APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1991, p. 1489–1497
0099-2240/91/051498-09$02.00/0

Vol. 57, No. 5

Ultrastructural Changes in an Obligately Barophilic Marine Bacterium after Decompression

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Received 15 January 1991/Accepted 28 February 1991

The bacterial isolate MT-41 from 10,476 m, nearly the greatest ocean depth, is obligately barophilic. The purpose of this study was to describe the morphological changes in MT-41 due to nearly isothermal decompression followed by incubation at atmospheric pressure. Two cultures were grown at 103.5 MPa and 2°C and then decompressed to atmospheric pressure (0.101 MPa). One of the cultures was fixed just before decompression. The other culture, kept at 0°C, was sampled immediately and four more times over 168 h. The number of CFU (assayed at 103.5 MPa and 2°C) declined with incubation time at atmospheric pressure. Decompression itself did not lead to immediate morphological changes. The ultrastructure, however, was altered with increasing time at atmospheric pressure. The first aberrations were intracellular vesicles and membrane fragments in the medium. After these changes were plasmolysis, cell lysis, the formation of extracellular vesicles, and the formation of ghost cells. Intact cells in the longest incubation at atmospheric pressure had the normal cytoplasmic granularity suggestive of ribosomes but had few and poorly stained fibrils in the bacterial nucleoids. From the practical standpoint, samples of hadal deep-sea regions need to be fixed either in situ or shortly after arrival at the sea surface even when recovered in insulated sampling gear. This should prevent drastic structural degradation of sampled cells, thus allowing both accurate estimates of deep-sea benthic standing stock and realistic morphological descriptions.

The growth rate of bacteria in the logarithmic phase of growth under given nutrient conditions is a function of temperature (T) and pressure (P) (22). Barophilic bacteria have a maximum value of k(T,P), the exponential growth rate constant, at high pressure (>1 atm; 1 atm = 1.01325 bars = 1.01325×10^5 N/m^2 = 0.101325 MPa) (2, 10, 11, 22, 26). Studies of barophiles from different capture depths show that k(T,P) is often correlated with the capture depth of each isolate (3, 10, 11, 22, 26). The maximum value of k(T,P) was found in one study to be within 5 MPa of the capture depth pressure (22) for six isolates, each from a different depth. The pressure gradient in the sea, therefore, should have major ecological and evolutional roles.

The survival of deep-sea bacteria is also a function of temperature and pressure and is of interest for several reasons. First, sensitivity to warming and to decompression may be a depth-dependent property. The possibility for this is seen in a comparison of the thermal sensitivity of bacterial isolate CNPT-3 from 5,782 m with that of the isolate MT-41 from 10,476 m. CNPT-3 loses colony-forming ability at atmospheric pressure slowly at 10°C and rapidly at 20°C (24), whereas isolate MT-41 loses it even at 0°C as an apparent consequence of decompression itself from 103.5 MPa to atmospheric pressure (25). Thus, the isolate from 10,476 m is more sensitive to complete decompression and to warming than is the isolate from 5,782 m. Both isolates come from habitats with a temperature close to 2°C (15). Second, sensitivity to warming and to decompression constrains the possible dispersal paths of barophilic bacteria through the various temperature-pressure regimes of the oceans. Third, warming (19, 24) and decompression change biological structures. This means that these structural changes affect the accuracy of studies relying on morphological analysis of organisms in samples of the deep sea. Most deep-sea samples contain organisms killed by decompression or by heat stress during the transit of sampling gear through the warm sea surface. Cell structure changes can accompany death and can compromise determinations of standing stock or of microscopic anatomy. Fourth, the effects of decompression and warming led to the development of laboratory techniques for the study of barophiles (23). Finally, the study of extreme decompression that is possible with organisms such as strain MT-41 may result in clearly visible damage, giving clues to some of the mechanisms of pressure adaptation and decompression sensitivity. Knowledge of the morphology of dying cells is essential for an understanding of the mechanisms of cell death (19).

Strain MT-41, obligately barophilic at all growth temperatures, was collected as described previously (27) from a 10,476-m depth in the Challenger Deep of the Mariana Trench. It grows only at pressures in excess of 40 MPa and is probably confined to ocean depths greater than 3,800 m—the average depth of the sea. When incubated at atmospheric pressure at 0°C, 90% of the CFU are lost in 6 h. Here we report a study of the morphological changes after the decompression of strain MT-41 from 103.5 MPa to atmospheric pressure at 0°C.

MATERIALS AND METHODS

Organism, growth conditions, and Gram stain. Strain MT-41 was maintained in type 2216 marine broth (Difco, Detroit, Mich.) at pH 7.0, 2°C, and 103.5 MPa. It grows with a generation time of about 35 h under these conditions (22, 27). The strain was transferred every 3 to 6 months to fresh medium. The transfer procedure occasionally included the selection of single colonies out of pour tubes of either silica gel (25) or gelatin (25) incubated at 103.5 MPa and 0°C. The pour tube techniques were also used to assay at high

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pressure for colony-forming ability in cultures exposed to atmospheric pressure. Clump formation at cell concentrations of about 2×10^7 cells per ml and growth at 103.5 MPa at 2°C are characteristics (27) of strain MT-41. Cell concentrations determined with colony-forming ability and with particle counts (see below) agreed at the titers used in the experiments described here.

Stains used in the standard Gram stain procedure were obtained from Sigma Chemical Co., St. Louis, Mo., and were certified by the Biological Stain Commission, Inc., Rochester, N.Y. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 served as gram-positive and -negative controls, respectively.

Determination of the concentration of cells. The number of cells in diluted samples of cultures in which large clumps were not present was determined by using a model H Coulter Counter (Coulter Electronics, Hialeah, Fla.) with an aperture tube with a 30-μm-diameter orifice. The diluent was an aqueous solution of 0.85% NaCl, 0.05% sodium azide, and 0.025% Formalin filtered with 0.22-μm-pore-diameter filters. Correction was made for coincidence counting where necessary.

Fixation of cells. All chemicals were of electron microscopy grade from Polysciences, Inc. (Warrington, Pa.). Solutions were passed through 0.22-μm-pore-size filters before use. All materials, including pipettes, centrifuge tubes, buffers, and fixatives, were chilled (2 to 4°C) to prevent shock to cells due to temperature change.

Samples of cultures were fixed by adding glutaraldehyde to a final concentration of 1% (vol/vol) directly to the samples. These were then placed on ice for 30 min. Fixed material was washed in chilled 0.01 M sodium cacodylate buffer (pH 7.2, 0°C) by centrifugation (2,800 × *g* for 10 min) at 2°C and suspension in buffer. After another centrifugation, pellets were suspended in 1% osmium tetroxide in buffer and incubated in an ice bath for 30 min. Then the samples were washed two times with buffer and warmed to room temperature. The fixed cells were embedded as described previously (13) at 45°C in 2% (wt/vol) Noble agar (Difco) that had been filtered through 0.22-μm-pore-diameter filters. Centrifuged (8 × *g*) and cooled pellets were cut into 1-mm^3 cubes with a sterile scalpel and dehydrated in a 10% graded ethanol-water series with the final two steps in absolute alcohol (10 min for each step). The cubes were infiltrated with complete Spurr resin (Polysciences, Inc.) in a vial rotated slowly overnight. The blocks were then placed in an oven at 70°C to polymerize the resin.

Sectioning and microscopy. Thin sections were cut with a diamond knife and collected on uncoated, 300-mesh copper grids. Sections were poststained for 15 min with 4% (wt/vol) uranyl acetate (aqueous, saturated solution) and for 2.5 to 3 min with 0.1% lead citrate. Specimens were viewed and photographed with either a Hitachi H-500 STEM or a Zeiss EM9S-2 electron microscope.

Fixation of cultures at high pressure. The Landau-Thibodeau method was used to fix cells under pressure (14). A two-chamber device allowed for cells to be grown in a nutrient medium at high pressure in one chamber separated by a glass coverslip partition from a solution of fixative. A stainless steel ball was in the chamber with the fixative. After 2 weeks and without alteration of the pressure of the incubation, the culture was mixed with the fixative by vigorously shaking the pressure vessel, thereby causing the steel ball to break the glass partition. The culture containing fixative was then decompressed for further processing. Osmium tetroxide was chosen as the single fixative with the Landau-Thibodeau method, since it is presumably more stable than glutaraldehyde over the incubation period of 2 weeks used for the growth of MT-41.

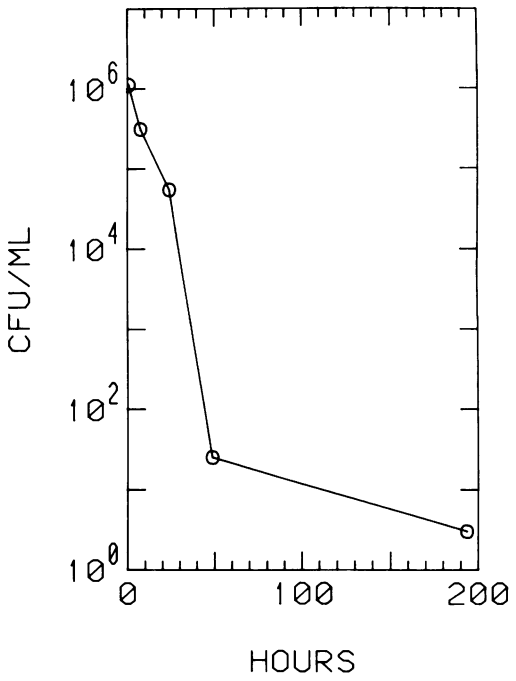
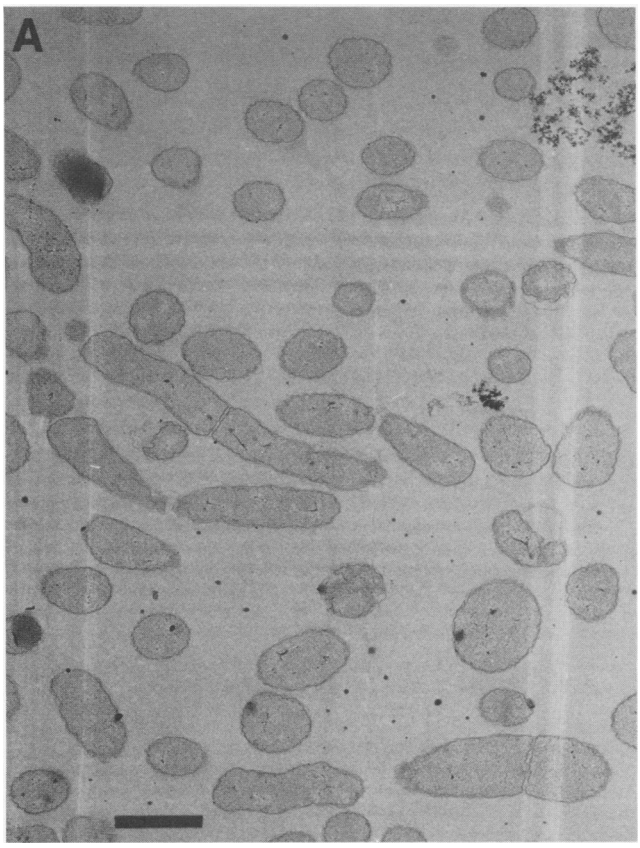


FIG. 1. Loss of colony-forming ability by strain MT-41 in liquid culture at atmospheric pressure and 0°C after transfer from 103.5 MPa and 2°C. The ultrastructural studies were done on samples taken at the same time as those for the assay of colony-forming ability.

The chamber was chilled in a freezer for more than 1 h and then placed on ice just before use to avoid temperature shock to the cells. The inoculated culture medium was poured into one chamber of the device. The fixative, 2% osmium tetroxide in 0.2 M sodium cacodylate buffer (pH 7.2 at 101.29 kPa and 0°C), and a sterile steel ball were put into the other chamber of the device. Care was taken to remove air from the chambers of the incubation-fixation device. Then the culture was put into a prechilled pressure vessel in an ice bath and compressed to the appropriate pressure.

The growth of the culture in the incubation-fixation device was estimated from the growth of a parallel culture (same inoculum) sampled with decompression as previously described (27, 28). Cultures were fixed after the desired amount of growth by vigorously shaking the pressure vessel housing the incubation-fixation device, which caused the steel ball to break the glass partition between the fixative and culture and permitted the two solutions to mix (14). The vessel was rocked to mix the solutions and was left on ice for 1 h before decompression, decantation to remove glass fragments, and centrifugation to isolate the fixed cells. The cells were washed with cacodylate buffer, embedded in agar, and processed for electron microscopy. A culture begun at the same time in an identical incubation-fixation chamber was fixed immediately after, rather than before, decompression.

Uninoculated and sterilized type 2216 marine broth in the incubation-fixation devices was not contaminated after incubations at high pressures.



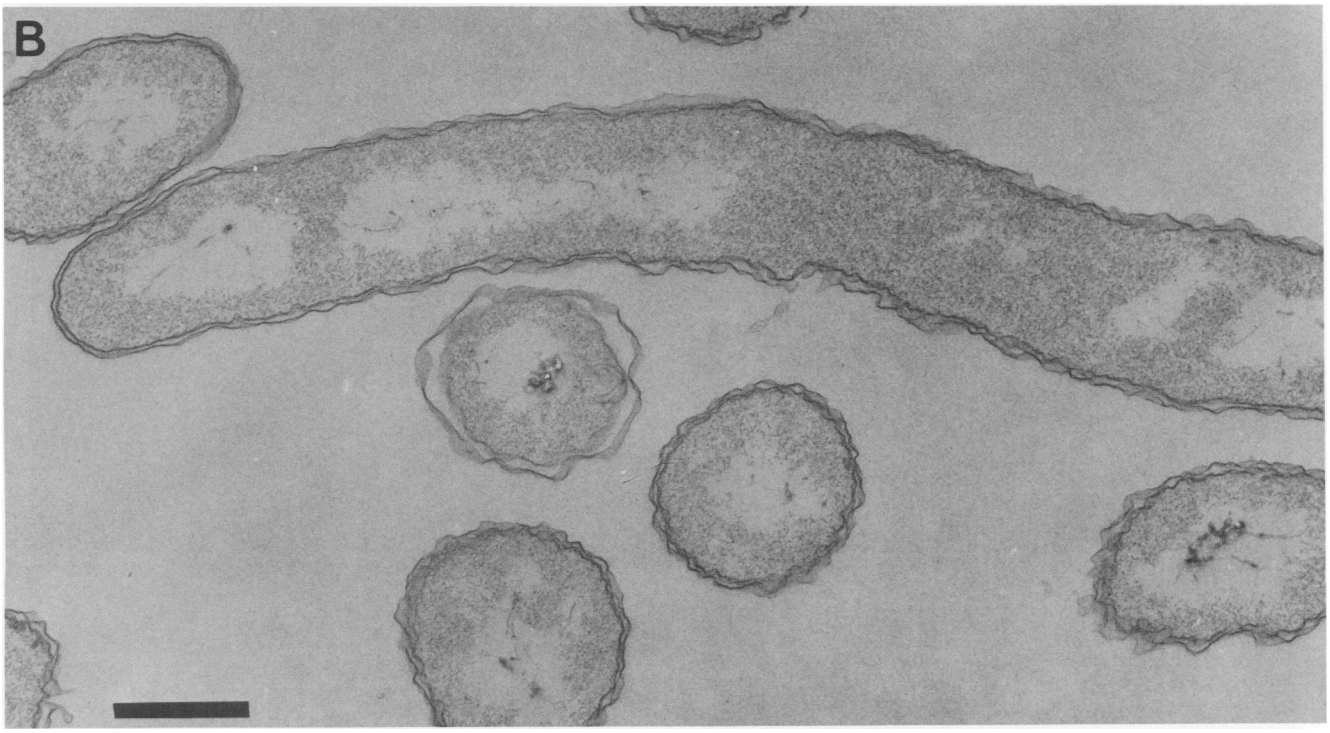
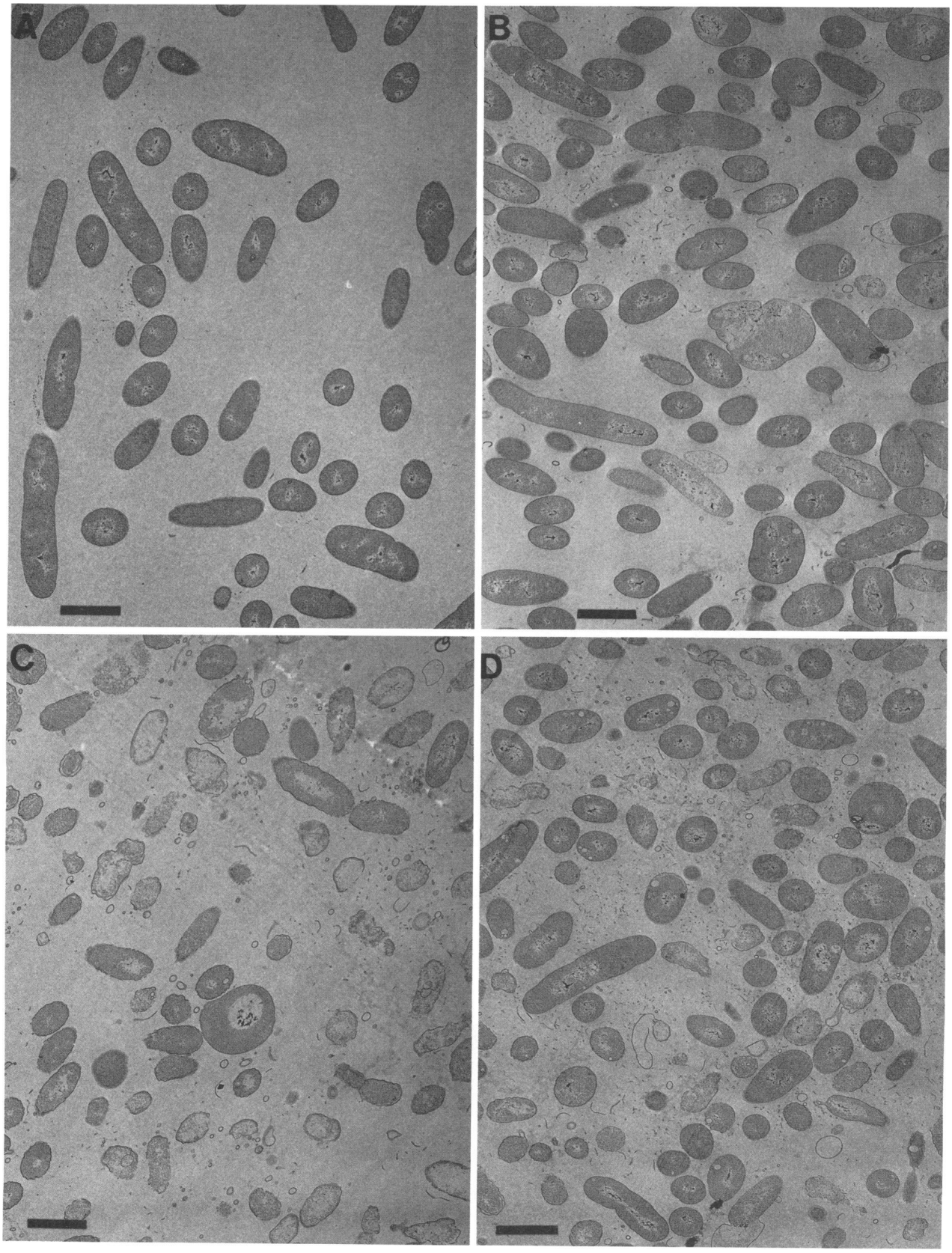


FIG. 2. Photomicrographs of thin sections of cells of MT-41 fixed with osmium tetroxide at 103.5 MPa before decompression. (A) Low-power scan (bar, 2 μm) of the cells shows cells fixed in the cell division stage with separate cytoplasmic membranes but daughter cells sharing a common outer membrane and periplasmic space. (B) Long cell in longitudinal section revealing a membrane and other fine structure features characteristic of gram-negative bacteria (bar, 0.5 μm).


A
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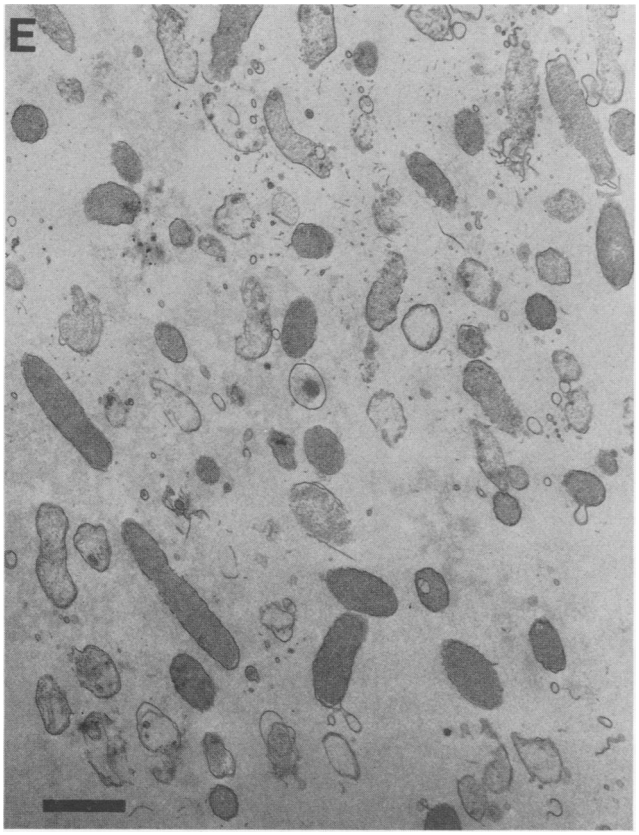

FIG. 3. Photomicrographs taken at low magnification (bars, 2 μm). Samples of the culture used for the results in Fig. 1 were fixed with both glutaraldehyde and osmium tetroxide. (A) Cells fixed immediately after decompression are intact, showing membrane integrity and defined nucleoids. Intracellular vesicles are small. (B) Cells were fixed 7 h after decompression. Twenty-five percent of the cells were capable of forming colonies. The sections of the cells show evidence of plasmolysis, the presence of intracellular membrane fragments, and the occurrence of enlarged vesicles located both intra- and intercellularly. (C) Cells were fixed 24 h after decompression. Intact cells, "ghosts," and cellular debris are evident, as are exaggerations of all of the changes noted in panel B. Five percent of the cells were capable of forming colonies. (D) Cells were fixed 48 h after decompression. Ghosts and cellular debris predominate the field of view. An occasional cell is intact with well-defined nucleoids. Only 20 cells per 10^6 cells, however, could form colonies. (E) Cells were fixed 168 h after decompression. Intact cells are rare, and nucleoids are not obvious. The granules in the intact cells may be evidence for the presence of ribosomes. Only 2 cells per 10^6 cells could form colonies. It is unlikely that any of the cells in panels D and E were potential CFU.

RESULTS

Two 500-ml cultures, one with a small inoculum and the other with a large inoculum, were grown at 103.5 MPa and 2°C for 5 days and then decompressed. Both cultures were sampled for electron microscopy, for determination of particle counts with a Coulter Counter, and for assay of CFUs. Samples for fixation and for particle counts were processed immediately. The CFUs developing at 103.5 MPa and 2°C were not ready for counting until 10 weeks later, because the colonies formed slowly. When the CFUs were counted we found out that the large-inoculum 500-ml culture with a CFU count of 1.0×10^6 per ml when it was decompressed also had 1.1×10^6 CFUs per ml. The plating efficiency for the other 500-ml culture was 30%. A variable plating efficiency is often encountered with deep-sea barophiles. Although we do not yet know the cause for this, one factor may be the size of the initial inoculum.

The fixed samples from the experiment in which plating efficiency was nearly 100% were used for this study. In that experiment CFU were inactivated during incubation at 0°C and atmospheric pressure (Fig. 1), as previously found (25). The kinetics of inactivation were first order for the first few hours of exposure to atmospheric pressure. The number of cells (Coulter counts) did not diminish during the incubation. We attributed the deviation from first-order kinetics that begins approximately at 48 h to the occurrence of clumps of cells in MT-41 cultures. Samples for the study of ultrastructural changes during incubation at atmospheric pressure were taken at the same time and from the same culture as were those for the assay of colony-forming ability and particle concentrations. To determine effects of decompression per se, a separate experiment was done with a culture grown under pressure and fixed before decompression.

No apparent morphological changes on decompression. The photographs in Fig. 2 are of thin sections of cells fixed at 103.5 MPa before decompression. A comparison of the micrographs of cell sections in Fig. 2 (cells fixed before decompression) with those in Fig. 3A (cell fixed immediately after decompression) shows no discernible substantive differences. The cells in Fig. 3A were prepared by fixation with both glutaraldehyde and osmium tetroxide, whereas those in Fig. 2 were prepared by fixation with osmium tetroxide alone. The differences in the appearance of the cells fixed under pressure (with osmium tetroxide only) and those fixed immediately after decompression to atmospheric pressure (with both osmium tetroxide and glutaraldehyde) are expected—differences in contrast and in membrane contour—and seem to be attributable to the fixatives themselves (12). Thus, the ribosomes in Fig. 2 do not appear to be as dark as those in Fig. 3 and 4. Similarly, the fibrillar structure in the nucleoids is not as apparent in Fig. 2 as it is in Fig. 3 and 4. Although the contours of envelopes of cells treated with osmium tetroxide only at high pressure (Fig. 2) were more wavy than those of cells fixed with both osmium tetroxide and glutaraldehyde at atmospheric pressure (Fig. 3A), this effect is seen in studies done entirely at atmospheric pressure (7, 17). In summary, there was no evidence of an immediate effect of decompression from 103.5 MPa, as is also the case with an obligate barophile decompressed from 75 MPa (4).

Since the Coulter counts and viable counts were virtually identical, it seems unlikely that there was an effect of decompression that caused a fraction of the cells to rupture immediately upon decompression. We do not have a basis, however, for believing that Coulter counts are a reliable indicator of intact cells.

Changes in cell structure after decompression. The photomicrographs shown in Fig. 3 and 4 are of sections of cells from the culture where cells were losing colony-forming ability as in Fig. 1. Photographs taken at low magnification (×5,100) (Fig. 3) show ultrastructure in cells of strain MT-41 kept at 0°C for 0, 7, 24, 48, and 168 h after decompression. Figure 4 shows photographs of the same preparations taken at magnifications of ×31,000 to ×34,000. The data in Fig. 1

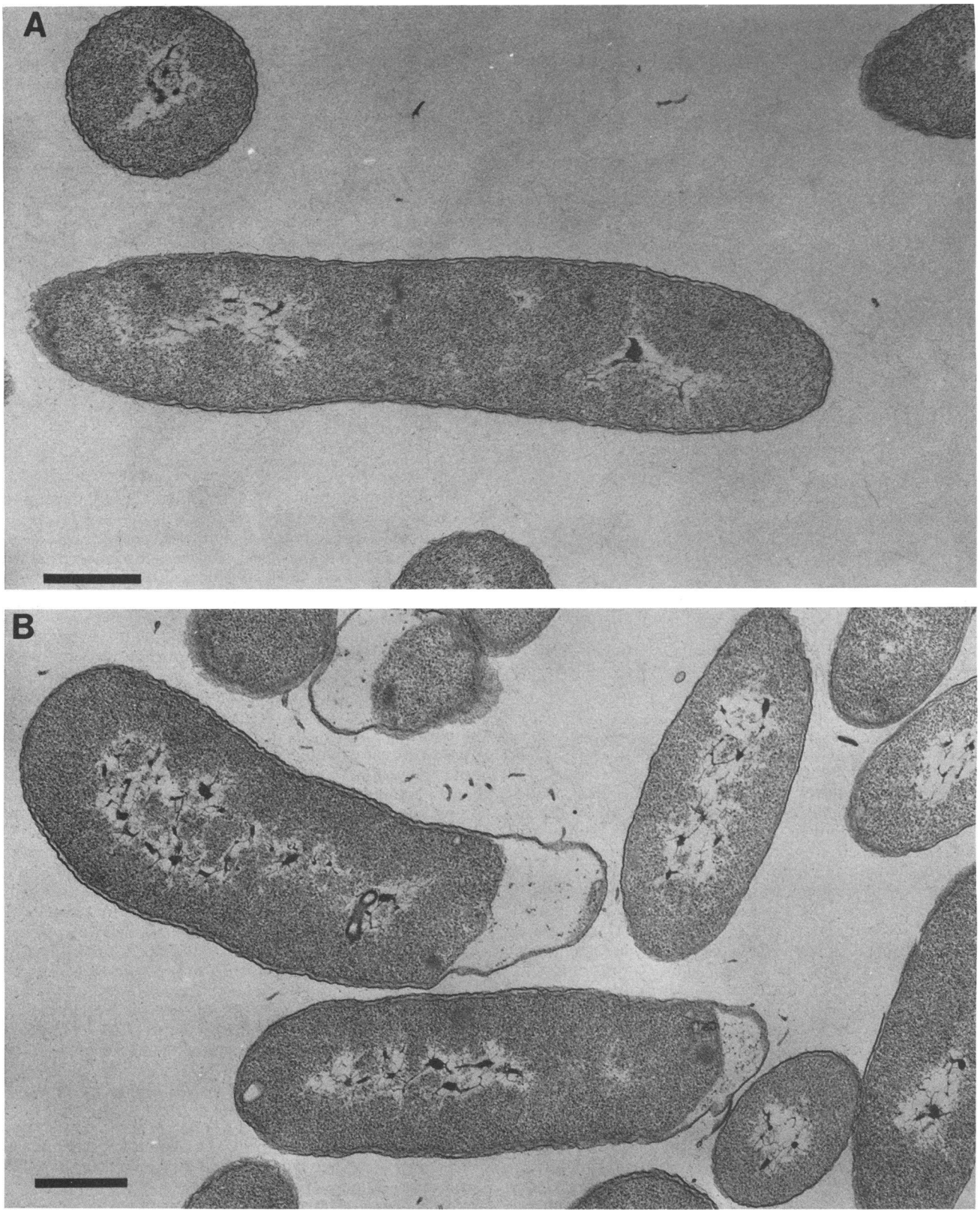

FIG. 4. Photomicrographs (as in Fig. 3 but at a higher magnification) of thin sections of cells of MT-41 fixed with both glutaraldehyde and osmium tetroxide as a function of time after decompression (bars, 0.5 μm). (A) Cells were fixed immediately after decompression from 103.5 MPa. (B) Cells were fixed 7 h after decompression. Shrinkage of cytoplasm is pronounced in some cells and vacuoles appear in many cells. (C) Cells were fixed 24 h after decompression. Loss of membrane integrity occurred by more than one process, since plasmolysis is seen in one cell yet fragmentation of membranes without plasmolysis is seen in another cell. (D) Cells were fixed 48 h after decompression. Intact bacteria were still present, but their nucleoids did not stain well and appeared more fibrous and less dense than those in panels A and B. (E) Cells were fixed 168 h after decompression. The section through one apparently intact cell shows a cytoplasmic granularity expected from ribosomes and a nucleoid with an appearance quite different from those in panels A through D.

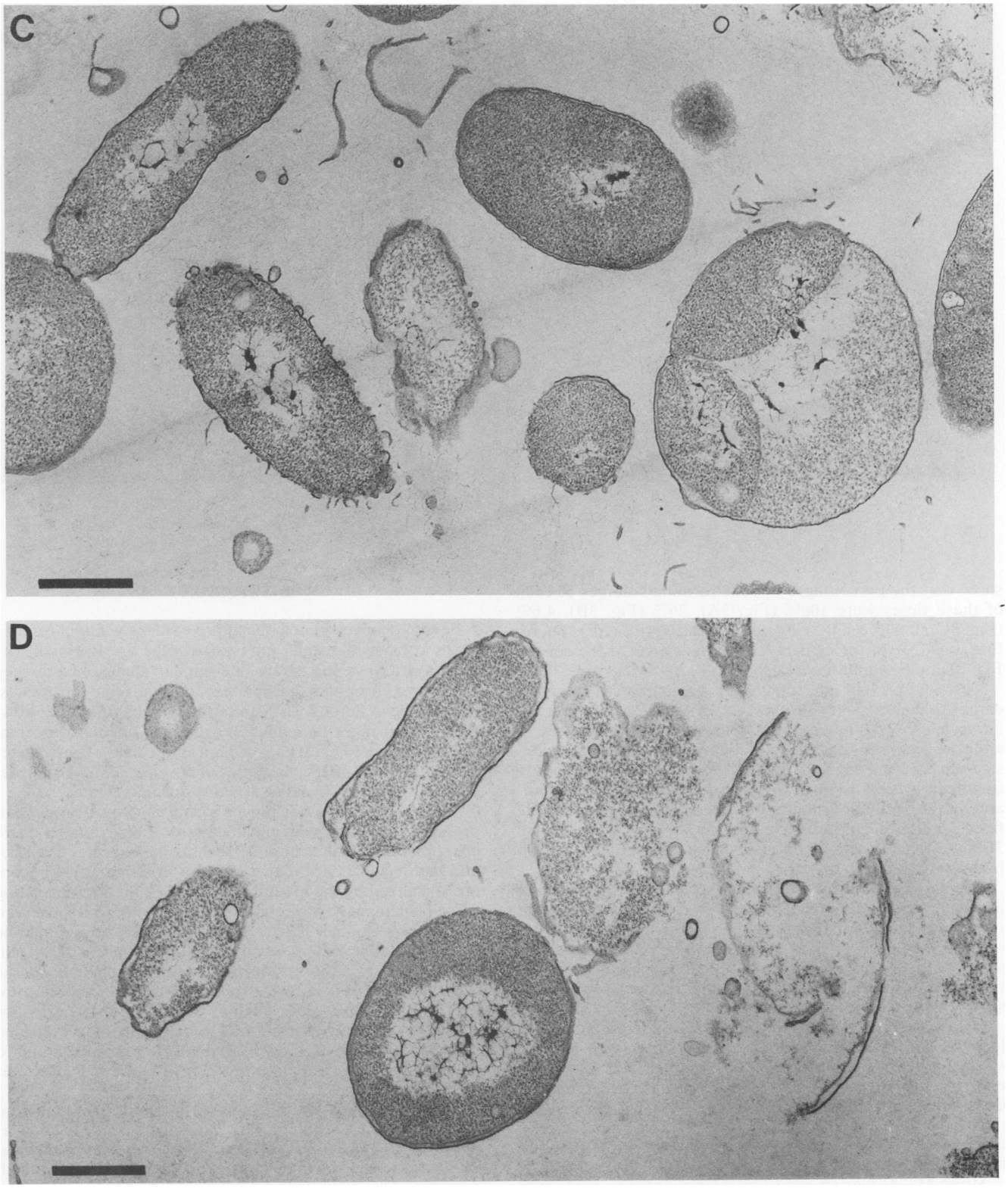

FIG. 4—*Continued*.

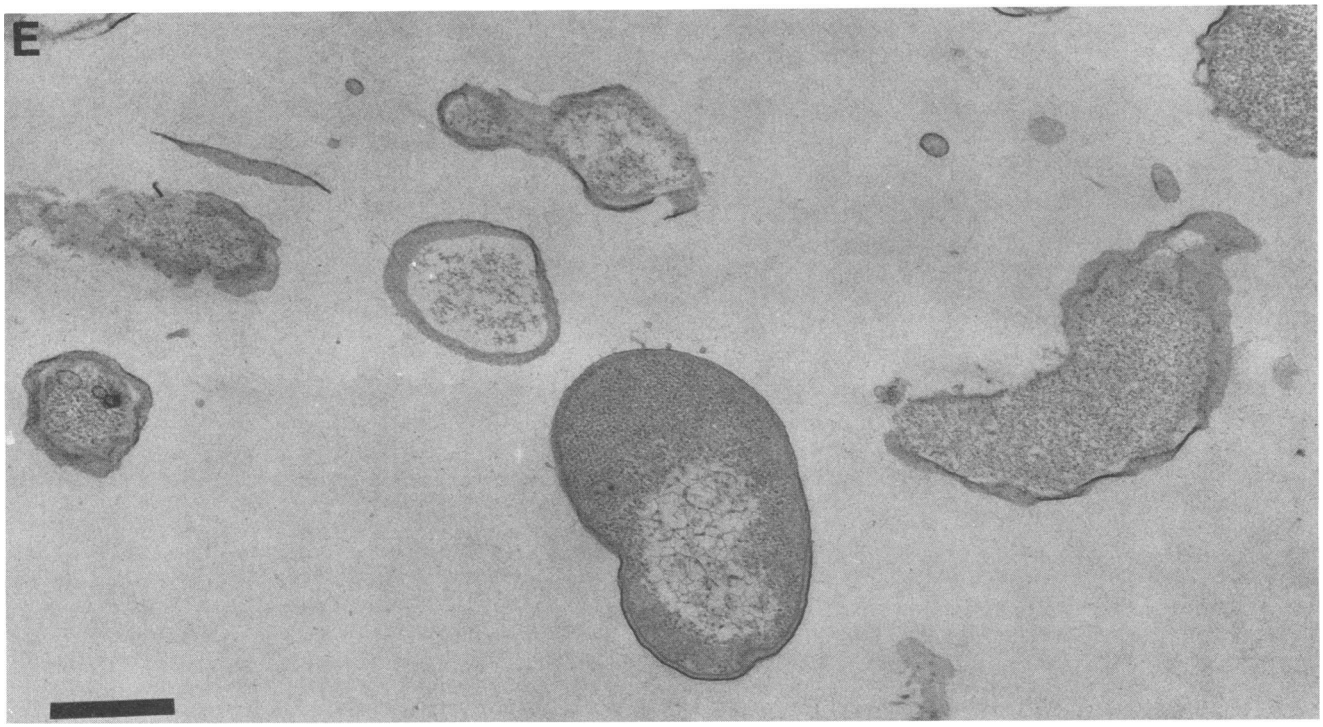

FIG. 4—*Continued.*

show that the percentages of cells forming colonies at each of these times were 100% (Fig. 3A), 26% (Fig. 3B), 4.6% (Fig. 3C), 0.002% (Fig. 3D), and 0.00025% (Fig. 3E). Therefore, only 5% of the cells in Fig. 3C and none of the cells in Fig. 3D or E would have been viable.

Vesiculation (18) and membrane fragmentation were the earliest changes seen (Fig. 3B and 4B). Although intracellular vesicles were apparent in preparations fixed immediately after decompression (Fig. 3A), their number increased considerably during the 7 h after decompression. Membrane fragments were absent in the culture sample fixed immediately after decompression but were abundant in the sample fixed 7 h after decompression. Plasmolysis was evident (Fig. 3B and C and 4B and C) in the samples exposed for 7 and 24 h to atmospheric pressure.

The granules in the cytoplasm of intact cells after 168 h at atmospheric pressure (Fig. 4E) were taken as evidence that ribosomes were not dissociated by decompression. Ribosomal dissociation in vitro has been observed after compression (9). If decompression caused loss of ribosomal structure, then ultrastructural evidence for it should have been seen (16).

It appeared that nuclear material stained less densely the longer cells were exposed to atmospheric pressure. Faintly stained thin fibrils were present in intact cells from all exposures to atmospheric pressure. Since osmium tetroxide does not interact with nucleic acids (13), the changes seen might be due to a diminution of protein synthesis or a degradation of nucleic acid with concomitant loss of associated nuclear proteins.

Strain MT-41 was shown to be a gram-negative bacterium by the ultrastructure of the cell envelope (Fig. 4A), by its ability to undergo plasmolysis (Fig. 4B and C) (5, 13), and by Gram staining.

DISCUSSION

Severe changes in cell shape are evident when barophilic bacteria from midocean (abyssal) depths are warmed. The results in this report show that similar changes were seen when a psychrophilic, barophilic bacterium from the greatest (hadal) ocean depths was isothermally decompressed; however, the changes happened slowly. The effects of decompression from 103.5 MPa were not immediate. The changes in morphology of the barophile *Colwellia hadaliensis*, decompressed from 75 MPa, are also not immediate.

The interpretation of changes in morphology after decompression was done with qualifications. For example, there are supramolecular structures such as the capsule, fimbriae, pili, and flagellae (18) that, if present in strain MT-41, were not visualized by the methods used. Also, the events leading to cell death may have had nothing to do with observed morphology change. That is, a pressure effect on a metabolic process could have led to cell death. Thymineless death and lactose killing, although not necessarily applicable to the case of strain MT-41, are two examples of metabolic processes that could conceivably be induced by pressure changes. With such precautions in mind, the morphological changes documented should prove useful to evaluate and to develop hypotheses for the mechanisms of cell death after decompression.

The only clearly documented (8) process killing cells on decompression is evident with gas vacuolate cells such as *Microcyclus aquaticus*. When cultures are supersaturated with a gas, the vacuoles become filled with gas at high pressure. The gas in vacuoles expands upon rapid decompression and breaks the cell envelope. Experiments with nonvacuolate bacteria reveal virtually complete survival after rapid decompression (8). The disruption of such ex-

panding vacuoles could not, therefore, explain the death of cells of isolate MT-41, a nonvacuolate bacterium. Cells of MT-41 were intact after decompression.

Other mechanical effects of decompression are imaginable from the standpoint of changes in cellular dimensions. Estimates of the compressibility of bacterial cell wall components and of bacterial cytoplasm are, however, not readily available. Furthermore, components of the cell envelope—especially phospholipid bilayers—may have anisotropic compressibilities (1). It is conceivable that the difference between the volumetric compressibility of cell envelope components and that of cytoplasm could be about 50×10^{-11} Pa. If that is the case, one would expect that the membranes of the cell envelope would expand more than either the peptidoglycan layer or the cytoplasm upon decompression. Thus, a more compressible cell envelope component, the lipid bilayer in particular, would be expected to cause problems on compression rather than on decompression. The data in this report show a long time course for morphological changes in cells transferred from 103.5 MPa to atmospheric pressure and thereby further support the conclusion that mechanical disruption of cells due to differences in the compressibilities of cell constituents is not a direct consequence of decompression.

Finally, there is a practical ramification of the observed degradation in cell morphology at 0°C and 0.1 MPa. A previous study (24) shows that cold deep-sea samples warmed for periods of less than 1 h to 25 to 32°C contain bacteria with altered cell shapes. This is significant because the surface waters over many of the deepest parts of the oceans have temperatures in excess of 25°C (6). Based on the results reported here, hadal deep-sea samples, even when retrieved cold, should be treated with a preservative immediately upon retrieval to ensure fixation of the cell structure of bacteria in the samples. One type of abyssal metazoan is known to be more sensitive to decompression (20, 21) than are abyssal bacteria. It would seem reasonable to expect that similar if not more stringent sampling constraints will be necessary to preserve the cellular structures in metazoa during retrieval from abyssal and hadal regions of the deep sea. Certain types of ultrastructural studies may be possible only with in situ fixation.

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation.

We thank R. Van Boxtel and A. S. Dietz for comments, discussions and technical help. N. Holland is thanked for his generous help and advice with sectioning and electron microscopy and for critical comments during the study.

REFERENCES

1. **Braganza, L. F., and D. L. Worcester.** 1986. Hydrostatic pressure induces hydrocarbon chain interdigitation in single-component phospholipid bilayers. Biochemistry **25**:2591–2596.
2. **Deming, J. W., and R. R. Colwell.** 1981. Barophilic bacteria associated with deep-sea animals. BioScience **31**:507–511.
3. **Deming, J. W., H. Hada, R. R. Colwell, K. R. Luehrsen, and G. E. Fox.** 1984. The ribonucleotide sequence of 5s rRNA from two strains of deep-sea barophilic bacteria. J. Gen. Microbiol. **130**:1911–1920.
4. **Deming, J. W., L. K. Somers, W. L. Straube, D. G. Swartz, and M. T. MacDonell.** 1988. Isolation of an obligately barophilic bacterium and description of a new genus, *Colwellia* gen. nov. Syst. Appl. Microbiol. **10**:152–160.
5. **de Petris, S.** 1965. Ultrastructure of the cell wall of *Escherichia coli*. J. Ultrastruct. Res. **12**:247–262.
6. **Dietrich, G., K. Kalle, W. Krauss, and G. Siedler.** 1980. General oceanography. John Wiley and Sons, Inc., New York.
7. **Glaubert, A. M., and M. J. Thornely.** 1969. The topography of the bacterial cell wall. Annu. Rev. Microbiol. **23**:159–198.
8. **Hemmingsen, B. B., and E. A. Hemmingsen.** 1978. Tolerance of bacteria to extreme gas supersaturations. Biochem. Biophys. Res. Commun. **85**:1379–1384.
9. **Infante, A. A., and P. N. Graves.** 1971. Stability of free ribosomes, derived ribosomes and polysomes of the sea urchin. Biochim. Biophys. Acta **246**:100–110.
10. **Jannasch, H. W., and C. D. Taylor.** 1984. Deep-sea microbiology. Annu. Rev. Microbiol. **38**:487–514.
11. **Jannasch, H. W., and C. O. Wirsen.** 1984. Variability of pressure adaptation in deep sea bacteria. Arch. Microbiol. **139**:281–288.
12. **Kellenberger, E.** 1990. Intracellular organization of the bacterial genome, p. 173–186. *In* K. Drlica and M. Riley (ed.), The bacterial chromosome. American Society for Microbiology, Washington, D.C.
13. **Kellenberger, E., A. Ryter, and J. Sechaud.** 1958. Electron microscope study of DNA-containing plasms. II. Vegetative and mature phage DNA as compared with normal bacterial nucleoids in different physiological states. J. Cell Biol. **4**:671–676.
14. **Landau, J. V., and L. Thibodeau.** 1962. The micromorphology of *Amoeba proteus* during pressure-induced changes in the sol-gel cycle. Exp. Cell Res. **27**:591–594.
15. **Mantyla, A. W., and J. L. Reid.** 1978. Measurements of water characteristics at depths greater than 10 km in the Marianas Trench. Deep-Sea Res. **25**:169–173.
16. **Morgan, C., H. S. Rosenkranz, B. Chan, and H. M. Rose.** 1966. Electron microscopy of magnesium-depleted bacteria. J. Bacteriol. **91**:891–895.
17. **Murray, R. G. E., P. Steed, and H. E. Elson.** 1965. The location of the mucopeptide in sections of the cell wall of *Escherichia coli* and other gram-negative bacteria. Can. J. Microbiol. **11**: 547–560.
18. **Rogers, H. J.** 1983. Bacterial cell structure, p. 77–83. American Society for Microbiology, Washington, D.C.
19. **Wyllie, A. H.** 1987. Cell death. Int. Rev. Cytol. **17**(Suppl.):755–785.
20. **Yayanos, A. A.** 1980. Measurement and instrument needs identified in a case history of deep-sea amphipod research, p. 307–318. *In* F. D. Diemer, F. J. Vernberg, and D. Z. Mirkes (ed.), Advanced concepts in ocean measurements for marine biology. University of South Carolina Press, Columbia.
21. **Yayanos, A. A.** 1981. Reversible inactivation of deep-sea amphipods (*Paralicella caperesca*) by a decompression from 601 bars to atmospheric pressure. Comp. Biochem. Physiol. **69A**: 563–565.
22. **Yayanos, A. A.** 1986. Evolutional and ecological implications of the properties of deep-sea barophilic bacteria. Proc. Natl. Acad. Sci. USA **83**:9542–9546.
23. **Yayanos, A. A., and E. F. DeLong.** 1987. Deep-sea bacterial fitness to environmental temperatures and pressures, p. 17–32. *In* H. W. Jannasch, R. E. Marquis, and A. M. Zimmerman (ed.), Current perspectives in high pressure biology. Academic Press, Inc., New York.
24. **Yayanos, A. A., and A. S. Dietz.** 1982. Thermal inactivation of a deep-sea barophilic bacterium, isolate CNPT-3. Appl. Environ. Microbiol. **43**:1481–1489.
25. **Yayanos, A. A., and A. S. Dietz.** 1983. Death of a hadal deep-sea bacterium after decompression. Science **220**:497–498.
26. **Yayanos, A. A., A. S. Dietz, and R. Van Boxtel.** 1979. Isolation of a deep-sea barophilic bacterium and some of its growth characteristics. Science **205**:808–810.
27. **Yayanos, A. A., A. S. Dietz, and R. Van Boxtel.** 1981. Obligately barophilic bacterium from the Mariana Trench. Proc. Natl. Acad. Sci. USA **78**:5212–5215.
28. **Yayanos, A. A., A. S. Dietz, and R. Van Boxtel.** 1982. Dependence of reproduction rate on pressure as a hallmark of deep-sea bacteria. Appl. Environ. Microbiol. **44**:1356–1361.