

Colicin E1 has High Antimicrobial Activity against *Listeria Monocytogenes* in Culture and on Packaged Ham

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Summary and Implications

Colicins are gram-negative bacteriocins produced by, and effective against *Escherichia coli* and closely related species. This study evaluated the activity of a purified pore-forming, Colicin E1 (ColE1) against *L. monocytogenes* in broth and on RTE product surfaces. Colicins were effective at reducing *Listeria* populations at doses as low as 0.1 µg/mL in broth culture, and up to 5.5 log CFU/g on cured ham slices. Colicins may be an effective intervention for the control of *Listeria* in ready to eat meat products.

Introduction

Listeria monocytogenes is a food-borne pathogen responsible for listeriosis, a severe gastrointestinal illness with a mortality rate of 30%. Numerous cases of food-borne illness have been linked to the consumption of ready-to-eat (RTE) products contaminated with *L. monocytogenes*. In response to this risk, the Food Safety and Inspection Service (FSIS) and the Food and Drug Administration (FDA) have set strict regulatory standards for RTE products, including a “zero tolerance” for *L. monocytogenes* on all RTE foods. Costs associated with the implementation of the currently available anti-listerial intervention strategies, as well as their limited efficacy, clearly indicate a critical need for a cost-effective intervention capable of high levels of reduction of *L. monocytogenes* on RTE products. The current study evaluated the activity of Colicin E1 (ColE1) against *L. monocytogenes* in both pure culture and on RTE product surfaces.

Materials and Methods

Broth Culture Evaluation

Five isolated strains of *Listeria 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 a RTE poultry product, H7762 and H7764 both isolated from frankfurters) all carrying the serotypes (1/2a, 4b) for human clinical illness.

For each strain, 100 µL of frozen stock culture was individually added to 9.9mL of sterile broth and incubated at 37° C for 24 hours.

Broth Culture Evaluation

After 24h incubation, the 5 *L. monocytogenes* cultures were diluted 1:100 into fresh broth and allowed to grow to 4 log₁₀ CFU/mL. From these cultures 7.9 mL aliquots were placed into 10 mL culture tubes containing either 0, 0.1, 1, or 10µg ColE1/mL culture. These cultures were then incubated at 37° C and optical density and CFU/mL of *L. monocytogenes* determined initially, and after 1, 3, and 6 hours of incubation.

RTE Meat Product Evaluation

RTE Ham slices were purchased from a local supermarket and sterilized by irradiation to avoid potential pre-contamination with *Listeria* or other bacteria. Ham slices weighing 3g (surface area of approximately 232cm²) were aseptically sliced in half, completely submerged in a *L. monocytogenes* inoculum consisting of the 5 strains used for broth culture evaluation for 10 minutes to allow for bacterial attachment, then drained briefly, and transferred into sterile vacuum bags containing 1mL of various ColE1 doses (0, 1, 5, 10 and 25µg) in 10mM Tris, pH 7.6. The bags were then massaged (Seward Stomacher 3500, Worthing, West Sussex, UK) for 60 seconds, vacuum packaged and placed into refrigeration at 4°C. Ham slices were sampled at 0, 1, 3, 7 and 14 days. For enumeration of *L. monocytogenes*, ham slices were aseptically cored (surface area =12.5 cm²) and the cores were serially diluted in 10mL peptone water, plated on MOX agar and incubated at 37°C for 24 hours. All samples were prepared in triplicate for each refrigeration temperature-dose-day sampling point.

Results and Discussion

Broth Culture Evaluation

All strains were sensitive to ColE1, however significant variation in strain susceptibility existed (Figure 1). Significant reductions in *L. monocytogenes* populations were seen in all of the strains tested with inclusion of 0.1µg ColE1/mL, with the exception of FSIS 1126, after 1 hr. Strain H7762 was the most susceptible strain, as 0.1 µg ColE1/mL culture resulted in approximately 3.5 log₁₀ CFU/mL less *L. monocytogenes* than in the untreated control after 7h of growth. Even among the more resistant *L. monocytogenes* strains, 10µg ColE1/mL culture resulted in an approximately 3-4.5 log₁₀ CFU/mL reduction in *Listeria* counts after 3h of incubation.

RTE Meat Product Evaluation

Growth of *L. monocytogenes* was reduced at every ColE1 dosage level (Figure 2) in the ham slices; however, the efficacy of the doses was dependant on the initial inoculum level. Despite this, the 5.0µg ColE1 dose was sufficient to reduce *L. monocytogenes* levels below our limits of detection for over 24 hours, regardless of the inoculation level of the ham slices. When 25µg ColE1 was applied on ham slices inoculated with 4 log₁₀ CFU/mL, *Listeria* was not detected for the entirety of the 14d study. Ham slices inoculated with 7 log₁₀ CFU/mL had detectable *L. monocytogenes* growth at this same ColE1 treatment after 3d. In the samples inoculated with 7 log₁₀ CFU/mL, ColE1 application at 5, 10 and 25µg still caused a 4 log₁₀ CFU/cm²

reduction in *L. monocytogenes* compared to the control slices at the completion of the 14d study. *Listeria monocytogenes* is a prevailing environmental pathogen which poses tremendous challenges for the RTE food industry. Based on the efficacy of ColE1 against *L. monocytogenes* demonstrated in this study, it appears that ColE1 is more efficacious against *Listeria* than any of the previously reported bacteriocins. The ability of minute quantities of ColE1 to eliminate detectable *L. monocytogenes* from a RTE product contaminated at levels above the average seen among contaminated RTE products in the industry strongly supports its potential as an anti-*Listerial* agent for use in food.

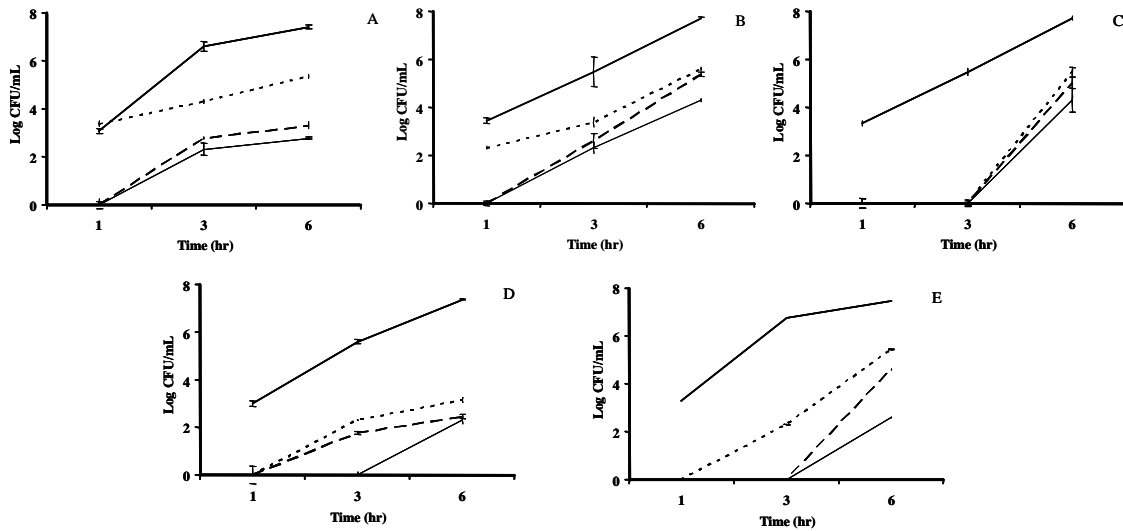


Figure 1. The effects of ColE1 on *Listeria monocytogenes* in broth culture. Changes in growth (Log₁₀ CFU/mL) were measured at 1, 3 and 6 hrs after treatment with different concentrations of ColE1 added to broth. Error bars represent SE (n = 3). Graph A = *L. monocytogenes* FSIS 1126; Graph B = *L. monocytogenes* Scott A; Graph C = *L. monocytogenes* NADC H7769; Graph D = *L. monocytogenes* NADC H7762; and Graph E = *L. monocytogenes* NADC H7764.

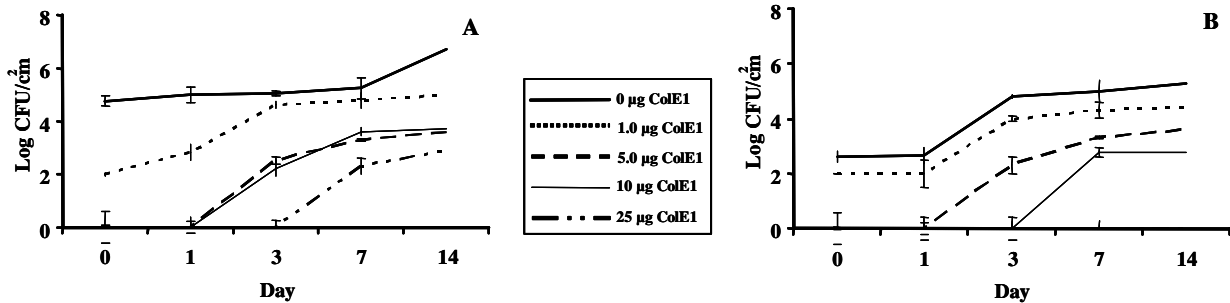


Figure 2. Effect of Colicin E1 on *L. monocytogenes* populations on refrigerated ham slices. A) ham slices inoculated in a 7 log CFU/mL *Listeria* cocktail, B) ham slices inoculated in a 4 log CFU/mL *Listeria* cocktail.