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Reduction of *Escherichia coli* O157:H7 in Fresh Spinach Using Bovamine® Meat Cultures as a Post–harvest Intervention and Its Impact on Sensory Properties

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ABSTRACT

To determine the inhibitory actions of lactic acid bacteria (LAB) toward *E. coli* O157:H7 in spinach, fresh spinach was inoculated with a 1 x 10^6 CFU/ml target population of the pathogen and then treated either with sterile distilled water or a four-strain LAB cocktail (2.0 × 10^8 CFU/ml). Both treatments were stored at 7°C for 24 h and then compared to an inoculated control to determine pathogen reductions. Reductions achieved by water alone and LAB were significant at 0.88 log CFU/g ($P < 0.0001$) and 1.03 log CFU/g ($P < 0.0001$) respectively, in comparison to the control sample. The improved reduction achieved by LAB over water was significant ($P = 0.0363$), indicating that LAB was the most effective intervention in the study. A triangle test was implemented to determine if LAB results in a difference in the sensory properties of fresh spinach when compared to water-treated spinach. Two spinach samples were rinsed with water and considered identical. The third spinach sample was rinsed with the LAB cocktail at a target concentration of 2.0 × 10^8 CFU/ml. A total of 40 panelists participated in the study and 16 correctly identified the LAB spinach as being the one odd sample. A total of 18 and 20 samples should be identified correctly as the odd sample in order to be statistically significant at the levels of 0.05 and 0.01, respectively. These results indicate that a significant difference does not exist ($\alpha = 0.05$ and 0.01) when LAB is applied to fresh spinach, making it an acceptable intervention from the standpoint of consumer acceptance.

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INTRODUCTION

Escherichia coli O157:H7 is a virulent pathogen that has been associated with produce in 21% of the foodborne outbreaks that occurred between 1982 and 2002 *(2)*. While *E. coli* O157:H7 is often associated with raw or undercooked ground beef *(15)* awareness of the potential for fresh fruit and vegetable consumption to cause illnesses from this pathogen has increased in recent years *(7, 8)*. In the mid-1990s, fresh produce was recognized as a vector for foodborne illness caused by *E. coli* O157:H7 *(7)*.

Because of the nature of its production, spinach is vulnerable to pathogenic contamination at every step in the production process. According to Warriner et al. *(21)*, post-harvest handling is believed to be the primary source of contamination. However, the same study also identified soil, water and harvest equipment as factors that may lead to the contamination of spinach plants during the growing process. As a result, it is necessary that spinach safety is emphasized throughout the entire production process.

Because fresh spinach production lacks a thermal kill step, reliance is placed on post-harvest wash interventions to control microbial populations. Up to 90% of spinach processors utilize sodium hypochlorite (chlorine) washes as the primary barrier against pathogenic contamination *(3)*. While chlorine is known to be an effective antimicrobial agent, numerous factors affect the efficacy of chlorine applied to fresh spinach, including water temperature, pH and contact time *(16)*. In general, it is understood that the ability of chlorine to inactivate microorganisms present on the surface of spinach leaves is not exceptional *(13)*. Warriner et al. *(21)* stated that the efficacy of chlorine is capable of reducing total microbial populations by no more than 2 logs. Beuchat *(4)* discovered that 200 parts per million (ppm) chlorinated water and deionized water were equally efficacious at killing, removing or inactivating *E. coli* O157:H7 on the surface of lettuce leaves. Lang et al. *(11)* observed reductions of *E. coli* O157:H7 on lettuce leaves of only 1.10 logs in comparison to the control after treatment with 200 ppm chlorine. These minimal reductions, in combination with the lack of a thermal processing step, indicate the need for additional interventions to be developed.

The use of lactic acid bacteria (LAB) as an intervention to control microbial growth in the food industry is not a new strategy. There are multiple properties associated with bacteria belonging to the LAB family that prove to be lethal to other bacteria, including some pathogens. Metabolism of LAB results in the production of bactericidal compounds, including hydrogen peroxide, bacteriocins, carbon dioxide and organic acids *(9, 17, 18)*. Production of organic acids, including lactic, propionic and acetic acid, induce lethal effects by acting on the cytoplasmic membrane of the bacterial cell *(9)*. Additionally, the creation of an acidic environment that is considered unfavorable for pathogenic growth aids in the control of *E. coli* O157:H7 *(9)*. The effects of such compounds on the sensory characteristics of fresh spinach are unknown, and consumer acceptance must be determined before LAB can be implemented as a post-harvest intervention in spinach production.

Lactic acid bacteria have been successfully utilized to control *E. coli* O157:H7, and other pathogens in raw meat products *(9)*, in cooked meat products *(1)* and in cattle *(5, 22, 23)*. These studies report that the use of NP51, alone or in combination with other LAB, has been effective in controlling the pathogen. Therefore, LAB may be an effective intervention for the spinach industry as well.

The overall objective of this study was to determine if Bovamine® Meat Cultures, a commercially produced LAB product, can be effectively implemented as a post-harvest intervention to reduce levels of *E. coli* O157:H7 in fresh spinach and to determine if the application of Bovamine® Meat Cultures to fresh spinach resulted in a statistical difference in sensory characteristics between treated and control spinach.

MATERIALS AND METHODS

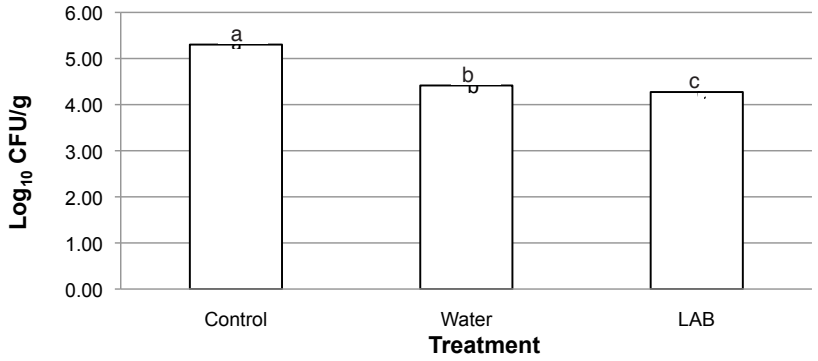
A cocktail mixture of four *E. coli* O157:H7 strains was used: A4 966, A5 528, A1 920 and 966. All strains were isolated from cattle and are maintained in the stock culture collection at Texas Tech University. The cocktail was prepared by making frozen concentrated cultures of each culture as described by Brashears et al. *(6)*. Briefly, one vial from each strain was obtained from the -80°C stock culture. A sterile loop was used to add the strains to separate tubes of brain heart infusion broth (BHI) (EMD, Gibbstown, NJ). The strains were incubated overnight at 37°C, transferred into fresh BHI tubes and incubated an additional night at 37°C. The concentration of each strain was determined to be at the appropriate numbers by plating on tryptic soy agar (TSA) (EMD, Gibbstown, NJ) and incubating for 24 hours at 37°C. All four strains were then transferred to fresh BHI and allowed to grow at 37°C overnight before being centrifuged for 10 minutes at 4,000 × g. The pellet was resuspended in BHI containing 10% glycerol and all four strains were then combined in equal portions to create the four-strain cocktail. The cocktail was then stored as a frozen culture at -80°C in 1-ml portions at a concentration of 1.0×10^9 CFU/ml in the Texas Tech University inventory.

Bovamine® Meat Cultures used in this study were obtained from Nutrition Physiology Corporation (Guymon, OK). This commercially available LAB product is comprised of four LAB strains: *Lactobacillus acidophilus* (NP 51), *Lactobacillus cristpatus* (NP 35), *Pediococcus acidilactici* (NP 3) and *Lactobacillus lactis* subsp. *lactis* (NP 7) *(19)*. Isolates NP 51 and NP 35 were originally isolated from cattle, while NP 3 was isolated from cooked hot dogs and NP 7 from alfalfa sprouts *(19)*. The culture was commercially prepared and packaged in 10-g portions in a freeze-dried form prior to shipping to Texas Tech University.

Pathogen reduction study

Fresh bagged baby spinach was obtained from a local grocery store and weighed into a sterile poultry rinsate bag (VWR, West Chester, PA) to ensure that total weight was approximately 500 g. The four-strain cocktail of *E. coli* O157:H7 was diluted 1:1000 in buffered peptone water (BPW) (OXOID, Basingstoke, Hampshire, England) to obtain a final concentration of 1.0×10^6 CFU/ml and an inoculum volume of 5 L. The pre-weighed spinach was submerged in the inoculum and allowed to soak for 20 minutes to facilitate attachment. Using a sterile tongs, the inoculated spinach was spread evenly across sterile drying racks in a biological hood (Fisher Hamilton model #54L925, Two Rivers, WI) and allowed to dry for one hour. A LAB

FIGURE 1. Composite least squares means *E. coli* O157:H7 levels in each spinach treatment held at a target temperature of 7°C for 24 hours



[a,b,c] indicates treatments that differ ($P < 0.05$).

[1]LAB is representative of the Bovamine® Meat Cultures lactic acid bacteria treatment.

wash with a concentration of 2.0×10^8 CFU/ml was prepared by combining 5 g of freeze-dried Bovamine® Meat Culture with 495 ml of sterile distilled water. The concentration of LAB was determined by making serial dilutions in buffered peptone water and plating on Lactobacilli MRS Agar (MRS) (EMD, Gibbstown, NJ). The MRS agar plates were incubated at 37°C for 24 to 48 hours. A control wash consisting of 500 ml of sterile distilled water was also prepared. Upon completion of drying, 100 g of the dry, inoculated spinach was added to the LAB rinse and 100 g to the control water rinse in sterile poultry rinsate bags. The bags were agitated for 1 minute at 230 rpm on an automatic orbital shaker (KS 260 Basic, IKA, Wilmington, NC). A third set of 100 g of dry, inoculated spinach was placed directly into a sterile Whirl-Pak (Nasco, Fort Atkinson, WI) bag to serve as the background control for this experiment. Following agitation, both rinse treatments were allowed to soak during the 0, 5 and 10 minute sampling time points. After 10 minutes, each rinse was drained in a sterile colander and transferred to sterile Whirl-Pak bags, using sterile tongs. All samples were stored at 7°C between sampling intervals.

From each rinse and the background control, 10 g of spinach was collected at 0, 5 and 10 minutes and at 1, 4, 8 and 24 hours. The exact sample weight was recorded and used to determine colony forming units (CFU) on a per gram basis. At each time point, the sampled spinach was stomached (Seward Model 400, Bohemia, NY) with 90 ml of buffered peptone water at 230 rpm for 2 minutes. Homogenized samples were serially diluted and quantitatively analyzed for *Escherichia coli* O157:H7, using a Neo-Grid™ Method (Neogen, Lansing, MI). Neo-Grid™ filters were placed on CHROMagar (CHROMagar, Paris, France) containing tellurite at a level of 2.5 mg/L. Tellurite was added to reduce the interference from other bacteria. CHROMagar plates were incubated at 37°C for 24 ± 2 hours. Mauve colonies were counted as presumptive positive for *E. coli* O157:H7 and agglutinated at random for confirmation, by use of a latex agglutination kit (Remel, Lenexa, KS).

This study was classified as a complete randomized block design. The Statistical Analysis System (SAS) software was used to analyze the data. All data were subjected to the PROC MIXED and PROC UNIVARIATE commands. The Least Squares (LS) means obtained from SAS were used to identify statistically significant differences between each individual treatment and the control. Additionally, the LS means of the water and LAB washes were compared to identify if one treatment was significantly more effective than the other. The Shapiro-Wilk value provided by the PROC UNIVARIATE procedure was used to determine normality of the data. The experimental procedure was replicated a total of three times.

Sensory study

Fresh bagged baby spinach was obtained from a local grocery store. All bags were combined to minimize the effects of natural variability and randomize the product. The combined spinach was then divided into three samples. One sample was rinsed with Bovamine® Meat Culture at a concentration of 2.0×10^8 CFU/ml. The remaining two samples were rinsed with tap water and considered to be identical. All 3 samples were drained in separate colanders and distributed into sample cups labeled with their respective three-digit sample number. The samples were placed on a tray, covered with aluminum foil and held in the refrigerator at 4°C before serving to panelists.

Forty consumer panelists were chosen at random to participate in the sensory study. All panelists were presented with the three samples simultaneously in a triangle test. They were instructed to evaluate each sample from left to right and identify the one sample they perceived to be different. The order in which the samples were presented to the panelists was randomized in order to decrease bias. Panelists were provided with a cracker, water and expectorant cup to clear their palate between samples. An answer sheet was supplied and panelists were encouraged to include comments.

Statistical significance of sensory data was evaluated using published statistical tables *(14)*. These tables were utilized to determine if statistically significant differences existed in the sensory characteristics of spinach treated with lactic acid producing-bacteria by comparing the number of responses identifying the correct "odd" sample to alpha values of 0.05 and 0.01. Additionally, the number of discriminators was calculated using methods described by Lawless and Heymann *(12)*. Discriminators are defined as those individuals who saw the true difference and selected the correct "odd" sample. It is speculated that the remainder of participants who selected the LAB sample merely guessed and were not able to perceive the true difference.

RESULTS

Pathogen reduction study

No interactions were detected among the treatments in this study. With both treatments, the total num-

TABLE 1. Summary of triangle test sensory data to determine statistical significance between tap water–treated fresh spinach and fresh spinach treated with Bovamine® Meat Cultures at an α–level of 0.05 and 0.01

α–Level	Correct Responses Required	Correct Responses	Decision	Interpretation
0.05	18[a]	16 < 18	Accept Null	No Detectable Difference
0.01	20[a]	16 < 20	Accept Null	No Detectable Difference

[a]Reject the assumption of "no difference" if the number of correct responses is greater than or equal to the tabled value.

bers of *E. coli* O157 declined over the 24 hour sampling period. *E. coli* O157:H7 populations recovered from the control maintained fairly consistent levels at just above 5 log CFU/g throughout the entire 24 hour study.

Because there were no time by treatment interactions, Fig. 1 represents the LS means of all data points composited for each treatment. As illustrated by this figure, both water ($P < 0.0001$) and LAB ($P < 0.0001$) resulted in significant reductions in comparison to the control. Water reduced *E. coli* O157:H7 numbers by 0.88 log CFU/g, while LAB was successful at reducing it by 1.03 log CFU/g (Fig. 1). The improved reduction of LAB was significantly different from that of water ($P = 0.0363$). This indicates that LAB was significantly more effective than water at reducing *E. coli* O157:H7 populations on baby spinach leaves when the composite LS means of each treatment were compared over the 24 hour sampling period.

Sensory study

Of the 40 panelists, 40% *(16)* correctly selected the LAB spinach as being the one "odd" sample. For a population of 40 panelists, the numbers of correct responses required for statistical significance at the $\alpha = 0.05$ and $\alpha = 0.01$ were 18 and 20, respectively. These values were determined using an equation outlined in Table T8 of the third edition of *Sensory Evaluation Techniques (14)*. The null hypothesis for this triangle test states that no difference exists between the control spinach and the spinach treated with lactic acid bacteria. Therefore, because the 16 correct responses obtained is less than the required responses of 18 and 20, there was no statistical significance and the null hypothesis was accepted at the $\alpha = 0.05$ and $\alpha = 0.01$ levels. These results are summarized in Table 1. Calculations to determine the number of discriminators estimated that 4 (10%) panelists perceived the true difference and selected the LAB sample as a result. These results suggest that a mere 25% (4 out of 16) of panelists who selected the LAB spinach truly detected a difference in the sensory properties of fresh spinach treated with Bovamine® Meat Cultures.

DISCUSSION

While little research has been conducted evaluating the effectiveness of LAB as an intervention for fresh spinach, the use of LAB in ground beef has been investigated. Smith et al. *(19)* utilized the same combined cultures included in Bovamine® Meat Cultures as an intervention to reduce the presence of *E. coli* O157:H7 in ground beef. The cultures were added to ground beef at a level of 10^9 CFU/g and stored at 5°C for 14 days. The combined cultures significantly reduced *E. coli* O157:H7 levels by 2.0 logs and 3.0 log cycles after 3 and 5 days of storage, respectively.

Given the proven effectiveness of these LAB cultures in other food products, Bovamine® Meat Cultures may have great potential for application in the spinach industry, as well. The LAB treated spinach was evaluated for a mere 24 hours and resulted in reductions of 1.55 log CFU/g compared to the control at the 24 hour sampling time (data not shown). Because LAB have the potential to produce inhibitory products over time, it is possible that longer exposure times could result in additional reductions in the spinach, making the present 24 hour study preliminary in nature. Additionally, these cultures may be an effective pre-harvest intervention to be applied to the crops prior to harvest. Furthermore, if the LAB-treated spinach had been held at 7°C for longer than 24 hours, perhaps the population of *E. coli* O157:H7 would have continued to decline in comparison to the control spinach and ultimately achieved reductions similar to those found by Smith et al. *(19)* in ground beef. Additionally, differences in the nutrient availability of the two products may play a role in the effectiveness of Bovamine® Meat Cultures. Meat is a nutrient dense environment with a high water activity *(10)*, while the surface of spinach leaves has low water availability and is rather nutrient poor in comparison to the internal surfaces of the plant *(20)*. A high level of nutrients and available water present in the food matrix will improve the metabolic activity of LAB and the resultant production of antimicrobial compounds will also increase.

Before application to the spinach, the LAB concentration was determined to be 7.5 $\log_{10}$ CFU/ml (3.0×10^7 CFU/ml) (data not shown). This value is the mean concentration of all three replications and is nearly 1 log CFU/ml less than the target concentration of 8.3 $\log_{10}$ CFU/ml (2.0×10^8 CFU/ml). This may be the result of adding the Bovamine® Meat Cultures to sterile distilled water.

Because solutes have been removed from distilled water, the osmotic pressure is greater outside the LAB cell relative to inside the cell. As a result, water will diffuse into the cell, potentially causing lysis.

Given that the LAB treatment was only applied as a rinse and at one concentration (2.0×10^8 CFU/ml), perhaps an improvement in performance could be achieved with a different application method or concentration level. For example, the implementation of a spray intervention may result in differing levels of success. Additionally, the Bovamine® Meat Cultures may be capable of the same degree of reduction in *E. coli* O157:H7 populations at concentrations lower than 2.0×10^8 CFU/ml. These items must be addressed before a definitive conclusion can be drawn about the effectiveness of LAB as a post-harvest intervention in the production of fresh spinach. The present study does not support LAB as an effective post-harvest intervention for fresh spinach. However, the results obtained do provide a foundation for future investigations.

Sensory results on the spinach were also similar to those of previous reports, including a 2002 study conducted by Amézquita and Brashears *(1)* which evaluated the effects of LAB on ready-to-eat meat products. They also executed a triangle test to determine whether an isolate *Pediococcus acidilactici* resulted in a significant difference between LAB treated and control frankfurters. Triangle tests were conducted on the frankfurters 9 times throughout the 56-day storage period. The number of correct responses obtained during each test was less than the number required for statistical significance. Therefore, they concluded that the application of *P. acidiliactici* did not result in a significant difference between treated and control frankfurters. Their findings support the results of the current spinach study, in which we did not have significant sensory changes in the product after the application of the cultures.

The lack of statistical significance obtained with this triangle test supports the use of LAB as a post-harvest intervention in the production of fresh spinach, from a consumer acceptance standpoint. The results of this study indicate that there is great potential for future research. As a result of metabolism and fermentative activities, LAB produce multiple by-products that have the potential to adversely affect the sensory properties of fresh spinach, particularly during shelf-life. For this reason, it is necessary to evaluate sensory changes throughout the shelf-life to determine if the production of metabolites over time will result in a statistically significant difference and decrease the consumer acceptance of product treated with Bovamine® Meat Culture.

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REFERENCES

1. Amézquita, A., and M. M. Brashears. 2002. Competitive inhibition of *Listeria monocytogenes* in ready-to-eat meat products by lactic acid bacteria. *J. Food Prot.* 65:316–325.
2. Aruscavage, D., K. Lee, S. Miller, and J. T. LeJune. 2006. Interactions affecting the proliferation and control of human pathogens on edible plants. *J. Food Sci.* 71:89–99.
3. Behrsing, J., S. Winkler, P. Franz, and R. Premier. 2000. Efficacy of chlorine for inactivation of *Escherichia coli* on vegetables. *Postharvest Biol. Technol.* 19:187–192.
4. Beuchat, L. R. 1999. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces applied to lettuce and the effectiveness of chlorinated water as a disinfectant. *J. Food Prot.* 62:845–849.
5. Brashears, M. M., M. L. Galyean, G. H. Loneragan, J. E. Mann, and K. Killinger–Mann. 2003. Prevalence of *Escherichia coli* O157:H7 and performance by beef feedlot cattle given *Lactobacillus* direct-fed microbials. *J. Food Prot.* 66:748–754.
6. Brashears, M. M., S. S. Reilly, and S. E. Gilliland. 1998. Antagonistic action of cells of *Lactobacillus lactis* toward *Escherichia coli* O157:H7 on refrigerated raw chicken meat. *J. Food Prot.* 61:166–170.
7. Delaquis, P., S. Bach, and L.-D. Dinu. 2007. Behavior of *Escherichia coli* O157:H7 in leafy vegetables. *J. Food Prot.* 70:1966–1974.
8. Johannessen, G. S., G. B. Bentsson, B. T. Heier, S. Bredholt, Y. Wasteson, and L. M. Rørvik. 2005. Potential uptake of *Escherichia coli* O157:H7 from organic manure into crisphead lettuce. *Appl. Environ. Micro.* 71:2221–2225.
9. Kostrzynska, M., and A. Bachand. 2006. Use of microbial antagonism to reduce pathogen levels on produce and meat products: a review. *Can. J. Microbiol.* 52:1017–1026.
10. Labadie, J. 1999. Consequences of packaging on bacterial growth. Meat is an ecological niche. *Meat Sci.* 52:299–305.
11. Lang, M. M., L. J. Harris, and L. R. Beuchat. 2004. Survival and recovery of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on lettuce and parsley as affected by method of inoculation, time between inoculation and analysis, and treatment with chlorinated water. *J. Food Prot.* 67:1092–1103.
12. Lawless, H.T., and H. Heymann. (ed). 1999. Sensory evaluation of food. Kluwer Academic/Plenum Publishers, Norwell, MA.
13. Lin, C.-M., S. S. Moon, M. P. Doyle, and K. H. McWatters. 2002. Inactivation of *Escherichia coli* O157:H7, *Salmonella enterica* serotype Enteritidis, and *Listeria monocytogenes* on lettuce by hydrogen peroxide and lactic acid and by hydrogen peroxide with mild heat. *J. Food Prot.* 65:1215–1220.
14. Meilgaard, M., and G. V. Civille (ed). 1999. Sensory evaluation techniques, 3rd edition. CRC Press LLC, Boca Raton, FL.
15. Pickering, L. K., T. G. Obrig, and F. B. Stapleton. 1994. Hemolytic–uremic syndrome and enterohemorrhagic *Escherichia coli*. *Pediatr. Infect. Dis. J.* 13:459–476.
16. Pirovani, M., A. Piagentini, D. Gemes, and S. Arkwright. 2004. Reduction of chlorine concentration and microbial load during washing-disinfection of shredded lettuce. Int. *J. Food Sci. Tech.* 39:341–347.
17. Sanz, Y., I. Nadal, and E. Sánchez. 2007. Probiotics as drugs against human gastrointestinal infections. Recent Patents on Anti–Infect. Drug Disc. 2:148–156.
18. Schillinger, U., R. Geisen, and W. H. Holzapfel. 1996. Potential of antagonistic microorganisms and bacteriocins for the biological preservation of foods. *Trends Food Sci. Tech.* 7:158–164.

19. Smith, J., J. E. Mann, K. Harris, M. F. Miller, and M. M. Brashears. 2005. Reduction of *Escherichia coli* O157:H7 and *Salmonella* in ground beef using lactic acid bacteria and the impact on sensory properties. *J. Food Prot.* 68:1587–1592.
20. Suslow, T. 2002. Production practices affecting the potential for persistent contamination of plants by microbial foodborne pathogens, p. 241–256. *In* S. E. Lindow, E. I. Hect–Poinar, and V. J. Elliot. (eds.), Phyllosphere microbiology. APS Press, St. Paul, MN.
21. Warriner, K., F. Ibrahim, M. Dickinson, C. Wright, and W. M. Waites. 2003. Interaction of *Escherichia coli* with growing salad spinach plants. *J. Food Prot.* 66:1790–1797.
22. Younts, S. M., M. L. Galyean, G. H. Loneragan, N.A. Elam, and M.M. Brashears. 2004. Dietary supplementation with *Lactobacillus*- and *Propionibacterium*-based direct-fed microbials and prevalence of *Escherichia coli* O157 in beef feedlot cattle and on hides at harvest. *J. Food Prot.* 67:889–893.
23. Younts–Dahl, S. M., G. D. Osborn, M. L. Galyean, J. D. Rivera, G. H. Loneragan, and M. M. Brashears. 2005. Reduction of *Escherichia coli* O157 in finishing beef cattle by various doses of *Lactobacillus acidophilus* in direct-fed microbials. *J. Food Prot.* 68:6–10.