ABSTRACT

The antibacterial activity of lactoferricin B on enterohemorrhagic *Escherichia coli* O157:H7 in 1% peptone medium and ground beef was studied at 4 and 10°C. In 1% peptone medium, 50 and 100 μg of lactoferricin B per ml reduced *E. coli* O157:H7 populations by approximately 0.7 and 2.0 log CFU/ml, respectively. Studies comparing the antibacterial effect of lactoferricin B on *E. coli* O157:H7 in 1% peptone at pH 5.5 and 7.2 did not reveal any significant difference (P > 0.5) at the two pH values. Lactoferricin B (100 μg/g) reduced *E. coli* O157:H7 population in ground beef by about 0.8 log CFU/g (P < 0.05). No significant difference (P > 0.5) was observed in the total plate count between treatment and control ground beef samples stored at 4 and 10°C. The antibacterial effect of lactoferricin B on *E. coli* O157:H7 observed in this study is not of sufficient magnitude to merit its use in ground beef for controlling the pathogen.

Enterohemorrhagic *Escherichia coli* O157:H7 has emerged as a foodborne pathogen of major public health concern in the United States (9). Cattle have been implicated as a principal reservoir of *E. coli* O157:H7 (5) with undercooked ground beef being a major vehicle of human infection (11). The United States Department of Agriculture has established a zero-tolerance policy for *E. coli* O157:H7 in ground beef. Effective methods for killing *E. coli* O157: H7 in ground beef would greatly reduce the likelihood of outbreaks of this pathogen and decrease economic losses to the meat industry due to product recalls.

Lactoferrin is an iron-binding glycoprotein that is present in most exocrine secretions of mammals including milk and colostrum (8, 10). Recently, Bellamy et al. (1) reported that pepsin digestion of bovine lactoferrin generates a 25- amino acid peptide called lactoferricin B that exerts antimicrobial activity against a variety of pathogenic microorganisms, including foodborne pathogens. Therefore, the objective of this study was to evaluate the potential of lactoferricin B for reducing *E. coli* O157:H7 populations in ground beef.

MATERIALS AND METHODS

Bacterial culture. Five strains of *E. coli* O157:H7 (E06 [milk isolate], E08 [meat isolate], E10 [meat isolate], E16 [meat isolate], and E22 [calf feces isolate]) were used for the study. The strains of *E. coli* O157:H7 were cultured individually in 250-ml Erlenmeyer flasks containing 100 ml of sterile tryptic soy broth (Difco Laboratories, Detroit, Mich.) at 37°C for 24 h with agitation (150 rpm). Following incubation, the bacteria were sedimented by centrifugation (4,000 × g for 20 min) in separate tubes and washed and resuspended in 0.1% peptone solution. The optical density (OD) of the solution was determined (OD of 0.5 at 640 nm represented approximately 109 CFU/ml). Each culture was serially diluted with 9 ml of sterile, 0.1% peptone to yield an approximate bacterial population of 107 CFU/ml. The bacterial population in each culture was confirmed by plating 0.1-ml portions of appropriately diluted culture on tryptic soy agar (Difco) plates and incubating the plates at 37°C for 24 h. An approximately equal population of the five strains was mixed, and 1.0 ml of the solution was used as the inoculum.

Antimicrobial activity of lactoferricin B against *E. coli* O157:H7 in 1% peptone medium. The effect of lactoferricin B (50 and 100 μg/ml) on the five-strain mixture of *E. coli* O157:H7 was evaluated in 1% peptone medium at 4 and 10°C at pH 5.5 and 7.2. Lactic acid (30% solution wt/vol, Sigma Chemicals, St. Louis, Mo.) was used to adjust the pH of the peptone medium to 5.5. Lactoferricin B (98% pure) was synthesized commercially (Boston Biomolecules, Woburn, Mass.). Stock solutions of lactoferricin were prepared in sterile deionized water and filter-sterilized (0.22-μm filter, Nalgene, Rochester, N.Y.). One milliliter of the five-strain mixture of *E. coli* O157:H7 (107 CFU) was added to 9 ml of sterile 1% peptone solution (pH 5.5 or 7.2) containing 50 or 100 μg of lactoferricin B per ml (treatment). Controls were prepared by adding 1.0 ml of inoculum to 9 ml of sterile 1% peptone solution containing no lactoferricin B. The solutions were incubated in a water bath at 4 or 10°C for 24 h. Following incubation, a 1-ml portion of the inoculated solution was serially (1:10) diluted in 9 ml of sterile 0.1% peptone water, and 0.1-ml portions from appropriate dilutions were surface-plated in duplicate on tryptic soy agar plates and incubated at 37°C for 24 h. Representative colonies from the plates were then confirmed as *E. coli* O157:H7 by *E. coli* O157:H7 latex agglutination assay (Remel Microbiology Products, Lenexa, Kans.). Duplicate samples of each treatment and control were assayed in all the experiments, and the entire study was replicated twice.

Antimicrobial activity of lactoferricin B against *E. coli* O157:H7 in ground beef. Fresh top round beef steaks (pH 5.6)
purchased from a local grocery store were used for making ground beef. Inoculation of *E. coli* O157:H7 in ground beef was done according to Doyle and Schoeni (3). Batches of 100 g of coarsely ground (1/16-in. [17.4-mm] cutting plate) beef were inoculated by adding 1 ml of the five-strain mixture of *E. coli* O157:H7 (10⁷ CFU/ml) followed by addition of 1.0 ml of deionized water containing 10 mg of lactoferricin (treatment). Control samples were prepared in the same way without the addition of lactoferricin. The inoculated meat was mixed at slow speed in a meat mixer (model A-120, Hobart Manufacturing Co., Ohio) followed by grinding through a ⅛-in. (3.2-mm) cutting plate (Hobart no. 12 grinder) to assure uniform distribution of inoculum. Portions of 10 g each of ground meat (treatment and control) were transferred to separate stomacher bags and stored at 4 or 10°C. The population of *E. coli* O157:H7 and total plate count were determined on three samples (treatment and control) at days 0, 1, 2, 3, 4, and 5 of storage at 4°C and at days 0, 1, 2, and 3 of storage at 10°C.

**Bacteriological analyses.** To each stomacher bag containing 10 g of ground meat, 90 ml of 0.1% peptone water was added and was macerated by a stomacher (model 400, Seward, England) at normal speed for 1 min. One milliliter of the meat homogenate was serially (1:10) diluted with 9 ml of 0.1% peptone water, and 0.1-ml portions of appropriate dilutions were spread on duplicate plates of Sorbitol MacConkey agar no. 3 agar (Oxoid Division, Unipath Co., Ogdenburg, N.Y.) with 0.1% 4-methylumbelliferyl-β-D-glucuronide (MUG) (Oxoid) (for *E. coli* O157:H7) and tryptic soy agar (for total bacterial count). The plates were incubated at 37°C for 24 h. Representative sorbitol-negative and MUG-negative colonies from SMA+MUG plates were confirmed as *E. coli* O157:H7 by *E. coli* O157:H7 latex agglutination assay. The bacterial counts were reported per gram of ground meat.

**Statistical analysis.** The data were analyzed by paired *t* test using the general linear model of the Statistical Analysis Systems procedures (12). The differences among means at the 5% (*P* < 0.05) level were determined by the least significant difference test.

**RESULTS AND DISCUSSION**

The effect of lactoferricin B on *E. coli* O157:H7 in 1% peptone solution was evaluated at 50 and 100 µg/ml because these concentrations represent the levels of lactoferrin normally present in bovine milk and colostrum, respectively (6). At a concentration of 50 µg/ml, only a reduction of approximately 0.7 log CFU *E. coli* O157:H7/ml was ob-
observed; however, at 100 μg/ml, lactoferricin B had greater antibacterial activity, reducing E. coli O157:H7 by approximately 2.0 log CFU/ml (P < 0.05) (Fig. 1). Therefore, a concentration of 100 μg of lactoferricin B per ml was used in subsequent experiments. Studies comparing the antibacterial effect of lactoferricin B (100 μg/ml) in 1% peptone solution at pH 5.5 and 7.2 at 4 and 10°C revealed no significant differences at the two pH values (P > 0.05) (Fig. 2). In ground beef, throughout the storage period at 4 and 10°C, the counts of E. coli O157:H7 were significantly higher (P < 0.05) in control samples than those recovered from the samples containing lactoferricin B (100 μg/g). Lactoferricin B reduced the E. coli O157:H7 population in ground beef by about 0.8 log CFU/g (Figs. 3 and 4). No significant difference in total plate count (P < 0.05) was observed between the treatment and control samples (Figs. 5 and 6). Further, no significant difference in the antibacterial activity of lactoferricin B was observed between the samples stored at 4 and 10°C.

Although the effect of lactoferricin B on E. coli O157:H7 counts in the 1% peptone medium and ground beef was significant statistically (P < 0.05), the level of reduction observed in ground beef may not be practically meaningful. In a recent study on the effect of lactoferrin and its peptides on E. coli O157:H7, Shin et al. (13) observed that lactoferrin B (100 μg/ml) was very effective (>5.0 log CFU/ml reduction) in killing the pathogen in 1% peptone solution; however, these investigators evaluated the effect of the peptide on E. coli O157:H7 at 37°C, whereas our experiments were conducted at 4 and 10°C that represent the normal storage temperatures for meat at home and retail levels. Further, lactoferricin B used in our studies was commercially synthesized, whereas Shin and coworkers (13) purified the peptide from fresh skim milk. Finally, we evaluated lactoferricin B against a five-strain mixture of E. coli O157:H7, whereas Shin et al. (13) determined the effect of the peptide on individual E. coli O157:H7 isolates. In another study on the antibacterial activity of lactoferricin B on Salmonella Enteritidis, Facon and Skura (4) reported that although lactoferricin exhibited a bactericidal effect on the pathogen in 1% peptone medium, no significant effect was observed in chicken skin extract, infant formula, and in tryptic soy broth, which is a complex medium compared to 1% peptone. Similarly, Jones et al. (7) observed that greater than 500 μg of lactoferricin B per ml was required to exert a bactericidal effect on E. coli, when the medium contained milk or infant formula. Bellamy et al. (2) reported that the bactericidal activity of lactoferricin B was inhibited by calcium and magnesium and this may explain the lack of significant antibacterial effect of lactoferricin in richer media, in foods, and in ground beef as observed in this study. Further, addition of EDTA at approved levels to negate the inhibitory effect of calcium and magnesium on lactoferricin B did not increase the antibacterial effect of the peptide in foods or complex media (4). The degree of antibacterial effect of lactoferricin B on E. coli O157:H7 observed in this study does not merit its use for controlling the pathogen in ground beef.

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REFERENCES


