Research Note

Inhibitory Activity of Colicin E1 against *Listeria monocytogenes*

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ABSTRACT

Colicins are gram-negative bacteriocins produced by and effective against *Escherichia coli* and related species. Colicin E1 (ColE1) is composed of three functional domains, which collectively have a pore-forming effect on targeted bacteria. ColE1 binding and translocation domains are highly specific in contrast to the pore-forming domain, implying that ColE1 could be broadly effective. In this study, the activity of ColE1 against *Listeria monocytogenes* was evaluated in broth and on surfaces of ready-to-eat products. Individual strains of *L. monocytogenes* were examined in broth containing ColE1 at 0, 0.1, 1, or 10 μg/ml. Although strain differences in sensitivity to ColE1 existed, growth was significantly reduced in all strains at doses as low as 0.1 μg/ml. Sterilized ham slices were submerged in a five-strain *L. monocytogenes* cocktail (either 7 or 4 log CFU/ml) and placed in vacuum packages containing 0, 1, 5, 10, 25, or 50 μg of ColE1. Ham slices were then stored at 4 or 10°C, and samples were removed and examined for *L. monocytogenes* after 1, 3, 7, and 14 days. Reduction of *L. monocytogenes* by ColE1 was dependent on initial inoculum concentration and storage temperature. For slices stored at 4°C, treatment with 25 μg reduced *Listeria* growth below detection limits for the slices inoculated with 4 log CFU/ml for the entire 14 days, whereas for the 7-log CFU/ml slices, growth was detected at 7 days postinoculation. For slices stored at 10°C, 10 μg/ml ColE1 significantly inhibited growth of *L. monocytogenes* for up to 3 days for both inoculation groups. These data indicate that ColE1 is highly effective against *Listeria*.

*Listeria monocytogenes* is a foodborne pathogen responsible for human listeriosis, a severe gastrointestinal illness with a mortality rate of 30% (28). Numerous cases of foodborne illness have been linked to the consumption of ready-to-eat (RTE) products contaminated with *L. monocytogenes*. Among the many categories of RTE foods, deli meats have been identified as products of highest risk for causing listeriosis on both a per serving and a per annum basis (37). The resilience of this pathogen has allowed for it to become ubiquitous in the food manufacturing and processing environments (18). *L. monocytogenes* resists the deleterious effects of high salt concentrations, pH extremes, freezing, and drying common to the manufacture of RTE meat products (22). Because of the widespread nature of *L. monocytogenes*, RTE products can become contaminated with this pathogen during peeling, slicing, and repackaging and at the retail and consumer levels during storage and preparation.

In response to this risk, the U.S. Food and Drug Administration and the Food Safety and Inspection Service (FSIS) have set strict regulatory standards for RTE products, including a zero tolerance for *L. monocytogenes* on all RTE foods. Several antimicrobial methods are currently being used and others have been evaluated for potential application to food products and packaging to meet these new FSIS standards. These methods include but are not limited to steam pasteurization, hot water pasteurization, radiant heating and high-pressure processing, UV and ionizing radiation, and the application of antimicrobial organic acids such as lactates, diacetates, and propionates. Costs associated with the implementation of the currently available methods and their limited efficacy clearly indicate a critical need for a cost-effective intervention capable of reducing high concentrations of *L. monocytogenes* on RTE products.

Colicins are antimicrobial proteins produced by and effective against *Escherichia coli* and other members of the *Enterobacteriaceae* family (6, 24). Pore-forming colicins function as potent bacteriocidal agents via formation of depolarizing ion channels in the cytoplasmic membrane (25, 31). Because of the efficacy of these proteins against various *E. coli* strains (32, 34), we sought to determine whether these bacteriocins could be efficacious against *L. monocytogenes*. The current study was conducted to evaluate the activity of colicin E1 (ColE1) against *L. monocytogenes* in both pure culture and on the surfaces of RTE products.

MATERIALS AND METHODS

Colicin production and purification. ColE1 was produced and purified by the method of Stahl et al. (34). ColE1 was produced from an *E. coli* K-12 strain containing plasmid pColE1-K53 (National Collection of Type Cultures, Public Health Laboratory Service, London, UK). Colicin expression was induced with mitomycin C, and the ColE1 was purified from the cell-free supernatant by ion exchange chromatography using Q Sepharose.
**Broth culture evaluation.** Five isolated strains of *L. monocytogenes* were grown and evaluated for sensitivity to ColE1. The five strains included one human clinical isolate (2045 Scott A) and four meat product isolates (FSIS 1126 isolated from a beef carcass, H7769 isolated from an RTE poultry product, and H7762 and H7764 both isolated from frankfurters). All of these isolates are serotypes (1/2a and 4b) associated with human clinical illness. All strains were obtained from National Animal Disease Center (NADC; Ames, Iowa) and the Food Safety Research Laboratory (Iowa State University, Ames). Growth curves for the individual strains were constructed to determine the time necessary to reach 4 log CFU/ml under the growth conditions utilized. For each of the five cultures examined, frozen stock cultures (9.5 log CFU/ml) were thawed at room temperature, and 100 μl of the resuspended stock culture was added to 9 ml of sterile TSBYE broth containing 0.6% yeast extract (TSBYE; Becton Dickinson, Sparks, Md.). Cultures were incubated at 37°C for 24 h, and then 1 ml of the culture was plated onto 99 ml of fresh TSBYE and changes in optical density and growth were determined hourly over a 24-h incubation period. Optical density was determined at 600 nm (Spectronic 20D spectrophotometer, Thermo Spectronic Inc., Madison, Wis.), and growth was determined by serial dilution and plating onto modified Oxford *Listeria* selective agar (MOX; Becton Dickinson) followed by incubation at 37°C for 24 h.

**Colicin sensitivity testing.** For each strain, 100 μl of frozen stock culture was individually added to 9.9 ml of sterile TSBYE and incubated at 37°C for 24 h. These cultures were then diluted 1:100 into fresh TSBYE and allowed to grow to 4 log CFU/ml. From these cultures, 7.9-ml aliquots were placed into 10-ml culture tubes containing 0, 0.8, 8, or 80 μg of ColE1 in 100 μl of 10 mM Tris, pH 7.6; the final concentrations of the ColE1 were 0, 0.1, 1, and 10 μg/ml of culture. These cultures were then incubated at 37°C, and optical density (at 600 nm) and concentration of *L. monocytogenes* were determined initially and after 1, 3, and 6 h of incubation. Concentration of *L. monocytogenes* was determined by serial dilution, plating in triplicate onto MOX agar, and incubating for 24 h.

**Acquired resistance evaluation.** To determine whether surviving *L. monocytogenes* had altered metabolism or had acquired resistance to ColE1, for each of the five strains tested single isolates of *L. monocytogenes* that survived the 10-μg/ml ColE1 dose in the broth culture study were isolated from the MOX plates. These isolates were then restreaked onto fresh MOX agar and allowed to grow at 37°C for 24 h. Regrown isolates were then identified as *L. monocytogenes* using AccuProbe *L. monocytogenes* culture identification tests (Gen-Probe Inc., San Diego, Calif.). Isolates were then analyzed for growth in the same manner as the original strains in TSBYE with either no ColE1 (control) or 1 μg/ml. Optical density and concentration of *L. monocytogenes* were determined initially and after 1, 3, and 6 h of incubation at 37°C as for the colicin sensitivity evaluation. Resistance isolation and regrowth was repeated three times, with five isolates per strain in each replication. The original ColE1 sensitivity testing and the acquired resistance growth studies were each conducted on three separate occasions.

**RTE product evaluation.** For each of the five stock cultures, 100 μl was added to 9.9 ml of sterile TSBYE and incubated at 37°C for 24 h. Each culture was then diluted 1:100 in fresh TSBYE and grown to either 4 or 7 log CFU/ml. For each culture concentration, 10 ml of culture was transferred into 90 ml of sterilized 0.1% peptone water, and the five individual strains were then combined (450 ml total) and used to inoculate ham slices. To ensure that the anticipated inoculation concentrations were obtained, a sample from each pooled inoculum culture was serially diluted and plated onto MOX agar.

Ham slices were sterilized by irradiation to avoid potential precontamination with *Listeria* or other bacteria (15). Ham slices weighing 50 g (surface area of approximately 232 cm²) were aseptically sliced in half, completely submerged in the five-strain *L. monocytogenes* inoculum for 10 min to allow bacterial attachment, drained briefly, and transferred into sterile vacuum bags containing 1 ml of various ColE1 doses in 10 mM Tris, pH 7.6. The bags were then massaged (Stomacher 3500, Seward, Worthington, UK) for 60 s, vacuum packaged, and stored under refrigeration. Slices stored at 4°C were treated with ColE1 concentrations of 0, 1, 5, 10, and 25 μg, and samples were removed for evaluation at 0, 1, 3, 7, and 14 days. Slices stored at 10°C were treated with ColE1 concentrations of 0, 1, 10, and 50 μg, and samples were removed for evaluation at 0, 1, and 3 days. For enumeration of *L. monocytogenes*, ham slices were aseptically cored (surface area of 12.5 cm² weighing 2.5 g), and the cores were serially diluted in 10 ml of peptone water, plated on MOX agar, and incubated at 37°C for 24 h. All samples were prepared in triplicate for each refrigeration temperature, dose, and day sampling point.
TABLE 1. Optical density (at 600 nm) at 0, 1, 3, and 6 h after treatment of Listeria monocytogenes strains with ColE1

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Within a given strain and time point, means with different letters are significantly different (P < 0.01).

RESULTS

Broth culture evaluation. All strains were sensitive to ColE1; however, there was significant variation in strain susceptibility (Table 1 and Fig. 2). Significant reductions in L. monocytogenes populations were found in all of the strains tested (with the exception of FSIS 1126) in response to ColE1 at 0.1 µg/ml after 1 h (Fig. 2). Strain H7762 was the most susceptible strain; ColE1 at 0.1 µg/ml of culture resulted in approximately a 3.5-log reduction of L. monocytogenes as compared with the untreated control after 7 h of growth. Even among the more resistant L. monocytogenes strains, ColE1 at 10 µg/ml of culture resulted in an approximately 3- to 4.5-log reduction in Listeria counts after 3 h of incubation. In all strains, the highest dose of ColE1 used (10 µg/ml) was not able to eliminate all of the Listeria in any of the cultures.

Acquired resistance evaluation. All five strains were evaluated for susceptibility of regrown isolates to further ColE1 treatment. In all strains, growth of control isolates was not significantly altered from the original strain data, suggesting that no change in growth or metabolism occurred after treatment with ColE1 (Fig. 3). Growth of the five retreated isolates was extremely similar to that of the original strains treated with ColE1 at 1 µg/ml, and in certain cases the retreated strains appeared to have become more susceptible. In evaluation of strains Scott A and...
Acquired resistance of *L. monocytogenes* to ColE1. Changes in growth (log CFU per milliliter) of *L. monocytogenes* isolates previously treated with ColE1 were determined at 1, 3, and 6 h after retreatment with ColE1 at either 0 or 1 µg/ml. Error bars represent standard error (n = 3). (A) *L. monocytogenes* FSIS 1126. (B) *L. monocytogenes* Scott A. (C) *L. monocytogenes* NADC H7762. (D) *L. monocytogenes* NADC H7764. (E) *L. monocytogenes* NADC H7764.

H7769, growth of retreated isolates was significantly lower (P < 0.01) after 6 h than was growth of the original strains.

RTE product evaluation. For the ham slices stored at 4°C, growth of *L. monocytogenes* was reduced at every ColE1 dosage (Fig. 4A and 4B); however, the efficacy of the doses was dependent on the initial inoculum concentration. The 5.0-µg/ml ColE1 dose was sufficient to keep *L. monocytogenes* on the ham slices to below the limit of detection for more than 24 h, regardless of the inoculation concentration. When 25 µg/ml ColE1 was applied to ham slices inoculated with 4 log CFU/ml (Fig. 4B), no *Listeria* was detectable for the entirety of the 14-day study. Ham slices inoculated with 7 log CFU/ml and treated with 25 µg/ml ColE1 had detectable *L. monocytogenes* growth after 3 days. In the samples inoculated with 7 log CFU/ml, ColE1 application at 5, 10, and 25 µg/ml still caused a 4-log reduction in *L. monocytogenes* on the inoculated slices as compared with the control slices at the completion of the 14-day study.

Ham slices stored at 10°C were used to evaluate the efficacy of ColE1 against *L. monocytogenes* on RTE products stored under insufficient refrigeration (46°C). The 10- and 50-µg/ml ColE1 doses eliminated detectable *L. monocytogenes* for over 24 h, regardless of initial inoculum concentration. When the ham slices were stored at 10°C (Fig. 3C and 3D), ColE1 significantly inhibited growth of *L. monocytogenes* (P < 0.01) at doses as low as 1 µg/ml. After 3 days of storage at this elevated temperature, ham slices treated with 1 µg/ml ColE1 had ~3-log (Fig. 3C) and ~2.2-log (Fig. 3D) lower counts compared with the control slices inoculated at 7 and 4 log CFU/ml, respectively. After 3 days, doses of 10 and 50 µg/ml ColE1 still significantly reduced the growth of *L. monocytogenes* by 3 and ~4 log CFU/cm² from the initial inoculum concentrations of 7 and 4 log CFU/ml, respectively. There were no significant differences between the results achieved with these two doses at any time point over the duration of this study.

**DISCUSSION**

*L. monocytogenes* is a prevalent environmental pathogen that poses tremendous challenges for the RTE food industry. This pathogen will grow at temperatures ranging from 1 to 45°C and within a pH range of 4.1 to 9.6 and has been documented to survive in cured products up to 120 days (22, 27). Current intervention strategies for controlling *L. monocytogenes* in RTE meat products are the addition of antimicrobials, chemical preservatives, generally recognized as safe substances, or a combination of two or more of these or similar compounds (28, 30). The most common of these compounds used to combat *L. monocytogenes* in RTE products are organic acids and their salts (40).

Sodium, potassium, or other salts of lactic, acetic, and other organic acids have had significant antimicrobial activity against *L. monocytogenes* in broth and in meat products (3, 7, 13, 35, 40). Although organic acids have been effective anti-*Listeria* agents, the concentrations required for this activity are extremely high. In several studies, *L. monocytogenes* on RTE meat products survived in refrigerated storage despite the presence of organic acids that have bacteriostatic activity against *L. monocytogenes* (3, 26, 38). Further limitations to the use of organic acids are their variable efficacy and objectionable sensory attributes. The antimicrobial activity of these acids is dramatically influenced by the presence of other antimicrobials or other ingredients commonly used in RTE meat manufacture (2, 3, 9, 17, 36), and studies have indicated that RTE meat prod-
FIGURE 4. The efficacy of ColE1 against *L. monocytogenes* in RTE products was determined as reductions of *L. monocytogenes* (log CFU per square centimeter) on ham slices after treatment with different concentrations of ColE1. Ham slices were originally inoculated with 7 log CFU/ml (A and C) or 4 log CFU/ml (B and D). Inoculated ham slices were refrigerated at 4°C for 14 days (A and B) or at 10°C for 3 days (C and D). Error bars represent standard error (n = 3).

ucts formulated or treated with organic acids had lower overall consumer acceptability compared with untreated products (16).

In the hopes of overcoming the limitations of organic acids, bacteriocins are being examined as potential interventions for *L. monocytogenes* control on RTE food products (11, 21). One such bacteriocin, nisin, inhibited growth of *L. monocytogenes* on the surface of bologna by 2.4 to 3.8 log CFU/cm² at a concentration of 125 μg/ml (16). Enterocin, another gram-positive bacteriocin of the pediocin family, has reduced *L. monocytogenes* in broth culture by 3 log CFU/ml when added at 4 μg/ml (14). Other pediocins have had variable anti-*Listeria* activity, ranging from 0.5- to 4-log reductions when added at concentrations greater than 500 μg/ml (1, 14, 20, 36).

Based on the efficacy of ColE1 against *L. monocytogenes* as demonstrated in this study, it appears that ColE1 is more efficacious against *Listeria* than are any of the previously reported bacteriocins. In our study, ColE1 was effective at reducing *L. monocytogenes* populations in pure culture, significantly reducing growth at doses of 0.1 μg/ml (Fig. 2). Among the five strains evaluated, up to 5.5-log reductions were observed with ColE1 concentrations as low as 1 μg/ml.

No other research has examined ColE1 for anti-*Listeria* activity. Because bacteriocins are frequently thought of as a means used by similar bacteria to compete for resources, it was surprising to see this high level of efficacy of an *E. coli*–derived bacteriocin against *Listeria*. ColE1 is cytotoxic to *E. coli* and other closely related bacteria because of the formation of ion channels that depolarize the cytoplasmic membrane (8). ColE1 is composed of three functional domains, which collectively cause a pore-forming effect on targeted bacteria (10). In gram-negative bacteria, pore-forming colicins must accomplish three tasks: binding to an outer membrane receptor, translocating across the periplasmic space, and inserting into the cytoplasmic membrane to form a highly conductive ion channel (12, 31, 39). For binding and translocation purposes in gram-negative bacteria, ColE1 uses the vitamin B₁₂ receptor BtuB and members of the Tol and OMP protein complexes (4, 10, 19, 33, 39). Future studies examining binding and attachment sites of ColE1 to *L. monocytogenes* are imperative to elucidate the mechanism of action of this bacteriocin against gram-positive organisms.

In our RTE product evaluation, significantly lower concentrations of ColE1 were required to inhibit growth of *L. monocytogenes* than were required in previous antimicrobial studies (14, 17). With properly refrigerated ham slices that initially had approximately 2.5 log CFU/cm², treatment with 10 μg/ml ColE1 kept *L. monocytogenes* concentrations below the detection limit for 3 days (Fig. 4B), and 25 μg/ml ColE1 kept *L. monocytogenes* populations to undetectable levels for the entire 14-day study. Increasing the refrigeration temperature from 4 to 10°C had dramatic effects on product stability and ColE1 efficacy. At 10°C, 50 μg/ml ColE1 reduced *L. monocytogenes* populations by 4 (Fig. 4C) and 3 (Fig. 4D) log CFU/cm² compared with the untreated control after 3 days of incubation for the 7 and 4 log CFU/ml treatments, respectively. At this time point,
concentrations of *L. monocytogenes* reached approximately 9 log CFU/cm² on control slices. Common *L. monocytogenes* concentrations in RTE products are 1 to 5 log CFU/g of product (37). In this study, our lower inoculum concentration (4 log CFU/ml) provided initial contamination (approximately 4.4 log CFU/g) that would be at the higher end of this product range. The ability of minute quantities of ColE1 to eliminate detectable populations of this product range. The ability of minute quantities of ColE1 to eliminate detectable *L. monocytogenes* from an RTE product contaminantated at above the average concentrations found among contaminated RTE products strongly supports the use of this bacteriocin as an anti-*Listeria* agent for use in food. Further supporting evidence of the value of ColE1 as a potent anti-*Listeria* agent is the lack of acquired resistance, as demonstrated in this study (Fig. 3A through 3E). In previous studies, *L. monocytogenes* has spontaneously become highly resistant to bacteriocins such as nisin and pediocin (5, 23).

Although we have demonstrated the efficacy of ColE1 against *L. monocytogenes*, the safety of this protein for human consumption is of particular importance for its use on food. Murinda et al. (29) compared the cytotoxicity of ColE1, nisin, and pediocin in mammalian cell culture and found that ColE1 was significantly less cytotoxic than were both nisin and pediocin. This finding and the long history of exposure of humans to colicins produced by commensal organisms in the human gastrointestinal tract (32) suggest that there should be no concerns for the use of this protein as a biopreservative in products meant for human consumption.

ColE1 effectively reduced populations of *L. monocytogenes* in broth culture and on RTE meat product surfaces. Although strains differed in their sensitivity to ColE1, growth was significantly reduced (*P < 0.01*) in pure cultures of all strains tested with a ColE1 dose of 0.1 µg/ml. The mechanism of action of ColE1 against gram-positive organisms such as *L. monocytogenes* has not yet been elucidated. An understanding of this mechanism could lead to broader applications of ColE1 against many other bacterial pathogens. ColE1 is a safe and highly effective anti-*Listeria* agent, and its application to RTE meat products could provide greater protection against *L. monocytogenes* throughout the food processing chain.

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REFERENCES


