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Short Communication

Synergistic Effects of Lactic Acid and Sodium Dodecyl Sulfate to Decontaminate *Escherichia coli* O157:H7 on Cattle Hide Sections

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Abstract

The objective of this study was to investigate the antibacterial properties of chitosan acetate (CA), sodium dodecyl sulfate (SDS), lactic acid (LA) and their synergism when combined against a nontoxigenic strain of *Escherichia coli* O157:H7. Treatments that significantly reduced the concentration of *E. coli* O157:H7 *in vitro* by more than two logs were further investigated using a cattle hide decontamination model. *In vitro* treatments included CA (1% chitosan in 1% acetic acid vol/vol), SDS (1% vol/vol), SDS (2% vol/vol), LA (1% vol/vol), CA-SDS combination (1% chitosan in 1% acetic acid vol/vol mixed with 1% SDS vol/vol), and LA-SDS combination in two different concentrations (1% LA mixed with 1% SDS vol/vol, and 1% LA mixed with 2% SDS vol/vol). Butterfield's Phosphate Buffer water was used as a control. The antibacterial effect of 1% CA solution alone and in combination with 1% SDS *in vitro* resulted in a 1.8 and 1.7 log colony-forming units (CFU)/mL reduction, respectively ($p < 0.05$). Only 1% LA, 1% SDS, 2% SDS and their combinations resulted in a >2 log reduction in *E. coli* O157:H7. On hide sections, both 1% LA–1% SDS and 1% LA–2% SDS combinations significantly ($p < 0.05$) reduced *E. coli* O157:H7 concentration by 4.6 and 4.7 log CFU/ cm^2 greater than the control, respectively. There was no significant difference in the antibacterial effect of 1% LA compared to the control, 2% SDS compared to the control, or 1% LA compared to 2% SDS. Hence, the antibacterial efficacy of 1% LA against *E. coli* O157:H7 on hide sections was significantly enhanced when combined with 1% SDS. Results of this study support the use of low concentration LA-SDS combination as a hide wash to reduce the risk of *E. coli* O157:H7 contamination.

Introduction

Escherichia coli O157:H7 is often incriminated in foodborne outbreaks associated with beef consumption (Vogt and Dippold, 2005; Currie et al., 2007). Ruminants are reservoirs of *E. coli* O157:H7, and carcass contamination may occur through direct contact or aerosols during removal of hides contaminated with feces containing *E. coli* O157:H7 (Byelashov and Sofos, 2009).

Antibacterial properties of natural compounds such as chitosan acetate (CA) have been studied (Friedman and Juneja, 2010). The effect of chitosan on *E. coli* O157:H7 on hides has not been studied. Similarly, the food additive sodium dodecyl sulfate (SDS) enhances the antibacterial properties of organic acids (Zhao *et al.*, 2009). The objective of this study was to investigate the antibacterial properties of CA, SDS, and lactic acid (LA) against *E. coli* O157:H7 *in vitro* and on inoculated cow-hide sections.

Materials and Methods

Inoculum preparation

A frozen stock culture of *E. coli* O157:H7 (ATCC **43888)** stored at −80°C was transferred onto sheep blood agar and incubated for 18 h at 37°C three consecutive times. The culture was stored at 4°C and subcultured twice on sheep blood agar before each experiment. After the second pass, two colonies were transferred to 25 mL of tryptic soy broth (TSB) and incubated for 18 h at 37°C. Concentration of the pure culture was then adjusted to 10^8 CFU/mL by dilution.

Reagent preparation

Eight treatments were evaluated *in vitro*: (1) 1% CA (chitosan in 1% acetic acid vol/vol, pH 3.7); (2) combination of 1% CA and 1% SDS vol/vol (pH 4.2); (3) 1% SDS (pH 3.2); (4) 2% SDS (pH 3.2); (5) 1% lactic acid (pH 2.7); (6) combination of 1%

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Table 1. Post-treatment Concentration of *Escherichia coli* O157:H7 (ATCC 43888) *in vitro* and on Experimentally Inoculated Cow Hide Sections

		In vitro *experiment*		*Hide decontamination model*	
Treatment type	*pH*	*Mean[a] survival (log CFU/mL) (Standard deviation)*	*Mean killed (log CFU/mL)*	*Mean[a] survival (log CFU/cm^2) (Standard deviation)*	*Mean killed (log CFU/cm^2)*
Control (Butterfield's Phosphate Buffer water)	7.2	8.0 A (0.6)	0.0	6.0 A (0.2)	0.0
Chitosan acetate in acetic acid (1%/1%)	3.7	6.2 B (0.5)	1.8		
Chitosan acetate in acetic acid (1%/1%)—1% SDS	4.2	6.3 B (0.5)	1.7		
1% Lactic acid	2.7	4.4 C (0.4)	3.6	5.7 A (0.8)	0.3
1% SDS	3.2	2.1 D (0.1)	5.9		
2% SDS	3.2	<2.0[b] D	>6.0	5.6 A (0.3)	0.4
1% Lactic acid—1% SDS	2.7	<2.0[b] D	>6.0	1.4 B (0.9)	4.6
1% Lactic acid—2% SDS	2.7	<2.0[b] D	>6.0	1.3 B (0.7)	4.7

[a]Means with different letters are significantly different ($p < 0.05$).
[b]Concentrations were calculated by using the method's detection limit.
CFU, colony-forming units; SDS, sodium dodecyl sulfate.

LA and 1% SDS vol/vol (pH 2.7); (7) combination of 1% LA and 2% SDS vol/vol (pH 2.7); (8) Butterfield's Phosphate Buffer water (BB, pH 7.2) as a negative control. Treatments that significantly reduced the concentration of *E. coli* 0157:H7 *in vitro* by more than 2 logs were further investigated using a cattle hide decontamination model. Only the latter five treatments were used in the hide decontamination model. Morpholinepropanesulfonic acid (MOPS) was used for neutralization of low pH after chemical treatments (Park and Chen, 2011).

In vitro *experiment*

Each treatment was conducted in four independent replicates at room temperature. For each replicate, 9 mL of the solution was mixed with 1 mL of the *E. coli* O157:H7 inoculum. Samples were taken after 5 min and diluted (1:10) in 0.05M MOPS (pH 7.4). Neutralized samples were serially diluted in BB and volumes of 0.1 mL were spread plated, in duplicate, onto ct-SMAC agar (Sorbitol MacConkey agar with cefixime and tellurite) and incubated at 37°C for 24 h. Absence of colony growth on plates of the first dilution (10^{-1}) indicated that the bacterial concentration was below the detection limit (2.0 log CFU/mL).

Hide Decontamination Model

Three cow hides were harvested at a slaughter plant, and 10-cm × 10-cm sections were cut from the ventral midline. Hide sections were positioned hair sides facing each other (Antic *et al*., 2010), placed in sterile bags and stored at −20°C before being thawed at room temperature 2 h prior to each experiment. Inoculations and treatments were performed in four independent replicates in a biosafety cabinet. Hide sections were inoculated with 1 mL of adjusted culture at 10^8 CFU/mL using a pipette over the midline of a 5-cm × 10-cm area. The inoculum was then distributed uniformly by five vertical followed by five horizontal strokes (Carlson *et al*., 2008) using a sterile disposable L-shaped rod and left to dry for 5 min. Each inoculated area was uniformly treated by 12 hand sprays that delivered 10 g of dispensed volume. A 5 min reaction time was allowed prior to sampling using a sterile dry sponge stick by applying five horizontal followed by five vertical motions. The sponge was then hand-massaged for 1 min in a sterile bag containing 25 mL of 0.05 M MOPS and the eluted contents enumerated as described in the *in vitro* experiment. Absence of growth on plates of the first dilution (10^{-1}) indicated that the bacterial concentration was below the detection limit (0.7 log CFU/cm^2).

Statistical analysis

The surviving log CFU/mL or log CFU/cm^2 of hide were analyzed using a one-way analysis of variance with multiple comparisons at 5% level of significance. A treatment's disinfecting effect was estimated as the difference in *E. coli* O157:H7 concentration between the treatment and control (BB).

Results and Discussion

Table 1 summarizes the *in vitro* and hide decontamination experiments. All seven *in vitro* treatments significantly reduced the survival of *E. coli* O157:H7 ($p < 0.05$). Treatments containing CA resulted in <2 log CFU/mL reduction hence were not included in the hide experiment. Treatments 1% SDS, 2% SDS, or either in combination with 1% LA reduced surviving *E. coli* O157:H7 concentrations by >2 log CFU/mL but did not differ significantly among each other ($p > 0.05$).

On hide sections, only the combination of LA and SDS significantly reduced the concentration of *E. coli* O157:H7 by 4.6 logs compared to the control. The lack of antibacterial effect of either chemical independently on hides may be due to the complex hide surface and normal microbiota (2.9–3.5 log CFU/cm^2, data not shown). Increasing the concentration of SDS to 2% in combination with 1% LA did not result in additional antibacterial effect. As a surfactant, SDS denatures proteins and destroys bacterial cell membranes at a low pH (Zhao *et al*., 2009). Synergism between LA and SDS may be explained by the ability of LA to increase the permeability of the bacterial cell membrane by releasing lipopolysaccharides, which may render bacteria more sensitive to SDS (Alakomi *et al*., 2000). Although a pH < 3 is bactericidal against Gram-negative bacteria (Tanner *et al*., 1992), the minimal antibacterial effect on hide sections by 1% LA at pH 2.7 and 2% SDS at pH 3.2 indicates that the pH alone was not effective in this

study. In conclusion, SDS enhanced the antibacterial effect of 1% LA against *E. coli* O157:H7 on hide sections. The combination may be a safe hide wash, given its low concentration.

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Disclosure Statement

No competing financial interests exist.

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