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Analysis of hydrostatic pressure effects on transcription in *Escherichia coli* by DNA microarray procedure

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Abstract Hydrostatic pressure is a well-known physical stimulus, but its effects on cell physiology have not been clarified. To investigate pressure effects on *Escherichia coli*, we carried out DNA microarray analysis of the entire *E. coli* genome. The microarray results showed pleiotropic effects on gene expression. In particular, heat- and cold-stress responses were induced simultaneously by the elevated pressure. Upon temperature stress (including both temperature up- and down-shifts) and other environmental stresses, gene expression adjusts to adapt to such environmental changes through regulations by several DNA-binding proteins. An *E. coli* mutant, which deleted the *hns* gene encoding one of the regulator proteins, exhibited great pressure sensitivity. The result suggested that the H-NS protein was a possible transcriptional regulator for adaptation of the high-pressure stress.

Keywords DNA microarray · *Escherichia coli* · H-NS · Pressure regulation · Stress response

Electronic Supplementary Material Supplementary material (a list of all of the genes with altered expression levels by elevated pressure and the ratio data) is available for this article at http://dx.doi.org/10.1007/s00792-004-0414-3

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Hydrostatic pressure is a particular physical stimulus in a deep-sea environment. We are interested in the pressure-stress responses of cells living under high-pressure conditions and investigate pressure effects on bacterial cells. We use *Escherichia coli* instead of a deep-sea bacterium, because several piezophilic bacteria have been isolated from the deep sea, and most deep-sea bacteria are classified as γ-Proteobacteria, including the well-known bacterium, *E. coli* (Jones and Inoue 1994; Kato et al. 1995, 2000; Purss and Drilica 1989). In the evolutionary process, *E. coli* is believed not to have evolved the high-pressure adaptation mechanisms and thus, *E. coli* cells appear to respond to pressure stress as other experienced stresses. Furthermore, many genetic techniques and the genome data are available to study the adaptation mechanisms to high pressure. Therefore, *E. coli* can be used as an efficient tool to compare pressure effects and has already revealed stress responses in cells (Welch et al. 1993).

Elevated pressure causes several characteristic physiological changes in *E. coli*, for instance, extended lag phase, cell filamentation, and ceased DNA, RNA, and protein synthesis (Zobell and Cobet 1962, 1964; Yayanos and van Boxtel 1969; Tamura et al. 1992; Kato et al. 1994). In addition, pressure stress solidifies cellular membrane lipids, thereby decreasing cellular membrane fluidity, which effect appears to overlap the other environmental stresses, especially low-temperature

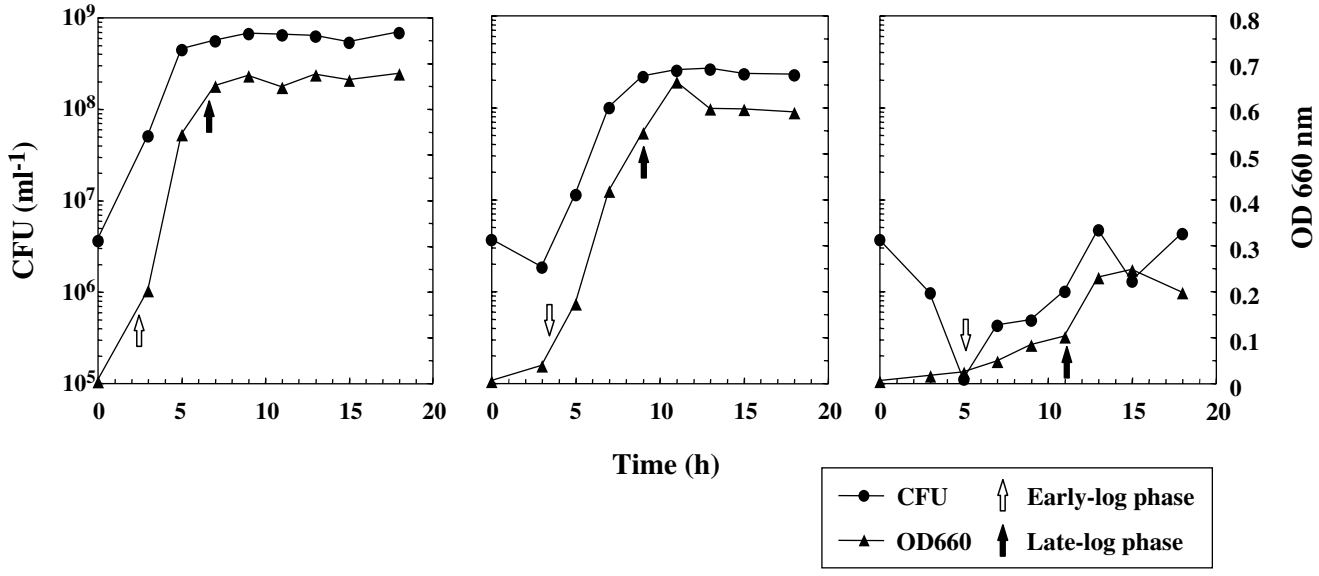

Fig. 1 Time-course analysis of *Escherichia coli* cell growth under each pressure condition. Growth was monitored by the number of colony forming units [(CFU), *filled circle*] and optical density at 660 nm (*filled diamond*). Each sampling point was indicated by *arrows*. Cells were harvested at the times indicated by *open arrows* (early-log phase) and *closed arrows* (late-log phase)

conditions (Cossins and Macdonald 1984; Hurme and Rhen 1998; Jones and Inoue 1994; Macdonald 1984; Vigh et al. 1998). Previously, the pressure stress has been discussed as being a mimic of cold stress. Under extreme temperature conditions, bacterial cells sense such an environment through alterations of lipid fluidity, protein flexibility, and nucleic acid conformation (Hurme and Rhen 1998; Jones and Inoue 1994; Vigh et al. 1998). The solidified membrane fluidity is recovered by decreasing the melting temperature of lipids included in membrane in order to increase branched-fatty acids upon cold stress and, similar to cold adaptation, deep-sea bacteria increase unsaturated fatty acids in high-pressure environments (Delong and Yayanos 1985, 1986; Marr and Ingraham 1962; Morris 1971; Sinensky 1974). In addition, previous studies revealed that translation was blocked due to a decrease in polysomes upon cold shock,

Table 1 Number of pressure-affected genes under each growth condition

Gene category[a]	Number of classified genes[b]	Early-log phase ratio				Late-log phase ratio			
		30 MPa		50 MPa		30 MPa		50 MPa	
		≧2[c]	≦0.5[d]	≧2	≦0.5	≧2	≦0.5	≧2	≦0.5
Amino acid metabolism	118	6	3	2	2	4	9	3	5
Biosynthesis of cofacters, prosthetic groups, carriers	125	6	2	6	1	14	2	14	1
Cell envelope	188	6	14	4	12	25	7	22	6
Cellular processes	101	16	3	7	3	14	7	16	12
Central intermediary metabolism	148	15	13	7	13	16	13	8	14
Energy metabolism	347	30	64	10	44	38	24	22	39
Fatty acid/phospholipid metabolism	58	2	3	3	2	15	2	16	3
Nucleotide metabolism	114	5	9	5	8	25	6	24	3
Regulatory functions	103	4	6	6	5	8	12	7	9
Replication	89	2	2	2	3	21	2	17	3
Transport/ binding protein	356	41	28	16	30	25	25	23	32
Translation	143	2	7	2	3	79	2	77	4
Transcription	47	1	1	3	1	14	3	10	1
Other categories	228	15	12	15	8	23	20	23	19
Hypothetical	1,966	99	52	54	36	93	150	83	146
Total	4,131	250	219	142	171	414	284	365	297

[a]Genes were categorized based on the role of products, modified from Riley and Labedan (1996)
[b]Number of genes classified in the category and spotted on the DNA chip
[c]Number of the genes for which the mean of the ratio Cy5/Cy3 was more than 2
[d]Number of the genes for which the mean of the ratio Cy5/Cy3 was less than 0.5

Table 2 Highly expressed genes

Gene	Early-log phase				Late-log phase			
	30 MPa		50 MPa		30 MPa		50 MPa	
	Ratio[a]	SD	Ratio[b]	SD	Ratio	SD	Ratio	SD
242#1	13.02	1.21						
246#1							20.83	4.72
246#2							18.94	12.25
aceA	21.21	3.88	10.25	0.68				
aceB	12.45	3.79						
aslB							EH[c]	
betT	10.54	3.71						
deaD					11.93	2.16		
dhnA							12.04	2.01
dnaX							EH	
fdnH							EH	
fruB	38.30	H[d]	10.26	4.08				
fruK	40.27	4.25					13.75	4.51
glpA					15.13	6.98		
glpB					12.95	3.10		
glpD					16.85	5.29		
glpK					15.70	0.12		
glpQ					14.92	3.10		
glpT					14.99	4.07		
mgtA							EH	
oppD					14.59	1.42	10.28	4.09
phoB			10.48	1.83				
phoR			35.66	18.63				
potA							10.05	1.77
poxB	13.79	H						
prfA					14.45	1.22	20.27	H
prsA							10.34	1.43
pstB							11.96	1.78
pstS							27.86	26.90
ptfB	33.70	5.45						
pstB			11.96	1.78				
queA							EH	
rbsA	25.50	12.86			13.33	2.23		
recR							EH	
rplI					10.70	8.98		
sodA					14.30	7.24		
thiI			EH				EH	
yceI	12.19	H						
ycgC							11.81	7.44
ydiU	12.82	0.89						
yhbE					10.71	0.47		
yqgC							12.20	3.71
yzgL					14.18	5.08		

[a]Mean of the ratio of Cy5 (30 MPa)/Cy3 (0.1 MPa)
[b]Mean of the ratio of Cy5 (50 MPa)/Cy3 (0.1 MPa)
[c]Both ratio data were extremely high (> 1,000)
[d]SD value was nonexistent, because the other ratio data were extremely high (> 1,000)

and that direct inhibition of ribosome function by antibiotics (e.g., chloramphenicol) caused a cold shock-like response (Dammel and Noller 1995; Thieringer et al. 1998; VanBogelen and Neidhardt 1990; Yamanaka 1999). Those results suggest that ribosomes are one of the cold-stress sensors in *E. coli*. Notably, the binding of aminoacyl-tRNA and/or the transfer process of aminoacyl-tRNA to peptidyl-tRNA on ribosomes is destabilized, protein biosynthesis is inhibited by elevated pressure (Baierlein and Infante 1974; Gross et al. 1993; Infante and Baierlein 1971), and the lag-phase extension by elevated pressure appears to correspond to the acclimation phase before resuming cell growth after cold shock is experienced. Several results and physiological observations are suspected to show the similarity between pressure stress and cold stress. However, the proteome analysis showed not only the increase of cold shock proteins (CSPs), but also the increase of heat shock proteins (HSPs) (Welch et al. 1993). Therefore, the relationship between pressure stress and cold stress remains unclear in genetics studies.

Obviously, one of the major response mechanisms to changing environment is transcriptional regulation. The comparison of transcriptional alterations with those after other environmental stresses is expected to clarify the effects of pressure on *E. coli* cells and, therefore, we carried out whole-gene expression analysis using the DNA microarray procedure. To eliminate the influence of the growth phase on RNA expression profiles, we set two sampling points based on the growth profiles under each pressure condition and prepared samples for DNA microarray analysis. *E. coli* strain W3110 was cultivated at 37°C in LB medium, and the first harvest point was defined as the log phase (Fig. 1, closed arrows), and the

Table 3 Highly repressed genes

Gene	Early-log phase				Late-log phase			
	30 MPa		50 MPa		30 MPa		50 MPa	
	Ratio[a]	SD	Ratio[b]	SD	Ratio	SD	Ratio	SD
(*fimD*)							0.081	L[d]
(*gltD*)					0.022	L	0.029	0.004
(*yagR*)					0.012	L	0.019	L
(*yagR*)					0.012	L		
465#5							0.085	0.002
467#1					0.053	0.019	0.076	0.001
576#14							0.054	0.001
ansB			0.070	L				
appY	EL[c]							
arcL					0.028	L	0.049	0.001
cadA	0.025	L			EL			
cadB	EL		0.038	L				
cbpA							0.098	0.041
cinA			0.097	0.049	0.090	0.055		
dcuB	0.084	L						
deoC			0.070	L				
dmsA	0.049	0.025						
dmsB	0.043	0.011	0.071	L				
dpaL					0.066	0.020	0.068	0.010
dps							0.031	0.002
fimA	0.089	0.014	0.097	L				
fimD	EL							
frdA	0.091	0.034						
frdB	0.099	0.048						
ftn	0.071	0.012						
gadA							0.015	0.002
gatD	0.047	0.010			EL		0.037	0.003
gatR							0.049	0.022
gatZ			0.033	0.018				
glgS					0.052	0.011	0.039	0.003
hdeA			0.069	L			0.015	0.004
hdeB					EL		0.019	0.004
hdeD							0.024	L
hoxK	0.068	0.004						
hyaE							EL	
hybB	0.074	0.044						
hybC	0.056	0.005						
lamB	0.058	0.050	0.065	0.027				
malE	0.005	L			EL		0.055	0.009
malF	0.046	0.005	0.060	0.025				
malK	0.061	0.028	0.026	0.002				
malM	0.047	0.007	0.050	0.015				
malP	0.061	0.011	0.034	L				
nmpC	0.062	0.009						
narH							0.081	0.001
nmpC			0.046	0.022				
osmE							0.076	0.028
ptkC	0.044	0.005	0.024	L			0.022	0.007
slp					0.053	0.008	0.021	0.010
tnaA	0.042	0.009	0.022	L			0.070	0.013
tnaB							0.079	0.011
treB	0.036	0.006			EL			
treC	0.033	0.011	0.036	L				
tsx			0.071	L				
vboR	0.099	0.011						
wrbA					0.061	0.020	0.058	0.041
xasA							0.006	0.003
ybaS							0.041	L
ybdQ							0.078	0.012
yccJ					0.058	0.004	0.026	L
ycfR					0.030	L		
ychH							0.088	0.002
yfiA					0.064	0.024	0.021	0.002
yfiL							EL	
ygeW					0.015	0.003	0.023	L

Table 3 (Continued)

Gene	Early-log phase				Late-log phase			
	30 MPa Ratio[a]	SD	50 MPa Ratio[b]	SD	30 MPa Ratio	SD	50 MPa Ratio	SD
ygeY					0.005	L	0.006	L
ygeZ					0.044	0.012	0.038	0.023
ygfJ							0.066	L
ygfM							0.080	0.042
ygfO					0.058	L	0.048	L
ygfP					0.026	L	0.073	0.014
ygfT					0.059	L		
yhiE					0.097	0.025	EL	
yhiM							0.084	0.016
yhiW							0.068	L
yjiH					0.091	L		
yjjI	0.046	0.001						
yliH					0.074	L	0.015	L
yqiI					0.082	L		

[a]Mean of the ratio of Cy5 (30 MPa)/Cy3 (0.1 MPa)
[b]Mean of the ratio of Cy5 (50 MPa)/Cy3 (0.1 MPa)
[c]Both ratio data were extremely low (> 0.001)
[d]SD value was nonexistent, because the other ratio data were extremely low (> 0.001)

Table 4 Expression levels of temperature shock protein genes

Gene	Early-log phase		Late-log phase	
	30 MPa[a]	50 MPa[b]	30 MPa	50 MPa
Heat shock protein genes				
clpB	0.67	0.41↓[c]	0.19↓	0.13↓
clpP	1.40	0.94	0.87	0.60
dnaJ	1.11	0.49↓	0.89	0.91
dnaK	0.65	0.37↓	0.32↓	0.25↓
grpE	0.76	0.49↓	0.87	0.83
hslJ	0.39↓	0.44↓	0.37↓	0.46↓
hslU	0.59	0.61	1.00	1.06
hslV	0.67	0.47↓	1.17	0.90
htgA	2.82↑	ND[d]	ND	0.67
htpG	0.61	0.58	0.68	1.10
htpX	1.31	0.84	1.02	1.41
htrA	1.31	ND	1.59	1.94
htrC	1.23	1.67	1.25	2.19↑
ibpA	0.98	0.55	0.31↓	0.23↓
ibpB	1.03	0.83	0.23↓	0.37↓
lon	0.88	0.58	1.35	1.38
mopA	0.43↓	0.31↓	0.39↓	0.46↓
mopB	0.50↓	0.22↓	0.53	0.56
rpoH	1.31	1.49	0.94	1.13
Cold shock protein genes				
aceF	3.60↑	0.98	8.03↑	4.06↑
aceE	2.44↑	0.93	6.72↑	5.64↑
cspA	1.53	1.65	2.42↑	1.27
cspB	0.57	1.90	0.44↓	0.57
cspC	0.52	0.41↓	1.43	0.79
cspD	0.69	0.52	0.20↓	0.20↓
cspE	0.41↓	0.73	2.58↑	2.30↑
cspF	1.61	3.51↑	1.73	2.67↑
cspI	1.32	2.07↑	0.61	0.78
cspJ	1.77	2.69↑	0.61	0.70
gyrA	1.17	1.42	3.15↑	2.95↑
hns	0.47↓	0.30↓	0.59	0.45↓
hscA	1.61	1.55	3.62↑	1.92
hscB	2.29↑	1.74	9.44↑	9.70↑
infB	1.81	1.83	5.46↑	3.56↑
nusA	1.26	1.43	3.75↑	2.69↑
pnp	1.41	1.19	6.09↑	2.40↑
rbfA	0.97	1.37	4.44↑	1.22
tig	0.67	0.54	1.96	3.64↑

[a]The mean of the ratio of Cy5 (30 MPa)/Cy3 (0.1 MPa)
[b]The mean of the ratio of Cy5 (50 MPa)/Cy3 (0.1 MPa)
[c]Upward-pointing arrows indicate a ratio of more than 2.0, and downward-pointing arrows indicate a ratio of less than 0.5
[d]*ND* No data exist

Table 5 Alterations of *hns*-regulated gene expressions in the late-log phase

Gene	30 MPa	50 MPa	*hns*$^-$ mutant[a]
appA	+[b]		+
appB	+		+
appY		−	+
aslB			+
caiT			−
cirA			−
cpsB			+
cspB	−		+
cspC			+
cspE	+	+	−
cspI			+
cydA			+
cydB	+		+
dps	−	−	+
evgA	−	−	+
feoB			+
fimB		+	+
fimI			+
fliC	−		−
gadA	−	−	+
gltD	+		+
gltF			+
hdeA	−	−	+
hdeB	−	−	+
hdeD	−	−	+
hha	−	−	+
mopB			+
ompC		−	+
ompF	+	+	−
osmC		−	+
pflB	+	+	+
phoP		−	+
proX			+
rcsA			+
rpoS	−	−	+
tdcE			+

[a]Ratio data were derived with reference to the results of Hommais et al. (2001)

[b]+ Mean of the ratio HP/AP (in the late-log phase) or *hns* mutant/wild-type *E. coli* is more than 2.0, − mean of the ratio HP/AP (in the late-log phase) or *hns* mutant/wild-type *E. coli* is less than 0.5

second was stationary phase (Fig. 1, open arrows). The cultures grown at 0.1 MPa were harvested at 2.5 and at 6.5 h. The cultures grown at 30 MPa were harvested at 3.5 and at 9 h. The cultures grown at 50 MPa were harvested at 5 and 11 h. Preparation of the cDNA library and hybridization were performed according to the previously described procedures, and IntelliGene *E. coli* chip, version 1.0, was purchased from TaKaRa (Oshima et al. 2002). Purified RNA from pressurized cells was prepared for the Cy5-labeled cDNA library, and RNA from nonpressurized cells (cultivated at 0.1 MPa) was prepared for the Cy3-labeled cDNA library. Analytical rules for the microarray data were also previously described (Oshima et al. 2002). A mean of the ratio Cy5/Cy3, which was more than 2 or less than 0.5, was recognized as a significant alteration. To analyze an expression profile, we adopted a ratio data corresponding to a behavior (upregulated or downregulated) between two results. The numbers of the altered genes in the early- and late-log phase are listed in Table 1. In the early-log phase, 469 and 313 genes were altered at 30 and 50 MPa, respectively, and 698 and 662 genes were altered at 30 and 50 MPa in the late-log phase, respectively. In both growth phases, the altered genes were distributed throughout all functional classes, and genes involved in energy metabolism, transporter/DNA-binding protein, and translation at the late-log phase were particularly affected by elevated pressure.

The particular genes altered the expression levels by pressure were picked up from the microarray result. The highly expressed genes (ratio was more than 1,000) are shown in Table 2 and repressed genes (ratio was less than 0.001) are shown in Table 3. Highly upregulated genes seemed to have nothing in common in each growth condition (Table 2). On the other hand, downregulated genes seemed to have much in common in the same growth phase (Table 3)—some hypothetical genes were repressed in the late-log phase. The role of these genes on high-pressure growth is interesting. In our previous studies of physiological changes under high-pressure, we found that the *malB* operon was downregulated, since the promoter activities of both *malK-lamB* and *malEFG* were suppressed by elevated pressure (Sato et al. 1996). In this study, the *malB* operon was also inhibited (Table 3). This result confirms that the expression profiles corresponded to the previously reported physiological changes.

We analyzed the mRNA expression profiles from the aspect of regulation of temperature-inducible genes, and the expression profiles of major HSP and CSP genes are shown in Table 4. The heat shock-related genes showed the tendency of decreased expression under high-pressure conditions (Table 4), although increased HSP proteins were already reported (Welch et al. 1993). In general, HSPs are immediately produced upon heat shock for 15–20 min, and then, HSP mRNA is rapidly degraded to readjust to the normal environment (Lemaux et al. 1978; Yamamori et al. 1978; Yamamori and Yura 1980). The decreases in HSP mRNA suggest that the *E. coli* cells readjusted after the high pressure-induced heat shock response. It has previously been documented that many *E. coli* HSPs and CSPs are transiently induced by elevated pressure (Welch et al. 1993). The microarray result seemed to show the difference between the acute response and long-term stress upon high pressure. On the other hand, many cold shock response genes were upregulated in *E. coli* cells cultivated under both high-pressure conditions (Table 4). Upon cold stress, ATP synthesis as an energy source is activated, and *aceEF* genes, which encode the enzymes that convert pyruvate to acyl-CoA, are induced to produce the increase in the ability to synthesize acyl-CoA, which enhances citrate cycle activity (Thieringer et al. 1998; Yamanaka 1999). The microarray data showed that *aceEF* genes were upregulated (Table 4). This upregulation was suggested to contribute to the upregulation of the genes involved in energy metabolism, as mentioned above.

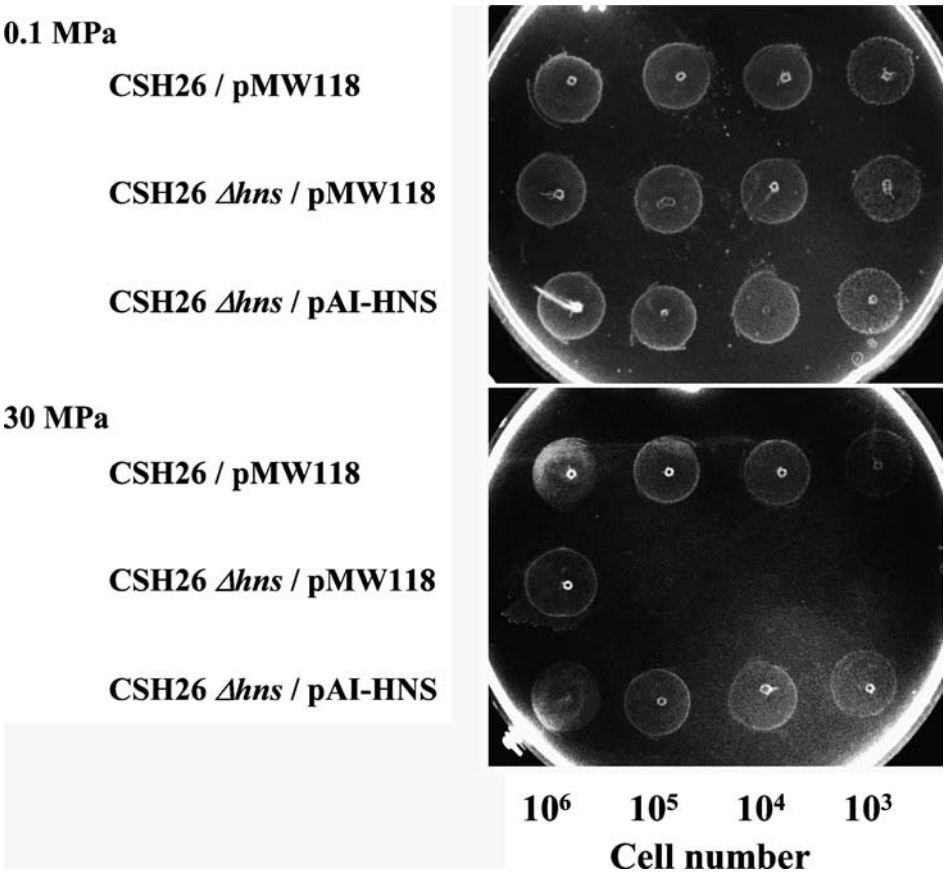

Fig. 2 Colony-forming assay under high-pressure conditions. Strains and cultivation pressure conditions are indicated on the *left*. Each strain was diluted to the indicated cell concentrations (*below*) and spotted on LB medium plates. After overnight cultivation, colony-forming ability was evaluated

The mechanisms of the alteration under temperature stress conditions have been well studied and the important roles of DNA-binding proteins determined in *E. coli* cells (Dersch et al. 1994; Jones and Inoue 1994; Hurme and Rhen 1998; Thieringer et al. 1998; Yamanaka 1999; Lopez-Garcia and Forterre 2000). The expression of several genes is regulated by DNA-binding protein with gyrase through alterations of DNA supercoiling and, probably, of local DNA structures (Lopez-Garcia and Forterre 2000). Actually, the elevated pressure increases the DNA-binding protein H-NS (Welch et al. 1993), and *cadAB* and *hdeABD* genes, which were repressed the expression levels by H-NS (Meng and Bennett 1992; Yoshida et al. 1993), were greatly down-regulated upon 30 and 50 MPa at the late-log phase (Tables 3 and 5). It was difficult to understand, because *hns* mRNA was exhibited a decrease of the expression level in our microarray analysis (Table 4). However, the mRNA regulation was considered to repress the excess expression of H-NS, and we considered that the DNA-binding protein H-NS played an important role in the adaptation to pressure stress in *E. coli*, based on the other H-NS regulated gene expression pattern (Table 5).

To investigate the contributions of the DNA-binding protein H-NS to high-pressure growth, the *hns* gene-deletion mutant was cultured under various pressure conditions. *E. coli* cells including the *hns* mutant and the parental cells displayed pressure sensitivity when growth was compared at 0.1 and 30 MPa (Fig. 2). Under atmospheric pressure conditions, the wild-type strain CSH26, the *hns*-deleted derivative, and the mutant harboring the pAI-HNS plasmid had the same growth characteristics. At 30 MPa, both the wild-type and complemented mutants formed colonies at all of the cell concentrations, although the *hns* mutants did not form colonies except on the spot of 10^6 cells. Thus, the Δ*hns* strain was indicated, at least, 1,000-fold more sensitive to elevated pressure than the wild-type strain. In the same way, in liquid LB medium, the growth rate of the wild-type *E. coli* was not decreased at the elevated pressure of 30 MPa (Fig. 3a). On the other hand, the mutant showed pressure-dependent decreases in growth from 10 MPa (Fig. 3b). In the wild-type *E. coli* cells, cell-doubling times based on the graphs of growth curves were 0.6 and 0.8 h at 0.1 and 30 MPa, respectively. In the *hns* mutant cells, the doubling times were 0.6, 0.8, 1.6, and 2.6 h at 0.1, 10, 20, and 30 MPa, respectively. A small amount of growth of the mutant cells at 30 MPa in the liquid medium was considered to depend on the cultivation conditions. With higher-pressure conditions, the growth of wild-type *E. coli* cells is inhibited, and they exhibit obviously filamentous shapes (Zobell and Cobet 1962, 1964). This result showed that the *hns* gene was essential for growth under high-pressure conditions.

Under stressful conditions, living cells alter their gene expression to maintain their intracellular environments.

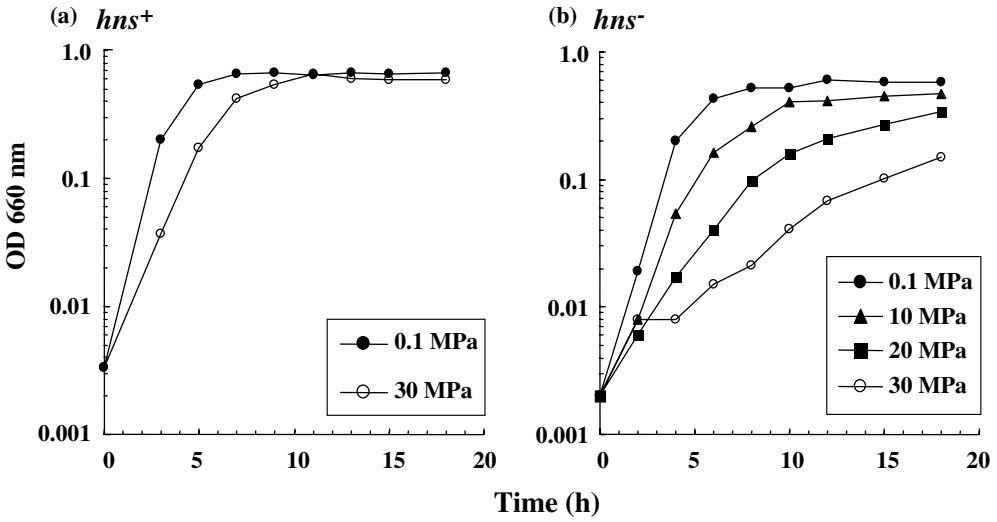

Fig. 3 Time-course analysis of *E. coli* cell growth under each pressure condition. **a** CSH26 (*hns*$^{+}$) and **b** CSH26 Δ*hns* (*hns*$^{-}$) strains were cultivated in LB medium, and the OD_{660} value was measured every 2 h. These strains were cultured at 0.1 (*filled circle*), 10 (*filled diamond*), 20 (*filled square*), and 30 MPa (*open circle*) at 37°C

The results of DNA microarray analysis revealed the pleiotropic effects of elevated pressure. The detailed microarray results showed that the characteristic pressure responses in the expression of several transporters and temperature-stress like responses. From the mutant analysis, a possible regulator to adapt to high-pressure was indicated. We expect that the present data will help to reveal the pressure effects on cells.

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