), as part of a comprehensive physical, chemical, geological, and biological study of the deep-sea benthic and epibenthic environment. ORB was moored near 32°34.75' N and 117°29.00' W at the center of an equilateral triangle (with one vertex directed due South), defined at the ocean bottom by acoustic transponders placed near the three vertices. The length of each leg was about 500 m. Samplings were from three different positions within the triangle.

Each barokam containing glutaraldehyde and evacuated with respect to air, was fastened in a horizontal position to RUM so that it would lie nearly on the ocean floor. The tube protruded in a direction away from RUM and was between 4 and 5 cm above the sediments. In two experiments, a copper tube (30 x 0.5 i.d. cm) was used. After touchdown, this tube was bent with the manipulator so that its open end would be in the water–sediment interface. When a sample was desired, the needle valve was opened by the manipulator, and water, at about 120 atm pressure, rushed into the barokam. When the microbial cells came into contact with the glutaraldehyde, fixation began immediately. Filling time was instantaneous. The manipulator then closed the needle valve and the cells were allowed to fix for at least an hour at the surrounding temperature (3.5 °C). It seems unlikely, even though there was an extremely brief period of decompression for bacteria which entered the barokam first, that the bacteria were affected morphologically (for a discussion of decompression and compression see reference 11). Because the baro-
kams filled almost instantaneously, there simply was not enough time for cells to change their sizes and shapes by altering growth patterns during or after the pressure shock. Evidence for this is provided in the figures which will be discussed later. RUM was brought to the surface shortly thereafter. The barokam was removed, put in ice, and transported to the shore-base laboratory within 3 to 6 h. In the laboratory the barokam was refrigerated at 5 °C until processed for the scanning electron microscope. A total of five samples were obtained.

All fixed samples were processed for SEM study with the technique of Paerl and Shimp (5). A Cambridge Stereoscan® Mark II scanning electron microscope was used to view each sample at an accelerating voltage of 20 kV.

Both types of samples, water and water plus sediments, showed bacteria having similar morphologies; see representative photomicrographs (Figs. 1a, b, c; 2). The cells were types most commonly observed and representative ones are shown. Most cells appeared to be coccoid and rod-like. A surprising observation was the few filamentous or chain-like cells which are normally present when bacteria are cultured in the laboratory under elevated pressure. Some filaments, however, were seen (Fig. 2). We feel most of the filaments were bacteria because of the extremely small sizes (less than 0.2 μm), but the possibility that fungal mycelia were present cannot be excluded.

Preparations of both types of samples, water and water plus sediments, showed particulate materials present (not stressed in figures), but the latter contained considerably more sediments. The possibility exists that not all the bacteria-like structures observed are bacteria or live organisms.

Although most of the bacteria appeared to be in aggregates or attached to particulate materials, there were many individual cells. Some appeared to be in colonial aggregates. The amount of free living or attached cells was difficult to estimate because sample collection and preparation may have dispersed aggregated bacteria, i.e. shaking the sample before filtration.

The observation of attached or aggregated cells was not unexpected since some marine bacteria are thought to be associated, either with particulate material or other cells (7, 8, 9). Paerl (4) recently provided evidence, via SEM, that the water of Lake Tahoe, California, contained much

![Fig. 1. Morphologies of deep-sea bacteria in waters collected above sediments (a) 3 November 1973; (b) 6 November 1973; (c) 6 November 1973. Arrows indicate bacteria.](image-url)
Fig. 2. Morphologies of deep-sea bacteria in water collected in areas of water–sediment interface, 6 November 1973. Arrows indicate bacteria.

detritus to which many bacteria were attached. He concluded that much of the cementing action in aggregation of particulate materials was caused by microbial activities. It is highly probable that similar activities may be involved at the water–sediment areas of the present study.

Many of the bacteria have diameters or widths less than 0.4 μm. (Note size of Nuclepore® filter pores which average 0.4 μm.) Some cell diameters approximated 0.2 μm. These diameters are generally smaller than those previously reported for deep-sea bacteria (6). In unpublished studies in our laboratories and those of others, these smaller bacteria are being observed. Recent use of membrane filters of different pore sizes gives information which shows that many bacteria in a seawater sample pass through a 0.45-μm pore-size filter. Also, it has long been known in marine microbiological studies that passage of a sample of seawater through a 0.45-μm membrane filter does not produce a sterile filtrate. Previous measurements of deep-sea bacteria have been obtained under light or phase microscopy aboard ship or in the laboratory at 1 atm pressure. Apparently, in those studies, the ascent through the water column concomitant with decreasing hydrostatic pressure in the environment had slight, if any, effects on cell morphology and size.

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