Survival of Human Enteric and Other Sewage Microorganisms Under Simulated Deep-Sea Conditions

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The survival of pure cultures of *Escherichia coli*, *Streptococcus faecalis*, *Clostridium perfringens*, and *Vibrio parahaemolyticus* under simulated deep-sea conditions of low temperature (4°C), seawater, and hydrostatic pressures ranging from 1 to 1,000 atm was determined over a period exceeding 300 h. The viability of *E. coli* and total aerobic bacteria in seawater-diluted raw sewage subjected to these deep-sea conditions was also measured. There was a greater survival of both *E. coli* and *S. faecalis* at 250 and 500 atm than at 1 atm at 4°C. *S. faecalis* was quite insensitive to 1,000 atm, whereas with *E. coli* there was a 10-fold die-off per 50-h exposure to 1,000 atm. In contrast, *V. parahaemolyticus* and *C. perfringens* were quite sensitive to pressures exceeding 250 atm, and with both of these species there was a total loss of viability of approximately 10⁴ cells per ml within 100 h at 1,000 atm and within 200 h at 500 atm. The viability of the naturally occurring fecal coliforms in sewage exposed to moderate pressures at 4°C was found to be similar to the survival patterns demonstrated with pure cultures of *E. coli*. The total numbers of aerobic bacteria in these sewage samples, however, stabilized at 500 and 1,000 atm after 100 h, and at 1 and 250 atm there was significant growth of sewage-associated bacteria, which apparently utilized the organic compounds in the seawater-diluted sewage samples. A preliminary classification of some of these bacteria indicated that approximately 90% (160 isolates) of the organisms that survived over a 400-h exposure to 500 and 1,000 atm were *Arthrobacter/Corynebacterium* species, and the representative organisms capable of growing at 1 and 250 atm in seawater at 4°C were gram-positive cellulose digesters and an unidentified gram-negative coccus. The significance of these results with respect to the contamination of the deep ocean with human pathogens and the possibility of sewage-associated microorganisms growing and competing with indigenous marine microbial flora in situ is discussed.

The disposal of human fecal wastes into coastal waters has been a common sanitary procedure for many centuries. The hazardous consequences from this practice are well documented, particularly near large urban centers where persistent dumping of sewage in marine environments has caused an increase in the frequency of disease in the resident animal population and a decrease in the numbers of both fish and benthic fauna, coincidental to creating a potential public health problem (9, 10, 25, 34, 38-40). Increased public awareness of these hazards and the future necessity for more efficient utilization of seafood resources within our continental shelf have prompted the examination of alternative waste disposal methods. There is, however, considerable pressure to continue the use of ocean disposal rather than either land disposal or some form of "complete" treatment, primarily because of cost. It has been reported that the use of long pipes or barges to transport either raw sewage or digested sludge into deep waters up to 250 miles (ca. 400 km) offshore would be significantly more economical than the alternatives of treatment plants or land disposal (10). It is apparently presumed that the rate of die-off of fecal microorganisms and the rate of degradation of sewage nutrients in pressure affected waters will be comparable to rates in coastal waters. There is considerable evidence contraindicating this presumption (14, 15, 26, 27).

Solar radiation, predation, antibiosis, chelation, and sedimentation have all been reported...
to effect the rapid die-off of *Escherichia coli* and other terrestrial microorganisms in offshore surface waters and in shallow inshore marine environments (7, 20-22, 28, 30). The disposal of sewage in oceanic waters at depths exceeding 1,500 m could result in a dispersion of sewage microorganisms throughout the water column and in sediments at depths exceeding 3,000 m. At these depths, most of the factors responsible for the die-off of coliforms in shallow waters would be, at best, only minimally effective. Thus, in pressure-affected waters, the combined effect of low temperature (3 to 4 C) and moderate hydrostatic pressures (250 to 1,000 atm) would result in little or no microbial activity (14, 15, 30). Moreover, it has been reported that *E. coli* can survive in seawater at 0 C for over 30 days (13), and, since increasing pressures theoretically have an effect similar to a lowering of the temperature (PV = nRT), it is possible that the combination of low temperature and moderate pressure could result in the long-term survival of some species of nonmarine bacteria. Indeed, it has been reported that *E. coli* and *Vibrio alginolyticus* are more resistant to pressure damage (300 to 800 atm) at temperatures near their minimum than at their optimum growth temperature (3). Psychrophilic microorganisms, in contrast, were found to become irreversibly damaged when exposed to pressures above their maximum for growth at in situ ocean temperatures (3). It is possible, therefore, that fecal bacteria could persist for longer periods in pressure-affected waters than in coastal environments.

In this report, the effect of simulated deep-sea conditions of low temperature (4 C), pressure (1 to 1,000 atm), and seawater on the survival of pure cultures of *E. coli*, *Streptococcus faecalis*, *Clostridium perfringens*, and *Vibrio parahaemolyticus* were measured over a period of up to 3 weeks. Fluctuations in the total numbers of bacteria and the natural coliform population from seawater-diluted raw sewage maintained under similar deep-sea conditions were also measured.

**MATERIALS AND METHODS**

**Bacterial strains and sewage samples.** *E. coli* C-600, *S. faecalis* (Oregon State University culture collection), *C. perfringens* type A (ATCC 12916), and *V. parahaemolyticus* (ATCC 17802) were employed in this investigation. The raw-sewage samples were obtained from the Corvallis municipal sewage disposal plant as their untreated influent, which consisted of both industrial and domestic wastes. Samples were collected in sterile, 500-ml wide-mouth bottles.

**Preparation of cells and pressure techniques.** *E. coli* and *S. faecalis* were cultivated in Trypticase soy broth (BBL) at 20 C. *V. parahaemolyticus* was grown in Lib-X broth (3) at 20 C. *C. perfringens* was cultivated in TPGY broth [Trypticase [BBL], 5%; peptone [Difco], 0.5%; glucose, 0.1%; yeast extract [Difco], 0.5%; and sodium thioglycolate, 0.1%; pH adjusted to 7.0] at 30 C. All cultures were harvested at the late log phase by centrifugation at 12,500 × g for 10 min in a Sorval RC-2B refrigerated centrifuge. The cells were washed three times in 4% artificial seawater (ASW) (Rila salts, 4% equal to the concentration of Na⁺ and other major and minor inorganic constituents, including heavy metals, found in natural seawater) and finally resuspended in 500 ml of sterile 4% ASW to a final concentration of approximately 10⁷ bacteria per ml as estimated by optical density readings at 600 nm with a Bausch & Lomb Spectronic 20 colorimeter. Natural seawater was not used because water samples could not be obtained from depths exceeding 200 m. Surface oceanic water would likely be contaminated by detritus, microbial metabolites, etc., all of which could influence the survival of microorganisms, and thus would not be representative of seawater obtained from depths equivalent to the pressures employed in this investigation. Raw-sewage samples were initially diluted 1:1 in 8% sterile ASW and then diluted further in 4% sterile ASW to give a final concentration of sewage of 1:20 in 4% ASW.

Washed-cell suspensions and diluted-sewage samples were pressurized in 10-ml sterile, disposable, plastic syringes (Becton-Dickinson & Co.) capped with epoxy cement-sealed sterile hypodermic needles. Two syringes for each sample at each pressure were placed into pressure vessels and pressurized (31). All samples were incubated at 4 C in a circulating water bath-incubator (Amino). The pressures used in this study were 1, 250, 500, and 1,000 atm. All enumerations were performed with duplicate samples for each pressure. The stability of each pressure vessel to maintain the original pressure was checked prior to release for sampling.

**Enumeration of bacteria.** All serial dilutions were made with 3.5% ASW at pH 7.5. The initial 10⁻¹ dilution was made with 10 ml of the sample in a 90-ml dilution blank, whereas 9-ml blanks were used for subsequent dilutions. *E. coli* and *S. faecalis* were enumerated on Trypticase soy agar (BBL), whereas *V. parahaemolyticus* was enumerated on Lib-X agar (3) by using the spread plate technique with a 0.2-ml inoculum in duplicate. *C. perfringens* was plated on TPGY agar (1.5% agar) by the spread plate technique with a 0.2-ml inoculum. Plates were incubated in Gas-Pak anaerobic jars (BBL, H₂-CO₃ gas generator) at 30 C for up to 48 h. The anaerobic jars were maintained dry by placing approximately 250 g of Drierite (W. A. Hammond Drierite Co.) in the bottom of the jar.

Three tube most-probable-number counts were used to determine the presumptive and confirmed numbers of fecal coliforms in sewage samples. Lactose and EC broths (Difco) were used in accordance with established procedures (2). The "total counts" from sewage were measured with plate count agar (Difco) using the pour plate technique. Plates were incubated
at 20 C for up to 5 days. Total psychrophilic bacterial counts for the sewage samples held at 1 and 250 atm were determined on plate count agar. Spread plates with a 0.2-ml inoculum were incubated at 4 C for 10 days.

Preliminary classification of sewage microorganisms. Bacterial isolates from sewage that survived over 400 h of exposure to both 500 and 1,000 atm at 4 C and isolates from samples that were capable of growth at 1 and 250 atm at 4 C in 4% Rila were tentatively classified to the genus level. All colonies on plates having counts between 30 and 100 were purified by streaking on Trypticase soy agar (BBL). Between 40 and 120 representative isolates from each sample were selected for final classification. All isolates were Gram stained and observed by phase microscopy. Gram-negative rods were classified further in accordance with the scheme of Shewan et al. (36) and Carpenter et al. (8). Gram-positive bacilli were differentiated by ability to sporulate and utilize cellulose, cellular morphology, and motility. Suspected Corynebacterium and Arthrobacter species were cultured and identified by the procedures of Cure and Keddie (12) and Crombach (11). Isolates belonging to the Micrococcaceae were identified by Gram reaction and cellular morphology. Presumed azotobacters were identified by cellular morphology and ability to grow on nitrogen-free media (16, 32). All isolates suspected of belonging to the Actinomycetales were classified as actinomycetes. These included acid-fast Mycobacterium and Nocardia species. Photomicrographs were made with a Leitz-Orthomat microscope and camera system.

RESULTS

Survival of pure cultures. Washed cells of E. coli, S. faecalis, V. parahaemolyticus, and C. perfringens were suspended in 4% ASW to a final concentration of approximately 10^8 cells per ml. The survival patterns for these organisms at 4 C under various pressures for a period exceeding 2 weeks are shown in Fig. 1 through 4. Clearly, the viability of E. coli (Fig. 1) and S. faecalis (Fig. 2) remained quite stable under these simulated deep-sea conditions. E. coli showed greater stability at 250 and 500 atm than at 1 atm, and at 1,000 atm there was approximately a 6-log decrease in the number of viable cells over a period of 300 h. In contrast, there was less than a 2-log decrease in the number of viable streptococci at 1 and 1,000 atm after 350 h and no significant reduction (less than 0.5 log) at 250 and 500 atm. Both C. perfringens and V. parahaemolyticus were far less resistant to the combined effect of low temperature, pressure, and salinity than E. coli or S. faecalis (Fig. 3, 4). Surprisingly, V. parahaemolyticus showed greater survival at 1 and 250 atm and at 4 C than would have been predicted from previous reports (24), and there was only approximately a 2-log decrease in the number of vibrios after 300 h at 1 and 250 atm. V. parahaemolyticus, however, was very sensitive to pressures exceeding 500 atm at 4 C, and there was a reduction of 10^8 cells per ml in less than 40 h of exposure to 1,000 atm. This extreme sensitivity to pressures exceeding 250 atm is similar to previous reports for V. alginolyticus held at 15 C (3) and for V. parahaemolyticus held at 15 and 25 C (35). Figure 4 shows the survival of C. perfringens under simulated deep-sea conditions. Within 200 h, there was a 10^4 reduction in the number of viable clostridia per ml at 250, 500, and 1,000 atm, whereas there was approximately a 3-log reduction of cells after a 300-h exposure to 1 atm. Two other C. perfringens strains, isolated from environmental samples, showed similar die-off rates at 250 atm.

Survival of microorganisms in raw sewage. The seawater-diluted sewage samples were subjected to various pressures at 4 C for up to 24 days. Table 1 shows the number of presumptive and confirmed fecal coliforms per milliliter for three different sewage samples. In general, the
confirmed coliform counts declined rapidly. In all samples, the number of confirmed E. coli declined more rapidly than did the presumptive counts. Obviously, the organisms responsible for gas formation in lactose broth were not E. coli. Microscope observations of some of these positive lactose fermenters from 1 and 250 atm showed a predominance of gram-positive, spore-forming bacilli, presumably including Clostridium sp.

Table 2 shows the results of 20 C standard plate counts from the three sewage samples. At 1 and 250 atm, there was an initial decline in the numbers of organisms, followed by an increase after 200 h. This is particularly apparent in sewage sample 3, where the counts increased from $3.7 \times 10^4$ to $4.1 \times 10^7/ml$ in 350 h. At 500 and 1,000 atm, the numbers of organisms rapidly declined within 30 h and then stabilized at approximately 1 to 5% of the original population at 500 atm and approximately 0.1% of the original population at 1,000 atm. The effects of various pressures on the numbers of bacteria from sewage sample 3 are clearly shown in Fig. 5. It is apparent that at 250 and 500 atm there was approximately a 1-log reduction in counts within the first 100 h. This decrease probably conforms to the 1-log decrease noted in the coliform counts within 100 h from the same sample (Table 1). At 1,000 atm, there was a 2-log decrease after 100 h, while there was a simultaneous 4-log reduction in the number of coliforms. Obviously, the most-probable-number lactose broth method for enumerating coliforms is too selective for high-pressure-exposed enteric bacteria.

Psychrotrophic and barotolerant sewage bacteria. The data from Fig. 5 indicate that the raw-sewage samples harbored microorganisms other than fecal coliform types and that some of these bacteria were capable of growing in seawater at 4 C and at 250 atm of pressure. Furthermore, there was a significant number of organisms that survived over a 400-h exposure to 500 and 1,000 atm to a greater extent than E. coli. Representative isolates that grew at 4 C and also isolates that survived over a 400-h exposure to 500 and 1,000 atm of pressure were provisionally classified to genus or related genera. Table 3 summarizes these data and shows the frequency of bacterial types either capable of growing in dilute sewage at 1 and 250 atm or surviving over a 400-h exposure to 500 and 1,000 atm. The predominant bacterial types recovered from the 500- and 1,000-atm samples were Corynebacterium/Arthrobacter types. The 500-atm samples did harbor approximately 10%
### Table 1. Presumptive coliform and confirmed E. coli most-probable-number counts per milliliter of sewage sample diluted in artificial seawater and held under different pressures at 4°C for up to 460 h.

| Sampling time (h) | Test     | 1 Atm   | 250 Atm | 500 Atm | 1,000 Atm |
|------------------|----------|---------|---------|---------|-----------|
|                  |          | 1*      | 2*      | 3*      | 1         | 2         | 3         | 1         | 2         | 3         |
| 0                | Presumptive Confirmed | 1,100   | 43      | 110     | —        | 110       | 1,100     | 43      | 110       | 1,100     | 43      | 110       |
|                  |          | 43      | 2.3     | 46      | —        | 46        | 43        | 2.3     | 46        | 43        | 2.3     | 46        |
| 30               | Presumptive Confirmed | 460     | 43      | —       | —        | —         | 93        | 9.3     | 80        | 0.09      | <0.001  | —         |
|                  |          | 24      | 2.3     | —       | —        | —         | 4.4       | 0.8     | 34        | 0.01      | <0.001  | —         |
| 56               | Presumptive Confirmed | —       | —       | —       | —        | —         | —         | —       | —         | 0.02      | <0.001  | —         |
|                  |          | —       | —       | 11      | —        | 4.6       | —         | —       | —         | 0.02      | 0.004   | —         |
| 132              | Presumptive Confirmed | 230     | 4.6     | —       | 240      | —         | 23        | 0.93    | 19        | <0.001    | <0.001  | —         |
|                  |          | 2.3     | 0.4     | 4.3     | —        | —         | 4.3       | <0.001  | 0.9       | <0.001    | <0.001  | —         |
| 150              | Presumptive Confirmed | —       | —       | 46      | —       | —         | 9.3       | —       | —         | —         | 0.09    | <0.001    |
|                  |          | —       | —       | 11      | —       | 4.6       | —         | —       | —         | —         | —       | —         |
| 220              | Presumptive Confirmed | 240     | 4.3     | —       | 240      | —         | 15        | 0.93    | —         | <0.001    | <0.001  | —         |
|                  |          | 1.5     | 0.2     | 1.5     | —        | —         | 0.4       | 0.009   | —         | <0.001    | <0.001  | —         |
| 270              | Presumptive Confirmed | —       | —       | 93      | —       | —         | 11        | —       | —         | 11        | —       | —         |
|                  |          | —       | —       | 2.4     | —       | 0.7       | —         | —       | 0.5       | —         | —       | —         |
| 350              | Presumptive Confirmed | 240     | 4.3     | 110     | 230      | —         | 11        | 0.4     | 0.2       | 12        | <0.001  | <0.001    |
|                  |          | 2.3     | 0.15    | 9.3     | 4.3      | —         | 0.5       | 0.09    | <0.001    | 0.5       | <0.001  | <0.001    |
| 460              | Presumptive Confirmed | —       | —       | 46      | —       | 4.6       | —         | 10      | —         | —         | —       | —         |
|                  |          | —       | —       | 2.4     | —       | 0.2       | —         | 0.5     | —         | —         | —       | —         |

*a* Raw sewage samples.

*b* — No data.
TABLE 2. Total 20 C bacterial plate counts per milliliter of sewage samples diluted in artificial seawater and held under different pressures at 4 C for up to 580 h

| Sampling time (h) | 1 Atm | 250 Atm | 500 Atm | 1,000 Atm |
|------------------|-------|---------|---------|-----------|
|                  | 1*    | 2*      | 3*      | 1         | 2         | 3         | 1         | 2         | 3         |
| 0                | 740   | 40      | 370     | 740       | 40        | 370       | 740       | 40        | 370       |
| 30               | 370   | 25      | —       | 250       | —         | —         | 120       | 21        | 90        |
| 56               | —     | —       | 290     | —         | —         | 47        | —         | —         | —         |
| 100              | —     | —       | —       | —         | —         | —         | 26        | 4.4       | 15        |
| 132              | 58    | 7.8     | —       | 80        | —         | —         | —         | —         | —         |
| 150              | —     | —       | 1,000   | —         | 80        | —         | —         | —         | —         |
| 220              | 80    | 6.4     | —       | 100       | —         | —         | 7.1       | 0.9       | 10        |
| 270              | —     | —       | 2,700   | —         | —         | 620       | —         | —         | —         |
| 350              | 200   | 18      | 4,100   | 400       | —         | 1,300     | 5.2       | 2.7       | —         |
| 460              | —     | —       | 2,600   | —         | 970       | —         | —         | 7.8       | —         |
| 580              | —     | —       | 2,800   | —         | —         | 1,900     | —         | —         | —         |

* Raw sewage samples.
* —, No data.

![Graph](http://aem.asm.org/)

FIG. 5. Viability of total aerobic bacteria from seawater-diluted raw sewage sample 3 held at various pressures at 4 C as determined by standard plate count at 20 C.

oxidase-negative lactose fermenters and presumptive azotobacters, whereas these organisms were absent from the 1,000-atm samples. The representative isolates responsible for the growth in the 1- and 250-atm-held sewage samples were presumptive azotobacters, spore-forming Bacillus sp., and a gram-positive motile rod capable of utilizing cellulose and tentatively classified as a Cellulomonas sp. All of these cultures were found to grow equally well in distilled water or seawater media and at both 0 and 25 C.

Figure 6a through e shows some of the typical bacterial isolates from 1,000- and 250-atm-incubated sewage samples. In Fig. 6a through e are representative gram-positive coryneforms isolated from 1,000-atm samples; Fig. 6f shows a Nocardia sp. also isolated at 1,000 atm. Figure 6g through i shows the three dominant bacterial types capable of growing in seawater-diluted sewage at 250 atm; Fig. 6g shows a frequently isolated gram-negative coccus. Figure 6h shows a probable Cellulomonas sp., and the organism in Fig. 6i is presently unidentified. Photomicrographs of the 1,000-atm coryneforms were made with 36-h cultures grown in EYGA broth (12) incubated at 22 C, whereas the isolates shown in Fig. 6g through i were cultured in Lib-X broth and incubated at 5 C for 72 h.

DISCUSSION

There are no pressure-affected marine environments below the thermocline having ambient temperatures exceeding 4 to 5 C. Therefore, the synergistic stress imposed by the combination of low temperature and moderate hydrostatic pressure will be the primary physical factor influencing the ability of microorganisms, as well as other fauna, to survive and grow in the deep sea. The known psychrophilic bacteria capable of growing at in situ deep-ocean temperatures invariably show a marked diminution in growth rate and biochemical activity with increasing pressure (3, 30, 40). Very few bacterial isolates have been reported to grow at pressures exceeding 500 atm at temperatures below 5 C (30). Moreover, it has been reported that marine bacteria having optimum growth temperatures close to in situ temperatures are irreversibly injured by pressures just exceeding the maximum pressure for growth (3). In contrast, some mesophilic species show greater viability with increasing pressures.
if there is a concomitant decrease in the temperature below their optimum (3, 18, 19). Similarly, it has been reported that E. coli shows a significantly slower die-off rate in seawater held at 0°C (13) or 4°C (7) than near or above optimum growth temperatures. In this report, there was little or no loss of viability of E. coli and S. faecalis after a 300-h exposure to 250 and 500 atm at 4°C, whereas at 1 atm there was approximately a 1-log die-off per 100 h for E. coli. S. faecalis was also insensitive to long-term exposure to 1,000 atm. Moreover, some apparent soil arthrobacter/corynebacteria and actinomyces-like organisms present in raw sewage were found to be unaffected by long-term exposure to 500 and 1,000 atm. Thus, microorganisms can tolerate higher pressures at temperatures below their minimum for growth than at temperatures within their growth range. It is possible that enzymatic activity, macromolecular synthesis, or substrate transport mechanisms are pressure sensitive at temperatures within the range of activity for these systems, whereas at temperatures below the minimum for activity no damage is incurred. It is apparent, therefore, that the primary physical parameter affecting microorganisms in the deep ocean is temperature, and as the pressure is increased (approximately 100 atm per 1,000 m depth) the resulting physical effect on organisms is analogous to a further decrease in the temperature within the limits whereby pressure is not physically inactivating macromolecules or their synthetic systems (23, 29, 30).

It is not wholly possible to ascertain whether the patterns of survival observed for enteric bacteria under ideal laboratory deep-sea conditions could be extrapolated to natural oceanic conditions. Obviously, in shallow near-shore environments or within the euphotic zone in off-shore waters, many of the factors known to influence the survival of coliforms in seawater, such as solar irradiation, predation, etc., would presumably be effective. Below the thermocline, sedimentation would be the most important factor in the removal of suspended bacteria and other particulates from the water column. Moreover, any antibacterial process involving metabolically active marine fauna would decrease with increasing depths, thus increasing the likelihood that the observations made in this report would be applicable to situ deep waters. Jannasch et al. (15) presented further supporting evidence based on their observation that food contained within the sunken research submersible Alvin was in a well-preserved state when recovered after 10 months at a depth of 1,500 m. Similarly, only a fraction of the organic substrates incubated with deep-water samples at depths to 5,300 m for 1 year was utilized compared with the 1-atm control (14). It is assumed that the marine bacteria present in deep waters have drastically reduced growth and metabolic rates due to the combination of low temperature and high pressure. Therefore, it is quite likely that some mesophilic bacteria associated with domestic sewage would remain viable but in an inactive state for an indeterminate period. There is some evidence that terrestrial microorganisms do accumulate in deep sediments. Bacillus stearothermophilus has been isolated from sediments at depths greater than 1,000 m (4), and Clostridium sporogenes and C. sticklandii were found in sediments obtained from the Cariaco Trench (37). Presumably, the survival of these Bacil-

**Table 3. Frequency of isolation of representative bacterial types from seawater-diluted raw-sewage samples held at various pressures at 4°C**

| Sample variable         | Isolation temp (C) | No. of isolates | Gram-negative rods | Arthrobacter/ corynebacteria | Gram-positive cocci | Gram-positive spore-formers | Cellulose digesters | Actinomycetes | Others |
|-------------------------|-------------------|----------------|-------------------|----------------------------|-------------------|-----------------------------|---------------------|---------------|--------|
| 1,000 atm, 480-h exposure | 20                | 120            | 0                 | 0                          | 104               | 1                           | 0                   | 14            | 1      |
| 500 atm, 480-h exposure  | 20                | 40             | 0                 | 5*                        | 28                | 2                           | 1                   | 0             | 3      |
| 250 atm, 480-h exposure  | 4                 | 60             | 0                 | 0                          | 3                 | 1                           | 9                   | 22            | 0      |
| 1 atm, 480-h exposure    | 4                 | 40             | 2*                | 0                          | 1                 | 0                           | 3                   | 20            | 0      |

* Confirmed to be E. coli.
* Isolates were primarily gram-negative, large cocci (Fig. 6g) and an unidentified organism (Fig. 6i).
* Both isolates were fluorescent pseudomonads.
laceae is favored by their ability to sporulate, but the facts still remain that the apparent natural bacterial cropping processes in the water column during particle descent, and in the sediments, may not be as rapid and effective as expected, even in relatively shallow waters. Moreover, the isolation of terrestrial bacteria from sediments below 1,000 m indicates that sedimentation is probably the most important factor responsible for the removal of microorganisms from the water column. Since the rate of sedimentation for particles the size of bacteria or bacterial aggregates is quite slow and in the range of 0.1 to 1 m/day (17), a necessary prerequisite before sewage microorganisms could accumulate in deep sediments would be

Fig. 6. Photomicrographs of representative bacterial isolates surviving over 400-h exposure to 1,000 atm of pressure at 4 C (a through f) and isolates capable of growing in seawater-diluted sewage at 250 atm at 4 C (g through i). Magnification, x2,016.
their ability to remain viable for many years in the water column. Apparently some terrestrial sporeformers and, based on the present report, *Arthrobacter/Corynebacterium* species, actinomycetes, and possible *S. faecalis, E. coli*, and other related human enteric pathogens could fulfill this prerequisite.

The persistent dumping of sewage on or just above the sediment in relatively shallow waters between 1,000 and 3,000 m would likely result in the accumulation of sewage microorganisms and sewage-associated organics and in the contamination of the indigenous fauna with potential human pathogens. This condition could eventually lead to anaerobiosis particularly if the rate of build-up of sewage and associated microorganisms exceeds the relatively slow rates of organic turnover observed in deep sediments (2, 27). Furthermore, the disposal of raw sewage into deep water at areas of upwelling could result in the recycling of potentially dangerous enteric microorganisms into surface waters of high productivity and eventually to the contamination of food-fish and invertebrates (6, 38, 39). Moreover, it has been reported that microorganisms are routinely captured by bubbles formed in the water surface and then ejected into the atmosphere (5). This could result in the eventual contamination, through aerosols, of large coastal urban centers. Presumably, the observations by ZoBell and Mathews (41) that viable marine bacteria were carried 30 miles (ca. 48.3 km) inland could be explained by this phenomenon.

Although the data presented in this report represent simulated deep-sea conditions only, there are some indicative problems that deserve further study. It was shown, for example, that bacteria associated with sewage had the capacity to grow under oceanic conditions equivalent to depths of 2,500 m. The question is could similar organisms grow and compete with indigenous marine bacteria in situ in water and sediments at depths exceeding 1,000 m, and what impact would this have on normal mineralization processes? Secondly, based on this investigation, neither *E. coli* nor *S. faecalis* should be employed as indicators of recent fecal pollution in deep waters or sediments. Moreover, *S. faecalis*, contrary to popular belief, can survive for long periods at 1 atm in seawater at 4 C and thus might not be a suitable indicator organism even from surface oceanic waters. Only vegetative *C. perfringens* cells were shown to have a relatively rapid die-off rate at all pressures at 4 C and thus could be the most suitable indicator for all off-shore marine environments. It is also not known whether specific human enteric pathogenic bacteria and viruses, when exposed to deep-ocean environments, would have survival patterns similar to the indicator organisms tested.

Based on this preliminary investigation, it is quite apparent that the dumping of raw or partially treated sewage into deep waters could have potentially dangerous ecological and public health consequences. Therefore, we strongly recommend an alternative method of waste recycling.

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