Research Note

Inactivation of Escherichia coli O157:H7 on Cattle Hides by Caprylic Acid and β-Resorcylic Acid

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ABSTRACT

Two naturally occurring, generally recognized as safe compounds, namely, caprylic acid (CA) (1%) and β-resorcylic acid (BR) (1%), and their combination, applied at 23 and 60 °C were evaluated for their antimicrobial effects against Escherichia coli O157:H7 on cattle hides in the presence and absence of bovine feces. Fresh cleaned cattle hides were cut into pieces (5 cm²), air dried, and inoculated with a five-strain mixture of nalidixic acid–resistant (50 μg/ml) E. coli O157:H7 (~8.0 log CFU). The hide samples were air dried under a biosafety hood for 2 h and sprayed with 95% ethanol, 1% CA, 1% BR, or a mixture of 1% CA and 1% BR at 23 or 60 °C. The hide samples were kept at 23 °C, and E. coli O157:H7 populations were determined at 2 and 5 min after treatment. Both CA and BR were effective in decreasing E. coli O157:H7 populations on hides by 3 to 4 log CFU/cm² (P < 0.05). Sterile bovine feces had no effect on the decontaminating property of CA and BR on cattle hides (P > 0.05). Results of this study indicate that CA and BR could potentially be used to decontaminate cattle hides, but follow-up research undertaken under slaughterhouse conditions is warranted.

Enterohemorrhagic Escherichia coli O157:H7 is a significant concern for the beef industry. Cattle act as the major reservoir host of this pathogen, where it colonizes the terminal rectum, particularly an anatomical area within the terminal rectum referred to as the recto-anal junction (35). E. coli O157:H7 is shed in the feces, thereby leading to pathogen contamination and persistence on the hide (5, 10, 27, 38). Pathogens from hides of contaminated animals can spread to other animals during transport and lairaging through direct body contact or indirectly by contact with contaminated floors and surfaces (38). The prevalence rate for E. coli O157:H7 on cattle hides ranges from 11% (22) to 76% (7). Since E. coli O157:H7 may persist on cattle hides for extended periods of time, strategies that reduce the fecal load of the pathogen in animals may not be effective to prevent carcass contamination on a long-term basis (8). Moreover, hide prevalence of E. coli O157:H7 has been reported as a more accurate predictor for carcass contamination than fecal prevalence of the pathogen (9).

Although carcass muscle surfaces are sterile, pathogen transfer from hide to meat surface can occur during slaughter and dressing operations. Since carcass contamination with pathogens is strongly correlated to hide contamination (5, 6, 11, 13, 14), it is critical to decrease E. coli O157:H7 populations on cattle hides in order to reduce the risk of foodborne outbreaks from beef products. Moreover, treatments that effectively eradicate or reduce E. coli O157:H7 on hides would be crucial for the successful implementation of hazard analysis critical control points (HACCP) programs by the meat industry (39, 43, 44).

Caprylic acid (CA; octanoic acid) is a natural, 8-carbon, medium-chain fatty acid present in breast milk, bovine milk, and coconut oil (26, 25, 46). β-Resorcylic acid (BR; 2,4-dihydroxybenzoic acid) is a phytophenolic compound widely distributed among the angiosperms and is a secondary metabolite that plays a key role in the biochemistry and physiology of plants (24). CA (CFR 184.1025) (28, 45) and BR (CAS RN no. 89-86 [Everything Added to Food in the United States]) (24, 29) are approved for use in foods by the U.S. Food and Drug Administration. Previous research conducted in our laboratory revealed that CA and BR were effective in inactivating a variety of pathogenic bacteria in different matrices (3, 28–31, 34).

The objective of the present study was to investigate the efficacy of CA, BR, and their combination (CA+BR) applied at 23 and 60 °C in the presence or absence of bovine feces for reducing E. coli O157:H7 on cattle hides.

MATERIALS AND METHODS

Bacterial strains. All bacteriological media were obtained from BD (Sparks, MD). Five isolates of E. coli O157:H7 were used for the study (obtained from Dr. Michael P. Doyle, Center for Food Safety, University of Georgia, Griffin). E. coli O157:H7 strains used in this study include E16 (meat isolate), E10 (meat isolate), E8 (meat isolate), E22 (calf feces isolate), and E6 (milk isolate). All the aforementioned strains possess both stx₁ and stx₂. The strains E8, E10, E16, and E22 belong to pulsed-field gel...
electrophoresis profile pattern II, whereas E6 belongs to pulsed-field gel electrophoresis profile pattern I (32). All strains of the pathogen were induced to resistance to nalidixic acid (NA) (50 μg/ ml), as described by Zhao et al. (47). For confirming resistance to the antibiotic, the cultures were streaked on tryptic soy agar (TSA; Difco, BD) supplemented with 50 μg of NA per ml (Sigma-Aldrich Chemical Co., St. Louis, MO), and growth was checked after incubation at 37°C for 24 h.

Inoculum preparation. Each bacterial isolate was cultured separately in 10 ml of sterile tryptic soy broth (TSB; Difco, BD) supplemented with 50 μg of NA per ml at 37°C for 24 h with agitation (150 rpm). Following incubation, the cultures were sedimented by centrifugation (4°C, 8,000 × g for 10 min), washed twice, and resuspended in 10 ml of sterile phosphate-buffered saline (PBS) (pH 7.3). The bacterial population in each culture was determined by plating 0.1-ml portions of serial dilutions (1:10 in PBS) on duplicate TSA + NA plates with incubation at 37°C for 24 h. Equal volumes containing approximately equal populations from each of the five strains were combined, and 500 μl of the suspension was used as the inoculum (~8 log CFU/0.5 ml).

Inoculation and treatment of hide samples. The antibacterial efficacy of CA and BR (both from Sigma-Aldrich Chemical Co., St. Louis, MO) to reduce E. coli O157:H7 on cattle hide was determined as described previously (2, 4, 20). Fresh cattle hides were collected from slaughtered cows at a local slaughterhouse. Pieces of the hides (5 by 5 cm) were cut, cleaned with 70% ethanol, and air dried under a laminar flow hood prior to the experiment. E. coli O157:H7 (~8.0 log CFU/0.5 ml) was applied in drops onto each hide piece and spread even using a sterile spreader. The inoculated hides were dried in a laminar flow hood (inside temperature, 23°C) for 2 h. After drying, each hide piece was sprayed with a spray bottle (Fisher Scientific, Pittsburgh, PA) containing 25 ml of sterile deionized water or 95% ethanol, or 1% CA, or 1% BR, or a mixture of 1% CA and 1% BR that was dissolved in 95% ethanol. Each treatment solution was tempered at 23 or 60°C prior to application on hide. Hide samples were kept in a vertical position during spraying. The average distance from the spray nozzle to the hide was 5 cm, and the spray bottle was held at an angle of 90° during the entire spraying time. Control treatment samples were not subjected to any spraying. The control and treated hide samples were kept at room temperature (23°C), and the surviving E. coli O157:H7 population was determined at 2 and 5 min after treatment. Since fecal matter, a common contaminant present on cattle hide, could potentially reduce the efficacy of CA and BR for inactivating E. coli O157:H7 (1, 19), the experiment was repeated in the presence of 1% autoclaved bovine feces added on hide samples. Briefly, an E. coli O157:H7 inoculum was prepared in PBS containing 1% sterile feces (wt/vol) and applied onto each hide piece, which was allowed to dry and subjected to the treatments as before. Three replicate samples of cattle hide were included for each treatment and control at each temperature, and the entire experiment was repeated four times.

Enumeration of E. coli O157:H7. A sterile cellulose sponge (Dukal Corporation, Ronkonkoma, NY) premoistened with 10 ml of maximum recovery diluent (Oxoid Division, Unipath Co., NY) was used to swab each hide sample five times, and the sponge was placed in a sterile stomacher bag (Fisher Scientific Co. LLC, Hanover Park, IL) containing 40 ml of maximum recovery diluent and pummeled in a stomacher (Tekmar, Inc., Cincinnati, OH) for 1 min. The diluent was serially diluted in PBS, and appropriate dilutions were plated on TSA + NA plates and incubated at 37°C for 24 h. Representative colonies of bacteria from sorbitol MacConkey agar plus methylumbelliferyl-β-D-glucuronate (SMA+MUG) were confirmed as E. coli O157 by E. coli O157 latex agglutination test (Oxoid Division, Unipath Co., Ogdensburg, NY).

Statistical analysis. Data from the four independent replicate experiments were pooled. The effects of bovine feces, CA and BR, sampling time, and temperature and their interaction on E. coli O157:H7 counts were analyzed using PROC MIXED of SAS (version 9.2; SAS Institute, Cary, NC). Variation among replicates was used as the error term. Data were expressed as least squares means, and differences were considered significant at P values of <0.05.

RESULTS
In the present study, we evaluated the efficacy of CA and BR and CA+BR for reducing E. coli O157:H7 on cattle hides at 23 and 60°C. In order to mimic the potential fecal contamination of cattle hides and determine its effect on the decontaminating property of the molecules, the bactericidal effects of CA and BR were determined in the presence and absence of autoclaved bovine feces. However, since CA and BR were equally effective against E. coli O157:H7 in the presence and absence of autoclaved bovine feces (data not shown), only the results obtained from hide samples containing autoclaved bovine feces are presented here (Fig. 1 and 2). The antibacterial activity of CA, BR, and CA+BR, applied at 23°C against E. coli O157:H7 on cattle hides, is provided in Figure 1. Approximately 6.0 log CFU of E. coli O157:H7 per cm² was recovered from the control hide samples, which were not subjected to any treatment. The E. coli O157:H7 population recovered from the water-sprayed hide samples remained the same as that of control samples, whereas ethanol control treatment reduced E. coli O157:H7 counts on hides by ~1.5 log CFU/cm². E. coli O157:H7 populations recovered from the hide samples at 2 and 5 min after treatment were not significantly different (P > 0.05). However, hide treatment with 1% CA, 1% BR, and CA+BR reduced E. coli O157:H7 population...
on hide samples by \(-3\) to \(4.0\) log CFU/cm\(^2\) \((P < 0.05)\). Among all the treatments, hide treatment with CA + BR was found to be more effective than CA or BR alone at 23°C (Fig. 1). The efficacy of CA, BR, and CA + BR for inactivating \(E.\ coli\ O157:H7\) on cattle hides at 60°C is depicted in Figure 2. In comparison to water-treated and untreated hide samples, the treatment with CA, BR, and CA + BR at 60°C brought about a reduction of \(-4\) log CFU/cm\(^2\) \((P < 0.05)\). However, no differences in \(E.\ coli\ O157:H7\) counts were observed among the treatments containing CA, BR, or CA + BR \((P > 0.05)\).

**DISCUSSION**

The U.S. Department of Agriculture Food Safety Inspection Service declared a zero tolerance policy for \(E.\ coli\ O157:H7\) in nonintact raw beef products in the 1990s, and six additional serogroups of Shiga toxin–producing \(E.\ coli\) (serogroups O26, O45, O103, O111, O121, and O145) were declared adulterants in nonintact raw beef products in September 2011. At 23°C, thereby renewing the interest in developing effective treatments for preventing or reducing carcass contamination by pathogens. Since cattle hide contamination with \(E.\ coli\ O157:H7\) and other pathogens has been positively correlated to carcass contamination, effective treatments that reduce the pathogens on hide surface are critical for improving the microbiological safety of beef products. This is especially important since washing of cattle hides with water has only minimal effect in reducing the pathogen load. Moreover, spraying with water has been reported to increase total microbial and \(E.\ coli\ O157:H7\) populations on hide by releasing bacteria encapsulated in dirt and fecal material present on hide.

A number of approaches for decreasing \(E.\ coli\ O157:H7\) on cattle hides have been reported in the literature, including washing cattle before slaughter, dehairting, and the use of antimicrobials. The various antimicrobials investigated for decontaminating cattle hides comprise cetylpyridinium chloride, sodium hydroxide, trisodium phosphate, acidified chlorine, phosphoric acid, organic acids, mineral acids, commercial detergents, disinfectants, and sanitizers, ozonated and electrolyzed oxidizing water, shellac, and bacteriophage application. For example, spraying of cattle hides with 2% lactic acid resulted in 1 to 5% citric acid, 1.5% acetic acid, and bacteriophage application led to a 2.3, 1.8, and 2.0 log CFU/cm\(^2\), respectively. Carlson and coworkers reported that treatment of hides with 10% acetic acid and lactic acid at 23°C reduced \(E.\ coli\ O157:H7\) counts by 0.7 and 2.9 log CFU/cm\(^2\), respectively. Similarly, spraying of cattle hides with a 23% shellac-in-ethanol solution yielded an \(-2.1\)-log reduction in \(E.\ coli\ O157:H7\) counts on artificially inoculated cattle hides. Most of these treatments have generally yielded about 2.0- to 3.0-log reductions in pathogen counts under commercial slaughter conditions.

We investigated the efficacy of CA and BR for rapidly decreasing \(E.\ coli\ O157:H7\) on cattle hide for potential application on unskinned, hoisted carcass before bleeding. Hence, treatment time points of 2 and 5 min were chosen for study. In addition to our assays at room temperature (23°C), we investigated the hide-decontaminating effect of CA and BR at a higher temperature (60°C), since high-temperature treatment has been previously found to be more effective in decreasing \(E.\ coli\ O157:H7\) counts. For example, Carlson et al. reported that spraying of cattle hides with 10% acetic acid at 23 and 55°C decreased \(E.\ coli\ O157:H7\) counts by 0.7 and 2.1 log CFU/cm\(^2\), respectively. Similarly, hides treated with 10% lactic acid at 23 and 55°C resulted in \(E.\ coli\ O157:H7\) reductions of 2.9 and 4.3 log CFU/cm\(^2\), respectively. Likewise, we observed that BR was more effective in decreasing \(E.\ coli\ O157:H7\) on hides at 60°C (4-log reduction) than at 23°C (3-log reduction). Moreover, the hide-decontaminating activity of CA + BR was more effective \((P < 0.05)\) than the treatment solution containing CA or BR alone at room temperature (Fig. 1), whereas all the treatments were equally effective \((P > 0.05)\) at 60°C (Fig. 2). We also observed that CA and BR were equally effective in reducing \(E.\ coli\ O157:H7\) on hides in the presence and absence of autoclaved bovine feces. Although CA and BR are products with generally recognized as safe status compounds for application in the food products, they could potentially be irritants at high concentrations. Therefore, further studies on the safety of CA and BR at the concentration used (1%) are warranted. In conclusion, the results of this study suggest the potential use of CA and BR for decontaminating cattle hide and justify further investigations to validate their efficacy and safety in slaughterhouses.

**REFERENCES**


A comparison of antimicrobial efficacy of multiple beef hide decontamination strategies to reduce levels of 


