Genes of *Escherichia coli* O157:H7 That Are Involved in High-Pressure Resistance

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Seventeen *Escherichia coli* O157:H7 strains were treated with ultrahigh pressure at 500 MPa and $23 \pm 2^\circ\text{C}$ for 1 min. This treatment inactivated 0.6 to 3.4 log CFU/ml, depending on the strain. The diversity of these strains was confirmed by pulsed-field gel electrophoresis (PFGE) analysis, and there was no apparent association between PFGE banding patterns and pressure resistance. The pressure-resistant strain *E. coli* O157:H7 EC-88 (0.6-log decrease) and the pressure-sensitive strain ATCC 35150 (3.4-log decrease) were treated with a sublethal pressure (100 MPa for 15 min at $23 \pm 2^\circ\text{C}$) and subjected to DNA microarray analysis using an *E. coli* K-12 antisense gene chip. High pressure affected the transcription of many genes involved in a variety of intracellular mechanisms of EC-88, including the stress response, the thiol-disulfide redox system, Fe-S cluster assembly, and spontaneous mutation. Twenty-four *E. coli* isogenic pairs with mutations in the genes regulated by the pressure treatment were treated with lethal pressures at 400 MPa and $23 \pm 2^\circ\text{C}$ for 5 min. The barotolerance of the mutants relative to that of the wild-type strains helped to explain the results obtained by DNA microarray analysis. This study is the first report to demonstrate that the expression of Fe-S cluster assembly proteins and the fumarate nitrate reductase regulator decreases the resistance to pressure, while sigma factor (RpoE), lipoprotein (NlpI), thioredoxin (TrxA), thioredoxin reductase (TrxB), a trehalose synthesis protein (OtsA), and a DNA-binding protein (Dps) promote barotolerance.

Escherichia coli O157:H7 is one of a few bacteria that emerged in the past 2 decades as significant food-borne pathogens. This bacterium causes hemorrhagic colitis and hemolytic-uremic syndrome, which may lead to renal failure and death (48). Most *E. coli* O157:H7 infections occur through consumption of contaminated food and water. Ground beef, milk, apple juice, produce, and foods that have been stored, cooked, and handled improperly are potential transmission sources of *E. coli* O157:H7. The incidence of this pathogen in many food sources and its low infectious dose are causes of major concern among food processors and regulatory agencies.

Nonconventional technologies, such as those utilizing ultrahigh pressure (UHP), have been introduced as alternatives to heat and other traditional food preservation methods. These alternative technologies have the potential to produce safe food without adversely affecting its sensory properties or its freshness attributes. Currently, UHP is used commercially to process fruit juices, purees, guacamole, desserts, sauces, oysters, rice dishes, and packaged cured ham (54). One of the challenges associated with UHP technology is that different strains of pathogenic bacteria, such as *E. coli* O157:H7, exhibit great variability in resistance to the treatment (13).

Several research groups investigated the mechanism of action of UHP against bacterial cells. According to a recent study, high pressure induces oxidative stress and the SOS response in *E. coli* (2, 4). The loss of *rpoS*, a gene that codes for the sigma factor of the RNA polymerase involved in stationary phase, decreased the resistance of *E. coli* O157:H7 to high

pressure (51). Exposure to a stress may alter a cell's response to a different type of stress. Pressure-damaged *E. coli* O157:H7 cells are more acid sensitive than untreated cells (46). It was suggested that sensitization to acid may involve a loss of protective or repair functions; thus, genes involved in repairing acid damage may have been suppressed by the pressure treatment. The cell membrane is believed to be the prime target for UHP; however, the nature of membrane damage and its relation to cell death may depend on the species and phase of growth (45). Recently, *E. coli* was grown at 30 and 50 MPa, and the transcriptional effects of these pressures were analyzed using a DNA microarray (30). According to that report, heat and cold stress responses were induced simultaneously by the elevated pressures. In addition, an *hns* mutant exhibited great pressure sensitivity. The *hns* gene encodes a histone-like nucleoid structuring protein (H-NS) which preferentially binds to curved DNA sequences, thus contributing to DNA compactness, acts as a versatile transcriptional repressor, and plays a role as an expression modulator of some genes posttranscriptionally by affecting mRNA stability and the efficiency of translation (56). Therefore, the H-NS protein is a likely transcriptional regulator for genes related to the adaptation of *E. coli* to pressure.

In the current study, DNA microarray analysis was carried out to reveal the genes involved in the resistance of *E. coli* O157:H7 to pressure. The gene transcriptional profiles of pressure-treated (sublethally) and untreated bacteria were analyzed to elucidate the potential physiological response of this pathogen to pressure treatment. Some of the results of the DNA microarray analysis were confirmed by assessing the pressure resistance (i.e., barotolerance) of selected *E. coli* knockout mutants.

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TABLE 1. Mutant and wild-type strains of nonpathogenic *Escherichia coli* tested in this study

Allele and function	Strain	Genotype or description	Reference
Stress response			
<i>ybdQ</i> ⁺	BW25113	<i>lacI</i> ^q <i>rmB</i> _{T14} Δ <i>lacZ</i> _{WJ16} <i>hsdR514</i> Δ <i>araBAD</i> _{AH33} Δ <i>rhaBAD</i> _{LD78}	17
<i>ybdQ</i> mutant		BW25113 Δ <i>ybdQ::km</i>	
<i>dps</i> ⁺	ZK126	W3110 <i>tna-2</i> Δ <i>lacU169</i>	5
<i>dps</i> mutant	SF2080	ZK126 <i>dps::kan</i>	
<i>cspA</i> ⁺	JC7623	<i>galK2 rpsL31 recB21 recC22 sbcB15</i>	9
<i>cspA</i> mutant	WB002	JC7623 Δ <i>cspA::cat</i>	
<i>ibpAB</i> ⁺	MC4100	<i>araD139</i> Δ (<i>argF-lac</i>) <i>U169 rpsL150 relA1 flbB5301 deoC1</i>	61
		<i>ptsF25 rbsR</i>	
<i>ibpAB</i> mutant	JGT17	MC4100 Δ <i>ibp1::kan</i>	
Thiol-disulfide redox system			
<i>trxA</i> ⁺	GJ1427	<i>thi rha nagA lacZ trkA405 kdp-200::[\lambda</i> dlac(Ap)]	55
<i>trxA</i> mutant	GJ1426	GJ1427 Δ <i>trxA</i>	
<i>trxB</i> ⁺	DHB4	MC1000 Δ <i>phoA</i> (PvuII) <i>phoR</i> Δ <i>malF3</i> F' [<i>lac</i> + (<i>lacI</i> ^q) <i>pro</i>]	16
<i>trxB</i> mutant	FA196	DHB4 Δ <i>trxB::P</i> _{araB} <i>trxB</i>	
Fe-S cluster status			
<i>iscU</i> ⁺ <i>hscA</i> ⁺ <i>fdx</i> ⁺ <i>sufABCDSE</i> ⁺	MG1655	F ⁻ wild type	21
<i>iscU</i> mutant	OD110	MG1655 <i>iscU110::cm</i>	
<i>hscA</i> mutant	OD114	MG1655 <i>hscA2::cm</i>	
<i>fdx</i> mutant	OD115	MG1655 <i>fdx-115::kan</i>	
<i>sufABCDSE</i> mutant	WO19	MG1655 Δ <i>sufABCDSE19::kan</i>	
<i>iscR</i> ⁺	MG1655	F ⁻ λ <i>ilvG rfb-50 rph-1</i>	57
<i>iscR</i> mutant	PK4854	MG1655 Δ <i>iscR</i>	
<i>fnr</i> ⁺	MG1655	F ⁻ λ <i>ilvG rfb-50 rph-1</i>	
<i>fnr</i> mutant	JRG5390	MG1655 Δ <i>fnr::cm</i>	
Spontaneous mutation			
<i>yafN-yafP</i> ⁺	SMR4562	Δ (<i>lac-proAB</i>) _{XIII} <i>thi ara</i> Rif ^r [F' <i>proAB</i> ⁺ <i>lacI33</i> Ω <i>lacZ</i>]	39
<i>yafN-yafP</i> mutant	SMR7491	SMR4562 Δ (<i>yafN-yafP</i>)602[F' Δ (<i>yafN-yafP</i>)602 <i>proAB</i> ⁺ <i>lacI33</i> Ω <i>lacZ</i>] ^{d+}	
Miscellaneous			
<i>rbsD</i> ⁺	OW1		52
<i>rbsD</i> mutant	YP17	OW1 <i>rbsD::TnphoA'</i> <i>km</i>	
<i>otsA</i> ⁺	MC4100	F ⁻ <i>araD139</i> Δ (<i>argF-lac</i>) <i>U169 rpsL150 relA1 flbB5301 deoC1</i>	25
		<i>ptsF25 rbsR</i>	
<i>otsA</i> mutant	ML1	MC4100 <i>otsA::Tn10</i>	
<i>rpoS</i> ⁺	ZK126	W3110 <i>tna2</i> Δ <i>lacU169</i> ; G ₀	69
<i>rpoS</i> mutant	ZK1000	ZK126 Δ <i>rpoS::Kan</i> ^r	
<i>rpoE</i> ⁺	BW25113		23
<i>rpoE</i> mutant		BW25113 Δ <i>rpoE</i>	
<i>hns</i> ⁺ <i>stpA</i> ⁺	M182	Δ (<i>lacIPOZYA</i>)74 <i>galU galK strA</i>	68
<i>hns</i> mutant		M182 Δ <i>hns::kan</i> ^r	
<i>stpA</i> mutant		M182 Δ <i>stpA::tet</i> ^r	
<i>hns stpA</i> mutant		M182 Δ <i>stpA::tet</i> ^r Δ <i>hns::kan</i> ^r	
<i>eno</i> ⁺	K10	[<i>garB10 fhuA22 ompF627</i> (T ₂ ^r) <i>adL701</i> (T ₂ ^r) <i>relA1 pit-10 spoT1</i>	15
		<i>rmB2 mcrB1 creC510</i>]	
<i>eno</i> mutant	DF261	[<i>garB10 fhuA22 ompF627</i> (T ₂ ^r) <i>fadL701</i> (T ₂ ^r) <i>eno-2 relA1 pit-10</i>	
		<i>spoT1 rmB2 mcrB1 creC510</i>]	
<i>nlpI</i> ⁺ A	RM4606	<i>sup</i> ⁰ F ⁺	42
<i>nlpI</i> A mutant	WU62	RM4606 <i>nlpI::cm</i>	
<i>nlpI</i> ⁺ B	LF82		11
<i>nlpI</i> B mutant		LF82 Δ <i>nlpI</i>	
<i>yfiD</i> ⁺	W3110		67
<i>yfiD</i> mutant	JRG4033	W3110 Δ <i>yfiD::kan</i> ^r	

MATERIALS AND METHODS

Strains and sample preparation. Strains of *E. coli* O157:H7 were kindly provided by J. LeJeune, Ohio State University, Wooster, Ohio. These are FRIK-526, FRIK-528, FRIK-579, CL-56, EC-84, EC-88, EC-91, EC-92, EC-96, EC-100, EC-103, 93-001, and O-2191831. Additionally, *E. coli* O157:H7 ATCC 35150 and *E. coli* O157:H7 ATCC 43889 were used in this study. For comparative purposes, *E. coli* K-12 (a nonpathogenic strain) and O157:H12 (a nontoxicogenic strain; Richter International, Inc., Columbus, Ohio) were investigated. Strains EC-84, EC-88, EC-91, EC-96, EC-100, and EC-103 were isolated from cattle. The remaining pathogenic

strains were originally isolated from human patients involved in disease outbreaks. For the study comparing isogenic pairs, 24 nonpathogenic *E. coli* mutants and their wild-type counterparts were kindly provided by the sources listed in Table 1. All strains were grown from a 0.1% inoculum in tryptose broth (TB; BD Difco, Sparks, Md.) at 35°C for 18 h. All cultures were transferred at least twice before experimentation.

Barotolerance of pathogenic and nonpathogenic *Escherichia coli* strains. *Escherichia coli* strains, including O157:H7, O157:H12, and K-12, were grown in 10 ml TB to stationary phase ($\sim 1 \times 10^9$ CFU/ml, or an *A*₆₀₀ value of ~ 0.9), washed in

sterile phosphate buffer (12.5 mM, pH 7.2), and then resuspended in 10 ml phosphate buffer. Each sample was then aseptically transferred to a sterile stomacher bag (Fisher Scientific, Pittsburgh, Pa.) and vacuum packaged, and the bag was heat sealed. The sample was pressure treated at 500 MPa and $23 \pm 2^\circ\text{C}$ for 1 min in a high-pressure processor (Quintus QFP-6; Flow Pressure Systems, Kent, Wash.). The initial temperature of the pressure-transmitting fluid was controlled to account for compression heating (3 to $4^\circ\text{C}/100$ MPa). An untreated control was held at $23 \pm 2^\circ\text{C}$ and atmospheric pressure (0.1 MPa) while each treated sample was being pressurized. After pressure treatment, samples were serially diluted using 0.1% peptone water, plated on tryptose agar plates, and incubated at 35°C for 48 h to enumerate the viable counts. The barotolerance of the wild-type strains and mutants of nonpathogenic *E. coli* was determined as described earlier, with modifications: stationary-phase cultures were directly pressurized in TB at 400 MPa and $23 \pm 2^\circ\text{C}$ for 5 min.

Statistical analysis of barotolerance results. For the barotolerance study of the pathogenic strains, the log survivor ratio was calculated as $\log N/N_0$, where N is the CFU/ml of treated sample and N_0 is the initial (before treatment) CFU/ml. The pressure resistance of the isogenic nonpathogenic *E. coli* pairs was evaluated by calculating the differential log survivor ratio (DLSR), as follows: $\log(N/N_0)$ of mutant $-\log(N/N_0)$ of wild type, where N is the CFU/ml of treated sample and N_0 is the initial (before treatment) CFU/ml. A positive DLSR indicates a barotolerance that is greater for the mutant than for the wild type, and a negative DLSR indicates the opposite. Data shown are results of at least three independent replications. Means were compared and standard deviations were calculated using commercial statistical programs (JMPin [SAS Institute Inc., Cary, N.C.] and SigmaPlot, version 9.0 [SPSS Inc., Chicago, Ill.]).

PFGE. Pulsed-field gel electrophoresis (PFGE) was performed as reported by Jenkins et al. (31). Extracted DNAs were digested with XbaI (Invitrogen, Carlsbad, Calif.).

Culture preparation for DNA microarray analysis. *Escherichia coli* O157:H7 EC-88 (pressure resistant) and *E. coli* O157:H7 ATCC 35150 (pressure sensitive) were cultured, and cell suspensions were prepared as indicated earlier. The bags of cell suspensions were pressure treated at 100 MPa and $23 \pm 2^\circ\text{C}$ for 15 min. The untreated control suspensions were kept at atmospheric pressure (0.1 MPa) and $23 \pm 2^\circ\text{C}$ during pressure processing of the treated suspensions.

RNA isolation. Total RNAs were isolated from control and pressure-treated *E. coli* O157:H7 cells by use of a commercial kit (Trizol method; Invitrogen, Carlsbad, Calif.) as described by the manufacturer. Briefly, a pressure-treated or untreated *E. coli* O157:H7 cell suspension was centrifuged ($10,000 \times g$ for 10 min at 25°C), and 1 ml of Trizol reagent was added to the cell pellet. Trizol-treated cells were kept on ice, glass beads (~ 0.1 g; 0.1-mm diameter) were added to the cell suspension, and the suspension was subjected to shaking for 10 s at maximum speed (mini-bead beater; Bio-Spec Products, Inc., Bartlesville, Okla.). The cell extract was recovered after centrifugation ($10,000 \times g$ for 10 min). RNAs were extracted with chloroform, precipitated with ethanol, and resuspended in diethyl pyrocarbonate-treated water. The RNA preparations were purified further using clean-up columns (RNeasy; QIAGEN Inc., Valencia, Calif.). RNase-free DNase I was added to the columns to eliminate DNA contamination. The purity of the RNAs was determined by measuring the absorbance ratio A_{260}/A_{280} . In addition, each RNA sample was subjected to electrophoresis on a formaldehyde gel to confirm the integrity of the 16S and 23S rRNAs.

DNA microarray analysis. Total RNAs of pressure-treated and control samples were subjected to DNA microarray analysis using an *E. coli* K-12 antisense gene chip (Affymetrix, Inc., Santa Clara, Calif.). The time between the end of pressure treatment and the addition of the Trizol reagent to the treated cells was approximately 15 min. After the pressure treatment and RNA isolation, cDNAs were synthesized according to the protocol supplied by Affymetrix and described by Soupene et al. (58). The resulting cDNAs were purified with a PCR purification kit (QIAGEN, Inc.). The cDNAs (1.5 μg) were then fragmented with DNase I, and the 3' termini of the fragments were labeled with biotin-ddUTP, using a terminal deoxynucleotide transferase. The hybridization of the fragmented, biotinylated cDNAs to an *E. coli* K-12 antisense gene chip and the washing, staining, and scanning of the chip were carried out as described by the chip supplier (Affymetrix expression analysis technical manual). The arrays were scanned with a Gene Chip Scanner 3000 (Affymetrix, Santa Clara, Calif.) at the Heart and Lung Research Institute, Microarray-Genetics Core Laboratory (The Ohio State University, Columbus, Ohio).

DNA microarray data analysis. Fluorescence intensities of the hybridized chips and numerous quality control parameters were stored in a database. Image analyses and normalization were done using commercial and web-based software (Gene Chip operating software v. 1.1 [Affymetrix] and Gene Publisher [http://www.cbs.dtu.dk/services/GenePublisher/]) (35). The quality of the images was inspected, and images were set up properly (gridding, segmentation, intensity

FIG. 1. Log survivor ratios [$\log_{10}(N/N_0)$] of *Escherichia coli* strains that were grown to stationary phase at 35°C , washed and resuspended in phosphate buffer, and treated for 1 min at 500 MPa and 23°C . Error bars represent 1 standard deviation ($n \geq 3$).

extraction, and background correction) as recommended by the Heart and Lung Institute DNA Microarray Core staff (The Ohio State University, Columbus, Ohio). Inspection ensured that images were free from defects (e.g., amorphous blobs or masking). Fluorescence intensities for each chip were normalized by applying a single scaling factor of a target intensity of 1,500, which assumes that the total amount of mRNA in a cell is constant. Before performing statistical analysis, a filtering process was applied to narrow the gene pool analyzed. The selection criteria to filter out noncritical genes were as follows. (i) All intergenic regions were eliminated. (ii) Genes with absent calls were eliminated. (iii) Average signal intensities and $\log_{10}$ changes [$\log(\text{treated mean intensity}/\text{control mean intensity})$] were calculated from a minimum of three independent samples of the filtered genes, and genes for which the log change (x) was in the range of $-0.15 < x < 0.15$ were eliminated. The final filtered genes were statistically evaluated for differential expression levels. Therefore, average signal intensities for each gene in the control and treated samples were determined and compared statistically using the t test (Excel; Microsoft Inc., Redmond, Wash.). A volcano plot was constructed to test the relationship between P values and expression changes. The Benjamini and Hochberg false discovery rate (FDR) was applied to correct for multiple testing (14). These differentially expressed genes were identified and their sequences were examined for homology with genes of known function by using public domain software programs, including GenProtEC, PubMed (http://www.ncbi.nlm.nih.gov), and Affymetrix Inquiry (Affymetrix, Inc.). Certain genes linked to the physiological process of interest were identified.

RESULTS

Barotolerance of *Escherichia coli* O157 strains. The barotolerance of *E. coli* strains, including O157:H7, O157:H12, and K-12 strains, was determined by comparing viabilities before and directly after a 1-min lethal treatment at 500 MPa and $23 \pm 2^\circ\text{C}$ (Fig. 1). *Escherichia coli* ATCC 35150 was the least resistant to high pressure, with a 3.4-log reduction, while EC-88 was one of the most resistant strains, with only a 0.6-log reduction ($P < 0.05$).

Genotyping by pulsed-field gel electrophoresis. The 16 *E. coli* O157 strains and *E. coli* K-12 were compared by PFGE analysis. The genetic similarities among these strains, as judged by the PFGE banding patterns, ranged from 34 to 85% (data

TABLE 2. Genes that were down-regulated in response to UHP (100 MPa for 15 min at 23 ± 2°C) in *Escherichia coli* O157:H7 EC-88

Gene and functional category ^b	Relevant function	P value (t test)	Corrected P value (FDR) ^a	Fold change
Stress response				
<i>uspA</i>	Universal stress protein A (UspA), linked to resistance to DNA damage	0.00075	0.019	1.8
<i>yjiT</i>	Putative stress flavoprotein (UspD)	0.0083	0.0495	1.9
<i>ybdQ</i>	Universal stress protein, flavoprotein (UP12)	0.00074	0.022	2.1
<i>ynaF</i>	Putative electron transfer flavoprotein (UP03)	0.029	0.095	2.4
<i>dps</i>	Stress response DNA-binding protein	0.0073	0.047	2.5
<i>yecG</i>	Putative electron transfer protein (UspC)	0.84	*	1.0
<i>pphB</i>	Serine/threonine-specific protein phosphatase 2	0.674	*	1.0
<i>ibpB</i>	Small heat shock protein	0.98	*	1.0
Thiol-disulfide redox system				
<i>trxB</i>	Thioredoxin reductase, FAD/NAD(P) binding	0.70	*	1.1
<i>grxB</i>	Glutaredoxin 2	0.15	*	1.2
<i>grxC</i>	Glutaredoxin 3	0.95	*	1.0
Fe-S cluster status				
<i>sufS</i>	Selenocysteine lyase, PLP dependent	0.0028	0.034	1.9
<i>sufA</i>	Fe-S assembly protein	0.18	*	1.3
<i>sufB</i>	Putative transport protein associated with Fe-S cluster assembly	0.12	*	1.3
<i>sufC</i>	Putative transport protein associated with Fe-S cluster assembly	0.18	*	1.3
<i>sufD</i>	Required for stability of Fe-S component of FhuF protein	0.18	*	1.3
<i>sufE</i>	Stimulator of SufS activity	0.13	*	1.2
Spontaneous mutation				
<i>dinB</i>	Error-prone DNA polymerase IV	0.97	*	1.0
<i>yafP</i>	Conserved protein with possible role in spontaneous mutation	0.45	*	1.1
Miscellaneous				
<i>eno</i>	Enolase	0.00090	0.020	2.0
<i>yfiD</i>	Putative formate acetyltransferase	0.0093	0.049	2.2
<i>rpoS</i>	Sigma S factor of RNA polymerase, major sigma factor during stationary phase	0.26	*	1.1
<i>stpA</i>	DNA-bending protein with chaperone activity	0.40	*	1.1
<i>ygiR</i>	Putative NAD(P)-binding dehydrogenase	0.0088	0.049	1.4
<i>ygcO</i>	Putative 4Fe-4S ferredoxin-type protein	0.0098	0.0499	1.4
<i>rhn</i>	tRNA processing exoribonuclease BN	0.0071	0.047	1.5
<i>yniA</i>	Protein kinase-like protein	0.0043	0.043	1.9
<i>ycbB</i>	Carboxypeptidase	0.0049	0.044	2.2
<i>ynhG</i>	ATP synthase subunit	0.0085	0.049	2.3
<i>yneA</i>	Sugar transport protein (ABC superfamily, periplasmic binding protein)	0.0062	0.044	2.3
<i>yccJ</i>	Unknown CDS	0.0036	0.038	3.0
<i>yehH</i>	Involved in peptidyl tRNA hydrolase activity	0.000082	0.015	3.5

^a *, genes did not pass the filtering process.^b The genes were grouped according to EcoCyc (22).

not shown). There was no apparent association between pressure resistance and genetic similarity. *Escherichia coli* O157:H7 EC-88 and ATCC 35150, which were very different in barotolerance, also produced diverse PFGE banding patterns. Therefore, these two strains were further analyzed for gene expression in response to UHP, using DNA microarray analysis.

Gene expression analysis after pressure treatment. More than 100 genes responded to sublethal pressure treatment of stationary-phase, barotolerant *E. coli* O157:H7 EC-88; however, only 36 genes passed the Benjamini and Hochberg corrections for multiple testing (14). Responses of genes with corrected *P* values of <0.05 were considered significant (Tables 2 and 3). In addition, genes with regulatory or functional similarity were analyzed, de-

spite their marginal significance (marginal values were corrected *P* values of ≥0.05 to <0.10). The sublethal pressure treatment (100 MPa for 15 min at 23 ± 2°C) was sufficient to induce a pressure stress response but not so severe as to inactivate the cells or prevent transcription.

The DNA-binding protein genes *uspA*, *yjiT*, *ybdQ*, *ynaF*, and *dps* were significantly down-regulated, by 1.8-, 2.0-, 2.1-, 2.4-, and 2.5-fold, respectively (Table 2). In contrast, *yecG* had no change in transcription. Expression of the stress response genes *hslV*, *ibpA*, *pphA*, and *cspA* significantly increased, by 1.5-, 1.9-, 2.0-, and 3.9-fold, respectively, due to the pressure treatment (Table 3); however, *ibpB* was not affected. In addition, genes involved in the thiol-disulfide redox system, *trxB*, *grxB*, *grxC*, *sufS*, *sufA*, *sufB*, *sufC*, *sufD*, *sufE*, *dinB*, and *yafP* were also down-regulated, by 1.1-, 1.2-, 1.3-, 1.3-, 1.3-, 1.2-, 1.0-, and 1.1-fold, respectively.

TABLE 3. Genes that were up-regulated in response to UHP (100 MPa for 15 min at 23 ± 2°C) in *Escherichia coli* O157:H7 EC-88

Gene and functional category ^b	Relevant function	P value (t test)	Corrected P value (FDR) ^a	Fold change
Stress response				
<i>hslV</i>	Peptidase component of the HslUV protease	0.0014	0.023	1.5
<i>ibpA</i>	Small heat shock protein	0.0053	0.043	1.9
<i>pphA</i>	Serine/threonine-specific protein phosphatase 1, signals protein misfolding	0.000094	0.0084	2.0
<i>cspA</i>	Major cold shock protein 7.4, transcription antiterminator of <i>hns</i> , single-stranded DNA-binding property	0.0011	0.020	3.9
Thiol-disulfide redox system				
<i>trxA</i>	Thioredoxin 1, redox factor, carrier protein	0.039	0.0991	1.4
<i>grxA</i>	Glutaredoxin 1	0.011	0.051	1.4
<i>trxC</i>	Thioredoxin 2	0.083	0.14	2.2
<i>nrdI</i>	Stimulates ribonucleotide reduction	0.0089	0.048	1.5
<i>nrdH</i>	Glutaredoxin-like protein	0.036	0.0995	1.6
<i>nrdE</i>		0.64	*	1.1
<i>nrdF</i>		0.72	*	1.0
Fe-S cluster status				
<i>fnr</i>	Transcriptional regulator of aerobic and anaerobic respiration and osmotic balance (cyclic AMP-binding family)	0.0053	0.045	1.5
<i>iscR</i>	Repressor of the <i>iscRSUA</i> operon, involved in assembly of Fe-S clusters	0.0054	0.042	1.8
<i>iscU</i>	Fe-S cluster template protein	0.10	*	1.2
<i>hscA</i>	Chaperone (Hsp70 family), involved in assembly of Fe-S clusters	0.98	*	1.0
<i>fdx</i>	[2Fe-2S] ferredoxin, electron carrier protein, involved in assembly of Fe-S clusters	0.89	*	1.0
Spontaneous mutation				
<i>yafO</i>	Conserved protein with possible role in spontaneous mutation	0.0044	0.042	1.5
<i>yafN</i>	Conserved protein with possible role in spontaneous mutation	0.022	0.082	1.6
Miscellaneous				
<i>nlpI</i>	NlpI lipoprotein	0.00062	0.028	1.9
<i>rbsD</i>	Membrane-associated component of high-affinity D-ribose transport system, membrane	0.0023	0.034	4.0
<i>rpoE</i>	Sigma E factor of RNA polymerase, response to periplasmic stress	0.039	0.098	1.5
<i>hns</i>	Transcriptional regulator, DNA-binding protein HLP-II, increases DNA thermal stability	0.49	0.49	1.5
<i>otsB</i>	Trehalose-6-phosphate phosphatase, osmoregulation	0.028	0.097	2.1
<i>yebF</i>	Conserved protein	0.0011	0.023	1.4
<i>yibF</i>	Putative glutathione S-transferase enzyme	0.00062	0.022	1.5
<i>yafQ</i>	Conserved protein	0.0058	0.043	1.6
<i>ycfJ</i>	Membrane protein	0.0077	0.047	1.6
<i>rpsT</i>	30S ribosomal subunit protein S20	0.0062	0.043	1.6
<i>infA</i>	Protein chain initiation factor IF-1	0.0025	0.032	1.8
<i>rpsU</i>	30S ribosomal subunit protein S21	0.0023	0.032	2.1
<i>rhoL</i>	<i>rho</i> operon leader peptide	0.0031	0.035	3.4
<i>glyY</i>	Glycine tRNA ³	0.00038	0.023	5.8

^a *, genes did not pass the filtering process.^b The genes were grouped according to EcoCyc (22).

grxA, and *nrdH*, were marginally up-regulated, by 1.4-, 1.4-, and 1.6-fold, respectively, while *nrdI* was significantly up-regulated (1.5-fold). In contrast, *trxB*, *grxB*, *grxC*, *nrdE*, and *nrdF* showed no evident change in expression. The Fe-S-related genes *fnr* and *iscR* were significantly up-regulated, by 1.5- and 1.8-fold, respectively, while *sufS* was significantly down-regulated, by 1.9-fold. Interestingly, the entire *suf* operon (*sufABCDSE*) appeared to be down-regulated, while *iscU*, *hscA*, and *fdx* had no significant change. The transcription of genes involved in spontaneous mutation (*dinB* and *yafP*) did not change in response to the pressure treatment. However, *yafO* and *yafN*, also involved in spontaneous mutation, were significantly and marginally up-

regulated, respectively, by 1.5- and 1.6-fold. Miscellaneous genes such as *nlpI* and *rbsD* were significantly up-regulated, by 1.9- and 4.0-fold, respectively, while *rpoE*, *hns*, and *otsB* were marginally up-regulated, by 1.5-, 1.5-, and 2.1-fold, respectively. On the other hand, *eno* and *yfiD* were significantly down-regulated, by 2.0- and 2.2-fold, respectively, and *rpoS* and *stpA* showed no change. Additional miscellaneous genes were significantly up-regulated; these are *yebF*, *yibF*, *yafQ*, *ycfJ*, *rpsT*, *infA*, *rpsU*, *rhoL*, and *glyY*. The sublethal pressure treatment down-regulated the following additional miscellaneous genes significantly: *ygiR*, *ygcO*, *rbn*, *yniA*, *ycbB*, *ynhG*, *yneA*, *yccJ*, and *yehH*.

The pressure-sensitive strain ATCC 35150 also showed tran-

FIG. 2. DLSRs for *Escherichia coli* isogenic pairs (mutants and corresponding wild-type strains) that were grown to stationary phase and pressure treated at 400 MPa for 5 min at $23 \pm 2^\circ\text{C}$. The DLSR equals $\log(N/N_0)_{\text{mutant}} - \log(N/N_0)_{\text{wild type}}$, where N is the CFU/ml of treated sample and N_0 is the initial (before treatment) CFU/ml. Positive DLSRs indicate barotolerance levels that are greater for the mutant than for the wild type, and error bars represent 1 standard deviation. *, the mutant was significantly different ($P < 0.05$) from its wild-type counterpart.

scriptional changes in response to the pressure treatment similar to those of EC-88. However, after performing the Benjamini and Hochberg FDR method for multiple testing, no significant changes (corrected P values of <0.05) in the expression of ATCC 35150 genes in response to the pressure treatment were confirmed (data not shown). The raw DNA microarray data for both strains are available at The Ohio State University Food Safety Laboratory website (<http://www.fst.osu.edu/foodsafetylab/index.htm>).

Barotolerance of *Escherichia coli* mutants. The barotolerance levels of available isogenic *E. coli* K-12 pairs covering various genotypic backgrounds were compared after pressure treatment at 400 MPa for 5 min. Barotolerance was expressed as the DLSR, as described earlier (Fig. 2). The 400-MPa treatment allowed the most discrimination between the isogenic pairs. The *dps*, *trxA*, *trxB*, *otsA*, *nlpI* B, *rpoS*, *rpoE*, and *nlpI* A mutants were significantly ($P < 0.05$) more sensitive to UHP than their wild-type counterparts, with DLSRs of -1.7 , -1.6 , -0.8 , -0.5 , -1.1 , -1.2 , -2.9 , and -3.0 , respectively. Individually, the *hns* and *stpA* mutants showed no differences in barotolerance compared to their wild-type counterparts; however, an *hns stpA* double mutant became much more pressure sensitive than the wild type, with a DLSR of -3.0 . The *fdx*, *iscU*, *hscA*, *sufABCDSE*, and *fnr* mutants were more resistant to UHP than their wild-type counterparts; the corresponding DLSRs were 2.0, 2.2, 2.2, 2.2, and 3.0.

DISCUSSION

The current study demonstrated considerable variability among *E. coli* O157:H7 strains in response to high pressure (Fig. 1). Strain variability was reported frequently when pathogens were treated with minimal or nonthermal preservation methods. A genetic typing technique using PFGE did not discriminate properly between pressure-resistant and pressure-sensitive *E. coli* O157:H7 strains (data not shown). Similarly, a previous study demonstrated variability among *Listeria monocytogenes* strains in the response to a pulsed electric field treatment, but strain resistance to a pulsed electric field was not associated with the PFGE pattern (37).

Food-borne pathogenic and spoilage microorganisms are commonly more processing resistant in the stationary than in the exponential phase of growth; therefore, stationary-phase cells were analyzed in this study. Transcriptional analysis using DNA microarray methodology revealed considerable differences between barotolerant (EC-88) and barosensitive (ATCC 35150) *E. coli* O157:H7 strains. More than 100 genes responded to the sublethal pressure treatment, particularly in the pressure-resistant strain EC-88. Assessing the barotolerance of *E. coli* strains having mutations in genes believed important to pressure resistance helped us to interpret the results of the microarray analysis. However, discrepancies between the results obtained by the microarray analysis and those for the mutant barotolerance test were inevitable; the former mea-

sure only the transcriptional response to sublethal pressure, whereas the latter represents the outcome of the entire gene expression process as the result of a lethal pressure treatment. This discussion will be restricted to *E. coli* EC-88, the pressure-resistant strain that demonstrated significant transcriptional changes when treated with pressure at 100 MPa for 15 min. Genes responding to pressure were grouped according to primary function, but overlap between these groups was not avoidable. *Escherichia coli* O157:H7 is genetically unstable due to its many prophages (29). Therefore, an *E. coli* K-12 gene chip is suitable for measuring the conserved genes of *E. coli* O157:H7. However, *E. coli* O157:H7 prophages may be related to pressure resistance, and the responses of their genes were not measured in this study. Although the sensitive strain (ATCC 35150) did not produce any significant results with the DNA microarray analysis, it is still important that many of the same genes as those in the resistant strain were up- or down-regulated, just not at such pronounced levels, including those of the thiol-disulfide redox system.

Stress response genes. When *E. coli* is subjected to conditions that arrest its growth (e.g., starvation) or exposed to DNA-damaging agents (e.g., UV), genes for universal stress proteins are expressed. The well-characterized universal stress protein A (UspA) and its paralogues (UspC, UspD, and UspE) are coordinately regulated in response to DNA damage (26). This UspA family includes UP03 (encoded by *ynaF*) and UP12 (encoded by *ybdQ*); the latter is a putative substrate of the molecular chaperone GroEL (17). Treatment of *E. coli* O157:H7 with high pressure down-regulated the UspA family genes 1.8- to 2.4-fold, but the expression of *yecG*, which codes for the universal stress protein C (22), was not altered (Table 2). A *ybdQ* mutant was similar to the parent strain in pressure sensitivity (Fig. 2).

High pressure down-regulated *dps* 2.5-fold (Table 2). This gene encodes Dps, a DNA-binding protein produced by starved cells which plays a major role in protecting bacterial DNA from reactive oxygen species (5). Dps restricts iron uptake and sequesters intracellular iron during H₂O₂ stress to protect the cell from the Fenton reaction (47). Dps is regulated by the *rpoS* gene product and integration host factor (IHF [a DNA-bending protein]) in the stationary phase and by OxyR/IHF during exponential phase (6). High pressure may have down-regulated *dps* by altering the conformation of the DNA, inhibiting IHF's ability to facilitate the binding of RpoS to the *dps* promoter. Other IHF-regulated genes were affected by pressure treatment, as discussed below. Krzyzaniak et al. (36) reported DNA conformational changes in low-salt buffer when the solution was treated with high pressure at 600 MPa. Other researchers observed a condensation of *E. coli* nucleoids in response to high pressure at 200 MPa for 8 min (38). Reducing the transcription of *dps* in response to pressure may allow the chromosomal DNA to renature to its protective state without interruption by abundant DNA-binding proteins. It is likely that the cell's reserve of Dps is sufficient for reconfiguring the supercoiled protective state of the DNA. The *dps* mutant was significantly more sensitive to UHP than its wild-type counterpart (Fig. 2). This finding indicates that the lack of Dps sensitizes *E. coli* to the pressure treatment, probably by exposing DNA to conformational changes or by exposing the cells to oxi-

dative damage, perhaps via the Fenton reaction. As stated earlier, high pressure has been shown to induce oxidative stress (4).

Heat shock proteins (Hsp's) are important in the bacterial stress response. Many of these proteins are molecular chaperones that bind to nascent, misfolded, or damaged polypeptides and assist them in reaching a native conformation (27). Chaperones of *E. coli* include Hsp60 (GroEL), Hsp70 (DnaK), Hsp100, and the small heat shock proteins IbpA and IbpB. The latter category is believed to assist the refolding of denatured proteins in the presence of other chaperones (65). The *pphA* gene encodes a serine/threonine-specific protein phosphatase that induces the accumulation of heat shock proteins by signaling protein misfolding (40). The pressure treatment increased the transcription of *ibpA* and *pphA* approximately two-fold (Table 3). The transcription of *ibpB* did not change in response to the sublethal pressure treatment (Table 2). The *ibpAB* mutant was slightly sensitive to pressure, but it was not statistically different from its wild type in barotolerance (Fig. 2). High pressure may cause protein denaturation and aggregation in the bacterial cell. According to Mañas and Mackey (38), cytoplasmic proteins in *E. coli* were aggregated in response to pressure treatment. Increased transcription of some heat shock protein genes (Table 3) may represent the cell's response to protein denaturation by pressure, and the accumulation of these gene products may aid in repairing pressure damage. Although the barotolerance test of the *ibpAB* double mutant did not indicate increased pressure sensitivity, recent studies showed that heat shock cross-protected *E. coli* against pressure (3) and that high pressure induced 11 heat shock proteins in *E. coli* (66).

The cold shock protein CspA is an RNA chaperone which destabilizes RNA secondary structures formed at low temperatures, thus making them susceptible to ribonucleases (32). Such a function may be crucial for efficient translation of mRNAs at low temperatures and may also have an effect on transcription. In addition, CspA induces the transcription of a histone-like nucleoid structuring protein (H-NS) (10); this protein controls the expression of many genes regulated by environmental parameters such as pH, temperature, and osmolarity (8). Treating *E. coli* O157:H7 at 100 MPa up-regulated *cspA* 3.9-fold (Table 3). Experiments using reverse transcription–real-time PCR confirmed the up-regulation of *cspA* in response to the pressure treatment (data not shown). The pressure treatment may have affected RNA in a fashion similar to that caused by cold shock, causing increased transcription of *cspA*. Interestingly, the *cspA* mutant was not significantly sensitive to UHP compared to its wild-type counterpart (Fig. 2). High pressure has been shown to induce four cold shock proteins (66). Furthermore, *E. coli* is currently known to have nine CspA homologues (49).

Thiol-disulfide redox system. *Escherichia coli* contains two thioredoxins (Trx1 and Trx2) and three glutaredoxins (Grx1, Grx2, and Grx3). These are small proteins with two redox-active cysteine thiols, which, by thiol-disulfide (SH/S-S) interchange, reduce acceptor disulfides in the cell's key proteins (43). An example of the activity of these cytoplasmic thioredoxins is the reduction of periplasmic DsbC through membrane-bound DsbD, where the reduced DsbC can then be used in disulfide bond isomerization and proper protein folding in the periplasm (12). Recently, it was shown that the thioredoxin

system has chaperone properties that do not involve cysteine residues (34). The three most effective cytoplasmic disulfide-reducing proteins of *E. coli* are Trx1 (*trxA*), Trx2 (*trxC*), and Grx1 (*grxA*) (59). The sublethal pressure treatment marginally up-regulated *trxA* and *grxA* expression, by 1.4-fold (Table 3). Although there was no significant difference, *trxC*, which falls in the same functional category as *trxA* and *grxA*, appeared to be up-regulated as well. However, there was no change in expression of *trxB*, *grxB*, and *grxC* in response to pressure. Interestingly, *rhoL*, the *rho* operon leader peptide, which is located immediately downstream of *trxA* (22), was significantly up-regulated in response to pressure, by 3.4-fold.

Escherichia coli has a ribonucleotide reductase encoded by the *nrdHIEF* operon, and expression of this operon is triggered in response to oxidative stress. The *nrdHIEF* operon is up-regulated when different chemical oxidants (e.g., H₂O₂ and paraquat) are added or when major antioxidant defenses are lost (41). It was suggested that an enhanced ribonucleotide reductase may protect DNA against reactive oxygen species escaping from the antioxidant defenses. High-pressure treatment significantly and marginally up-regulated *nrdI* and *nrdH*, respectively. In fact, it appeared that the entire *nrd* operon was up-regulated due to pressure treatment.

Both *trxA* and *trxB* mutants were significantly more sensitive to UHP than were their wild-type counterparts (Fig. 2). The thioredoxin reductase (*trxB*) can reduce Trx1 in the cytosol. The reduced state of Trx1 can aid in disulfide isomerization to correctly fold a misoxidized protein (50). High pressure promoted β -lactoglobulin aggregation through thiol-disulfide interchange reactions (24). A study investigating the effects of pressure on the hydrophobicity and interactions of -SH groups of myofibrillar proteins showed that the number of free -SH groups grew with increasing pressure and treatment time (18). We propose that high pressure denatures proteins in a way that increases the accessibility of -SH or S-S to catalytic agents in the cell cytosol. According to Åslund and Beckwith (7), "disulfide stress" occurs when unwanted disulfide bonds are generated in microbial cells, which will ultimately impact the protein's activity as well as the redox homeostasis. In response to high pressure, *E. coli* up-regulated the thiol-disulfide redox system in a manner that increased barotolerance, perhaps by facilitating proper protein folding and/or maintaining redox homeostasis.

Iron-sulfur clusters. Iron-sulfur (Fe-S) clusters are prosthetic groups commonly found in proteins that participate in oxidation-reduction reactions. The protein FNR of *E. coli* is required for transcriptional regulation of many anaerobic metabolism genes in response to oxygen availability (53). Under anaerobic conditions, FNR is active and contains a [4Fe-4S] cluster. In the presence of oxygen, [4Fe-4S] · FNR is converted to [2Fe-2S] · FNR, resulting in monomerization and inactivation of FNR as a gene regulator. Prolonged exposure to oxygen converts [2Fe-2S] · FNR to apoFNR, an FNR that is devoid of Fe-S clusters (1). The assembly of iron-sulfur clusters in *E. coli* involves two independent systems, namely, ISC, encoded by *iscSUA-hscBA-fdx*, and SUF, encoded by *sufABCDSE* (63). Unlike the ISC system, the SUF system is required for Fe-S cluster assembly under iron starvation conditions and perhaps to provide a shielded pathway for donating sulfane sulfur to Fe-S clusters (44). An *E. coli* mutant in which the entire *isc*

operon was deleted grew very poorly and had a marked decrease in Fe-S protein activity compared to the wild type, while deletion of the entire *suf* operon showed no severe phenotypic effects (60). When both operons were deleted, the mutant exhibited synthetic lethality, which indicates that these two systems are essential for cell viability. However, the overproduction of *suf* operon products suppressed the defects in the *isc*-deleted strain.

The sublethal pressure treatment significantly down-regulated *sufS*, and the entire *suf* operon appeared to be down-regulated (Table 2). Additionally, the *sufABCDSE* mutant was significantly more barotolerant than its wild type (Fig. 2). The *suf* operon is believed to be regulated by two mechanisms. When the ferric uptake regulation protein Fur binds to Fe, it represses the *suf* operon. It is likely that pressure treatment increases the availability of iron ions in the cell, thus suppressing the *suf* operon, or at least one of its genes. Some Fe-S clusters are sensitive to environmental stresses, and the release of Fe from these proteins under deleterious conditions (e.g., pressure) may cause further cell damage via the Fenton reaction. Accordingly, the assembly of specific proteins that contain Fe-S clusters may render the bacterial cell pressure sensitive. In addition, these findings may indicate that pressure treatment enhances oxidative reactions, probably through modifying macromolecules and exposing sites (such as thiol-disulfide groups) which react quickly with these oxidative species. With oxygen solubility increasing under high pressure, it is also likely that pressure treatment sensitizes proteins containing Fe-S clusters to oxygen, causing the destruction of the Fe-S clusters and thus releasing Fe, which is disadvantageous to the cell. The second mechanism of regulating *suf* involves IHF, a DNA-binding protein, along with OxyR; both are required for *suf* activation. Similar to the *dps* regulation proposed earlier, pressure may have affected the promoter region of the *suf* operon, impeding proper binding of OxyR and IHF.

As stated earlier, FNR is an oxygen sensor that regulates genes involved in anaerobic respiration. Although the iron-sulfur cluster in active FNR is degraded by exposure to oxygen, a previous study showed that the level of FNR itself did not differ significantly between anaerobic and aerobic conditions (64). In the current study, *fnr* was significantly up-regulated in response to high-pressure treatment (Table 3). According to Trageser et al. (64), proteolysis of FNR occurs by a protease which is located outside the cytoplasmic membrane or activated upon disruption of the membrane. Therefore, it is likely that pressure treatment disrupted the cell membrane, thus exposing FNR to proteolysis. Since *fnr* is self-regulated, the proteolysis of FNR would up-regulate the gene. A strain with a mutation in *fnr* was much more resistant to UHP than its wild type (Fig. 2). As suggested earlier, Fe-S clusters in some proteins (e.g., FNR) are likely targets for high pressure, and the release of Fe by this treatment is disadvantageous to the cell. In addition, FNR may regulate genes that affect barotolerance, perhaps those encoding Fe-S-related proteins.

The ISC system (encoded by *iscSUA-hscBA-fdx*) is the primary manager of Fe-S cluster assembly in *E. coli* (63). Pressure treatment significantly up-regulated *iscR* (Table 3), a gene involved in repressing the *isc* operon. Compared to the wild type, the *iscR* mutant was not significantly more sensitive to UHP. Individual mutations in *iscS*, *iscU*, *hscB*, *hscA*, and *fdx*

caused changes in the growth rate, nutritional requirements, and Fe-S enzyme activities, with the *iscS* mutant showing the most severe phenotypic changes (62). The *iscU*, *hscB*, *hscA*, and *fdx* mutants had identical phenotypic changes, while the *iscR* mutant displayed no phenotypic differences from the wild type (62). Consistent with our hypothesis on the contribution of Fe-S clusters to pressure sensitivity, the *iscU*, *hscA*, and *fdx* mutants were significantly more resistant to UHP than were their wild-type counterparts (Fig. 2). In summary, this research suggests that UHP affects Fe-S proteins, Fe-S cluster assembly proteins, the Fe-S cluster status, and/or Fe availability, all of which affect the cell's redox homeostasis.

Genes involved in spontaneous mutation. The *dinB* operon (*dinB-yafN-yafP*) is known to be involved in spontaneous mutation, while the exact role of each *yaf*-encoded protein is unclear. However, a polar *dinB* (error-prone DNA polymerase IV) mutation caused a decrease in spontaneous mutation, whereas a nonpolar substitution and nonpolar deletion allele of *dinB* did not (39). Therefore, *yafN*, *yafO*, and *yafP* are partly or wholly responsible for phenotypes in spontaneous mutation, translesion synthesis, and adaptive mutation. Extremely pressure-resistant strains have been generated by multiple rounds of exposure to ultrahigh pressure followed by selection of survivors (28). The current research shows that *yafO* and *yafN* are significantly and marginally up-regulated due to pressure, respectively, while *dinB* and *yafP* transcripts show no significant change. The *yafN-yafP* mutant was similar to its wild type in pressure resistance.

Miscellaneous genes. The current study showed no statistical differences in the expression of *hns*, a transcriptional dual-regulator gene, and *stpA*, which codes for an H-NS-like protein, in response to sublethal pressure treatment (Tables 2 and 3). Similarly, *hns* and *stpA* mutants were not significantly different in pressure resistance from their wild-type counterparts. However, when both genes were deleted, the resulting double mutant was highly pressure sensitive (Fig. 2). Therefore, H-NS and StpA can independently contribute to barotolerance, and the basal level of either one of these DNA-binding proteins may regulate genes involved in barotolerance. According to Ishii et al., an *E. coli hns* mutant was more sensitive to high pressure than its wild-type counterpart (30). In conclusion, *hns*, independently or in combination with *stpA*, is critical to *E. coli* barotolerance.

Sigma E (*rpoE*), a periplasmic stress sigma factor, controls the folding of polypeptides in the bacterial envelope and the biosynthesis/transport of lipopolysaccharide. The conditions that cause unfolding of polypeptides are signaled by the RseA and RseB proteins (20). The NlpI protein is a recently discovered lipoprotein, and its exact role in the cell is under investigation. Ohara et al. (42) indicated that NlpI is possibly involved in cell division. In response to the sublethal pressure treatment, *rpoE* and *nlpI* were marginally and significantly up-regulated, respectively (Table 3). In addition, *rpoE* and *nlpI* mutants were significantly more sensitive to UHP than their wild-type counterparts. This suggests that high pressure induces periplasmic stress by denaturing polypeptides involved in the synthesis of the bacterial envelope. Interestingly, a deep-sea bacterium, *Photobacterium* sp. strain SS9, contains an *rpoE*-like locus that controls membrane protein synthesis and growth at high pressures (19). Lastly, it is plausible that NlpI is in-

involved in the repair of cell injury caused by high-pressure treatment.

According to Robey et al. (51), the stationary-phase sigma factor RpoS is involved in the resistance of *E. coli* O157:H7 to high pressure. The current study confirms this finding; an *rpoS* mutant was more sensitive to pressure than its wild-type counterpart (Fig. 2). The synthesis of trehalose may protect the bacterial cell against heat, cold, and osmotic stresses and oxygen radicals (33). The *otsAB* genes are involved in trehalose synthesis (22). Sublethal pressure treatment marginally up-regulated *otsB* (Table 3), and the *otsA* mutant was more sensitive to high pressure than its wild-type counterpart (Fig. 2). Since *E. coli* O157:H7 was not exposed to excessive heat or cold temperatures in this study, the changes in the expression of the trehalose synthesis genes suggest that the high-pressure treatment induces osmotic and/or oxidative stress. Lastly, UHP significantly up-regulated *rbsD*, a ribose mutarotase gene, and *glyY*, a glycine tRNA3 gene, by 4.0- and 5.8-fold, respectively. However, the *rbsD* mutant was similar to its wild-type counterpart in barotolerance. The up-regulation of *rbsD* and *glyY* in response to UHP is interesting, but the role of these genes in the resistance of *E. coli* O157:H7 to pressure is not well understood.

Conclusion. Alternative food preservation technologies such as UHP are promising, but the wide variation in resistance among bacterial strains should be addressed to ensure successful implementation of these technologies in the food industry. Ultrahigh pressure had a profound effect on the transcriptional profile of *E. coli* O157:H7. This study is the first to propose that stress-related DNA-binding proteins, protein aggregation, the thiol-disulfide redox system, and the Fe-S cluster status greatly influence the barotolerance of *E. coli* O157:H7. In addition, this is the first report to suggest that the *dinB* operon may be involved in the cell's adaptation to pressure. The importance of both *stpA* and *hns* as well as *nlpI*, *rpoE*, and *otsAB* in high-pressure resistance is proven in this study. Future research in our laboratory will investigate the detailed mechanisms involved in the barotolerance of *E. coli* O157:H7 and other food-borne pathogens.

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