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Inactivation of Shiga Toxin-Producing *Escherichia coli* (STEC) and Degradation and Removal of Cellulose from STEC Surfaces by Using Selected Enzymatic and Chemical Treatments[▽]

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Some Shiga toxin-producing *Escherichia coli* (STEC) strains produce extracellular cellulose, a long polymer of glucose with β-1-4 glycosidic bonds. This study evaluated the efficacies of selected enzymatic and chemical treatments in inactivating STEC and degrading/removing the cellulose on STEC surfaces. Six cellulose-producing STEC strains were treated with cellulase (0.51 to 3.83 U/15 ml), acetic and lactic acids (2 and 4%), as well as an acidic and alkaline sanitizer (manufacturers' recommended concentrations) under appropriate conditions. Following each treatment, residual amounts of cellulose and surviving populations of STEC were determined. Treatments with acetic and lactic acids significantly ($P < 0.05$) reduced the populations of STEC, and those with lactic acid also significantly decreased the amounts of cellulose on STEC. The residual amounts of cellulose on STEC positively correlated to the surviving populations of STEC after the treatments with the organic acids ($r = 0.64$ to 0.94), and the significance of the correlations ranged from 83 to 99%. Treatments with cellulase and the sanitizers both degraded cellulose. However, treatments with cellulase had no influence on the fate of STEC, and those with the sanitizers reduced STEC cell populations to undetectable levels. Thus, the correlations between the residual amounts of cellulose and the surviving populations of STEC caused by these two treatments were not observed. The results suggest that the selected enzymatic and chemical agents degraded and removed the cellulose on STEC surfaces, and the treatments with organic acids and sanitizers also inactivated STEC cells. The amounts of cellulose produced by STEC strains appear to affect their susceptibilities to certain sanitizing treatments.

Shiga toxin-producing *Escherichia coli* (STEC) strains are enteropathogens producing one or more toxins related to the Shiga toxins of *Shigella dysenteriae* serotype 1 (15, 19, 20). The pathogens cause human illness ranging from mild diarrhea to severe hemorrhagic colitis and hemolytic uremic syndrome (3, 11, 16, 21). The reservoirs of STEC are ruminants such as cattle, sheep, goats, etc., but cattle have been identified as the predominant source of STEC (1, 17). STEC infection can be transmitted through contaminated foods, especially raw and undercooked foods of animal origin (6, 17, 18, 33).

Cells of certain STEC strains produce cellulose as an extracellular component (5, 45). The cellulose is a long polymer of glucose, which is insoluble and inelastic, and has a high tensile strength (23, 44). The polymer forms subfibrils and crystallizes into microfibrils (12). The fibrils subsequently build insoluble layered sheets and form hydrogen-bonding networks (23). Cellulose-producing and other bacteria can be entrapped in the networks formed by the cellulose polymers (23, 41).

Cellulose is viscous and hydrophilic and protects bacterial cells from changes in moisture content, acidity, and toxin content in various environments (23). Bacterial cellulose has the capability to hold water to over 100 times its weight due to its structural and hydrophilic properties (23, 27). Williams and Cannon (41) reported that cellulose produced by *Acetobacter xylinus* grown on rotting fruit protected the bacterial cells from the detrimental effect of UV light. Cellulose along with curli, a protein projection on cell surface, has been shown to play an important role in biofilm formation (10, 34) and to protect the cells of *Salmonella* from desiccation and treatment with sodium hypochlorite at a concentration up to 30 ppm (39). Similar results were also observed by Solano et al. (31). The mechanical and chemical protection provided by cellulose to bacteria makes sanitation in the food processing environment a greater challenge. The objectives of this study were to evaluate the efficacies of selected enzymatic and chemical treatments in inactivating STEC and degrading and removing the cellulose on the STEC cell surface and to determine the correlation between the residual amounts of cellulose on and the surviving cell populations of STEC after the enzymatic and chemical treatments.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Six wild-type strains of STEC (all from our laboratory culture collection) were used in the study: 6-8 (O5:H−), 6-35 (O103:H2), 7-17 (O26:H11), 7-49 (O103:H2), 7-50 (O103:H2), and 7-51 (O103:H2). These strains are of human origin, and in addition to Shiga toxin production, they also carry other virulence genes, such as *eaeA* and *hlyA* (data not shown). Cultures of the STEC strains were grown on Luria-Bertani no-salt (LBNS) agar (10 g tryptone, 5 g yeast extract, 15 g agar per liter) at 28°C for 72 h. The resulting cultures were used in the following experiments.

Enzymatic treatment. Cellulase of *Aspergillus niger* (5 g; 1.02 U/mg [Sigma-Aldrich, Inc., St. Louis, MO]) was dissolved in 10 ml of sterile distilled water. The enzyme solution was dialyzed in sterile distilled water at refrigeration temperature for 18 h. The concentration of the dialyzed cellulase solution was determined as 6.25%. The STEC cultures on the surface of LBNS agar plates were treated with 15 ml of 0.05 M sodium acetate buffer (pH 5.0) containing different

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concentrations of cellulase (0.51, 2.12, or 3.83 U/15 ml). STEC cultures treated with 15 ml of sodium acetate buffer without cellulase were included in the study as controls. The treatments were conducted at 37°C for 2 h with gentle shaking on a platform shaker.

Acid treatments. Acetic acid (JT Baker, Phillipsburg, NJ) and lactic acid (Purac America, Inc., Lincolnshire, IL) were used in the study. Each of the STEC cultures on the surface of LBNS agar plates was treated with 15 ml of 2 or 4% acetic and lactic acid, respectively. STEC cultures treated with 15 ml of sterile distilled water were used as controls. The treatments were conducted at room temperature for 20 min with gentle shaking on a platform shaker.

Sanitizing treatments. Two commercial sanitizers were used to treat the STEC cells with cellulose. One of the sanitizers was alkaline (pH 13; Ecolab, St. Paul, MN), while the other one was acidic (pH 1.0 to 1.5; ZEP Manufacturing Company, Atlanta, GA). The active ingredients in the alkaline sanitizer included potassium hydroxide, phosphoric acid, and potassium hypochlorite, while the acidic sanitizer contained phosphoric acid as the active component. The working solutions of the sanitizers were prepared according to the manufacturers' recommendations. The alkaline sanitizer was diluted by mixing 10 ml of the commercial product with 368 ml of sterile water. The working solution of the acidic sanitizer was prepared by diluting the commercial product 4-fold with sterile water. Cells of the STEC cultures were treated with 15 ml of the diluted sanitizer solutions at room temperature for 7 and 15 min, respectively, with gentle shaking. STEC cultures treated with the same volume of sterile distilled water were used as controls.

Quantification of cellulose. After each treatment, 10 ml each of the treated and untreated control samples was collected for cellulose quantification using a colorimetric method developed by Updegraff (35) with modifications. The STEC cultures were placed in glass centrifuge tubes (17 by 118 mm) with conical bottoms. The cultures were centrifuged at 3,400 *g* for 25 min. The cell pellet of each culture was collected after the supernatant fluids were discarded. Three milliliters of an acetic-nitric reagent (150 ml 80% acetic acid and 15 ml concentrated nitric acid) was added to the cell pellet of each culture in the glass centrifuge tube and mixed by vortexing. The test tubes were covered with aluminum foil and placed in a boiling water bath for 30 min, after which the contents in the test tubes were recentrifuged at 3,400 × *g* for 15 min. Following centrifugation, the supernatants were discarded, and the pellets were washed twice with sterile distilled water. One milliliter of 67% sulfuric acid was then added to each centrifuge tube and allowed to stand for 1 h at room temperature. The acid solution in each centrifuge tube, sitting in an ice bath, was diluted with 4 ml of distilled water followed by addition of 10 ml of refrigerated anthrone reagent (0.2 g anthrone in 100 ml concentrated H_2SO_4) (Acros Organics, New Jersey, NJ). The centrifuge tubes were inverted gently, placed in a boiling water bath for 16 min, and then cooled rapidly in the ice bath. The absorbance of each sample at 620 nm (A_{620}) was recorded using the Novaspec II spectrophotometer (Pharmacia Biotech, Cambridge, United Kingdom). A standard curve of absorbance as a function of the cellulose (Sigma-Aldrich, Inc.) concentration was prepared. The quantities of cellulose remaining on the surface of STEC cells were calculated by comparing the absorbance values of the standard with the values of the tested samples. All reagents used in the quantification of cellulose were purchased from Fisher Scientific unless otherwise specified.

Inaction of STEC after the treatments. Immediately after the treatments described above, 1 ml of each treated STEC cell suspension and untreated control was mixed with 9 ml of double-strength Dey-Engley (DE) buffer following a previously reported protocol (28). The cell suspensions were left in the DE buffer at room temperature for 15 min before serial dilutions were made in 0.1% buffered peptone water. The last three dilutions of each cell suspension (0.1 ml) or the undiluted samples (0.5 ml) were plated in duplicate on tryptic soy agar (TSA) plates. The colonies on the surface of TSA plates were enumerated after 24 h of incubation at 37°C. The detection limit was therefore 2 CFU/ml or 0.30 log CFU/ml.

Statistical analysis. All samples were tested in duplicate and had appropriate controls. Each experiment was repeated in three independent trials. The data obtained were analyzed using the Student *t* test and general linear model of the Statistical Analysis Software (SAS) (26) at a 95% confidence level. Significant differences in the residual amounts of cellulose on STEC reflecting the efficacies of cellulose degradation and reduction achieved by different treatments were calculated by comparing the mean absorbance levels of the anthrone solutions at 620 nm. The influence of the treatments on the survival of the STEC was also determined using the same statistical protocol. Correlation coefficients between residual amounts of cellulose on STEC and surviving STEC populations after each treatment were calculated using the JMP software (25).

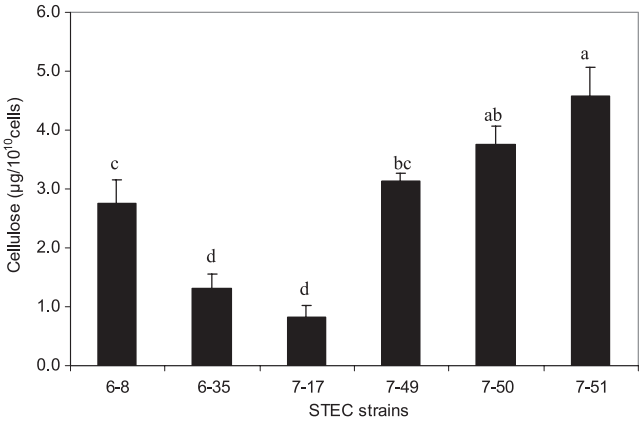

FIG. 1. Cellulose production by STEC strains on LBNS agar. The amounts of cellulose produced by STEC were measured using a colorimetric assay and presented as the absorbance values of anthrone solutions at 620 nm. Bars not labeled at the top with the same letters are significantly different in terms of the amounts of cellulose produced by different STEC strains ($P < 0.05$).

RESULTS

Quantities of cellulose expressed by STEC. The six wild-type STEC strains used in the study expressed different amounts of cellulose on their surfaces. The amounts of cellulose on 6-8, 7-49, 7-50, and 7-51 were significantly ($P < 0.05$) larger than the amount of cellulose produced by 7-17 (Fig. 1). Cells of strain 6-35 produced an average of 1.32 μg cellulose per 10^{10} cells, which was significantly ($P < 0.05$) smaller than the amounts of cellulose produced by the cells of strains 6-8, 7-49, 7-50, and 7-51 (Fig. 1). Cells of 7-51, the greatest cellulose producer among strains evaluated in this study, produced approximately 5.58 times more cellulose than did the cells of 7-17 (Fig. 1).

Treatments with cellulase. Treatments with cellulase significantly reduced ($P < 0.05$) the average amounts of cellulose on the six STEC strains (Table 1). The efficacy of the treatments increased as the concentration of cellulase increased (Fig. 2). The amounts of cellulose on strain 7-51 were reduced by 4.38, 4.94, and 5.09 μg per 10^{10} cells, respectively, when the cells were treated with 0.50, 2.16, or 3.83 U/15 ml of cellulase at 37°C for 2 h. The reductions in the average amounts of cellulose on 7-49 were relatively smaller—0.39 and 2.10 μg per 10^{10} cells, respectively—by the treatments with 0.50 or 2.16 U/15 ml of cellulase and 2.44 μg per 10^{10} cells by the treatments with 3.83 U/15 ml of cellulase (Fig. 2). Furthermore, treatments with 2.16 and 3.83 U/15 ml of cellulase reduced the amount of cellulose on 7-50 by 3.00 and 3.09 μg per 10^{10} cells, respectively. A similar declining trend was also observed with the amounts of cellulose on 6-8 (Fig. 2). Treatments with cellulase did not have any influence on the survival of STEC cells (Table 2).

Treatments with organic acids. Treatments with 2 or 4% acetic and lactic acid significantly ($P < 0.05$) reduced the average populations of the six STEC strains (Table 1) as well as the populations of individual STEC strains used in the study, except for 6-35 and 7-51 when treated with 2% acetic acid (Table 2). Treatments with 4% acetic acid and both concen-

TABLE 1. Average residual amounts of cellulose on and populations of the STEC strains ($n = 12$) after treatments with selected enzymatic and chemical agents

Treatment	Avg residual amt of cellulose ($\mu g/10^{10}$ cells)[a]	Avg surviving cell population (log CFU/ml)[a]
Cellulase (37°C, 2 h)		
Untreated control	2.83 A	8.80 A
0.50 U/15 ml	1.63 B	8.74 A
2.16 U/15 ml	0.40 C	—[b]
3.83 U/15 ml	0.37 C	8.67 A
Organic acids (28°C, 20 min)		
Untreated control	2.72 A	8.80 A
Acetic acid		
2%	2.11 AB	7.67 B
4%	1.62 BC	6.82 B
Lactic acid		
2%	1.65 BC	5.01 C
4%	0.99 C	3.70 D
Commercial detergents (28°C, 7 or 15 min)[c]		
Untreated control	2.72 A	8.80 A
Alkaline		
7 min	1.87 AB	ND[d] B
15 min	1.10 BC	ND B
Acidic		
7 min	1.37 BC	ND B
15 min	0.71 C	ND B

[a] Values that are not followed by the same letters within the same treatment category (cellulase, organic acids, or commercial sanitizers) are significantly different ($P < 0.05$).
[b] —, not determined.
[c] The manufacturers' recommended concentrations were used.
[d] ND, not detectable because mean values were below the detection limit (<0.30 log CFU/ml).

trations of lactic acid significantly reduced ($P < 0.05$) the average amounts of cellulose on the six STEC strains (Table 1). The average amounts of cellulose on the six STEC strains were reduced by 0.61 and 1.07 μg per 10^{10} cells, respectively, by the treatments with 2% acetic or lactic acid and by 1.10 and 1.73 μg per 10^{10} cells, respectively, by the treatments with 4% acetic or lactic acid (Table 1).

The amounts of cellulose on strain 7-51 were reduced by 2.36 and 3.00 μg per 10^{10} cells, respectively, by the treatments with 2% acetic or lactic acid (Fig. 3A and B). Treatments with 2% acetic acid reduced the average amounts of cellulose on 7-49 and 7-50 by 0.46 and 0.82 μg per 10^{10} cells, respectively (Fig. 3A), while the treatments with the same concentration of lactic acid reduced the average amounts of cellulose on 7-49 and 7-50 by 0.69 or 1.67 μg per 10^{10} cells (Fig. 3B). Treatments with 2% acetic or lactic acid reduced the amounts of cellulose on 6-8 by 0.21 or 0.83 μg per 10^{10} cells (Fig. 3A and B). The amounts of cellulose on 6-35 were reduced by 0.17 and 0.58 μg per 10^{10} cells, respectively, by the treatments with 2% acetic or lactic acid (Fig. 3A and B). The amounts of cellulose on 7-17 were reduced to 0.24 and 0.22 μg per 10^{10} cells by the treatments with 2% acid and lactic acid, respectively (Fig. 3A and B). Increasing the concentration of acetic and lactic acid from 2 to 4% further reduced the amounts of cellulose on the six individual STEC strains used in the study (Fig. 3A and B).

Treatments with sanitizers. Treatments with the two sanitizers reduced the average cell populations of the six STEC strains to undetectable levels (Table 1 and 2). The average amounts of cellulose on the six STEC strains were reduced by 0.85 and 1.35 μg per 10^{10} cells, respectively, after the cells were treated with the acidic or alkaline sanitizers at room temperature for 7 min (Table 1). When the treatments were extended to 15 min, the average amounts of cellulose on the six STEC strains were reduced by 1.62 and 2.01 μg per 10^{10} cells, respectively (Table 1).

Treatments with the alkaline sanitizer at room temperature for 7 min reduced the amounts of cellulose on strains 7-49 and 7-50 by 0.29 and 1.00 μg per 10^{10} cells, respectively. The treatments also reduced the average amounts of cellulose on 6-8, 6-35, and 7-51 by 0.62, 0.36, or 2.70 μg per 10^{10} cells (Fig. 4A). The amounts of cellulose on five out of the six STEC strains were below 1.0 μg per 10^{10} cells when the cells were treated with the acidic sanitizer for 15 min (Fig. 4B). Treatments with acidic sanitizer for 7 min reduced the average amounts of cellulose on 7-49 and 7-50 by 2.16 and 2.25 μg per 10^{10} cells, respectively. After the same treatment, the amounts of cellulose on 6-8, 6-35, and 7-51 were reduced by 1.49, 0.39, and 1.61 μg per 10^{10} cells, respectively (Fig. 4B). Extending the treatment time from 7 to 15 min with the acidic and alkaline sanitizers did not significantly improve ($P > 0.05$) the efficacy of cellulose degradation (Table 1).

Correlation between residual amounts of cellulose on and the surviving cell populations of STEC. As stated previously, treatments with cellulase and commercial detergents both degraded the cellulose on STEC. However, treatments with cellulase had no influence on the survival of STEC cells, and those with the commercial sanitizers decreased the populations of STEC cells to undetectable levels (Table 2). Correlations between the residual amounts of cellulose and surviving cell populations of STEC that resulted from these treatments were, therefore, not observed. However, STEC treated with acetic and lactic acid had residual amounts of cellulose that positively correlated with the surviving cell populations of individual STEC strains: the correlation coefficients (r) between the two parameters were 0.64 to 0.94, with confidence levels ranging from 83 to 99% (Table 3). The correlation coefficients (r)

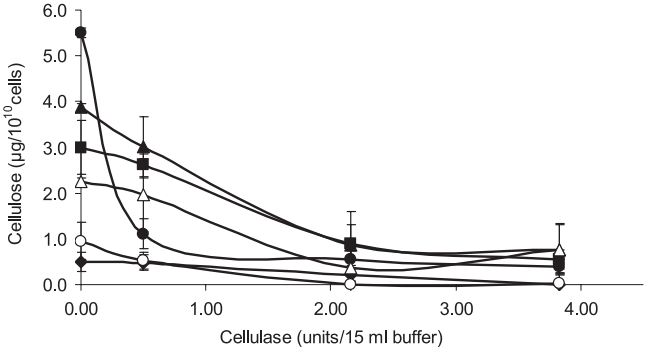

FIG. 2. Enzymatic hydrolysis of cellulose on STEC. STEC cells on LBNS agar were treated with 0.5, 2.16, and 3.83 U of cellulase per 15 ml of sodium acetate buffer for 2 h at 37°C, and the cellulose remaining on STEC surfaces after the treatments was measured using a colorimetric assay. ◆, 7-17; ■, 7-49; ▲, 7-50; ●, 7-51; △, 6-8; ○, 6-35.

TABLE 2. Cell populations of individual STEC strains after treatments with cellulase, selected organic acids, and commercial sanitizers

Treatment	Cell population (log CFU/ml) of STEC strain[a]:					
	6-8	6-35	7-17	7-49	7-50	7-51
Untreated control	9.00 aAB	9.09aA	8.77 aAB	9.07 aA	9.09 aA	8.83 aAB
Cellulase (37°C, 2 h)						
0.51 U/15 ml	8.62 aA	8.47 aA	8.69 aA	9.07 aA	9.09 aA	8.50 aA
3.83 U/15 ml	8.65 aABC	8.97 aA	8.55 aBC	8.90 abAB	8.61 abABC	8.34 aC
Organic acids (28°C, 20 min)						
Acetic acid						
2%	7.49 bA	8.59 aA	5.70 bB	7.61 bcA	8.27 bA	8.34 aA
4%	7.09 bA	6.63 bB	5.94 bC	6.97 cAB	7.17 bA	7.12 bA
Lactic acid						
2%	5.20 cA	5.70 bA	0.85 cB	5.28 dA	6.74 cA	6.75 bA
4%	4.10 dB	4.00 cB	0.73 cC	4.23 dAB	4.59 dA	4.57 cA
Commercial detergents (28°C, 7 or 15 min)						
Alkaline						
7 min	ND[b] eA	ND dA	ND cA	ND eA	ND eA	ND dA
15 min	ND eA	ND dA	ND cA	ND eA	ND eA	ND dA
Acidic						
7 min	ND eA	ND dA	ND cA	ND eA	ND eA	ND dA
15 min	ND eA	ND dA	ND cA	ND eA	ND eA	ND dA

[a] Means in the same column not followed by the same lowercase letters are significantly different in terms of STEC strains ($P < 0.05$). Means in the same row not followed by the same uppercase letters are significantly different in terms of treatments ($P < 0.05$).
[b] ND, not detectable because mean values were below the detection limit (<0.30 log CFU/ml).

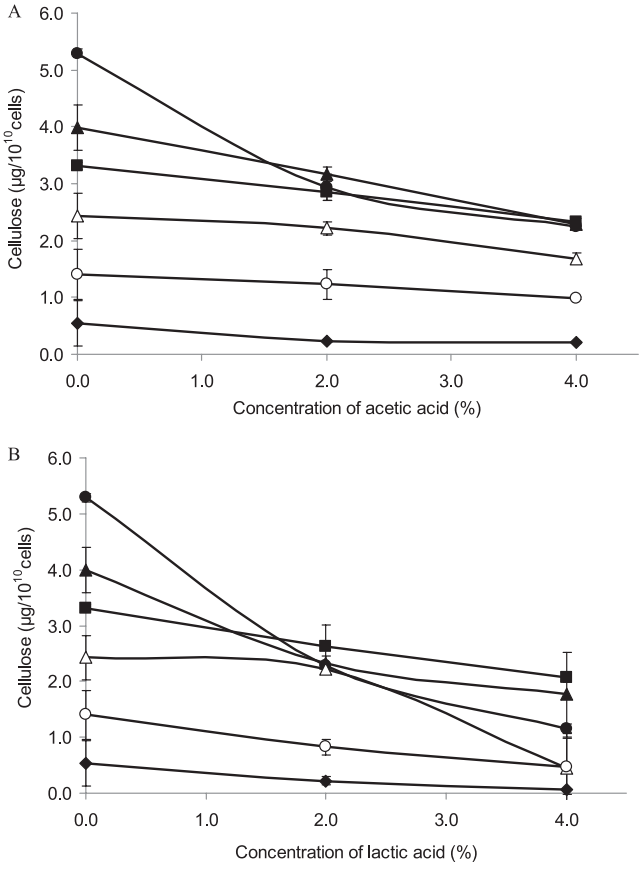

FIG. 3. Removal of cellulose on STEC using treatments with organic acids. Cultures of STEC on LBNS agar were treated with 2 and 4% acetic acid (A) or lactic acid (B) solutions for 20 min at 28°C, and the residual amounts of cellulose on STEC surfaces were measured using a colorimetric assay. ◆, 7-17; ■, 7-49; ▲, 7-50; ●, 7-51; △, 6-8; ○, 6-35.

between the residual amounts of cellulose on and the surviving cell populations of strains 6-8, 7-50, and 7-51 were 0.85 to 0.94 with 97 to 99% confidence levels (Table 3). The confidence levels of correlations between the residual amounts of cellulose on and the surviving cell populations of 6-35 and 7-49 were also in the range of 97 to 99%, while the confidence level of correlation was 94% for 7-17 under the treatment with lactic acid. The significance values of correlations between the residual amounts of cellulose on and surviving cell populations of 6-35, 7-17, and 7-49 under the treatments with acetic acid were 83 to 90% (Table 3).

DISCUSSION

Cellulose is a long polymeric chain of D-glucose monomers which are linearly linked together by β-(1-4) glycosidic bonds (23). The glycosidic bond is generated by the reaction between the hydroxyl group on a glucose molecule and the hemiacetal goup of another glucose molecule accompanying the water loss (37). According to Steinbüchel et al. (32), bacterial cellulose is relatively purer than plant cellulose, which is often associated with hemicelluloses, lignin, and waxy materials. Previous studies have shown that bacterial cellulose produced by *A. xylinum* was hydrolyzed by cellulase from *Trichoderma* and chemical agents such as sodium hydroxide and hydrochloric acid (13, 24, 29, 30). The present study evaluated the effectiveness of selected enzymatic and chemical treatments that have been or have the potential to be used by the food industry to control cellulose-producing bacterial pathogens such as STEC.

Cellulase is a cellulolytic enzyme which breaks down cellulose to glucose units by acting on the glycosidic bonds. The enzyme is produced by several fungi, including *Trichoderma* and *Aspergillus*, as well as cellulolytic bacteria such as *Sporocytophga myxococcoides*, *Clostridium*, and *Acetivibrio* (42).

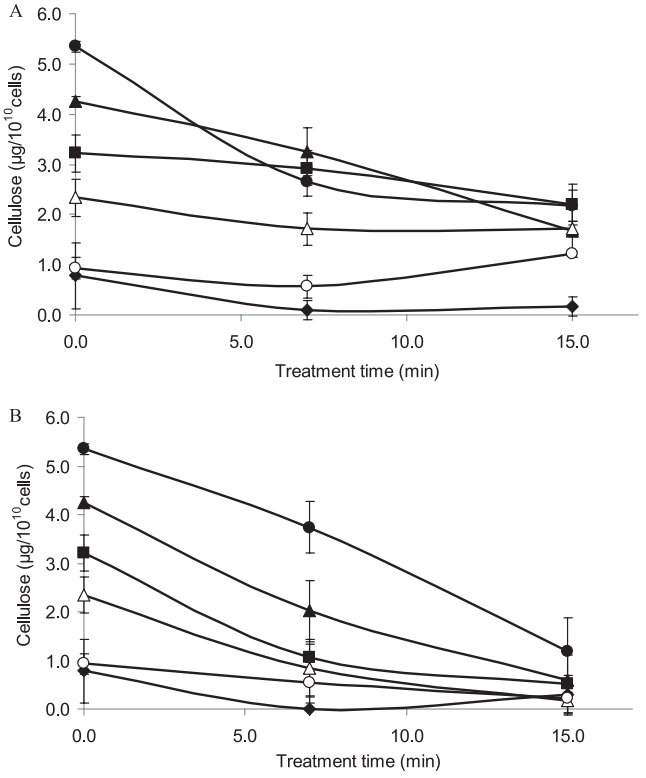

FIG. 4. Degradation of cellulose on STEC using treatments with commercial sanitizers. Cultures of STEC on LBNS agar were treated with a commercial alkaline sanitizer (A) or acidic sanitizer (B) for different lengths of time. The manufacturers' recommended concentrations were used. ◆, 7-17; ■, 7-49; ▲, 7-50; ●, 7-51; △, 6-8; ○, 6-35.

There are three different classes of cellulose hydrolytic enzymes, including endo-1,4-β-D-glucanase (endo-cellulase), exo-1,4-β-D-glucanase (exo-cellulase), and β-glucosidase based on the modes of chemical reactions (2). The cellulase used in the present study was an endo-cellulase from *A. niger*, which randomly hydrolyzes the chains of cellulose, reducing the length of cellulose chain or the degree of polymerization and releasing cellobiose and glucose (2, 4, 9). Hurst et al. (8) reported that purified cellulase from *A. niger* (0.25 μg) could hydrolyze 5 mg of cellulose to reducing sugars in 0.1 M sodium acetate buffer (pH 4.0) at 40°C in 60 min. In the present study, treatment with the cellulase of *A. niger* significantly reduced the amounts of cellulose on STEC (Table 1). The efficacies of the treatment increased as the concentration of cellulase increased (Fig. 2).

Strong inorganic acids, such as sulfuric, phosphoric, and hydrochloric acids, can break the β-(1-4) glycosidic bonds in cellulose polymers (43). The acid hydrolysis is initiated with the interaction between a proton from the acid and the glycosidic oxygen, forming a conjugate acid. The C-O bond is then cleaved, followed by the breakdown of the conjugate acid. Subsequent addition of water results in the release of a free sugar and a proton (22, 43). Acetic and lactic acids are weak organic acids, and their effects on degrading the cellulose polymer are limited. The reduction of cellulose on STEC by acetic and lactic acid treatments observed in this study could be the results of physical removal of cellulose from STEC cell surfaces. However, it has been stated that acid hydrolysis of cellulose is influenced by several factors, including but not limited to the type and location of glycosidic bonds in cellulose molecules (7). The possibility of hydrolyzing the glycosidic bonds at the end of the molecule is high, due to increased flexibility. Furthermore, acid breakdown of cellulose involves two stages, and the first step is to remove the amorphous regions as this portion of the cellulose polymer is readily accessible by acid. It is not clear, though whether the glycosidic bonds at the end and the amorphous regions of cellulose molecules are susceptible to treatments by weak organic acids such acetic and lactic acid.

The alkaline sanitizer used in the present study contained a mixture of potassium hydroxide and potassium hypochlorite, and the treatments with the sanitizer either significantly or numerically reduced the amounts of cellulose on the STEC strains (Table 1). Under alkaline conditions, chemical isomerization occurs at the reducing end of the cellulose molecule, which causes the carbonyl groups in the cellulose molecules to move along the carbon chain (36). Cellulose depolymerization subsequently occurs through a peeling-off reaction, and glucose units in the cellulose molecule are released one by one (14, 35). Shibazaki et al. (30) reported that treatment with 18% NaOH for 60 min at 22°C was effective in reducing the crystallite size of bacterial cellulose from 75 to 25 nm. Phosphoric acid is the active component in the acidic sanitizer. The phosphoric acid has been used for cellulose depolymerization or decrystallization (38, 40, 46). Under the treatment with phos-

TABLE 3. Correlations between the residual amounts of cellulose on and the surviving cell populations of STEC treated with acetic or lactic acid for 20 min at 28°C using the linear least-squares regression and linear models ($n = 6$)

STEC strain	Result from treatments for 20 min at 28°C with[a]:					
	Acetic acid (2 and 4%)			Lactic acid (2 and 4%)		
	Linear model	Correlation coefficient (r)	P value	Linear model	Correlation coefficient (r)	P value
6-8	$y = 2.39x + 2.88$	0.88	0.02	$y = 2.17x + 2.87$	0.90	0.02
6-35	$y = 2.72x + 4.74$	0.72	0.10	$y = 3.57x + 3.50$	0.91	0.01
7-17	$y = 3.97x + 5.18$	0.64	0.17	$y = 9.30x + 0.46$	0.79	0.06
7-49	$y = 1.85x + 2.34$	0.68	0.14	$y = 2.31x + 0.15$	0.85	0.03
7-50	$y = 0.78x + 5.55$	0.94	0.01	$y = 1.22x + 3.30$	0.93	0.01
7-51	$y = 0.44x + 6.57$	0.85	0.03	$y = 0.85x + 4.42$	0.88	0.02

[a] The linear models explain the relationships between the residual amounts of cellulose (x) and the surviving cell populations of individual STEC strains (y). The P values indicate the significance of the correlations. $P < 0.05$ indicates that r exceeded the critical value with 95% confidence.

phoric acid (>80%), cellulose swells (38), depolymerizes (38), and then become solubilized.

Linear model regression analysis performed in the study revealed a positive correlation between the residual amounts of cellulose on and the surviving cell populations of individual STEC strains (Table 3). When smaller amounts of cellulose were on their surface, STEC cells were more easily inactivated by the treatments with the two organic acids. As a result, relatively lower numbers of STEC cells survived the treatment process. On the contrary, higher numbers of STEC survivors were recovered from the cells with relatively greater amounts of cellulose on their surface after the treatments with the two organic acids.

In conclusion, treatments with enzymatic and certain chemical agents used in the present study were effective in inactivating STEC and degrading and removing the cellulose on STEC surface. Positive correlations were observed between the residual amounts of cellulose on and surviving cell populations of STEC following the treatments with acetic and lactic acids. STEC strains producing less cellulose appear to be more vulnerable to certain sanitizing treatments.

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