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Stress Response of *Escherichia coli* to Elevated Hydrostatic Pressure

TIMOTHY J. WELCH,[1] ANNE FAREWELL,[2] FREDERICK C. NEIDHARDT,[2] AND DOUGLAS H. BARTLETT[1]*

Center for Marine Biomedicine and Biotechnology, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California 92093-0202,[1] and Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, Michigan 48109-0620[2]

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The response of exponentially growing cultures of *Escherichia coli* to abrupt shifts in hydrostatic pressure was studied. A pressure upshift to 546 atm (55,304 kPa) of hydrostatic pressure profoundly perturbed cell division, nucleoid structure, and the total rate of protein synthesis. The number of polypeptides synthesized at increased pressure was greatly reduced, and many proteins exhibited elevated rates of synthesis relative to total protein synthesis. We designated the latter proteins pressure-induced proteins (PIPs). The PIP response was transient, with the largest induction occurring approximately 60 to 90 min postshift. Fifty-five PIPs were identified. Many of these proteins are also induced by heat shock or cold shock. The PIP demonstrating the greatest pressure induction was a basic protein of 15.6 kDa. High pressure inhibits growth but does not inhibit the synthesis of stringently controlled proteins. Cold shock is the only additional signal which has been found to elicit this type of response. These data indicate that elevated pressure induces a unique stress response in *E. coli*, the further characterization of which could be useful in delineating its inhibitory nature.

Pressure is a physical parameter which has influenced the evolution and distribution of life. For example, in the deep sea, obligatory barophilic bacteria which require pressures greater than 1,000 atm for optimal growth (1 atm = 1.01325×10^5 Pa = 1.01325 bars = 14.7 lb/in^2; 36) have been isolated. Although there is little information regarding the mechanisms of bacterial adaptation to high pressure, the fundamental basis of all pressure effects stems from the changes in volume which accompany biochemical and physiological processes. High pressure inhibits the rate or extent of reactions which result in an increase in the volume of activated complexes or end products. Conversely, high pressure promotes reactions which decrease overall system volumes. In many cases, the volume changes are due to changes in water structure around proteins, nucleic acids, ions, and enzyme substrates (27). Although numerous studies have investigated high-pressure effects on particular functions in *Escherichia coli*, a bacterial species that does not ordinarily grow at high pressure, controversy remains as to the key pressure points which limit the growth of bacteria at high pressure (14, 20, 22).

The effects of elevated pressure on *E. coli* are pleiotropic. Both rotation of the flagellum and flagellar filament polymerization are affected at pressures well below those which affect cell growth (16). The proton-translocating ATPase is also pressure sensitive (14). Because the capacity of this enzyme to promote proton translocation across the membrane is considerably more sensitive to elevated pressure than is its ability to catalyze ATP hydrolysis, cells under pressure expend more energy pumping protons. Moderate pressures in the range of 200 to 500 atm inhibit cell division more than cell growth, causing single cells to form long filaments (42). Moderate pressures also lengthen the lag period of *E. coli* cultures (41). When the uptake of radiolabeled thymine, leucine, and uracil is measured, the results indicate that DNA synthesis stops at around 500 atm, protein synthesis stops at around 580 atm, and RNA synthesis stops at around 770 atm (38). At certain pressures, cell division and DNA synthesis become partially synchronous, perhaps because elevated pressure inhibits new rounds of DNA replication. The sensitivity of protein synthesis to high pressure is likely caused by pressure inhibition of aminoacyl tRNA binding to the ribosomes and loose couple formation (6, 21). Moderate pressures are known to affect the quaternary structures of many proteins (33).

Although *E. coli* is not a bacterial species which is believed to have evolved specific mechanisms for coping with variation in hydrostatic pressure, many of the effects of pressure could overlap with those of stressors to which *E. coli* has evolved adaptational mechanisms. Identification of such overlaps could reveal fundamental aspects of the inhibitory effect of high pressure on cell metabolism. As a starting point for analysis of the biochemical effects of, and physiological responses to, elevated pressure, we chose to monitor the high-pressure protein responders synthesized in *E. coli*. In addition, the extensive gene-protein data base available for *E. coli* was used to identify proteins whose relative rates of synthesis are elevated under high pressure. The data show that elevated hydrostatic pressure induces a unique stress response in *E. coli* which results in higher levels of both heat shock proteins (HSPs) and cold shock proteins (CSPs), as well as of many proteins which appear only in response to high pressure.

MATERIALS AND METHODS

Bacterial strain, media and growth conditions. *E. coli* K-12 strain W3110 (26) cells were grown at 28°C in morpholine propanesulfonic acid (MOPS) minimal medium (17) that was modified for anaerobic growth by increasing the MOPS buffer concentration to 80 mM. Glucose (0.8% [wt/vol]) was added as a carbon and energy source. Cells were grown anaerobically to a steady state in 25-ml Monovett syringes (Sarstedt Inc., Newton, N.C.). All culture manipulations were done in an anaerobic glove box under nitrogen gas to minimize introduction of oxygen into the culture. It was necessary to culture *E. coli* anaerobically when incubating cells under elevated pressure because gases such as oxygen can become toxic at increased partial pressures (13). For the viability experiments, bacteria were diluted into L broth (5) and plated onto the same medium containing 1.5% agar (Difco Laboratories, Detroit, Mich.).

* Corresponding author.

High-pressure incubations. Cells were incubated at elevated hydrostatic pressure in stainless steel pressure vessels equipped with quick-connect fittings for rapid decompression-recompression as previously described (39).

Bacterial growth measurements. The optical density of the bacterial culture was measured with a Spectronic 20 spectrophotometer (Milton Roy Co., Rochester, N.Y.). Direct bacterial counts were determined by epifluorescence microscopy after staining with 4′,6-diamidino-2-phenylindole (23).

Determination of relative rates of protein synthesis. Relative rates of protein synthesis were determined by measuring incorporation of L-[4,5-^{3}H]leucine (final concentration, 20 μM; 165 mCi/mmol; Amersham, Arlington Heights, Ill.) into a cold 5% trichloroacetic acid-insoluble fraction. This fraction was collected by centrifugation as previously described (25). Radioactivity was determined by liquid scintillation counting.

Radioactive labeling of proteins. Proteins were pulse-labeled by addition of TRAN-^{35} S-label (1,089 Ci/mmol; 50 μCi/ml; ICN Biomedicals, Inc., Costa Mesa, Calif.) for 30-min periods at various times before and after pressurization. To expose the cells to the label without decompressing the culture, the label was diluted into MOPS medium and 1.5 ml was put into a small latex finger cot and sealed by clamping the loose end with a metal clamp such that no air spaces existed. The finger cots were then placed into 25-ml syringes containing the culture, and syringe needles were affixed to the inside syringe tops. After the syringes were placed inside pressure vessels, labeling could be started at any time before or after pressurization by inversion of the pressure vessel, which resulted in rupture of the latex balloon by the syringe needle. At the end of a labeling period, the cultures were quickly decompressed and labeling was stopped by addition of nonradioactive methionine (final concentration, 50 mM) and cysteine (final concentration, 8 mM). Control experiments verified that no protein labeling occurred until inversion of the pressure vessels.

One-dimensional SDS-PAGE. One dimensional protein separation was performed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (1). The resolving gel was 12.5% acrylamide. Protein samples were dissolved in Laemmli sample buffer (9). For fluorography, gels were treated with En^{3}Hance (Dupont, Boston, Mass.) before exposure to Kodak X-Omat AR film.

Two-dimensional PAGE. Both the preparation of cell extracts for two-dimensional (2D) PAGE and the 2D PAGE itself were performed by the methods of O'Farrell (18) with the modifications of VanBogelen and Neidhardt (31).

Immunoprecipitation. Immunoprecipitations were carried out as described in the PANSORBIN immunological applications handbook radioimmunoprecipitation protocol (Calbiochem, San Diego, Calif.).

PIP identification. Pressure-induced protein (PIP) isoelectric points (pI) and relative molecular masses were determined by plotting x or y coordinates versus reference spots of proteins with known pI or molecular weights (32). Preliminary identification of PIPs was accomplished by comparing PIP alphanumeric and coordinate positions with those present in the *E. coli* gene-protein data base (32), as well as by comparing Fig. 7 with previously published HSP (28) and CSP (8) autoradiograms.

RESULTS

Changes in OD, viability, and cell number after pressure upshift. W3110 cultures were divided into two portions at an optical density (OD) at 600 nm of 0.10 (designated time zero). At this time, one portion was incubated under the original atmospheric pressure conditions while the other was subjected to 546 atm of hydrostatic pressure. At various times, both cultures were sampled to determine OD, viability CFU, and cell numbers (direct cell counts). Although sampling of the high-pressure culture necessitated brief periods of decompression and recompression (approximately 30 s), it has previously been found that for bacterial cultures with generation times of several hours, brief decompression-recompression does not affect cell growth (37). Control experiments in which the ODs of a W3110 culture were measured at time zero and 1,360 min postshift to 546 atm indicated that the sampling regimen had no effect on cell growth.

Elevated pressure had a deleterious effect on all measured parameters of W3110 growth (Fig. 1). Upon pressurization, the OD displayed a lag of approximately 100 min before once more increasing at a lower, postshift rate. Conversely, no recovery in CFU or direct cell counts over time was observed postpressurization. The discrepancy between OD and cell number at high pressure is caused by a pressure-induced increase in cell size. Microscopic analysis of 4′,6-diamidino-2-phenylindole-stained cells revealed that by 1,360 min postpressurization, the 546-atm cells were an average of 2.3 times longer than the 1-atm control cells (Fig. 2). Also, the nuclear material inside the 546-atm cells was considerably more condensed than in the 1-atm cells.

Changing rates of protein synthesis after pressure upshifts. The effect of elevated pressure on protein synthesis in W3110 was studied by monitoring the incorporation of [^{3}H]leucine into protein after a pressure shift to 546 atm. Leucine was added to two cultures in sufficient quantity to support protein synthesis for more than 6 h. Label incorporation proceeded for 200 min to allow the intracellular [^{3}H]leucine time to equilibriate. After 200 min, one culture was pressurized to 546 atm and the other was left at 1 atm. Incorporation of [^{3}H]leucine into protein was determined for both cultures. The data in Fig. 3 show that exposure to a pressure of 546 atm dramatically inhibited protein synthesis. It was estimated from the leucine incorporation over a 6-h period that the overall rate of protein synthesis at 546 atm was only 13% of that of the 1-atm control.

Differential rate of protein synthesis after pressure upshift. The previous experiments verified that under the culture conditions employed, exposure of W3110 to 546 atm of hydrostatic pressure is highly inhibitory to cell division and protein synthesis. To investigate whether pressure treatments also resulted in synthesis of specific stress proteins, steady-state W3110 cultures were divided into five portions which were pressurized to 1, 272, 546, 819, and 1,092 atm. At 60 min postpressurization, all cultures were labeled for 30 min with sufficient [^{35}S]methionine to prevent its depletion during the labeling period. Extracts of each sample were prepared, and portions were processed in two ways. Trichloroacetic acid precipitation was performed to monitor the effects of different pressures on protein synthesis, as measured by [^{35}S]methionine incorporation. One-dimensional SDS-PAGE and autoradiography were performed to monitor the effect of pressure on the synthesis of specific proteins (Fig. 4). In this way, the effects of pressure on protein synthesis could be directly compared to the autogradiographic results. The [^{35}S]methionine incorporation data as percentages of the 1-atm value were 62, 30, 0.8, and 0.08% at 273, 546, 819, and 1,092 atm, respectively. These data indicated a linear decrease in the rate of protein synthesis with increasing pressure up to approximately 819 atm. At 1,092 atm, there was essentially no protein synthesis. Increased pressure also caused modulation of the rates of synthesis of specific proteins. While the number of protein species synthesized decreased with increasing pressure, some polypeptides ap-

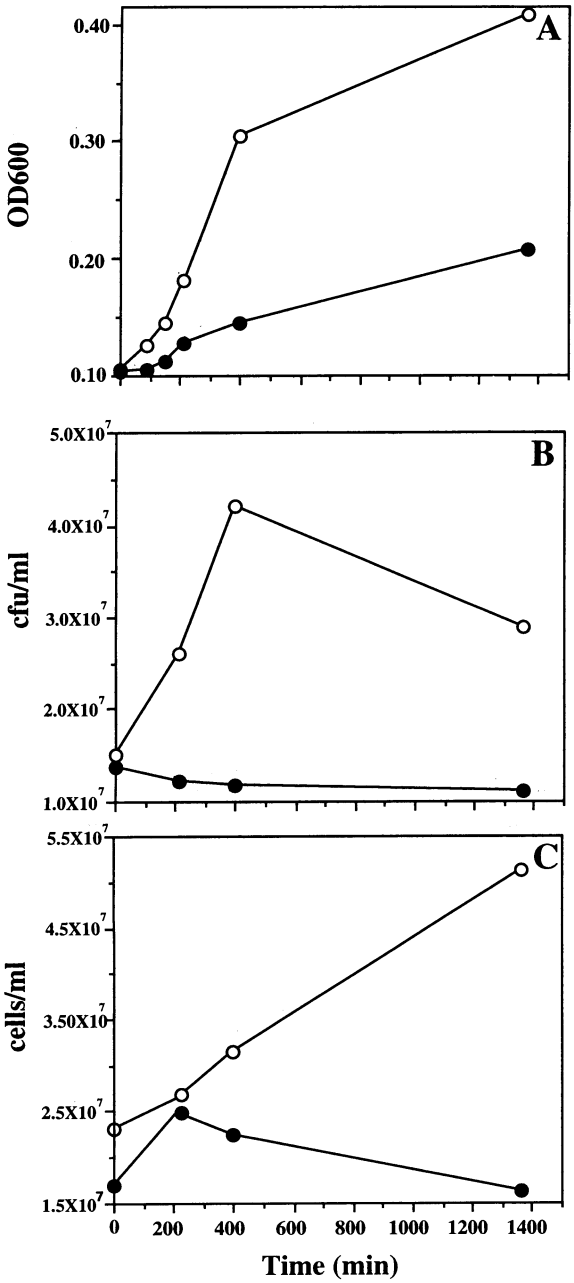

FIG. 1. Growth of strain W3110 in modified glucose-MOPS medium after hydrostatic pressure upshift. OD at 600 nm (OD600) (A), CFU (B), and epifluorescence direct counts (C) as a function of time after a shift to 546 atm (●) are compared with those of unpressurized cells (○).

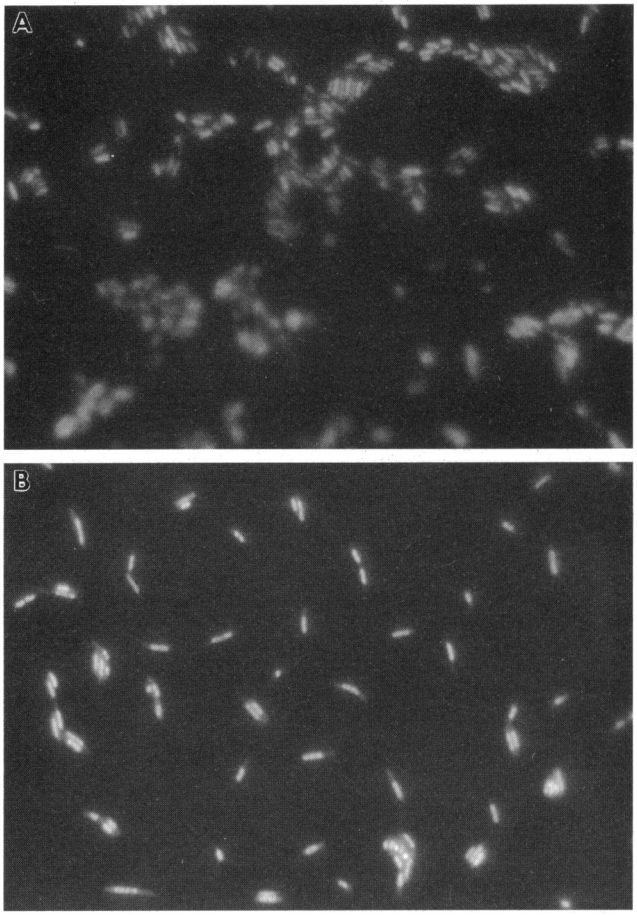

FIG. 2. Epifluorescence photomicrographs of 4′,6-diamidino-2-phenylindole-stained cells from low- and high-pressure-treated cultures used for the growth experiments whose results are shown in Fig. 1. Samples for photomicrographs were taken at 1,400 min. Panels: A, 1-atm-treated cells; B, 546-atm-treated cells.

peared to maintain their 1-atm synthesis rates relative to that of total protein synthesis. The rate of production of others was elevated relative to the rate of total protein synthesis. Proteins whose relative rates of synthesis increased with higher pressure were designated PIPs. In fact, while PIPs were operationally defined as being pressure inducible, they could include proteins whose rates of synthesis decrease with pressure to a lesser extent than those of most proteins, are unaffected by high pressure, or are elevated in response to high pressure. The magnitude of PIP induction generally correlated with the magnitude of the pressure shift. A similar finding has been made with the induction of HSPs in *E. coli* (35). The pressure giving the most dramatic PIP response was 819 atm, although 546 atm induced two proteins that were not as apparent at 819 atm. The induction of one prominent PIP, a 16-kDa protein, was remarkable in increasing in a barometer-like fashion up to 819 atm. Two additional major PIPs, with molecular masses of 66 and 56 kDa were evident, and their levels of synthesis also increased in a barometer-like fashion.

Time course of PIP induction following a shift to 546 atm. To investigate the effect of the duration of the pressure upshift on overall rates of protein synthesis and on the induction of specific PIPs, a series of cultures were pulse-labeled for 30-min intervals before upshift and at 0, 60, 120, and 180 min postshift. Extracts were prepared and processed by trichloroacetic acid precipitation to monitor [^{35}S]methionine incorporation, as well as by one-dimensional SDS-PAGE and autoradiography to monitor the kinetics of PIP synthesis (Fig. 5). A 546-atm pressure shift was selected because in the previous experiment it had yielded the strongest PIP response. During the labeling periods, protein synthesis decreased to 23% of the 1-atm value by 60 to 90 min and then slightly increased during the following two labeling periods (with respect to the 1-atm value, 31% at 0

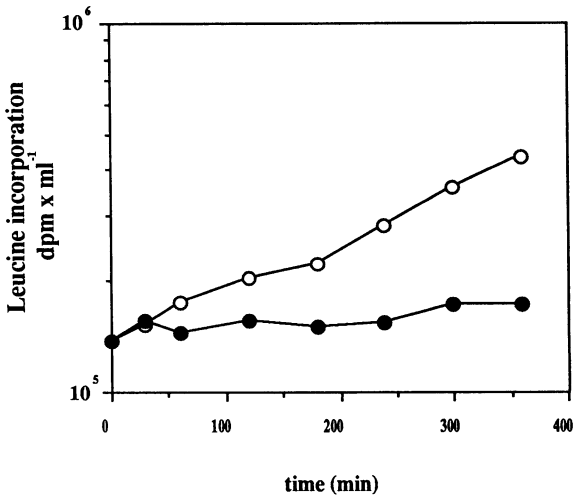

FIG. 3. Protein synthesis after hydrostatic pressure upshift. [^{3}H]leucine incorporation into protein, measured as a function of time after cells were shifted to 546 atm (●), was compared with label incorporation into protein in unpressurized cells (○).

to 30 min, 23% at 60 to 90 min, 28% at 120 to 150 min, and 32% at 180 to 210 min). Although the time course of PIP induction and repression was different for each PIP, all inductions were evident by either the 0- to 30- or 30- to 60-min period (Fig. 5). The transient nature of most inductions was seen by their repression by either 60 to 90 or 120 to 150 min. However, some PIPs maintained elevated levels of synthesis even 180 to 210 min postshift to high pressure.

High pressure induces HSPs. Many stresses that limit cell growth induce certain HSPs, particularly the heat shock chaperones DnaK and GroEL (32). We investigated whether pressure upshift would induce the synthesis of these HSPs. W3110 cells were heat shocked, and proteins were labeled for 8 min before and after a shift to 50°C. Samples were compared by SDS-PAGE with the labeled proteins synthesized before and after the shift to 546 atm. There was a striking similarity in mobility between the proteins induced by heat shock and those induced by high pressure. In particular, among the major PIPs, the 66- and 56-kDa PIPs migrated like HSPs DnaK and GroEL, respectively, while the 16-kDa PIP was not induced by heat shock. To determine whether DnaK or GroEL is specifically induced by high pressure, W3110 proteins labeled pre- and postshift to a high temperature and pre- and postshift to a high pressure were immunoprecipitated with polyclonal antisera to DnaK (Fig. 6B) and GroEL (Fig. 6C). The results indicate that both DnaK and GroEL are indeed PIPs and appear to be the 66- and 56-kDa major PIPs.

2D gel analysis and cataloguing of PIPs. To locate additional PIPs and to make use of the *E. coli* gene-protein data base (32) for preliminary PIP identification, 2D PAGE was performed. Extracts from W3110 cells labeled for 30 min immediately prior to pressure treatment, and beginning at 60 min postshift to 546 atm, were prepared, and equal amounts of labeled protein were subjected to both equilibrium and nonequilibrium 2D PAGE and autoradiography (Fig. 7). Fifty-five polypeptides had markedly higher rates of protein synthesis relative to total protein synthesis subsequent to the high-pressure upshift. The 16-kDa PIP, PIP 45 (I15.9), is a highly basic protein (approximate pI, 11.0) and resolved near several basic ribosomal proteins on nonequilibrium 2D PAGE. Of the

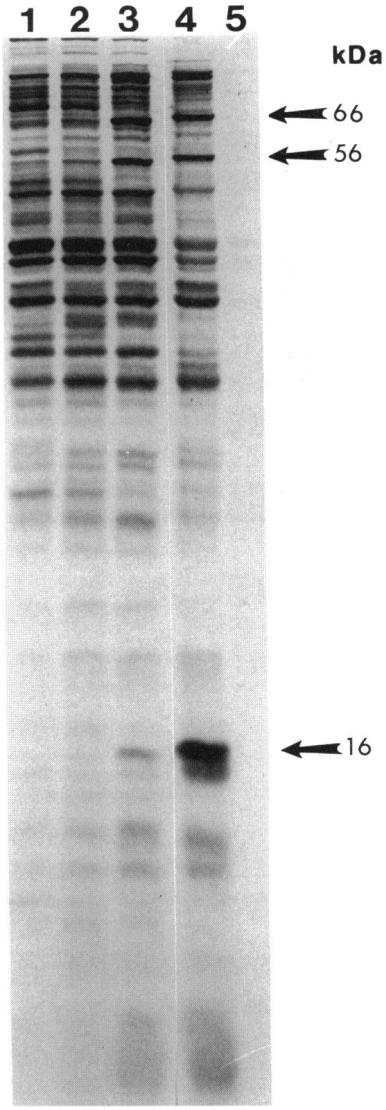

FIG. 4. Effect of hydrostatic pressure upshift on the pattern of protein synthesis. Cells from 1-atm cultures in balanced, anaerobic growth were pulse-labeled by addition of [^{35}S]methionine for a 30-min period at 60 min postshift to the indicated pressures. On the basis of [^{35}S]methionine incorporation data, equal counts from each of the first three pressure shift samples were subjected to SDS-PAGE and processed for fluorography. For the two highest pressure shift samples, incorporation was too low to load counts equal to the first three samples, and instead a maximum amount of protein was loaded onto the SDS-PAGE gel. Fluorography of lanes 4 and 5 required 10-fold longer exposure than the first three lanes. Lanes: 1, 1-atm shift; 2, 273-atm shift; 3, 546-atm shift; 4, 819-atm shift; 5, 1,092-atm shift. Arrows denote major PIPs.

55 PIPs, 18 appeared to share coordinates with previously identified proteins, and an additional 12 possible overlaps also existed. Paradoxically, 11 of the 55 PIPs were HSPs (ClpB, ClpP, Lon, RpoH, DnaK, GroEL, GroES, GrpE, G21.0, and F10.1), while 4 of the 55 PIPs were CSPs (G41.2, RecA, HNS, and F10.6). Another interesting feature of the pressure response is that it did not result in repression of stringently controlled proteins that are normally repressed during shifts down in growth rate (arrows in Fig. 7).

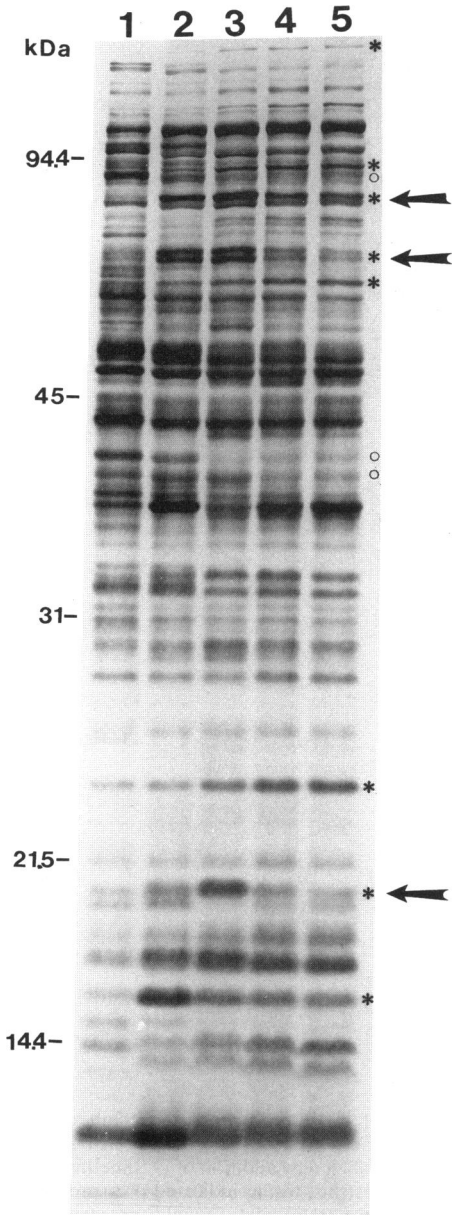

FIG. 5. Time course of induction of PIPs. Cells from 1-atm cultures in balanced, anaerobic growth were pulse-labeled by addition of [^{35}S]methionine for 30-min intervals before and after pressure upshift to 546 atm. On the basis of [^{35}S]methionine incorporation data, equal counts from each pressure shift were subjected to SDS-PAGE and processed for fluorography. Lanes: 1, 30 min preshift to time zero; 2, time zero to 30 min postshift; 3, 60 to 90 min postshift; 4, 120 to 150 min postshift; 5, 180 to 210 min postshift. Arrows denote major PIPs. Asterisks denote a larger number of PIPs. Open circles denote proteins whose relative rates of synthesis were repressed by elevated pressure.

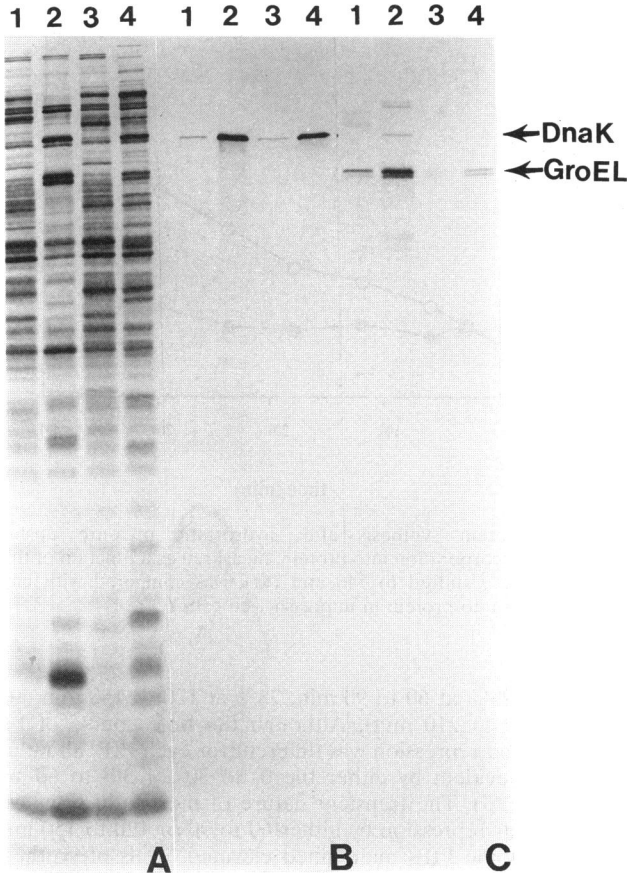

FIG. 6. Pressure upshift induces HSPs. Lanes 1 and 2, heat shock of *E. coli* W3110. Temperature shift experiments were done by labeling cells with [^{35}S]methionine for an 8-min period before and after a shift from 30 to 50°C. Lanes 3 and 4, pressure treatment of *E. coli* W3110. Pressure shift experiments were done by labeling with [^{35}S]methionine for 30 min preshift at 1 atm and 60 to 90 min postshift to 546 atm. Heat-shocked and pressure-treated samples were subjected to SDS-PAGE and processed for fluorography. (A) Lanes: 1, 30°C; 2, 50°C; 3, 1 atm; 4, 546 atm. Panel B shows the same samples as panel A immunoprecipitated with antiserum specific to DnaK. Panel C shows the same samples as panel A immunoprecipitated with antiserum specific to GroEL.

DISCUSSION

Extremes of pressure manifest their effects on cellular processes in many ways, including inhibition of protein and DNA synthesis, membrane-associated processes, and disruption of macromolecular quaternary structure (11, 24, 27, 38). The extent to which the inhibitions of these processes by pressure are interrelated remains unknown. The objective of this study was to identify proteins whose rates of synthesis are increased by pressure so that pressure stress could be placed in the context of existing information about other stress responses.

Under our culture conditions, exposure of *E. coli* W3110 to 546 atm inhibited cell division and held viable cell numbers at preshift levels. However, the OD of cultures did eventually increase under pressure. After a lag of approximately 100 min, OD increased at a substantially reduced rate. This increase was explained by cell filamentation occurring at high pressure. Our results regarding both the high-pressure maintenance of viable but nondividing bacteria and its enhancement of filamentous growth agree with the results of others (2, 38, 41, 42). Do the OD results suggest that *E. coli* can adapt to high pressure? While *E. coli* does not increase in cell number at 546 atm, it appears that biomass production can at least double. The cells appear to be capable of mounting a partially adaptive, albeit incomplete, response to high pressure. Considering the fact that *E. coli* does not experience pressure variation in its natural

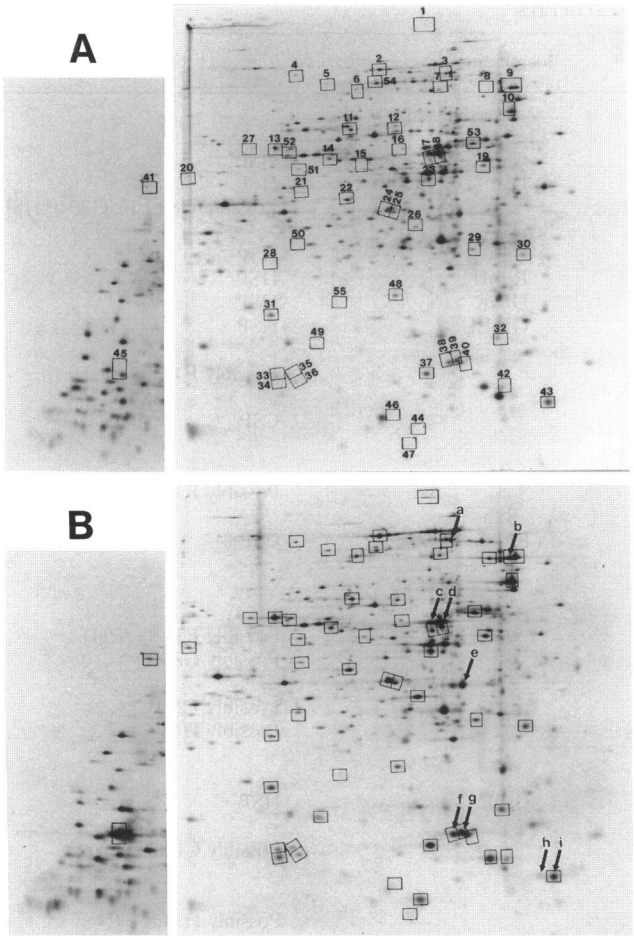

FIG. 7. Autoradiograms of 2D gels of PIPs. Samples were generated by labeling cells with [^{35}S]methionine for 30 min preshift at 1 atm (A) and 60 to 90 min postshift to 546 atm (B). Each panel is a composite of two gels run with the same extract: right, pH 5 to 7 ampholine equilibrium gel; left, pH 3 to 10 nonequilibrium gel. PIPs are marked by squares and are numbered as in Table 1. Arrows point to stringently controlled proteins. Spots: a, protein chain elongation factor G; b, ribosomal protein S1; c and d, protein chain elongation factor Tu; e, protein chain elongation factor TS; f and g, ribosomal protein S6; h, ribosomal protein L12; i, ribosomal protein L7. Spots f, g, and i correspond to PIPs 38, 39, and 43, respectively.

environment, the observed increase in OD following the initial lag is intriguing and suggestive of overlap between certain inhibitory aspects of high pressure and other environmental stresses for which *E. coli* has evolved adaptive responses. The possible roles of PIPs in the resumption of cell growth at elevated pressure await investigation.

Our finding of a correlation between rates of [^{35}S]methionine incorporation and hydrostatic pressure is qualitatively consistent with the results of others concerning the pressure sensitivity of protein synthesis. For example, Yayanos and Pollard reported a similar finding (38). However, under our conditions, *E. coli* W3110 demonstrated a pressure limit of approximately 800 atm, which is at odds with previously reported values ranging from 580 to 670 atm (21, 38). The extent to which strain, medium, and temperature differences contributed to these discrepancies is unknown, but all of these variables have been reported to affect the ability of bacteria to grow or synthesize protein at increased pressure (11, 19, 40). Another difference is the use of anaerobic cultures in this study. In addition, our high-pressure pulse-labeling technique is likely to provide greater sensitivity than the radiolabel accumulation methods employed in the past to measure translation rates. This is particularly true if differences in protein turnover rates between low- and high-pressure-treated cells exist.

To monitor the response of *E. coli* to high pressure without bias towards any particular aspect of cell physiology, we have chosen a global systems approach. Changes in the synthesis of proteins were identified from 2D gels, and protein coordinates were compared with those of proteins in the *E. coli* gene-protein data base (30, 32). This resulted in the identification of 55 PIPs. It is unlikely that additional experiments will result in the identification of additional PIPs, as the data suggest that the magnitude of the pressure stress appears to affect the magnitude of PIP induction rather than which PIPs are synthesized. The most profound induction occurs with PIP 115.6 (Fig. 4 and 7). Protein I15.9 has not been previously observed. Since its resolution by 2D PAGE requires nonequilibrium isoelectric focusing, it is possible that additional stresses not yet analyzed by this technique also induce this protein. The isoelectric focusing point of PIP I15.9 is 11.0.

Many of the other PIPs which have been identified, or for which putative identifications have been made (Table 1), present an interesting contradiction. High pressure induces more HSPs (n = 11) than most other conditions outside of those which precisely mimic a heat shock response, while also inducing more CSPs (n = 4) than most conditions outside of those which precisely mimic a cold shock response (32). HSPs and CSPs have inverse responses to a variety of conditions, including temperature and antibiotics which target the ribosome (8, 31). There are, however, commonalities between high-pressure effects and the effects of both decreases and increases in temperature. Both low temperature and high pressure inhibit an early step of translation (3, 6, 11). The cold shock response has been suggested to be an adaptive response to facilitate the expression of genes involved in translation initiation (8). Also, pressure, like low-temperature incubation, results in the continued synthesis of stringently controlled proteins involved in transcription and translation despite the growth rate decrease; this behavior suggests decreased translational capacity.

Pressure could turn on both HSPs and CSPs by affecting ribosomes, either by inducing all ribosomes to signal both stress responses partially or because of differential effects on ribosomes, depending on the state of the ribosome at the time of pressurization. There are several additional possible targets through which pressure could initiate a partial heat shock response. Both high temperature and high pressure destabilize the quaternary structures of proteins (7, 33), and an increased proportion of dissociated subunits could induce a heat shock sigma-32 factor-dependent heat shock response (4). Alternatively, pressure could directly affect the phosphorylation state or ATPase activity of HSP DnaK. These properties of DnaK also modulate the heat shock response (15).

The time course of synthesis of pressure-induced HSPs is dramatically different from that of temperature upshift. Within minutes of a shift of *E. coli* from 28 to 50°C, many HSPs are apparent (12, 34, 35), while at 28°C, pressure induction of the major HSPs requires 60 to 90 min, suggesting that the signal for heat shock induction is generated only slowly by high pressure. As with heat shock, the pressure response is also transient, GroES synthesis is repressed by 60 to 90 min postshift, GroEL is repressed by 120 to 150 min postshift, and

TABLE 1. *E. coli* W3110 PIPs

Serial no.[a]	Alpha-nunumeric designation[b]	Coordinates[c]	Identification[d]	Comment(s)
1	F157.4	67 × 126		
2	G98.0	53 × 116		
3	F84.1	72 × 113	ClpB	HSP
4	H94.0	31 × 115	Lon	HSP
5	G80.2	41 × 112		Possibly G80.1 or G80.0; HSP
6	G77.4	49 × 111		
7	E72.0	70 × 109	ClpB	HSP
8	C62.5	87 × 107	RpoH	HSP
9	B66.0	104 × 108	DnaK	HSP
10	B56.5	102 × 102	GroEL	HSP
11	G53.0	48 × 99		
12	G52.1	57 × 98		Possibly F48.8 G6PD
13	H48.8	25 × 95		
14	G41.2	41 × 90		CSP
15	G43.4	47 × 89		
16	F48.8	59 × 95		
17	F42.8	66 × 88		Possibly F39.6
18	E43.6	70 × 89		
19	C39.3	84 × 87	RecA	CSP
20	H43.0	3 × 89		
21	H37.5	33 × 83		
22	G36.7	45 × 79		
23	F38.7	60 × 82		Possibly F39.0 (ArgI)
24	G34.7	55 × 76		Possibly G34.2
25	F34.1	56 × 75		
26	F30.8	61 × 70		Possibly F29.0
27	H47.0	22 × 95		Possibly H47.4
28	H25.6	24 × 62		
29	C26.3	81 × 63		
30	B25.2	107 × 62	GrpE	HSP
31	H20.0	24 × 47		
32	C17.1	92 × 38		Possibly C17.2 (Dut)
33	H15.6	24 × 29		
34	H15.1	24 × 26		
35	H15.7	27 × 29		Possibly H14.0 L9
36	H15.3	29 × 27		
37	F14.7	63 × 28	HNS	CSP
38	D14.7	73 × 31	RpsF	Ribosomal protein S6A
39	C14.8	76 × 31	RpsF	Ribosomal protein S6B
40	C15.4	79 × 30	GroES	HSP
41	I60.9	54 × 104		Nonequilibrium gel
42	C13.1	93 × 25		Possibly C13.2
43	A13.0	117 × 22	RplL	Ribosomal protein L7
44	F10.6	60 × 14		CSP
45	I15.9	35 × 32		Nonequilibrium gel
46	F10.9	53 × 17		
47	F10.1	58 × 11		HSP
48	F21.5	67 × 51	ClpP	HSP
49	G16.6	35 × 39		
50	H29.5	31 × 68		
51	G42.3	43 × 87		
52	H46.9	28 × 93		
53	C46.1	83 × 92		Possibly C44.0
54	G77.5	53 × 111		Possibly G70.0 PFL
55	G21.0	42 × 50		HSP

[a] Numbers correspond to the numbers in Fig. 7.
[b] Alphanumeric designations are as previously described (32).
[c] Protein coordinates were assigned on the basis of the positions of these proteins on reference gels (32).
[d] Gene product identifications were based on overlapping reference gel coordinates, except for PIPs 9 and 10, whose identities were verified by immunoprecipitation.

DnaK remains high even 180 to 210 min postshift. During the heat shock response, such readjustments in the rates of protein synthesis require only 15 to 20 min (12). Therefore, both the upshift and downshift in rates of HSP synthesis are delayed during the pressure induction response. The more rapid stress response to temperature may be due to an increase in the Q_{10}.

The stress responses which are not seen in response to pressure upshift are informative. For example, there does not appear to be a DNA damage response (SOS response; 29) although high pressure is known to inhibit DNA synthesis (10, 38, 42). Indeed, we found that the nuclear material was considerably more compacted at high pressure than at low

pressure. Also, while it is known that pressures of the magnitude used in this study alter membrane structure and transport processes, there is little evidence of a carbon, nitrogen, or phosphate starvation response. It will be interesting to investigate the overlap between PIPs and responders to osmotic stress and acid stress which are not yet in the *E. coli* gene-protein data base.

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REFERENCES

1. **Ames, G. F.-L.** 1974. Resolution of bacterial proteins by polyacrylamide gel electrophoresis on slabs. J. Biol. Chem. **249:**634–644.
2. **Baross, J. A., F. J. Hanus, and R. Y. Morita.** 1975. Survival of enteric and other sewage microorganisms under simulated deep-sea conditions. Appl. Microbiol. **30:**309–318.
3. **Broeze, R. J., C. J. Solomon, and D. H. Pope.** 1978. Effects of low temperature on in vivo and in vitro protein synthesis in *Escherichia coli* and *Pseudomonas fluorescens*. J. Bacteriol. **134:**861–874.
4. **Craig, E. A., and C. A. Gross.** 1991. Is hsp70 the cellular thermometer? Trends Biochem. Sci. **16:**135–140.
5. **Davis, R. W., D. Botstein, and J. R. Roth (ed.).** 1980. Advanced bacterial genetics, p. 201. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
6. **Groß, M., and R. Jaenicke.** 1990. Pressure-induced dissociation of tight couple ribosomes. FEBS Lett. **267:**239–241.
7. **Jaenicke, R.** 1981. Enzymes under extreme conditions. Annu. Rev. Biophys. Bioeng. **10:**1–67.
8. **Jones, P. G., R. A. VanBogelen, and F. C. Neidhardt.** 1987. Induction of proteins in response to low temperature in *Escherichia coli*. J. Bacteriol. **169:**2092–2095.
9. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
10. **Landau, J. V.** 1966. Protein and nucleic acid synthesis in *Escherichia coli*: pressure and temperature effects. Science **153:**1273–1274.
11. **Landau, J. V., and D. H. Pope.** 1980. Recent advances in the area of barotolerant protein synthesis in bacteria and implications concerning barotolerant and barophilic growth. Adv. Aquat. Microbiol. **2:**49–76.
12. **Lemaux, P. G., S. L. Herendeen, P. L. Bloch, and F. C. Neidhardt.** 1978. Transient rates of synthesis of individual polypeptides in *E. coli* following temperature shifts. Cell **13:**427–434.
13. **Marquis, R. E.** 1984. Reversible actions of hydrostatic pressure and compressed gases on microorganisms, p. 273–301. *In* A. Hurst and A. Nasim (ed.), Repairable lesions in microorganisms. Academic Press Ltd., London.
14. **Marquis, R. E., and G. R. Bender.** 1987. Barophysiology of prokaryotes and proton-translocating ATPases, p. 65–73. *In* H. W. Jannasch, R. E. Marquis, and A. M. Zimmerman (ed.), Current perspectives in high-pressure biology. Academic Press Ltd., London.
15. **McCarty, J. S., and G. C. Walker.** 1991. DnaK as a thermometer: threonine-199 is site of autophosphorylation and is critical for ATPase activity. Proc. Natl. Acad. Sci. USA **88:**9513–9517.
16. **Meganathan, R., and R. E. Marquis.** 1973. Loss of bacterial motility under pressure. Nature (London) **246:**526–527.
17. **Neidhardt, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **119:**736–747.
18. **O'Farrell, P. H.** 1975. High resolution two dimensional electrophoresis of proteins. J. Biol. Chem. Res. **7:**40–46.
19. **Palmer, D. S., and L. J. Albright.** 1970. Salinity effects on the maximum hydrostatic pressure for growth of the marine psychrophilic bacterium, *Vibrio marinus*. Limnol. Oceanogr. **15:**343–347.
20. **Pope, D. H., and L. R. Berger.** 1973. Inhibition of metabolism by hydrostatic pressure: what limits microbial growth? Arch. Mikrobiol. **93:**367–370.
21. **Pope, D. H., N. T. Connors, and J. V. Landau.** 1975. Stability of *Escherichia coli* polysomes at high hydrostatic pressure. J. Bacteriol. **121:**753–758.
22. **Pope, D. H., W. P. Smith, R. W. Swartz, and J. V. Landau.** 1975. Role of bacterial ribosomes in barotolerance. J. Bacteriol. **121:**664–669.
23. **Porter, K. G., and Y. S. Feig.** 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. **25:**943–948.
24. **Schulz, E., H.-D. Ludemann, and R. Jaenicke.** 1976. High pressure equilibrium studies on the dissociation-association of *Escherichia coli* ribosomes. FEBS Lett. **64:**40–43.
25. **Smith, D. C., and F. Azam.** 1992. A simple, economical method for measuring bacterial synthesis in seawater using ^{3}H-leucine. Mar. Microb. Food Webs **6**(2)**:**107–114.
26. **Smith, M. W., and F. C. Neidhardt.** 1983. Proteins induced by anaerobiosis in *Escherichia coli*. J. Bacteriol. **154:**336–343.
27. **Somero, G. N.** 1992. Adaptations to high hydrostatic pressure. Annu. Rev. Physiol. **54:**557–577.
28. **Van Bogelen, R. A., M. A. Acton, and F. C. Neidhardt.** 1987. Induction of the heat shock regulon does not produce thermotolerance in *Escherichia coli*. Genes Dev. **1:**525–531.
29. **VanBogelen, R. A., P. M. Kelley, and F. C. Neidhardt.** 1987. Differential induction of heat shock, SOS, and oxidation stress regulons and accumulation of nucleotides in *Escherichia coli*. J. Bacteriol. **169:**26–32.
30. **VanBogelen, R. A., and F. C. Neidhardt.** 1990. Global systems approach to bacterial physiology: protein responders to stress and starvation. FEMS Microb. Ecol. **74:**121–128.
31. **VanBogelen, R. A., and F. C. Neidhardt.** 1990. Ribosomes as sensors of heat and cold shock in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87:**5589–5593.
32. **VanBogelen, R. A., and F. C. Neidhardt.** 1991. The gene-protein database of *Escherichia coli*: edition 4. Electrophoresis **12:**955–994.
33. **Weber, G., and H. G. Drickamer.** 1983. The effect of high pressure upon proteins and other biomolecules. Q. Rev. Biophys. **16:**89–112.
34. **Yamamori, T., K. Ito, Y. Nakamura, and T. Yura.** 1978. Transient regulation of protein synthesis in *Escherichia coli* upon shift-up of growth temperature. J. Bacteriol. **134:**1133–1140.
35. **Yamamori, T., and T. Yura.** 1980. Temperature-induced synthesis of specific proteins in *Escherichia coli*: evidence for transcriptional control. J. Bacteriol. **142:**843–851.
36. **Yayanos, A. A.** 1986. Evolutional and ecological implications of the properties of deep-sea barophilic bacteria. Proc. Natl. Acad. Sci. USA **83:**9542–9546.
37. **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.
38. **Yayanos, A. A., and E. C. Pollard.** 1969. A study of the effects of hydrostatic pressure on macromolecular synthesis in *Escherichia coli*. Biophys. J. **9:**1464–1482.
39. **Yayanos, A. A., and R. Van Boxtel.** 1982. Coupling device for quick high pressure connections to 1000 MPa. Rev. Sci. Instrum. **53:**704–705.
40. **Yayanos, A. A., R. Van Boxtel, and A. S. Dietz.** 1983. Reproduction of *Bacillus stearothermophilus* as a function of temperature and pressure. Appl. Environ. Microbiol. **46:**1357–1363.
41. **Zobell, C. E., and A. B. Cobet.** 1962. Growth, reproduction, and death rates of *Escherichia coli* at increased hydrostatic pressures. J. Bacteriol. **84:**1228–1236.
42. **Zobell, C. E., and A. B. Cobet.** 1963. Filament formation by *Escherichia coli* at increased hydrostatic pressures. J. Bacteriol. **87:**710–719.