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Sensitivity of *Escherichia coli* O157:H7 to Commercially Available Alkaline Cleaners and Subsequent Resistance to Heat and Sanitizers

Manan Sharma and Larry R. Beuchat*

Center for Food Safety and Department of Food Science and Technology, University of Georgia, Griffin, Georgia 30223-1797

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The effects of seven commercially available alkaline cleaners used in the food processing industry, 0.025 M NaOH, and 0.025 M KOH on viability of wild-type (EDL 933) and *rpoS*-deficient (FRIK 816-3) strains of *Escherichia coli* O157:H7 in logarithmic and stationary phases of growth were determined. Cells were treated at 4 or 23°C for 2, 10, or 30 min. Cleaners 2, 4, 6, and 7, which contained hypochlorite and <11% NaOH and/or KOH (pH 11.2 to 11.7), killed significantly higher numbers of cells than treatment with cleaner 3, containing sodium metasilicate (pH 11.4) and <10% KOH, and cleaner 5, containing ethylene glycol monobutyl ether (pH 10.4). There were no differences in the sensitivities of logarithmic and stationary-phase cells to the alkaline cleaners. Treatment with KOH or NaOH (pH 12.2) was not as effective as four out of seven commercial cleaners in killing *E. coli* O157:H7, indicating that chlorine and other cleaner components have bactericidal activity at high pH. Stationary-phase cells of strain EDL 933 that had been exposed to cleaner 7 at 4 or 23°C and strain FRIK 816-3 exposed to cleaner 7 at 23°C had significantly higher $D_{55°C}$ (decimal reduction time, minutes at 55°C) values than control cells or cells exposed to cleaner 5, indicating that exposure to cleaner 7 confers cross-protection to heat. Cells of EDL 933 treated with cleaner 7 at 12°C showed significantly higher $D_{55°C}$ values than cells of FRIK 816-3, indicating that *rpoS* may play a role in cross-protection. Stationary-phase cells treated with cleaner 5 or cleaner 7 at 4 or 12°C were not cross-protected against subsequent exposure to sanitizers containing quaternary ammonium compounds or sodium hypochlorite, or to cetylpyridinium chloride and benzalkonium chloride.

Exposure of bacterial cells to extreme pHs may result in cross-protection against stress environments that would otherwise be lethal (25). Acid-adapted stationary-phase cells of *Escherichia coli* O157:H7 are more resistant to heat than unadapted cells (3, 27). Leyer and Johnson (15) reported that acid-adapted *Salmonella enterica* serotype Typhimurium cells were more resistant to heat, salt (NaCl), the lactoperoxidase system, crystal violet, and polymyxin B than were unadapted cells. They also observed that outer membrane proteins expressed in acid-adapted cells were different from those expressed in unadapted cells and concluded that a change in the outer membrane proteins may be responsible for increased resistance to environmental stresses. Increased heat tolerance of acid-adapted cells correlates well with the synthesis of heat shock proteins by acid-adapted nonpathogenic *E. coli* (10).

Relatively little is known about the survival and potential for induction of cross-protection of *E. coli* O157:H7 upon exposure to alkaline environments. The pathogen may, however, be exposed to alkaline conditions in a variety of pre- and post-processing and handling environments resulting from the use of alkaline cleaners and sanitizers in food processing plants and the food service industry. Highly alkaline cleaners are used to remove heavy soils, particularly fats and proteins, from food contact surfaces in processing plants, including equipment such as that found in smokehouses and commercial ovens, mechanized or high-pressure systems, and areas which must be cleaned by hand (18). Studies using broth alkalinized with NaOH have shown that some *E. coli* O157:H7 cells are able to survive at pH 12 for up to 3 h and at pH 11 for up to 24 h (20). A nonpathogenic strain of *E. coli* survived for the same treatment time at pHs 11 and 12 but at a lower final population than *E. coli* O157:H7. Although this work was limited in the number of strains examined, an initial observation was that cells of *E. coli* O157:H7 may have greater resistance to alkali than cells of nonpathogenic *E. coli*.

Exposure of *E. coli* to alkaline conditions has been shown to induce synthesis of two heat shock proteins, DnaK and GroE (30). Similarly, cells of *Salmonella enterica* serotype Enteritidis grown in broth at pH 7 and then suspended in broth at pH 9.2 for 5 to 30 min had a $D_{55°C}$ (decimal reduction time, minutes at 55°C) value almost fourfold higher than that of cells not exposed to alkaline pH (11). Thermotolerance was rapidly induced when broth cultures were incubated at 37°C at pH 9.2 for 2 h and was dependent upon protein synthesis (12).

The *rpoS* gene has been reported to play an important role in the survival of *E. coli* and *Salmonella* cells exposed to chemical and physical stresses. *E. coli* O157:H7 cells deficient in the expression of the *rpoS* gene were more susceptible to acidic, osmotic, and heat stresses than were wild-type cells (4, 8). Other research has shown that *rpoS*-deficient *E. coli* survives in much smaller populations than wild-type *E. coli* in gelatin at low water activity (24). The *rpoS* gene may also aid in survival of *E. coli* O157:H7 in high-pH environments, providing cells with a simple mechanism for tolerating alkaline conditions they may encounter in the gastrointestinal system of a host

* Corresponding author. Mailing address: Center for Food Safety and Department of Food Science and Technology, University of Georgia, 1109 Experiment St., Griffin, GA 30223-1797. Phone: (770) 412-4740. Fax: (770) 229-3216. E-mail: lbeuchat@uga.edu.

TABLE 1. Formulation and pH of alkaline cleaners, NaOH, and KOH evaluated for lethality to *E. coli* O157:H7

Cleaner	Trade name	Manufacturer	Active ingredient	Concn of active ingredient	pH[a]
1	ProChlor	ZEP Manufacturing Co., Atlanta, Ga.	Potassium hydroxide Sodium metasilicate Sodium hypochlorite	<10% <10%	11.31
2	FS 4089	ZEP Manufacturing Co.	Potassium hydroxide Sodium hydroxide Sodium hypochlorite	<10% <10% <10%	11.64
3	FS 4490	ZEP Manufacturing Co.	Potassium hydroxide Sodium metasilicate	<10% <10%	11.35
4	K Foam SF Plus	CK Enterprises, Lee's Summit, Mo.	Postassium hydroxide Sodium hypochlorite	11% <5%	11.20
5	K Foam Lo	CK Enterprises	Ethylene glycol monobutyl ether	—[b]	10.41
6	Quorum Yellow	Ecolab, Inc., St. Paul, Minn.	Sodium hydroxide Total available chlorine	10% 2.5%	11.69
7	Enforce	Ecolab, Inc.	Sodium hydroxide Total available chlorine	11% 1.8%	11.71
	NaOH		Sodium hydroxide	0.025 M	12.17
	KOH		Potassium hydroxide	0.025 M	12.20

[a] pH is that of a working concentration of the cleaner solution at 23°C.
[b] —, concentration was not listed on label.

(28). However, studies evaluating the role of *rpoS* in *E. coli* O157:H7 upon exposure to alkaline cleaners and sanitizers commonly used in food processing environments have not been reported.

The objective of this study was to determine the survival characteristics of *E. coli* O157:H7 upon exposure to alkaline cleaners commonly used in food processing plants. Cells surviving exposure to alkaline cleaners were evaluated for changes in thermotolerance and resistance to sanitizers. The *rpoS* gene was examined for its role in protecting cells treated with alkaline cleaners and potential cross-protection of treated cells against subsequent exposure to heat and sanitizers.

MATERIALS AND METHODS

Strains used. Two strains of *E. coli* O157:H7 (EDL 933 and FRIK 816-3) were obtained from Charles Kaspar at the University of Wisconsin, Madison. Strain EDL 933 was isolated from a patient suffering from hemorrhagic colitis associated with the consumption of a hamburger sandwich (33). Strain FRIK 816-3 is an *rpoS*-deficient mutant of EDL 933 (4).

Preparation of cells for treatment with alkaline cleaner solutions. Cells of strains EDL 933 and FRIK 816-3 grown on tryptic soy agar (TSA) (BBL/Difco, Sparks, Md.) and TSA supplemented with 100 μg of ampicillin/ml (TSAA), respectively, were inoculated into 10 ml of tryptic soy broth (TSB) (BBL/Difco) and TSB supplemented with 100 μg of ampicillin/ml (TSBA), respectively. Cultures were incubated at 37°C for 24 h, then inoculated using a loop into 100 ml of TSB or TSBA and incubated at 37°C for 5 h or 24 h to attain logarithmic growth or stationary-phase cells, respectively. Cultures were centrifuged in 50-ml conical centrifuge tubes (VWR International, South Plainfield, N.J.) at 2,000 × *g* in a Centra CL2 centrifuge (International Equipment Company, Needham Heights, Mass.). The supernatant was decanted, and cells were resuspended in 100 ml of sterile 0.05% peptone (BBL/Difco) water. Cell suspensions (2 ml) were deposited in 15-ml conical centrifuge tubes (VWR International) and treated with alkaline cleaners, NaOH, KOH, and 0.05% peptone water (control) as described below.

Cells of *E. coli* O157:H7 strains EDL 933 and FRIK 816-3 in stationary phase were prepared for treatment with alkaline cleaners before exposure to heat treatment as described above, with minor modifications. Only 5 ml of each 24-h culture was centrifuged at 2,000 × *g*, and cells were resuspended in 5 ml of sterile 0.05% peptone water in a 50-ml conical centrifuge tube before treatment with cleaners, followed by treatment with heat.

Stationary-phase cells of EDL 933 and FRIK 816-3 were grown and harvested as described above for treatment with alkaline cleaners before exposure to sanitizer treatments, but also with modifications. Cells (40 ml) were centrifuged at 2,000 × *g* and then resuspended in 40 ml of 0.05% peptone in 50-ml conical centrifuge tubes before treatment with cleaners, followed by treatment with sanitizers.

Preparation of alkaline cleaner and sanitizer solutions. Seven commercially available alkaline cleaners used in the food industry were evaluated for their effectiveness in reducing populations of *E. coli* O157:H7 EDL 933 and FRIK 816-3 (Table 1). Alkaline cleaner treatment solutions were prepared to give either 100% of the working concentration as recommended by manufacturers or 25% of the working concentration when equal volumes of cleaner and cell suspension were combined. Three additional solutions, 0.025 M NaOH, 0.025 M KOH, and 0.05% peptone (control), were also evaluated. All solutions were adjusted to 4 or 23°C before use in treatment of *E. coli* O157:H7 cells.

For experiments involving exposure of cells to alkaline cleaners before heat or sanitizer treatment, cleaners 5 and 7 (Table 1) at 100% concentration and 0.05% peptone (control) at 4, 12, and 23°C were used. Cleaners 5 and 7 were selected for testing because they were shown in initial experiments to have the lowest and highest lethality, respectively, for both strains of *E. coli* O157:H7. Three sanitizers and two antimicrobial ingredients present in some sanitizers were tested. The active quaternary compounds in Quorum Clear were dimethyl benzylammonium chloride (5%) and dimethyl ethylbenzyl ammonium chloride (5%). The active ingredient in Quorum Green was sodium hypochlorite (5.25%). These sanitizers, obtained from Ecolab, Inc. (St. Paul, Minn.), were prepared to give an active ingredient concentration of 200 μg/ml. FS Amine B contains octyldecyl dimethyl ammonium chloride (2.25%), didecyl methyl ammonium chloride (1.35%), dioctyl dimethyl ammonium chloride (0.90%), and alkyl dimethyl benzyl ammonium chloride (3.00%) and was obtained from Zep Manufacturing Co. (Atlanta, Ga.). This sanitizer was prepared to give a total concentration of these ingredients of 150 μg/ml. Stock solutions of cetylpyridinium chloride (10 mg/ml) and benzalkonium chloride (1 mg/ml) (Sigma Chemical Company, St. Louis, Mo.), two antimicrobial compounds present in some alkaline sanitizers, were prepared. Solutions were diluted to yield a test concentration of 100 μg/ml. Sanitizers were prepared in sterile water with a total hardness equivalent to 250 μg of $CaCO_3$/ml. Solutions were adjusted to 4 or 12°C before use. Sterile water with a total hardness of approximately 250 μg of $CaCO_3$/ml was used as a control.

Exposure of *E. coli* O157:H7 cells to alkaline cleaner, hydroxide solutions, and peptone water. Logarithmic and stationary-phase cells of both strains of *E. coli* O157:H7 were exposed to seven alkaline cleaner solutions, NaOH, KOH, and 0.05% peptone at 4 and 23°C, and reductions in populations were determined. Suspensions (2 ml) of cells were combined with 2 ml of cleaner solutions to give either 100 or 25% final concentrations of cleaners, 0.025 M NaOH, 0.025 M KOH, and 0.05% peptone water in a 15-ml conical centrifuge tube and thoroughly mixed. Cells were suspended in each treatment solution for 2, 10, or 30 min while agitating at 150 rpm on an Innova 2000 platform shaker (New Brunswick Scientific Co, Edison, N.J.). Alkaline cleaner and hydroxide solutions were neutralized by adding 6 ml of 2× Dey-Engley (DE) neutralizing broth at pH 6.0 (BBL/Difco); 6 ml of 2× DE broth was also added to the cell suspension in

0.05% peptone water. The pH of treatment suspensions after the addition of 2× DE broth was 7.0 to 8.0.

Thermal treatment of *E. coli* O157:H7 after exposure to alkaline cleaners. Cell suspensions (5 ml) of each strain of a stationary-phase (24-h) culture prepared as described above were combined with 5 ml of cleaner 5 or cleaner 7 to give a concentration of 100% cleaner in the solution or 0.05% peptone and kept at 4, 12, or 23°C for 2 min without agitation. Ten milliliters of sterile 2× DE broth (pH 6.0) were added to the suspension, which was then placed in crushed ice to cool, followed by centrifugation at 2,000 × *g* for 10 min. The supernatant was separated from the cell pellet using a sterile 5-ml pipette. Cells were resuspended in 10 ml of sterile deionized water and centrifuged at 2,000 × *g* for 10 min; the supernatant was again removed using a pipette. Cells were resuspended in 5 ml of 0.05% peptone water and temporarily placed on ice until subjected to thermal treatment. Sterile capillary tubes (Kimble-Kontes, Vineland, N.J.) measuring 0.8 to 1.10 (inside diameter) by 90 mm were sealed at one end. The cell suspension (50 μl) was deposited in each tube using a sterile disposable 1-ml syringe (Becton Dickinson, Franklin Lakes, N.J.) equipped with a sterile deflected-point needle (Popper and Sons, Inc., Hyde Park, N.J.). The open end of each capillary tube was flame sealed, and tubes were placed on crushed ice. Chilled tubes containing cell suspension were adjusted to 23°C and submerged in a water bath (B. Braun, Burlingame, Calif.) at 55°C for 0, 3, 6, 12, 15, 18, 24, 28, or 32 min. The time elapsed between treatment with cleaners and heating cells was 20 to 30 min. At the end of each heating time, tubes were removed from the water bath and immediately placed in crushed ice. Each tube was then immersed in 70% ethanol, rinsed with sterile deionized water, placed in 5 ml of sterile 0.1% peptone water in a 15-ml conical centrifuge tube, and crushed using a sterile glass rod. Cell suspensions were placed on ice for up to 20 min before analysis for populations of *E. coli* O157:H7. At least three replicate experiments were performed for each treatment.

Treatment of *E. coli* O157:H7 with sanitizers after pretreatment to alkaline cleaners. Cell suspension (40 ml) of each strain of stationary-phase (24-h) culture was deposited in a 600-ml sterile beaker (Corning, Inc., Acton, Mass.). Forty milliliters of cleaner 5, cleaner 7, or 0.05% peptone were added to give 100% working concentrations of cleaner solutions and 0.05% peptone, and the mixtures were kept at 4 or 12°C for 2 min without agitation. Eighty milliliters of sterile 2× DE broth (pH 6.0) was added to each mixture, which was then centrifuged at 2,000 × *g* for 10 min. Supernatant was separated from the pellet using a 10-ml pipette, and cells were resuspended in 40 ml of sterile deionized water and centrifuged again at 2,000 × *g* for 10 min. The supernatant was decanted, cells were resuspended in 40 ml of 0.05% peptone, and 5 ml of the suspension was deposited in a 25- by 150-mm glass test tube (Corning, Inc.). Sanitizer solutions or sterile water (control) (5 ml) at 4 and 12°C were combined with the cell suspensions, and the mixture was held for 1 min before neutralization with 10 ml of 2× DE broth. The number of cells surviving in sanitizer and control suspensions was determined.

Microbiological analyses. Populations of *E. coli* O157:H7 in neutralized cleaner and hydroxide solutions, as well as 0.05% peptone water, after treatment for 2, 10, or 30 min at 4 or 23°C were determined by surface plating of undiluted samples (0.25 ml in quadruplicate or 0.1 ml in duplicate) or samples serially diluted in 0.1% peptone water (0.1 ml, in duplicate) on TSA and TSA containing 4% NaCl (TSAS) to determine the presence of injured cells. Plates were incubated at 37°C for 24 to 48 h before colonies were counted.

Populations of *E. coli* O157:H7 surviving treatment with alkaline cleaners at 4, 12, and 23°C for 2 min followed by heating at 55°C were determined by serially diluting suspensions of 0.1% peptone water containing the contents from crushed capillary tubes and surface plating on TSA using the procedure describe above. Plates were incubated at 37°C for 24 h before colonies were counted.

Populations of *E. coli* O157:H7 in surviving sequential treatments with cleaners and sanitizers were determined by surface plating of diluted samples (0.1 ml, in duplicate) on TSA and TSAS using the same procedures described above. Plates were incubated at 37°C for 24 to 48 h before colonies were counted.

Statistical analysis. All experiments were replicated at least three times. Populations of *E. coli* O157:H7 recovered from neutralized cleaners, hydroxide solutions, and peptone water in which cells were treated were subjected to analysis of variance and least-significant-difference tests (SAS Institute, Cary, N.C.) to determine significant differences ($P \leq 0.05$). Populations of *E. coli* O157:H7 surviving treatment with alkaline cleaners followed by heat treatment were plotted on the *y* axis against time (min) on the *x* axis. The linear regression function in SAS software was used to calculate equations for the best-fit lines, and $D_{55°C}$ values were determined for both strains. Populations of *E. coli* O157:H7 surviving treatment with alkaline cleaners followed by treatment with sanitizers were subjected to analysis of variance and least-significant-difference tests. Data presented represent mean values for at least three replicate experiments.

RESULTS AND DISCUSSION

Higher numbers of *E. coli* O157:H7 strain FRIK 816-3 (Table 2) and strain EDL 933 (Table 3) were killed by alkaline cleaners 2, 4, 6, and 7, all containing NaOH or KOH and sodium hypochlorite, than by cleaners that did not contain these ingredients. Treatment with a 100% concentration of cleaners 2, 4, 6, and 7 caused significant reductions ($P \leq 0.05$) compared to treatment with 0.05% peptone (control). These cleaners also killed significantly higher populations than cleaner 3, which contains < 10% KOH and sodium metasilicate, and cleaner 5, which contains ethylene glycol monobutyl ether. Cleaners at 100% concentration killed significantly higher numbers of *E. coli* O157:H7 than did the same cleaners at 25% concentration. Reductions in populations of logarithmic- and stationary-phase cells were similar, indicating that bactericidal activity was largely unaffected by inherent differences in the physiological state presumed to exist in the two types of cells. Reductions in populations of both strains treated at 4 and 23°C were similar. Statistical analysis to determine the combined effects of test factors revealed that reductions in populations of *E. coli* O157:H7 increased with increased time of exposure to alkaline cleaners and were significant for mean values over all treatment temperatures, growth phases, cleaners, and cleaner concentrations.

Death of *E. coli* O157:H7 upon exposure to alkaline cleaners was expected. Exposure of gram-negative bacteria to high pH destroys cell membranes and causes leakage of the internal contents of cells (19). Cell membranes may be disrupted by saponification of lipids or solubilization of proteins. Gram-negative cells are also susceptible to alkaline pH, in part because the thin peptidoglycan layer loses structural integrity and ruptures. Alkaline treatments with high concentrations of carbonate (CO_3^{-2}) and ammonia (NH_3) are effective in killing 6 $\log_{10}$ CFU of *E. coli* O157:H7 and *S. enterica* serotype Typhimurium DT 104 per g of manure over a period of 7 days (22). A study using a library of transposon insertional mutants in *Listeria monocytogenes* identified 12 mutants that showed delayed growth on brain heart infusion agar adjusted to pH 8.5 and brain heart infusion agar containing 5.5% NaCl (9), and several genes were identified that may play a role in alkali and salt tolerance. An increased sensitivity of one of the mutants to alkali was attributed to disruption of the *lmo668* gene; the sequence showed similarity to the *yadH* gene present in some gram-negative bacteria. The function of these genes is not well characterized, but it is thought that they code for membrane permeases that aid in maintaining the pH homeostasis of the cells by active transport of sodium or hydrogen ions across the membrane (9).

Death of *E. coli* O157:H7 caused by chlorinated alkaline cleaners (Tables 2 and 3) is caused by factors in addition to high pH. The 0.025 M NaOH and KOH solutions had a higher pH (12.2) than those of cleaner 2 (11.6), cleaner 4 (11.2), cleaner 6 (11.7), or cleaner 7 (11.7) but in many cases did not cause reductions of populations of cells as effectively as did the chlorinated alkaline cleaners. Higher pHs of treatment solutions did not necessarily correlate with a higher number of *E.*

TABLE 2. Reduction in population of *E. coli* O157:H7 (strain EDL 933) as affected by type and concentration of alkaline cleaner, NaOH, and KOH

Growth phase	Initial population (log_{10} CFU/ml)	Treatment temp (°C)	Treatment[b]	Reduction in population (log_{10} CFU/ml)[a]					
				2 min		10 min		30 min	
				100% concn	25% concn	100% concn	25% concn	100% concn	25% concn
Logarithmic	7.49	4	Control	a 0.06 d	0.06 c	a 0.06 e	0.06 d	+0.05 e	+0.05 c
			Cleaner 1	a 1.97 cd	b 0.04 c	a 6.09 ab	b 0.35 d	a 5.77 bc	b 0.39 c
			Cleaner 2	b 3.64 bc	a 3.83 a	a 6.93 a	b 5.99 a	a 7.08 ab	b 5.65 a
			Cleaner 3	a 1.10 d	b+0.28 c	a 3.66 bcd	a 0.28 d	a 3.82 cd	a 0.47 c
			Cleaner 4	a 4.46 ab	b 0.13 c	a 6.44 a	b 2.02 c	a 6.44 ab	b 2.46 b
			Cleaner 5	a 0.11 d	a 0.04 c	a 1.34 de	a 0.23 d	a 1.35 e	a 0.34 c
			Cleaner 6	a 5.57 ab	b 0.40 c	a 5.52 ab	b 2.89 bc	a 7.49 a	b 3.84 ab
			Cleaner 7	a 6.59 a	b 2.40 b	a 6.36 a	b 4.13 b	a 7.01 ab	b 3.65 b
			NaOH	3.68 bc		4.74 abc		6.44 ab	
			KOH	3.75 bc		2.99 cd		3.78 d	
	7.29	23	Control	a+0.04 e	+0.04 b	a+0.52 e	+0.52 c	+0.63 e	+0.63 c
			Cleaner 1	a 5.11 abc	b 0.89 ab	a 4.72 b	b+0.50 c	a 5.05 abc	b+0.12 c
			Cleaner 2	a 6.92 a	b 2.03 a	a 7.29 a	b 5.41 a	a 6.98 a	b 6.40 a
			Cleaner 3	a 1.22 de	a+0.44 b	a 2.86 cd	b+0.37 c	a 4.86 abc	a+0.33 c
			Cleaner 4	a 6.43 a	b+0.08 b	a 7.29 a	b 1.46 bc	a 6.98 a	b 3.46 b
			Cleaner 5	a 0.32 e	a+0.34 b	a 1.20 de	a+0.16 c	a 2.28 cd	a+0.40 c
			Cleaner 6	a 5.22 abc	b 0.98 ab	a 6.67 a	b 3.81 ab	a 6.98 a	b 4.49 ab
			Cleaner 7	a 5.95 b	b 2.40 a	a 6.91 a	b 3.26 ab	a 5.98 ab	b 4.11 ab
			NaOH	3.50 bcd		3.88 bc		2.41 cd	
			KOH	2.72 cde		3.28 bc		4.26 bc	
Stationary	8.59	4	Control	a+0.02 f	+0.02 a	0.05 c	0.05 c	0.11 c	a 0.11 bc
			Cleaner 1	a 0.30 f	a+0.08 a	a 0.82 c	a 0.07 b	a 1.58 c	a+0.22 c
			Cleaner 2	a 5.69 a	b+0.16 a	a 6.57 a	b 1.37 ab	a 8.04 a	b 1.85 ab
			Cleaner 3	a 0.64 ef	a+0.02 a	a 0.49 c	a 0.18 ab	a 1.57 c	a 0.17 bc
			Cleaner 4	a 3.25 bcd	b 0.27 a	b 0.13 c	a 1.47 a	a 8.59 a	b 1.71 abc
			Cleaner 5	a 0.05 f	a+0.10 a	a 0.21 c	a 0.13 ab	a 0.57 c	a 0.93 abc
			Cleaner 6	a 3.66 bc	b 0.11 a	a 4.34 b	b 0.05 b	a 7.68 a	b 0.41 bc
			Cleaner 7	a 3.99 b	b 0.02 a	a 3.87 b	b 0.42 ab	a 4.99 b	b 2.92 a
			NaOH	2.38 cd		2.98 c		4.16 b	
			KOH	2.03 de		3.96 b		3.92 b	
	8.59	23	Control	a+0.03 d	+0.03 b	+0.01 e	0.01 b	0.10 c	0.10 b
			Cleaner 1	a 0.87 cd	a+0.20 b	a 3.90 bcd	b 0.34 b	a 6.67 a	b+0.36 b
			Cleaner 2	a 4.37 a	b 1.91 a	a 6.81 ab	b 2.44 a	a 8.59 a	b 3.87 a
			Cleaner 3	a 0.35 d	a 0.35 b	a 2.39 de	a+0.17 b	a 3.29 b	a 0.14 b
			Cleaner 4	a 4.03 a	b 0.38 b	a 6.45 abc	b 1.11 ab	a 8.59 a	b 3.95 a
			Cleaner 5	a 0.35 d	b 0.22 b	a 3.33 cd	a+0.03 b	a 0.18 c	b 0.24 b
			Cleaner 6	a 4.12 a	b 0.15 b	a 4.96 abcd	b 0.99 b	a 7.86 a	b 3.71 a
			Cleaner 7	a 2.22 bc	b 1.16 ab	a 7.16 a	b 2.50 a	a 7.22 a	b 5.27 a
			NaOH	3.51 ab		3.60 cd		3.60 b	
			KOH	3.54 ab		2.62 de		4.12 b	

[a] Reduction or increase in population based on the initial population (0 min) in 0.05% peptone solution (control). cells were treated for 2, 10, and 30 min in 0.05% peptone (control), 100 and 25% concentrations of alkaline cleaners, 0.025 M NaOH or 0.025 M KOH. Within growth phase, treatment temperature, treatment time, and treatment concentration, mean values that are not followed by the same letter indicate significant differences caused by treatments ($P \leq 0.05$). Within growth phase, treatment temperature, treatment, and treatment time, mean values that are not preceded by the same letter indicate significant differences ($P \leq 0.05$) caused by treatment concentration.

[b] See Table 1 for description of cleaners.

coli O157:H7 bacteria killed, suggesting that chlorine and other cleaner components contribute to bactericidal activity of some alkaline cleaners. Active chlorine in alkaline cleaners helps to solubilize proteinacious and carbohydrate soils (18). Chlorine reacts with insoluble cross-linked proteins and oxidizes disulfide bonds, making the protein soluble. Chlorine also aids in solubilization of carbohydrate molecules (18). These mechanisms may adversely affect the structure and function of proteins in *E. coli* O157:H7 cells. The apparent bactericidal activity of chlorine in alkaline pH cleaners was unexpected, since its lethality is attributed largely to hypochlorous acid, which is most prevalent in hypochlorite solutions at pH 4 to pH 7. Other lethal mechanisms of chlorine that may not be pH dependent have been proposed, however, and include disruption of protein synthesis, oxidative decarboxylation of amino acids, and induction of lesions in DNA (7, 18). One or more of these mechanisms may be responsible in part for the bactericidal action observed in chlorinated alkaline cleaners examined in this study. Sublethal injury of *E. coli* O157:H7 cells resulting from treatment with 0.5 μg of chlorine/ml has been attributed to a decrease in the ability of cells to uptake nutrients. Membrane potential, respiratory activity, and mem-

TABLE 3. Reduction in population of *E. coli* O157:H7 (strain FRIK 816–3) as affected by type and concentration of alkaline cleaner, NaOH, and KOH

Growth phase	Initial population (log_{10} CFU/ml)	Treatment temp (°C)	Treatment[b]	Reduction in population (log_{10} CFU/ml)[a]					
				2 min		10 min		30 min	
				100% concn	25% concn	100% concn	25% concn	100% concn	25% concn
Logarithmic	7.14	4	Control	0.00 e	0.00 b	+0.27 e	+0.27 c	+0.29 e	+0.29 d
			Cleaner 1	a 3.15 d	b 1.67 a	a 4.00 bc	b 0.56 bc	a 6.42 ab	b 0.93 cd
			Cleaner 2	a 5.83 ab	b 3.12 a	a 5.09 ab	b 3.46 a	a 6.12 abc	b 5.96 a
			Cleaner 3	a 3.27 cd	b 0.03 b	a 5.16 ab	a 0.65 bc	a 3.58 bcd	b+0.30 d
			Cleaner 4	a 6.14 ab	b+0.30 b	a 6.65 a	b 0.20 bc	a 6.73 a	b 0.77 d
			Cleaner 5	a 0.23 e	a+0.05 b	a 1.07 de	a+0.32 c	a 1.57 de	a+0.07 d
			Cleaner 6	a 6.63 a	b+0.29 b	a 6.44 ab	b 1.42 b	a 5.54 abc	b 3.06 bc
			Cleaner 7	a 5.69 abc	b 2.00 a	a 6.44 ab	b 2.95 a	a 7.14 a	b 4.13 ab
			NaOH	3.96 bcd		2.46 cd		3.42 cd	
			KOH	4.70 abcd		5.46 ab		3.48 cd	
	7.29	23	Control	+0.36 d	+0.36 b	+0.54 f	+0.54 e	+0.52 e	+0.52 c
			Cleaner 1	a 2.99 abc	b+0.36 b	a 3.49 cde	b+0.12 cde	a 5.71 a	b 0.16 c
			Cleaner 2	a 4.32 ab	b 1.57 ab	a 6.39 ab	b 4.27 ab	a 7.28 a	b 3.71 ab
			Cleaner 3	a 1.28 abc	a+0.41 b	a 3.95 cd	b+0.21 cde	a 2.72 c	a+0.34 c
			Cleaner 4	a 4.66 a	b+0.09 b	a 7.28 a	b 2.21 bc	a 7.28 a	b 5.68 a
			Cleaner 5	a 0.41 cd	a+0.27 b	a 1.47 ef	a+0.42 de	a 3.21 bc	a 1.90 bc
			Cleaner 6	a 5.28 a	b 1.77 ab	a 6.52 ab	b 2.06 bcd	a 6.35 a	b 3.60 ab
			Cleaner 7	a 4.35 a	b 3.71 a	a 4.63 bc	b 5.09 a	a 5.68 ab	b 4.16 ab
			NaOH	4.45 a		4.56 bc		2.47 cd	
			KOH	2.26 abcd		2.35 de		2.91 c	
Stationary	8.31	4	Control	0.06 cd	0.06 ab	0.43 d	0.43 b	+0.54 g	+0.54 b
			Cleaner 1	a+0.01 cd	a 0.71 a	a 0.79 d	a 0.43 b	a 1.66 cde	b 0.16 b
			Cleaner 2	a 4.53 a	b 0.00 ab	a 5.78 b	b 0.08 b	a 7.89 a	b 0.18 b
			Cleaner 3	a+0.48 d	a 0.02 ab	a 0.35 d	a+0.31 b	a 1.42 def	a+0.33 b
			Cleaner 4	a 3.76 ab	b+0.24 ab	a 4.50 bc	b+0.09 b	a 5.94 b	b 0.61 b
			Cleaner 5	a 0.12 cd	a+0.50 b	a 0.74 d	a+0.18 b	a+0.44 fg	a 0.22 b
			Cleaner 6	a 4.46 a	b+0.15 ab	a 8.31 a	b 0.15 b	a 6.82 ab	b 0.04 b
			Cleaner 7	a 4.07 a	b 0.45 ab	a 4.28 c	b 1.67 a	a 5.87 b	b 2.84 a
			NaOH	1.88 bc		3.39 c		3.23 cd	
			KOH	1.40 cd		3.38 c		3.40 c	
	8.14	23	Control	+0.67 d	+0.67 b	+0.58 e	+0.58 b	+0.38 e	+0.38 c
			Cleaner 1	a 4.65 ab	b+0.31 ab	a 4.39 ab	b+0.37 b	a 4.52 abcd	b 0.27 bc
			Cleaner 2	a 6.23 a	b 0.58 a	a 7.11 a	b 2.94 a	a 6.21 abc	b 4.02 ab
			Cleaner 3	a 0.00 cd	a+0.15 ab	a 1.05 cd	a+0.65 b	a 2.60 de	b+0.36 c
			Cleaner 4	a 3.02 abc	b+0.43 b	a 4.62 ab	b 0.38 b	a 6.89 ab	b 4.22 ab
			Cleaner 5	a+0.34 cd	a+0.46 b	a 0.48 cde	a 0.52 b	a+0.25 e	b+0.41 c
			Cleaner 6	a 4.07 ab	b+0.31 b	a 5.25 ab	b 0.01 b	a 7.23 a	b 4.72 a
			Cleaner 7	a 2.86 abc	b 0.01 ab	a 4.74 ab	b 3.44 a	a 6.73 ab	a 4.75 a
			NaOH	4.76 ab		2.57 bcd		3.25 cd	
			KOH	1.90 bcd		3.10 bc		4.12 bcd	

[a] Reduction or increase in population based on the initial population (0 min) in 0.05% peptone solution (control). cells were treated for 2, 10, and 30 min in 0.05% peptone (control), 100 and 25% concentrations of alkaline cleaners, 0.025 M NaOH or 0.025 M KOH. Within growth phase, treatment temperature, treatment time, and treatment concentration, mean values that are not followed by the same letter indicate significant differences caused by treatments ($P \leq 0.05$). Within growth phase, treatment temperature, treatment, and treatment time, mean values that are not preceded by the same letter indicate significant differences ($P \leq 0.05$) caused by treatment concentration.

[b] See Table 1 for description of cleaners.

brane integrity are also adversely affected by chlorine (16). Greater sensitivity to hypochlorous acid was observed with *rpoS*-deficient *E. coli* cells than with wild-type cells, indicating that the *rpoS* gene may play a role in resistance to hypochlorous acid (7). These observations, coupled with our results, suggest that hypochlorous acid was not the primary bactericidal mechanism associated with chlorine in chlorinated alkaline cleaners in the present work.

Recovery of higher mean populations of *E. coli* O157:H7 strain EDL 933 and strain FRIK816-3 from 190 of 192 (99%) and 188 of 192 (98%) suspensions of treated cells, respectively, representing all combinations of test parameters on TSA compared to TSAS (data not shown), indicates that a portion of the treated cells were injured. However, only 12 and 15%, respectively, of the treated suspensions of strain EDL 933 and strain FRIK 816-3 cells showed significantly higher counts ($P \leq 0.05$) on TSA compared to TSAS. The observations that some of the cells exposed to highly alkaline conditions were sublethally injured is contrary to observations that stationary-phase cells of *E. coli* O157:H7, *S. enterica* serotype Enteritidis, and *L. monocytogenes* exposed to buffered NaOH were not sublethally injured (19). Taormina and Beuchat (31), on the other hand,

TABLE 4. $D_{55°C}$ (min) of *E. coli* O157:H7 as affected by temperature and type of alkaline cleaner[a]

Treatment temp (°C)	Strain	Result with treatment		
		Control	Cleaner 5	Cleaner 7
4	EDL 933	a 16.4 b	a 12.5 b	a 24.1 a
	FRIK 816–3	a 15.6 a	a 12.4 a	a 19.0 a
12	EDL 933	a 22.9 a	a 12.2 b	a 19.7 a
	FRIK 816–3	a 13.9 a	a 11.9 a	b 13.8 a
23	EDL 933	a 14.3 b	a 9.5 b	a 20.8 a
	FRIK 816–3	a 14.7 b	a 9.3 b	a 21.7 a

[a] Mean values in the same row that are not followed by the same letter are significantly different ($P \leq 0.05$); within the same treatment temperature and treatment, mean values not preceded by the same letter are significantly different ($P \leq 0.05$). See Table 1 for description of cleaners.

showed that treatment of logarithmic-growth-phase cells of *L. monocytogenes* at pH 10.0 caused sublethal injury. Our work shows that *rpoS*-deficient cells did not exhibit greater sublethal injury than wild-type cells, indicating that the *rpoS* gene does not play a major role in protecting cells from injury caused by alkaline cleaners.

Cells of *E. coli* O157:H7 strain EDL 933 had significantly higher ($P \leq 0.05$) $D_{55°C}$ values after treatment with cleaner 7 at 4 or 23°C compared to $D_{55°C}$ values of cells that had been treated with 0.05% peptone or cleaner 5 at 4 or 23°C (Table 4). Strain EDL 933 cells treated at 12°C with cleaner 7 or 0.05% peptone had $D_{55°C}$ values that were significantly higher than that of cells treated with cleaner 5. Cells of *E. coli* O157:H7 strain FRIK 816-3 treated with cleaner 7 at 23°C had a significantly higher $D_{55°C}$ value than cells that had been treated with 0.05% peptone or cleaner 5 at 23°C. Cells of FRIK 816-3 did not show significant statistical differences in $D_{55°C}$ values for cells treated with 0.05% peptone, cleaner 5, or cleaner 7 at 4 or 12°C. Treatment of cells with cleaner 7 at 12°C was the only combination of cleaner and temperature at which the $D_{55°C}$ value for wild-type-strain (EDL 933) cells was significantly higher than that for the *rpoS*-deficient *E. coli* O157:H7 strain FRIK 816-3.

Exposure of *E. coli* O157:H7 strain EDL 933 to cleaner 7 caused greater reductions in viable cells than exposure of cells to cleaner 5 or 0.05% peptone. A subpopulation of cells surviving treatment may have been physiologically older and therefore more resistant to heat injury than younger cells. More cells survived treatment with cleaner 5 and 0.05% peptone, but these cells were more sensitive to the effects of heat than were cells that survived treatment with cleaner 7. Treatment of cells with cleaner 7 at 4 and 23°C did not result in a statistically significant difference in $D_{55°C}$ values for the two strains. Cells were held at 23°C and then shifted to 12°C for a 2-min exposure to 0.05% peptone and cleaners. The increased $D_{55°C}$ value for strain EDL 933 over that for strain FRIK 816-3 exposed to cleaner 7 at 12°C may be attributable to a temperature-induced *rpoS*-mediated mechanism (13) that does not occur at 4 or 23°C. The temperature shift may have induced the expression of *otsA* and *otsB* genes, which control synthesis of trehalose in the cell (13). These genes are regulated by the *rpoS* gene, providing a reason for the same phenomenon not occurring in strain FRIK 816-3 at 12°C. Trehalose production is more prevalent when cells are shifted from 37 to 16°C than when shifted from 37°C to 4°C (13), suggesting that increased trehalose synthesis may have occurred when *E. coli* O157:H7 is shifted from 23 to 12°C but not when shifted from 23 to 4°C. Trehalose serves as a molecular chaperone that can protect cells by reducing heat-induced denaturation and aggregation of proteins in the cell.

The higher $D_{55°C}$ values for *E. coli* O157:H7 strain EDL 933 cells treated with cleaner 7 at 4 and 23°C support observations on the increased heat resistance of *S. enterica* serotype Enteritidis cells upon exposure to alkaline conditions (11). Results also support observations made for *L. monocytogenes*, which exhibited higher $D_{56°C}$ and $D_{59°C}$ values after exposure to tryptose phosphate broth at pH 12.0 than did cells treated at pH 7.3 (31). Cells of *Vibrio parahaemolyticus* that were adapted to an environment at pH 9.0 for 2 h showed increased resistance to heat, crystal violet, deoxycholic acid, and hydrogen peroxide (14).

E. coli O157:H7 surviving treatment with alkaline cleaners did not show increased resistance to sanitizers (Table 5). Populations of cells pretreated with cleaner 5 or 0.05% peptone showed greater reductions when subsequently treated with Quorum Green (200 μg/ml) than with Quorum Clear (200 μg/ml) or FS Amine B (150 μg/ml). Treatment with Quorum Green, Quorum Clear, and FS Amine B reduced counts more than treatment with cetylpyridinium chloride (100 μg/ml) and benzalkonium chloride (100 μg/ml), perhaps because they contain higher concentrations of the latter antimicrobials as well as additional bactericidal compounds. Quorum Green was more lethal than Quorum Clear or FS Amine B, indicating that hypochlorite in the sanitizer may be more effective than quaternary compounds in reducing populations of *E. coli* O157:H7 that had been pretreated with alkaline cleaners. Both strains of pretreated *E. coli* O157:H7 behaved similarly to subsequent treatment with a given sanitizer. The temperature at which cells were pretreated with cleaners did not have an effect on the reduction in populations upon treatment with sanitizers. Reductions in the number of cells pretreated with cleaner 7 were observed to be smaller than reductions in the number of cells pretreated with cleaner 5 or 0.05% peptone and then exposed to sanitizers (Table 6), but this does not necessarily indicate that cells pretreated with cleaner 7 exhibited cross-protection against bactericidal activity of sanitizers. Rather, these reductions may be smaller because the population of cells after pretreatment with cleaner 7 was smaller than populations after pretreatment with 0.05% peptone or cleaner 5. Overall, significantly higher populations of strain EDL 933 pretreated with alkaline cleaners and subsequently exposed to sanitizers were recovered on TSA than on TSAS, whereas no significant difference was observed in populations of strain FRIK 816-3 recovered on TSA versus those recovered on TSAS. It is unclear why wild-type cells (strain EDL 933) underwent more sublethal injury than *rpoS*-deficient cells (strain FRIK 816-3).

Lack of cross-protection of *E. coli* O157:H7 against sanitizers after pretreatment with cleaner 7 is not unexpected, because resistance of the pathogen to sanitizers may require exposure to specific sublethal stresses not imposed by this cleaner. The exposure time (2 min) to cleaner treatments may not have been sufficient to allow cells to adapt to these conditions. Zook et al. (34) reported that *E. coli* O157:H7 cells

TABLE 5. Populations and reductions of stationary phase on *E. coli* O157:H7 cells treated with sanitizers as affected by temperature of pretreatment with 0.05% peptone or cleaners 5 and 7

Treatment temp (°C)	Strain	Sanitizer	Population (log_{10} CFU/ml) and reduction after treatment with cleaners and sanitizers[a]					
			Control		Cleaner 5		Cleaner 7	
			Population	Reduction	Population	Reduction	Population	Reduction
4	EDL 933	Control	a 8.38	−0.23 a	a 8.33	−0.23 a	a 4.23	0.25 a
		Quorum Clear	c 2.51	5.64 a	c 3.33	4.78 b	b 0.63	3.84 b
		Quorum Green	d <0.60	8.15 a	d 0.76	7.34 a	b <0.60	3.96 b
		FS amine b	b 5.99	2.16 a	b 5.77	2.33 a	b 1.46	3.01 a
		Cetylpyridinium chloride	b 6.15	1.99 a	b 5.41	2.69 a	b 1.48	2.99 a
		Benzalkonium chloride	a 8.28	−0.13 b	a 8.23	−0.12 b	a 3.87	0.60 a
	FRIK 816–3	Control	a 8.36	−0.62 c	a 8.31	−0.01 a	a 1.79	−0.39 b
		Quorum Clear	d 2.19	5.55 a	c 3.03	5.20 a	b <0.60	2.19 a
		Quorum Green	c <0.60	7.74 a	d <0.60	8.29 a	b <0.60	2.19 b
		FS amine b	bc 6.85	0.89 a	b 6.04	2.26 a	b <0.60	2.19 a
		Cetylpyridinium chloride	c 6.45	1.29 a	b 6.07	2.22 a	b <0.60	1.66 a
		Benzalkonium chloride	a 8.18	−0.44 a	a 8.39	−0.09 a	a 3.39	−0.07 a
12	EDL 933	Control	a 8.28	−0.38 b	a 8.26	−0.38 b	a 3.77	0.02 a
		Quorum Clear	c 3.81	4.11 a	e 2.49	5.38 a	b <0.60	3.28 a
		Quorum Green	d 1.38	6.54 a	f <0.60	7.97 a	b <0.60	3.23 b
		FS amine b	b 5.70	2.21 b	c 5.79	2.04 b	b <0.60	3.56 a
		Cetylpyridinium chloride	b 5.90	2.01 b	d 5.50	2.37 b	b <0.60	3.73 a
		Benzalkonium chloride	a 7.71	0.20 b	b 7.81	0.06 b	a 2.99	0.80 a
	FRIK 816–3	Control	a 8.53	−0.20 a	a 8.38	−0.07 a	a 3.11	0.17 a
		Quorum Clear	c 3.25	5.08 a	c 3.46	4.85 a	b <0.60	3.28 a
		Quorum Green	d <0.60	8.31 a	d <0.60	8.17 a	b <0.60	3.17 b
		FS amine b	b 5.58	2.74 a	d <0.60	3.28 a	b <0.60	2.41 a
		Cetylpyridinium chloride	b 5.30	3.02 a	b 6.03	2.29 a	b <0.60	3.28 a
		Benzalkonium chloride	a 8.33	−0.01 a	a 8.41	−0.01 a	a 3.28	0.00 a

[a] The detection limit was 4 CFU/ml (0.60 log_{10} CFU/ml). Within treatment temperature, strain, and control or cleaner treatment, mean values that are not preceded by the same letter indicate significant differences caused by sanitizer treatment ($P \leq 0.05$). Mean values of reductions in the same row that are not followed by the same letter indicate significant differences ($P \leq 0.05$). Reductions indicate the differences between populations after pretreatment with 0.05% peptone (control) or cleaner and population after treatment with sterile water (control) or sanitizer.

exposed to a low concentration (12 mM) of hydrogen peroxide showed increased survival when subsequently exposed to 80 mM hydrogen peroxide compared to cells that were not exposed to 12 mM hydrogen peroxide. Acid-shocked and acid-adapted cells of *Salmonella* were not more resistant than non-shocked cells to antibiotics (2). The lack of increased sensitivity of *E. coli* O157:H7 to sanitizers after exposure to alkaline cleaners is not in agreement, however, with previous observations that *L. monocytogenes* is sensitized by cleaners to sanitizers and sanitizer components (32).

The *rpoS* gene has been reported to play an important role in the survival of *E. coli* upon exposure to chemical and physical stresses. Nonionic humectants, such as sucrose, glycerol, and lactose, induce expression of the RpoS protein in *S. enterica* serotype Typhimurium (6). *E. coli* O157:H7 mutants deficient in the *rpoS* gene showed no induction of an acid resistance mechanism and much lower levels of other acid resistance systems than wild-type strains (23). *E. coli* O157:H7 may use an *rpoS*-dependent mechanism to respond to acid stress, but the same mechanism is not evident in protecting cells against stress imposed by alkaline cleaners. High cell densities are sufficient to induce the expression of *rpoS* in *E. coli* in the absence of a chemical or nutritional stress (17). The level of RpoS increased eightfold when populations of *E. coli* increased from 8.3 log_{10} CFU/ml to 9.1 log_{10} CFU/ml. In our study, cells of both strains of *E. coli* O157:H7 in logarithmic growth phase may not have been exposed to stress conditions required for the expression of *rpoS*, and this may be a reason FRIK 816-3 and EDL 933 strains behaved similarly when exposed to alkaline cleaners. At a population of 8.59 log_{10} CFU/ml, stationary-phase cells of strain EDL 933 expressed RpoS but possibly not at a high enough level to distinguish it from the *rpoS*-deficient strain. Others have suggested that acid sensitivity of *E. coli* O157:H7 increases with cell density and that *rpoS*-deficient *E. coli* O157:H7 showed less acid sensitivity than wild-type strains at high cell densities (5). This behavior is generally in agreement with our observations on *rpoS*-deficient and wild-type strains of *E. coli* O157:H7 exposed to alkaline cleaner stress. Another possibility for the lack of observed differences between the strains of *E. coli* O157:H7 we tested is that the wild-type strain (EDL 933) may have had an attenuated RpoS function when approaching stationary phase. Loss of the RpoS function in stationary-phase cells could have conferred a growth advantage in stationary phase (21) to *E. coli* O157:H7, a condition which allows cells to scavenge nutrients from other cells in culture. Attenuation of *rpoS* function in *E. coli* cultures is more common in cells with extended doubling times (21). However, in our study, attenuation of the *rpoS* gene in strain EDL 933 is less likely because cultures were grown at 37°C and were not nutritionally limited.

Other mechanisms may enable *E. coli* O157:H7 to survive treatment with highly alkaline cleaners. Several proteins in-

TABLE 6. Populations and reductions ($\log_{10}$ CFU/ml) of *E. coli* O157:H7 on TS as affected by exposure to alkaline cleaner for 2 min before exposure to sanitizers

Treatment temp (°C)	Strain	Treatment[a]	Population ($\log_{10}$ CFU/ml)	
			After treatment[b]	Reduction
4	EDL 933	Control	8.15	0.97
		Cleaner5	8.11	1.01
		Cleaner7	4.47	4.65
	FRIK 816–3	Control	7.74	1.01
		Cleaner5	8.29	0.46
		Cleaner7	3.36	5.39
12	EDL 933	Control	7.92	0.92
		Cleaner5	7.82	1.01
		Cleaner7	3.79	5.05
	FRIK 816–3	Control	8.32	1.14
		Cleaner5	8.31	1.15
		Cleaner7	3.28	6.18

[a] See Table 1 for description of cleaners.
[b] Within treatment temperature and strain, initial populations were the same for all treatments. Cells were exposed to 0.05% peptone (control) or cleaners at 4 or 12°C for 2 min before neutralization with 2× DE broth and enumeration.

volved in the catabolism of maltodextrins, as well as tryptophan, arginine, glutamate, and cysteine, are expressed at high levels in nonpathogenic *E. coli* grown at alkaline pH (29) and may also contribute to the survival of *E. coli* O157:H7 exposed to alkaline cleaners. These proteins generate weak acids inside the cell which lower the internal pH upon exposure to a high external pH. The expression of several genes involved in the catabolism of arginine and glutamate is controlled by *rpoS*, suggesting a potential role for *rpoS* in response to exposure to alkaline conditions (1, 29). Other proteins which play a role in stabilizing disulfide bonds in periplasmic enzymes at extreme alkaline pH may also aid the cell in surviving exposure to alkaline cleaners (29). Exposure of cells of *E. coli* to pHs 8.5 to 9.5 increased their survival at pHs 10 to 11 (26). Whether or not any of these proteins play a role in survival of *E. coli* O157:H7 during exposure to alkaline cleaners is not known. However, if *E. coli* O157:H7 has a resistance mechanism for short-term exposure to alkaline cleaners, it is possible that the responsible effector is not *rpoS*.

In summary, we have shown that the composition and concentration of alkaline cleaners as well as treatment temperature and time are factors that influence lethality to *E. coli* O157:H7. In addition to high pH, chlorine, in combination of sodium hydroxide or potassium hydroxide, contributes to the bactericidal activity of alkaline cleaners. Wild-type cells of *E. coli* O157:H7 that survived treatment with alkaline cleaners containing sodium hydroxide and sodium hypochlorite at 4 and 23°C had increased thermal tolerance compared to cells exposed to 0.05% peptone or a cleaner containing ethylene glycol monobutyl ether. RpoS-deficient cells surviving treatment with cleaner 7 have more thermal resistance than cells surviving treatment with 0.05% peptone or cleaner 5 at 23°C. The *rpoS* gene does not appear to play a role in protecting *E. coli* O157:H7 from bactericidal alkaline cleaners or cells pretreated with alkaline cleaners and subsequently treated with sanitizers or sanitizer components, but it may a play a role in thermal protection of cells that are that are exposed to cleaner 7 at 12°C. Further investigation is needed to determine if cells of *E. coli* O157:H7 exposed to alkaline pH stress gain resistance to other stress conditions commonly encountered in food processing environments.

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