Reports

Death of a Hadal Deep-Sea Bacterium After Decompression

Abstract. *An obligately barophilic bacterium that was recovered from a depth of 10,476 meters in the Pacific Ocean slowly lost colony-forming ability (assayed at 101.3 megapascals and 2°C) during incubation at atmospheric pressure and 0°C.*

Studies of deep-sea bacteria reveal adaptations to high pressures (*1–10*). Bacteria that grow better (*1, 4, 10*) at deep-sea pressures than at atmospheric pressure are called barophilic, and those that grow only (*5, 9*) at deep-sea pressures are called obligately barophilic. The definition of barophily must be made at a reference temperature usually chosen to be that of the natural environment of the organism under consideration. The pressure allowing the maximum rate of reproduction of bacteria from the ocean at 2°C is proportional to the depth of origin of the bacteria (*7*). We report that an obligately barophilic bacterium died at atmospheric pressure as revealed by the loss of colony-forming ability (CFA).

Strain MT-41 is an obligate barophile from the Marianas Trench at a depth of 10,476 m (*5*). Pure cultures were maintained at 2°C and 1013 bars (1 bar = 0.1 megapascal) except for intervals of 30 to 60 minutes at atmospheric pressure during transfers to fresh medium (*5*). Cells were grown at a concentration of 10^7 cells per milliliter in marine broth (type 2216, Difco) having a *p*H of 7. The culture was decompressed and diluted 1:100 into three different cold media that had been filtered (0.22-μm pore) and autoclaved. The media were type 2216 marine broth (*p*H 7.0); fresh seawater (*p*H 7.8) originating from a depth of 3 m off the end of the Scripps pier and conveyed to the laboratory through polyvinyl chloride pipes; and artificial seawater (ASW) (*p*H 7.2) containing 24 g of NaCl, 5.3 g of $MgCl_2 \cdot 6H_2O$, 7 g of $MgSO_4 \cdot 7H_2O$, 1.0 g of KNO_3, 10 ml of 1*M* TES buffer, and 1 liter of water. The suspended cultures were incubated at 0°C for 170 hours, stirred before samples were removed, and checked periodically with a phase microscope for the development of aggregates of cells. Serial dilutions of each sample were made in the 2216 marine broth at 0°C. Two 0.1-ml portions of each dilution were placed in 4-ml polystyrene tubes at 0°C, and a freshly prepared mixture of 2 volumes of the 2216 broth (*p*H 7, 0°C) and 1 volume of gelatin (120 g per liter of ASW) (*p*H 7, 20°C) was added. The temperature of this mixture, which initially was 9°C, decreased to 0°C within 7 minutes after the mixture was placed on ice. Gelation occurred within 3 minutes. The gels were covered with Parafilm and incubated at 2°C and 1000 bars for 3 weeks. Colonies in the pour tubes were counted with the aid of a stereomicroscope. Isolate CNPT-3, a barophile found at a depth of 5700 m in the Pacific Ocean, grew at atmospheric pressure. A suspension of isolate CNPT-3, grown at 580 bars and 2°C, was made in ASW and kept at 0°C and 1 atm. Samples of this suspension were taken and assayed for CFA at 580 bars (*3*).

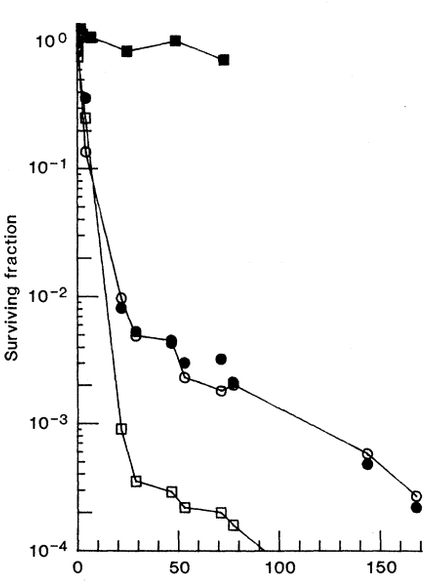

Fig. 1. A culture of isolate MT-41 was decompressed at 0 hours, and three incubations were begun at atmospheric pressure and 0°C. (□) A suspension of MT-41 in type 2216 marine broth; (●) a suspension in artificial seawater; and (○) a suspension in filtered seawater. (■) A suspension of isolate CNPT-3, a simply barophilic bacterium, in artificial seawater containing 1 percent type 2216 marine broth, shown for comparison. All suspensions of cells were incubated at 0°C and atmospheric pressure. Samples of the suspensions were incubated for colony-forming ability at high pressure as described in the text.

The obligate barophile, strain MT-41, lost CFA at atmospheric pressure and 0°C (Fig. 1). Strain MT-41 grows only at pressures greater than 346 bars (*5*). The survival of strain MT-41 was compared with that of strain CNPT-3 (*4, 6*) at 0°C in ASW (Fig. 1). Strain CNPT-3 grows at 2°C over the range of 1 to 800 bars in nutrient medium (*6*). The kinetics of thermal inactivation (*3*) of isolate CNPT-3 at temperatures of 10°C and higher are first-order. The kinetics of inactivation by decompression of isolate MT-41 were complex, possibly interpretable as the sum of two first-order processes. It seems likely that the kinetics are first-order over the first 10 to 15 hours but then deviate as a result of the clumping of some of the cells in the cell suspension.

The kinetics (Fig. 1) demonstrate how a decompression-sensitive bacterium such as strain MT-41 could be recovered from the Marianas Trench (*5*) in a sample that was decompressed to atmospheric pressure, although thermally insulated (recovered at 6°C), during retrieval from the sea; that is, significant numbers of bacteria died during a 4-hour interval—the length of time that the sample recovered from the Marianas Trench was at a pressure less than 500 bars before being compressed to 1013 bars on board the ship. Therefore, a large number of cells must have been present in the original sample.

Death after decompression has not been observed with bacteria from abyssal depths. Samples from the Pacific Ocean at abyssal depths of about 5900 m have not yet yielded (*7*) obligately barophilic bacteria, but rather barophilic bacteria such as isolate CNPT-3 (*4*). The latter were obtained from a sample that was retrieved without decompression and that grew at in situ pressures and temperatures for 5 months (*4*). Samples from the hadal parts of the Pacific have yielded obligately barophilic bacteria. More examples are needed to determine whether sensitivity to decompression will be correlated with the depth of origin of the bacteria.

It is nevertheless becoming clear that organisms are adapted to a pressure

range determined in part by the pressure of their normal environment (*7*) and by their position in the taxonomic hierarchy (*11, 12*). Thus, the bacterium CNPT-3 from an environment of 580 bars survived and grew at atmospheric pressure. Yet, a bacterium, isolate MT-41, from an environment of 1062 bars died at atmospheric pressure. A group of greater biological complexity, the amphipods (arthropods), from the same depth as bacterial strain CNPT-3, died at atmospheric pressure (*12*).

Pressures exceeding the maximum pressure permitting growth kills organisms. This has been shown for various prokaryotic cells (*2*). Our study shows that pressures lower than those permitting growth can also kill avacuolate microorganisms.

Sensitivity to decompression can arise from the presence of organs or organelles containing gases (*13*) and from the temporal aspects of pressure changes. Such factors acting in concert can lead to complex mechanisms of death due to decompression. Only in bacteria containing gas vesicles that expand on decompression, causing the cell wall and membrane to burst, is the mechanism of cell death clear (*13*). Methods (*14, 15*) that permit colonies to grow in the complete absence of decompression may allow for the discovery of gas-vacuolated bacteria in the deep sea.

A. ARISTIDES YAYANOS
ALLAN S. DIETZ
Physiological Research Laboratory, Scripps Institution of Oceanography, University of California, San Diego, La Jolla 92093

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16. Supported by a contract from Sandia National Laboratories and by grant OCE79-08972 from the National Science Foundation.

1 July 1982; revised 9 December 1982

Electron Microscope Tomography: Transcription in Three Dimensions

Abstract. *Three-dimensional reconstruction of an asymmetric biological ultrastructure has been achieved by tomographic analysis of electron micrographs of sections tilted on a goniometer specimen stage. Aligned micrographs could be displayed as red-green three-dimensional movies. The techniques have been applied to portions of in situ transcription units of a Balbiani ring in the polytene chromosomes of the midge* Chironomus tentans. *Current data suggest a DNA compaction of about 8 to 1 in a transcription unit. Nascent ribonucleoprotein granules display an imperfect sixfold helical arrangement around the chromatin axis.*

The objective of the tomographic method is the formation of an image of the internal structure of an object from a series of projections. Computerized axial tomography, made possible by advances in applied mathematics and computer technology (*1*), is well known in medical imaging and other fields. Application to electron microscopy has heretofore been limited to objects with known symmetry, such as crystalline materials (*2*) or those with a well-defined cylindrical form (*3*). In this report, we describe electron microscope tomography (EMT) of objects lacking any apparent internal symmetry or predetermined orientation and thus applicable to many cellular structures in situ.

A series of electron micrographs of a specimen tilted around a single arbitrary axis is prepared. The micrographs constitute a set of magnified projections viewed at various tilt angles. The projections are combined, via their Fourier transforms in the direction perpendicular to the tilt axis, to yield the three-dimensional image. Ideally, projections spanning 180° of tilt should be used; in practice, the specimen shape and the mechanical limitations of tilt stages and specimen grids preclude tilts beyond ±60°. The image constructed from incomplete data exhibits decreased resolution in the direction of the missing views (*4*); nonetheless, sufficiently detailed and informative reconstructed images are obtained to warrant further use of the technique.

The sequence of operations as applied to sectioned specimens can be outlined as follows. Sections up to 0.25 μm thick were mounted on clean copper grids with the support film omitted in order to achieve complete stain penetration. Carbon coating followed the staining procedure. A colloidal suspension of gold was applied to both surfaces of the stained plastic section. These spherical gold particles constitute the reference points for relating the different tilt views. Areas for tomographic reconstruction were chosen by studying stereo pairs. A tilt series was then obtained on a microscope equipped with a eucentric goniometer (consequently, the objective lens current did not change throughout the series). Micrographs were taken at intervals of 5° to 10° between ±60°.

In an earlier reconstruction from electron micrographs of paramyosin filaments (*3*), the rod-shaped objects had been carefully aligned in the microscope to be parallel to the tilt axis. The spatial correlation of the micrographs along the tilt axis was accomplished by visual registration of images of the cylindrical structure and gold particles. In the present case, the arbitrary orientation of the object necessitated a more elaborate procedure. Digitization of the micrographs preceded the alignment step; a Vidicon scanning camera was mounted above a light box equipped with a micrometer-controlled stage, and connected to an image-processing system (International Imaging Systems model 70). The rasterized image is 512 × 512 pixels, with resolution controlled by the distance from the negative to the camera. (In the present study 1 pixel was set equal to about 3 nm of specimen structure.) The Vidicon camera has the virtue of a high refresh rate (30 sec^{-1}), which facilitates interactive positioning of the negative.

For each micrograph, alignment and registration require the determination of the rotation angle which orients the micrograph with the projection of the tilt axis in a standard direction; in addition, two independent translations are required in order to achieve a common origin of coordinates. Our program (*5*) establishes best values of these parameters by a least-squares fit between the observed positions of the gold spheres, estimated from the digitized images of all micrographs in a tilt series, and their calculated positions. Additional useful parameters evaluated by the least-squares program include best values of the tilt angles (precise to within 0.1°), magnification ratios between tilted and untilted projections, and Cartesian coordinates of the gold spheres. When the translations and rotation angles are established, each micrograph is again digi-