CONTROL OF FOODBORNE PATHOGENS ON FRESH BEEF BY JENSENIIN G, A BACTERIOCIN PRODUCED BY *PROPIONIBACTERIUM THOENII (JENSENII)* P126

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Abstract- The combined effect of jenseniin G, a bacteriocin produced by *Propionibacterium thoenii (jensenii)* P126, with an organic acid mixture or an EDTA treatment against *Listeria monocytogenes* or *Escherichia coli* was examined on fresh beef cuts to increase inhibition spectrum of the bacteriocin. While combination of jenseniin G and the organic acid mixture reduced the number of *L. monocytogenes* attached to lean beef tissues from 4.78 to 3.49 log CFU/g, combination of 20 mM EDTA with jenseniin G did not show a significant reducing effect on the number of *E. coli* over the 3-day period.

I. INTRODUCTION

Meat and meat products are subjected to foodborne illnesses due to possible contamination with pathogenic bacteria [1,2]. Application of bacteriocins as food protection and preservation agents have prompted a promising approach to control growth of pathogenic bacteria associated with meat and meat products. The antibotulinal activity of jenseniin G, a bacteriocin produced by *Propionibacterium thoenii (jensenii)* P126; its heat and pH stability suggest its usefulness as a biological food preservative in thermally processed foods. One limitation for jenseniin G as a food preservative, as observed in other applications, is its narrow spectrum of activity. Jenseniin G is only active against related propionibacteria and some lactic acid bacteria and is sporostatic to botulinal spores [3,4].

Combination of jenseniin G with sublethal treatments that impair the barrier functions of the cell wall of Gram-positive and Gram-negative bacteria resulted in the sensitivity of organisms normally resistant to jenseniin G [5]. Similar results were obtained when sublethally injured Gram-positive and Gram-negative bacteria were treated with pediocin AcH and nisin [6].

The objective of this study was to determine whether combining jenseniin G with an acid or an EDTA treatment would increase its spectrum against two foodborne pathogens, *L. monocytogenes* and *E. coli* on fresh beef samples.

II. MATERIALS AND METHODS

Cultures and growth conditions: The jenseniin G producer *Propionibacterium thoenii* P126 and the jenseniin G-sensitive indicator *Lactobacillus delbrueckii* subsp. *lactis* ATCC 4797 were grown in sodium lactate broth (NLB) and lactobacillus MRS broth, respectively, as previously described [5]. *Esherichia coli* ATCC 25922 and *Listeria monocytogenes* ATCC 15313 were propagated in brain heart infusion (BHI) broth (BBL, Microbiology Systems, Cockeysville, MD) at 37˚C for 18-20 h. The identity of the strains was confirmed by Gram staining and biochemical assays. All cultures were stored in the appropriate growth medium containing 20% glycerol at -70^{0}C. Viable counts were performed according to standard methods on BHI, NLA, or MRS agar, as appropriate, and expressed in log10 CFU/ml.

Jenseniin G preparation: Partially purified preparations of jenseniin G were obtained as described by Ekinci and Barefoot [7]. Jenseniin G activity was detected by a spot-on-lawn method and quantitated by a modification of the critical dilution assay as previously described [5,7]. The assay culture was the jenseniin G-sensitive indicator, *L. delbrueckii*

subsp. *lactis* ATCC 4797. Bacteriocin titers were expressed as the reciprocal of the highest dilution exhibiting detectable inhibition and reported in activity units (AU) per milliliter [7].

Sample Preparation: Lean beef muscle tissue was obtained from a local supermarket. Meat was aseptically cut into 2.5 x 2.5 x 1.5 cm pieces, sterilized by U.V. light (2 x 15-watt germicidal bulbs, 35 cm distance from meat pieces, 60 min), stored in sterile whirlpack bags at -20^0C, and thawed at room temperature for 20-30 min before use. *L. monocytogenes* ATCC 15313 and *E. coli* ATCC 25922 were grown in BHI broth for 18 h at 37^0C and diluted to 10^3 to 10^4 CFU/ml with 0.1% peptone. Individual pieces of meat were inoculated by submersion in 10 ml of solution containing approximately 10^3 to 10^4 CFU/ml of each culture for 15 min at 25^0C and mixed manually at 5 min intervals. Each piece of inoculated tissue was submerged into 10 ml of sterile test solution for 30 min at 25^0C, attached to sterile clips, hung in a covered, sterile beaker, and held at 4^0C. Solutions were: a) control (0.1% peptone); b) organic acids (40% lactic acid, 16% propionic acid, and 16% acetic acid, pH 5.5) for *L. monocytogenes* (ACID) and 20 mM EDTA in 0.1% peptone for *E. coli* c) jenseniin G (JG) (10000 AU/ml); and d) Jenseniin G (10000 AU/ml) and mixture of organic acids for *L. monocytogenes* and jenseniin G (10000 AU/ml) and 20 mM of EDTA in 0.1% peptone for *E. coli*. Randomly selected pieces of beef tissue (each weighing approximately 7 g) were blended in a stomacher blender with 63 ml of 0.1% peptone for analysis at days 0, 1, and 3.

III. RESULTS AND DISCUSSION

Activity of jenseniin G in the presence of 20 mM EDTA against *E. coli* inoculated on the surface of beef cuts was tested during 3 days storage at 4°C. Although EDTA+jenseniin G combination reduced *E. coli* viable counts as compared to the other groups and to the initial count on the first day of storage ($p<0.05$), this effect was not apparent on day 3 (Fig. 1). This very limited activity of EDTA+jenseniin G combination in the present study might be due to relatively low concentration of the bacteriocin used for application on the beef surface. The storage period in the current study was also limited given the 3 day period. Further studies are needed to determine the effect of the higher concentrations of the bacteriocin jenseniin G on meat surfaces.

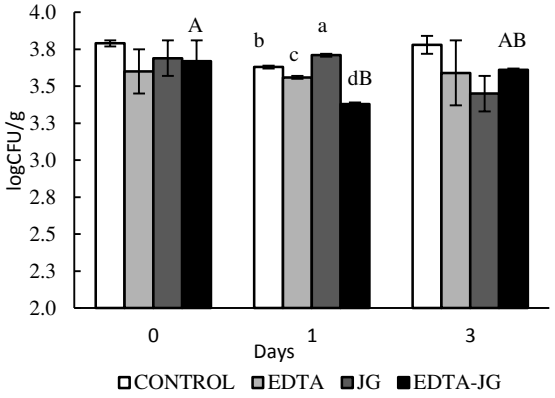

Fig. 1. *E. coli* counts (log CFU/g) of beef cuts during 3 days storage at 4°C. Bars represent the mean value ± standard error. (a-c): Within a day between sample groups, bars having common letters are not statistically different ($p>0.05$). (A, B): Within a sample group between storage days, bars having common letters are not statistically different ($p>0.05$).

Combination of lactic acid and acetic acids were selected for preservation treatments because they are generally recognized as safe (GRAS) food additives [8]. Combinations of lactic and acetic acids can be used for increased microbial inhibition and shelf-life extension [9,10]. Treatments based on organic acids are widely used in decontamination of carcasses [11]. In a previous study by Baker et al. [5], in vitro treatments of *L. monocytogenes* with jenseniin G and organic acid mixture caused 4.5 log reductions in the population of *L. monocytogenes* after seven days storage at 4˚C. In this study the effect of jenseniin G and an organic acid mixture against *L. monocytogenes* ATCC 15313 attached to lean beef tissue was examined at 4°C for up to 3 days (Fig. 2). While jenseniin G and the organic acid mixture alone were not effective on *L. monocytogenes*, the combination of them reduced the number of *L. monocytogenes* over the 3 day period from 4.78 to 3.49 CFU/g.

There could be many reasons for the inability of this treatment to reduce as much populations of *L. monocytogenes* attached to meat compared to in vitro treatments [5].

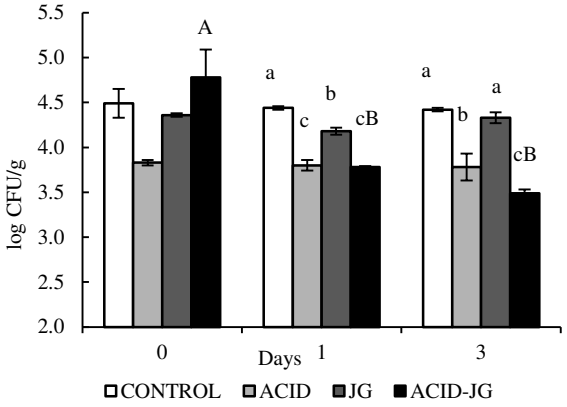

Fig. 2. *L. monocytogenes* counts (log CFU/g) of beef cuts during 3 days storage at 4°C. Bars represent the mean value ± standard error. (a-c): Within a day between sample groups, bars having common letters are not statistically different (p>0.05). (A, B): Within a sample group between storage days, bars having common letters are not statistically different (p>0.05).

Bacteriocins may be degraded by endogenous proteases associated with meat or may bind to adipose [12]. Concentration of bacteriocin on meat surface is also important. However, the amount of jenseniin G on the meat surface was not determined in this study after the treatment or storage for 3 days.

IV. CONCLUSIONS

An acid mixture or EDTA was used to increase antimicrobial activity of jenseniin G against *L. monocytogenes* or *E. coli* on fresh beef cuts, respectively. A slight effect of EDTA + jenseniin G combination against *E. coli* and a comparatively greater, but still not significant, effect of acid mix+jenseniin G against *L. monocytogenes*, was determined. Even though the enhanced antimicrobial activities of jenseniin G *in-vitro* has been reported in previous studies, the same effect, when applied on the fresh beef surface, was not observed in the current study. Meat has a very complex food matrix. In most cases, although activity of an antimicrobial agent was observed during in vitro studies, this activity might be decreased in real food applications, likely due to characteristics of the product and the possible interaction between the active agent and food components. There is a need for further investigation of higher concentrations of jenseniin G against foodborne pathogens in meats over longer storage periods.

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