INHIBITORY ACTIONS OF *LACTOBACILLUS LACTIS* ON *ESCHERICHIA COLI* O157:H7 DURING STORAGE AT 7°C IN BROTH AND ON RAW CHICKEN MEAT

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Story in Brief

The survival of *Escherichia coli* O157:H7 strain 43894 during storage at 7°C in Trypticase Soy Broth and on chicken breast pieces was evaluated. Cells of *Lactobacillus lactis* L(+) 1 were added to samples to determine if they had an inhibitory effect on *E. coli* O157:H7 strain 43894. Generally, *L. lactis* populations greater than 5.1 x 10^7 cfu/ml, had an antagonistic effect on *E. coli* O157:H7 populations during storage at 7°C in TSB broth. The total numbers of *E. coli* O157:H7 declined in samples containing *L. lactis* L(+) 1 while numbers in control samples remained constant. *Lactobacillus lactis* L(+)1 had a similar effect on *E. coli* O157:H7 on chicken breast pieces. This study suggests that *L. lactis* L(+)1 could be used as an effective treatment for the control of *E. coli* O157:H7 on raw poultry meat.

(Key Words: *Lactobacillus lactis, E. coli*O157:H7.)

Introduction

Due to the severity of the food borne illness caused by *Escherichia coli* O157:H7, it has become one of food industry's primary concerns in the safety of food products. Consuming food contaminated with *E. coli* O157:H7 can lead to a severe intestinal disorder such as hemorrhagic colitis which, if not treated, can result in hemolytic uremic syndrome (HUS) which may cause renal failure and possibly death. Foods of animal origin have been the primary source of *E. coli* O157:H7 and subsequent outbreaks. Doyle et al. (1987) reported that 1.5% of all poultry carcasses tested were positive for *E. coli* O157:H7. This is not acceptable to either the poultry processor or the consumer. It is imperative that an applicable approach to the control of *E. coli* O157:H7 in poultry products be developed. *L. lactis* has been shown to be inhibitory to psychrotrophic spoilage bacteria at refrigeration temperatures primarily due to the production of hydrogen peroxide (Gilliland et al., 1983). If *L. lactis* can exert inhibitory action on *E. coli* O157:H7 at refrigeration temperatures, it may be possible to add the lactobacilli to poultry carcasses in the early stages of processing (i.e., chill tank) to exert control of this pathogen. The purpose of this study was to determine if *L. lactis* can exert inhibitory

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action on *E. coli* O157:H7 at 7°C in a laboratory medium and on raw chicken meat.

Materials and Methods

Frozen concentrated cultures of *L. lactis* L+(1) were prepared by centrifugally removing the cells from 200 ml of an MRS broth culture that had been grown at 37°C for 24 hours. The cells were resuspended in 20 ml of cold sterile nonfat milk to yield a population of approximately 1 x 10^9cfu/ml. The resulting concentrated culture was dispensed in 2 g portions into cryogenic vials and frozen at -196°C in liquid nitrogen until needed.

Following thawing by submersion in water at room temperature, the frozen concentrated culture of *L. lactis* L+(1) was added to Trypticase Soy Broth (TSB) which previously had been inoculated with approximately 1.0 x 10^5 cfu/ml of *E. coli* 0157:H7 strain 43894. The broth inoculated with *E. coli* had been divided into four equal portions and placed into sterile bottles. An appropriate amount of concentrated culture of *L. lactis* was added to each of three of the bottles to yield populations of 1.0 x 10^7 cfu/ml, 5.0 x 10^7 cfu/ml, and 8.0 x 10^7 cfu/ml. The fourth bottle contained no *L. lactis* and served as a control. All bottles were held in a mixture of ice and water while they were prepared and before analyses. After preparation, the bottles were stored at 7°C.

The total numbers of *E. coli* O157:H7 were determined on days 0, 3, and 7 by plating appropriate dilutions using the pour plate method on Violet Red Bile Agar (VRBA). VRBA plates were incubated at 37°C for 24 hours. On day 0, the total numbers of lactobacilli present were determined by plating appropriate dilutions on MRS agar. MRS agar plates were incubated at 37°C for 48 hours.

Additional experiments were done to determine if *L. lactis* L(+)1 would exert antagonistic action on *E. coli* O157:H7 strain 43894 added to chicken breast pieces. Appropriate amounts of a concentrated culture of *L. lactis* L(+)1 were added to two separate beakers containing 250 ml of sterile water to yield a population of 5 x 10^7 and 1 x 10^8 cfu/ml of *L. lactis*. Two other beakers contained sterile water and served as dipping solutions for the two controls. Boneless chicken breasts were purchased from a local supermarket and cut into twelve approximately equal sized pieces (1 in^2) using a sterile knife on a sterile cutting board. Sterile tongs were used to handle the chicken pieces. Because chicken meat may have *E. coli* naturally present on the surface, three of the pieces were dipped into sterile water for 30 sec. The numbers from this treatment represented the background flora present on the chicken breast and served as the background control. The other nine pieces were dipped into water containing 1 x 10^5 cfu/ml of *E. coli* O157:H7 strain 43894 and held for 30 seconds. Three of the pieces which had been dipped into water containing the *E. coli* were dipped into water containing 5 x 10^7 cfu/ml of *L. lactis* L(+)1 and

held for 30 seconds. Another three pieces were dipped in a similar manner into water containing 1 x 10^8 cfu/ml of *L. lactis* L(+)1. The remaining three pieces were dipped in sterile water and served as a treatment control. Excess fluid was drained from each piece of chicken after each dip procedure and each individual chicken piece was placed into a sterile whirl pack bag and stored at 7^{o}C. One bag from each treatment was removed for analyses on days 0, 3, and 7. Numbers of *E. coli* and lactobacilli were determined as described in the previous paragraph.

Statistical analyses for both treatments were conducted as a 4 x 3 factorial arrangement of treatments in a randomized complete block design. Least significant difference analyses were used to compare means for significant differences at the 5% level of confidence. All data were analyzed with SAS programs PROC GLM and LSMEANS.

Results and Discussion

L. lactis populations of 5.1 x 10^7 and 9.4 x 10^7 cfu/ml had an inhibitory effect on total numbers of *E. coli* O157:H7 strain 43894 during storage in TSB broth at 7^{o}C (Table 1). On day 0 there were no significant differences (P>.05) among the initial populations of *E. coli* for the four treatments. After 3 days of storage at 7^{o}C, there were significant declines in numbers of *E. coli* O157:H7 for treatments containing 5.1 x 10^7 and 9.4 x 10^7 cfu/ml of *L. lactis* L(+)1. There was an additional significant decline (P<.05) in the numbers of cells of *E. coli* after 7 days of storage for the same two treatments. There was no significant decline (P>.05) in the number of *E. coli* for the treatment containing 1.6 x 10^7 cfu/ml of *L. lactis* L(+)1 suggesting that there must be sufficient numbers of *L. lactis* present to have an antagonistic effect on *E. coli* O157:H7 strain 43894. The number of cells of *E. coli* O157:H7 did not change (P>.05) for the control sample which contained no added lactobacilli.

L. lactis L(+)1 had a similar effect on *E. coli* O157:H7 on chicken meat. The numbers of *E. coli* O157:H7 declined during storage at 7^{o}C on the pieces of chicken which had been dipped in water containing 5 x 10^7 and 1 x 10^8 cfu/ml of *L. lactis* L(+)1 prior to storage (Table 2). The populations of *L. lactis* on the chicken pieces (1.1 x 10^6 and 1.7 x 10^6/g) were lower than those achieved in the broth samples because they represent the numbers of *L. lactis* L(+)1 that actually adhered to the chicken pieces. The total number of *E. coli* declined significantly (P<.05) after 3 days of storage for the chicken containing 1.7 x 10^6 *L. lactis* per g. Both populations of *L. lactis* had significant (P<.05) inhibitory action on the *E. coli* populations after 7 days of storage at 7^{o}C compared to the treatment control sample.

The treatment control and background control samples, which had no *L. lactis* added, actually exhibited increases in numbers of coliforms during

storage. These samples also had a foul odor and a slime layer present on the surface of the chicken after 7 days of storage indicating the growth of psychrotrophic spoilage organisms. No foul odor was noted on the samples that had been dipped in water containing the lactobacilli prior to storage. This suggests that *L. lactis* also exhibited inhibition on the growth of spoilage microorganisms on the chicken during refrigerated storage.

L. lactis L(+)1 has an antagonistic effect on *E. coli* O157:H7 strain 43894 during storage in TSB broth and on chicken pieces. The antagonism is probably due to the peroxide produced by these cultures during refrigerated storage. The *L. lactis* does not grow at refrigeration temperatures, but it does produce peroxide (Gilliland et al., 1983). The findings of this research could be beneficial to the poultry industry. *L. lactis* could be added to the chill tank to control *E. coli* 0157:H7 as well as psychrotrophic spoilage microorganisms.

Literature Cited

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Table 1. Total numbers of *Lactobacillus lactis* strain L+(1) and *Escherichia coli* O157:H7 strain 43894 during storage in Trypticase Soy Broth at 9°C.

		E. coli/ml[1, 2]		
Treatment	Lactobacilli/ml[1, 3]	Day 0	Day 3	Day 7
1	1.6×10^{7}	1.0×10^{5a}	7.8×10^{4ab}	9.6×10^{4a}
2	5.1×10^{7}	8.6×10^{4a}	2.6×10^{4bc}	1.2×10^{3d}
3	9.4×10^{7}	8.0×10^{4a}	1.0×10^{4c}	5.3×10^{2d}
Control	0	1.0×10^{5a}	1.0×10^{5a}	1.4×10^{5a}

[1] Each value represents the mean of three trials.

[2] Reported as colony forming units/ml on VRBA agar.

[3] Reported as colony forming units/ml on MRS agar on Day 0.

[a,b,c] Numbers with the same alphabetic superscripts, did not differ significantly (P>.05), all others were different.

Table 2. Total numbers of *Lactobacillus lactis* strain L+(1) and *Escherichia coli* O157:H7 strain 43894 on fresh chicken meat during storage at 7 °C.

Treatment	Lactobacilli/g[1, 3]	*E. coli*/g[1, 2] Day 0	Day 3	Day 7
1[4]	1.1×10^{6}	5.6×10^{3a}	6.8×10^{3a}	1.0×10^{2c}
2[4]	1.7×10^{6}	4.7×10^{3a}	4.7×10^{2b}	1.1×10^{2c}
Treatment control[4]	0	3.1×10^{3a}	7.7×10^{3a}	2.2×10^{5d}
Background control[5]	0	1.2×10^{1}	1.8×10^{1}	1.5×10^{4}

[1] Each value represents the mean of two trials.

[2] Reported ascolony forming units/g on VRBA agar.

[3] Reported as colony forming units/g on MRS agar on Day 0.

[4] All inoculated with *E. coli* 0157:H7.

[5] Uninoculated.

[a,b,c,d] Numbers with the same alphabetic superscripts, did not differ significantly (P>.05), all others were different.