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Stimulation of Starter Culture for Further Reduction of Foodborne Pathogens during Salami Fermentation

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ABSTRACT

This study was conducted to determine if stimulated meat starter culture (MSC; *Pediococcus acidilactici*) would further control *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Staphylococcus aureus* during salami fermentation. Manganese ion (0.005% of $MnSO_4$) was used as a stimulator for the growth and acid production of MSC. After 24-h salami fermentation, nonstimulated MSC and stimulated MSC reduced *E. coli* O157:H7 levels by 1.3 and 2.3 $\log_{10}$ units, respectively. Nonstimulated MSC reduced *L. monocytogenes* levels by 1.2 $\log_{10}$ units, whereas the stimulated MSC achieved a 2.2-$\log_{10}$ reduction after 24-h fermentation. In the case of *S. aureus,* nonstimulated MSC and stimulated MSC reduced *S. aureus* levels by 1.3 and 2.3 $\log_{10}$ units after 24-h fermentation, respectively. Stimulated MSC by $MnSO_4$ reduced those foodborne pathogens more effectively compared with nonstimulated MSC ($P < 0.05$).

Several reports have investigated *Escherichia coli* O157:H7 *(1, 5, 8, 9, 17), Listeria monocytogenes (12, 16, 22),* and *Staphylococcus aureus (2–4)* as important foodborne pathogens in fermented meat products. Outbreaks by these foodborne pathogens have made necessary the reexamination of current manufacturing practices, especially for products that do not receive any heat treatment. The safety of fermented dry sausage relies on the rapid growth of lactic acid bacteria (LAB) and a rapid pH decrease to minimize the risk of the growth of foodborne pathogen. Dry-cured salami is not cooked but is usually produced through fermentation followed by drying. Specific manufacturing processes may vary among companies and for different types of salami. For rapid growth of starter culture and rapid acid production, manganese ions have been reported and identified as strong stimulators for meat starter culture (MSC) *(19, 20).* Manganese ions are required in trace quantities for growth and metabolic activities of LAB *(20, 24).* They were found to accelerate growth and acid production by LAB *(24).* The effects of manganese ion on LAB include enhancement of lactic acid fermentation and protection from oxygen toxicity. To date, no studies have evaluated the indirect effect of manganese ion on the destruction of foodborne pathogens during salami fermentation. The effect of manganese ion for the further reduction of foodborne pathogens should be evaluated. This study aimed to demonstrate the effect of $MnSO_4$ (generally recognized as safe) as a manganese ion source on the growth and acid production of MSC (*Pediococcus acidilactici*), resulting in the potential inhibition of foodborne pathogens during meat fermentation.

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MATERIALS AND METHODS

Cultures and media used. *E. coli* O157:H7 (Eh 7-7), *L. monocytogenes* (Scott A), and *S. aureus* (S-18) were obtained from the Food Microbiology Culture Collection at Kansas State University. Each foodborne pathogen was transferred into brain heart infusion (BHI; Difco Laboratories, Detroit, Mich.) broth and incubated at 37°C aerobically for 24 h before use. The pathogens were stored on BHI slants at 4°C and transferred weekly. HP frozen starter culture (Diversitech Inc., Gainesville, Fla.) was used as MSC. *P. acidilactici* is the main LAB for HP starter culture. The frozen cultures were thawed before experiments. Sorbitol MacConkey agar plate (SMAC; Difco), *Listeria* selective agar (LSA; Oxoid, UK), and Baird-Parker agar (BP; Difco) were used for enumeration of *E. coli* O157:H7, *L. monocytogenes*, and *S. aureus*, respectively. Lactobacilli MRS (Difco) was used for enumeration of MSC. Plated SMAC, LSA, and BP were aerobically incubated at 37°C for 24 h, whereas plated MRS was anaerobically incubated at 37°C for 48 h. A stock solution of $MnSO_4$ (Sigma Chemical Co., St. Louis, Mo.) was prepared by adding 0.5 g to 10 ml of sterilized water. After mixing, the stock solution was filter sterilized through a 0.2-μm membrane filter.

Preparation of inoculum. Each foodborne pathogen was incubated into 9.0 ml of BHI broth and incubated at 37°C for 24 h. After incubation, 1 ml of each culture broth was transferred into two sterilized 500-ml polycarbonate centrifuge bottles (Nalgene, Rochester, N.Y.) containing 300 ml of BHI broth and incubated at 37°C for 24 h. Cells of each were harvested by centrifugation at 9,000 × *g* for 25 min at 4°C. Then the pellet was resuspended in sterile 0.1% peptone water. The resuspended cells were centrifuged and resuspended again as previously described.

Meat preparation. Salami samples were prepared with 15 kg of beef (75% lean, 25% fat at 10°C) obtained from the Meats Laboratory at Kansas State University (Manhattan, Kan.). The beef was first ground through a ⅜-in. and then through a ⅛-in. plate (Biro MFG. Co., Marblehead, Ohio) and transferred to a ribbon mixer (model I200DA70, Leland South West, Fort Worth, Tex.). The dry ingredients were mixed with meat for 2 min. The

TABLE 1. *pH changes and viable cell numbers (log CFU/g) of meat starter culture (MSC) and* Escherichia coli *O157:H7 during meat fermentation*[a]

	Treatments							
	E. coli[b]		*E. coli* mixed with nonstimulated MSC[c]			*E. coli* with stimulated MSC[d]		
Times (h)	*E. coli* (log CFU/g)	pH	MSC (log CFU/g)	*E. coli* (log CFU/g)	pH	MSC (log CFU/g)	*E. coli* (log CFU/g)	pH
0	7.01 ± 0.21	5.90 ± 0.02	7.10 ± 0.24	7.12 ± 0.31	5.90 ± 0.03	7.10 ± 0.26	7.11 ± 0.31	5.90 ± 0.02
8	6.98 ± 0.31	5.90 ± 0.04	6.75 ± 0.20	6.89 ± 0.16	5.80 ± 0.11	6.90 ± 0.24	6.85 ± 0.15	5.70 ± 0.14
16	6.79 ± 0.25	5.89 ± 0.11	8.63 ± 0.19	5.72 ± 0.21	4.80 ± 0.12	8.92 ± 0.31	5.40 ± 0.25	4.67 ± 0.10
24	6.40 ± 0.21	5.80 ± 0.11	8.90 ± 0.22	5.10 ± 0.20	4.45 ± 0.09	9.25 ± 0.23	4.00 ± 0.11	4.25 ± 0.11

[a] Data represent mean ± SD of three measurements.
[b] Pure culture system: *E. coli* O157:H7 inoculation.
[c] Mixed culture system: *E. coli* O157:H7 and nonstimulated MSC (*Pediococcus acidilactici*) mixed culture.
[d] Mixed culture system: *E. coli* O157:H7 and manganese ion (0.005% $MnSO_4$)–stimulated MSC (*P. acidilactici*) mixed culture.

following ingredients were added to meat: salt (2.3%), sucrose (1.5%), nonfat dry milk (1.5%), modern cure 0.2% (which contains 6.25% nitrite), black pepper (0.1%), garlic powder (0.1%), and knockwurst seasonings (0.8%).

Inoculation of individual pathogens. The meat batter was divided into three parts and separately inoculated with an overnight culture (37°C) of each foodborne pathogen culture. Before inoculation, the culture suspension of each pathogen was serially diluted in 0.1% peptone water. A preliminary study was performed with meat batter to establish dilution levels required to achieve inoculum levels of the pathogen of approximately 6.0 log CFU/g of meat. The inoculum was pipetted drop-wise over the entire surface area of the meat. The meat was then folded on top of itself and massaged with a gloved hand. Massaging was done for about 5 min to uniformly distribute the pathogen in the product. Meat inoculated with one type of pathogen was then separated into three parts. Three separated batches were treated with (i) each foodborne pathogen, (ii) foodborne pathogen with MSC, (iii) foodborne pathogen + MSC + 0.005% $MnSO_4$. MSC was mixed according to the manufacturer's instructions and added drop-wise to the batter. Three treated meat samples for each pathogen were stuffed into casings. Each small salami was approximately 50 g, 2.5 cm in diameter, and 7.5 cm in length.

Fermentation and sampling. The links fermented in the Alkar smoke house (Lodi, Wis.) for 24 h at 40°C in the condition at 96% humidity. Samples were taken at 0, 8, 16, and 24 h after incubation. The fermented products were cut using a alcohol-flamed knife *(11).* The pH of three samples for each pathogen-inoculated product was measured with a pH meter (Accumet 620, Fisher Scientifics, Pittsburgh, Pa.) after the meat was homogenized in 0.1% sterile peptone water (10-fold dilution). For viable cell counts, a 25-g sample was mixed with 225 ml of 0.1% peptone diluent in a stomacher bag and massaged in a stomacher (model 400, Seward Medical, London, England) for 2 min. The samples were 10-fold serially diluted and plated onto MRS for MSC (*P. acidilactici*), SMAC for *E. coli* O157:H7, LSA for *L. monocytogenes*, or BP for *S. aureus.* SMAC, LSA, and BP were aerobically incubated at 37°C for 24 h, whereas the plated MRS was anaerobically incubated at 37°C for 48 h.

Statistical analysis. Analysis of variance was performed on cell numbers and pH data using the general linear model procedure of SAS *(23).* Means of three replicates were reported in Tables 1 through 3. Cell counts were converted into logarithm values to determine the significance of differences at the 95% confidence limit ($P < 0.05$). Differences among treatments were examined for level of significance by Duncan's multiple range test.

RESULTS AND DISCUSSION

The growth of MSC and *E. coli* O157:H7 and pH changes were monitored through 24-h fermentation (Table

TABLE 2. *pH changes and viable cell numbers (log CFU/g) of meat starter culture and* Listeria monocytogenes *during meat fermentation*[a]

	Treatments							
	L. monocytogenes[b]		*L. monocytogenes* mixed with nonstimulated MSC[c]			*L. monocytogenes* with stimulated MSC[d]		
Time (h)	*L. monocytogenes* (log CFU/g)	pH	MSC (log CFU/g)	*L. monocytogenes* (log CFU/g)	pH	MSC (log CFU/g)	*L. monocytogenes* (log CFU/g)	pH
0	6.18 ± 0.13	5.90 ± 0.01	7.73 ± 0.11	6.13 ± 0.10	5.90 ± 0.03	7.70 ± 0.16	6.11 ± 0.15	5.90 ± 0.02
8	5.83 ± 0.23	5.90 ± 0.03	7.85 ± 0.21	5.83 ± 0.11	5.78 ± 0.11	7.94 ± 0.28	5.76 ± 0.14	5.70 ± 0.15
16	5.80 ± 0.19	5.89 ± 0.11	8.33 ± 0.26	5.60 ± 0.14	4.80 ± 0.14	8.50 ± 0.14	5.42 ± 0.27	4.67 ± 0.09
24	5.60 ± 0.17	5.80 ± 0.13	8.42 ± 0.23	5.02 ± 0.11	4.58 ± 0.11	8.71 ± 0.21	4.28 ± 0.21	4.40 ± 0.08

[a] Data represent mean ± SD of three measurements.
[b] Pure culture system: *L. monocytogenes* inoculation.
[c] Mixed culture system: *L. monocytogenes* and nonstimulated MSC (*Pediococcus acidilactici*) mixed culture.
[d] Mixed culture system: *L. monocytogenes* and manganese ion (0.005% $MnSO_4$)–stimulated MSC (*P. acidilactici*) mixed culture.

TABLE 3. *pH changes and viable cell numbers (log CFU/g) of meat starter culture (MSC) and* Staphylococcus aureus *during meat fermentation*[a]

	Treatments							
	S. aureus[b]		*S. aureus* mixed with nonstimulated MSC[c]			*S. aureus* with stimulated MSC[d]		
Time (h)	*S. aureus* (log CFU/g)	pH	MSC (log CFU/g)	*S. aureus* (log CFU/g)	pH	MSC (log CFU/g)	*S. aureus* (log CFU/g)	pH
0	6.02 ± 0.12	5.95 ± 0.19	7.07 ± 0.14	6.03 ± 0.21	5.90 ± 0.03	7.15 ± 0.16	6.11 ± 0.11	5.90 ± 0.07
8	6.34 ± 0.20	5.94 ± 0.03	7.10 ± 0.10	6.02 ± 0.13	5.60 ± 0.14	7.12 ± 0.21	5.97 ± 0.10	5.45 ± 0.13
16	7.02 ± 0.19	5.90 ± 0.09	8.55 ± 0.19	5.75 ± 0.15	4.80 ± 0.09	8.84 ± 0.15	5.48 ± 0.22	4.58 ± 0.12
24	7.20 ± 0.21	5.90 ± 0.10	8.87 ± 0.21	4.83 ± 0.21	4.50 ± 0.12	9.11 ± 0.25	3.82 ± 0.27	4.24 ± 0.12

[a] Data represent mean ± SD of three measurements.
[b] Pure culture system: *S. aureus* inoculation.
[c] Mixed culture system: *S. aureus* and nonstimulated MSC (*Pediococcus acidilactici*) mixed culture.
[d] Mixed culture system: *S. aureus* and manganese ion (0.005% $MnSO_4$)–stimulated MSC (*P. acidilactici*) mixed culture.

1). Without MSC, the salami pH changed from 5.9 to 5.8. Nonstimulated MSC reduced salami pH to 4.45 after 24-h fermentation, whereas manganese ion–stimulated MSC reduced the pH to 4.67 and 4.25 after 16- and 24-h fermentation, respectively. Manganese ion statistically significantly stimulated MSC to produce large amounts of acid in the presence of *E. coli* O157:H7. The numbers of *E. coli* O157:H7 were reduced by 1.3 $\log_{10}$ units by nonstimulated MSC, whereas the stimulated MSC reduced *E. coli* O157:H7 by 2.3 $\log_{10}$ units ($P < 0.05$). The acid produced by stimulated MSC reduced a higher number of *E. coli* O157:H7. Several reports demonstrated the effect of MSC on controlling *E. coli* O157:H7 *(6, 7, 13, 18).* The reduction of *E. coli* O157:H7 by MSC during meat fermentation depends on salami styles, kinds of MSC, and *E. coli* O157:H7 culture types. However, the reduction of the foodborne pathogen was about 1.0 $\log_{10}$ units by fermentation only *(6, 7, 13, 18).* Faith et al. *(10)* evaluated three kinds of meat batters (refrigerated, tempered/frozen/thawed, and frozen/thawed) to validate salami processing. The reductions of *E. coli* O157:H7 levels also ranged from 1.0 to 1.5 $\log_{10}$ units. As a preliminary experiment, manganese ion was evaluated for whether it could stimulate the foodborne pathogens. Manganese ion did not stimulate the growth of the foodborne pathogens (data not shown). Since early 1995, processors of dry and semidry fermented sausage have been required by the U.S. Department of Agriculture, Food Safety and Inspection Service to validate that processing of these products results in at least a 5-$\log_{10}$ reduction in *E. coli* O157:H7 levels *(21).* Therefore, manganese ion–stimulated MSC has a much stronger potential to achieve the target reduction of *E. coli* O157:H7 compared with nonstimulated MSC.

In the case of the inoculum of *L. monocytogenes,* pH decreased from 5.80 to 5.60 without MSC after 24-h fermentation. However, the pHs changed to 4.58 and 4.48 with nonstimulated and stimulated MSC, respectively. Manganese ion also stimulated MSC to reduce pH more quickly ($P < 0.05$) in the presence of *L. monocytogenes.* Initial counts of *L. monocytogenes* decreased from 6.18 to 5.60 $\log_{10}$ CFU/g at 40°C incubation for 24 h without MSC. However, *L. monocytogenes* levels changed to 5.02 and 3.98 $\log_{10}$ CFU/g with nonstimulated and stimulated MSC, respectively. Stimulated MSC further reduced *L. monocytogenes* levels by approximately 1.1 $\log_{10}$ units compared with nonstimulated MSC. Johnson et al. *(14)* also reported a 1.0-$\log_{10}$ reduction of *L. monocytogenes* after 24-h fermentation by nonstimulated MSC. *L. monocytogenes* levels were inhibited by stimulated MSC more easily than nonstimulated MSC ($P < 0.05$).

In the case of *S. aureus* inoculum, the initial pH changed from 5.95 to 5.90 (4.50 and 4.25 without MSA) with nonstimulated and stimulated MSC, respectively, after 24-h fermentation. These results also can be attributed to the stimulation of MSC. *S. aureus* levels reduced from 6.1 to 4.83 and 3.82 log CFU/g with nonstimulated and stimulated MSC, respectively. The numbers were significantly different between samples with and without manganese ion controlling for *S. aureus* during salami fermentation ($P < 0.05$). The moisture contents were not affected by the addition of manganese ion (data not shown) for each foodborne pathogen experiment.

As a preliminary experiment, we evaluated the meat to check background microflora. The samples were identified as *E. coli* O157:H7, *L. monocytogenes,* and *S. aureus* negative samples. Even though we used and evaluated one kind of each foodborne pathogen, the further reductions for the three foodborne pathogens were significant by stimulated MSC compared with nonstimulated MSA. As a previous report, we demonstrated the effect of manganese ion on the bacteriocin production by *P. acidilactici (15).* $MnSO_4$ strongly stimulated *P. acidilactici* to produce large amounts of bacteriocin. During salami fermentation, there were several antimicrobial ingredients (spices, NaCl, black pepper, and nitrite, etc.), which can inhibit the growth of those foodborne pathogens. These ingredients can interact with low acid and bacteriocin produced by stimulated MSC to further control those foodborne pathogens. Only 0.05% manganese ion strongly stimulated MSC to produce significantly high amounts of acid and bacteriocin for further reduction of three kinds of foodborne pathogens.

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REFERENCES

1. Besser, R. E., S. M. Lett, and J. T. Weber. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh pressed apple cider. JAMA 269:2217–2220.
2. Centers for Disease Control and Prevention. 1975. Staphylococcal food poisoning associated with dry salami. Morbid. Mortal. Weekly Rep. 24:374–379.
3. Centers for Disease Control and Prevention. 1980. An unusual outbreak of staphylococcal food poisoning associated with fermented salami–United States, p. 1–2. *In* Veterinary public health notes. Centers for Disease Control and Prevention, Atlanta.
4. Centers for Disease Control and Prevention. 1983. Staphylococcal food poisoning on a cruise ship. Morbid. Mortal. Weekly Rep. 32: 294–295.
5. Centers for Disease Control and Prevention. 1995. *Escherichia coli* O157:H7 outbreak linked to commercially distributed dry-cured salami–Washington and California. Morbid. Mortal. Weekly Rep. 44: 157–159.
6. Clavero, M. R. S., and L. R. Beuchat. 1996. Survival of *Escherichia coli* O157:H7 in broth and processed salami as influenced by pH, water activity, and temperature and suitability of media for its recovery. Appl. Environ. Microbiol. 62:2735–2740.
7. Clicioglu, M., N. G. Faith, D. R. Buege, and J. B. Luchansky. 1997. Viability of *Escherichia coli* O157:H7 in fermented, semidry, low-temperature-cooked beef summer sausage. J. Food Prot. 60:1158–1162.
8. Doyle, M. P. 1984. Hemorrhagic *Escherichia coli.* J. Food Prot. 47: 824–825.
9. Doyle, M. P. 1992. A new generation of foodborne pathogens. Dairy Food Environ. Sanit. 12:492–493.
10. Faith, N. G., N. Parniere, T. Larson, T. D. Lorang, C. W. Kaspar, and J. B. Luchansky. 1998. Viability of *Escherichia coli* O157:H7 in salami following conditioning of batter, fermentation and drying of sticks, and storage of slices. J. Food Prot. 61:377–382.
11. Fung, D. Y. C., R. K. Phebus, D. H. Kang, and C. L. Kastner. 1995. Effect of alcohol-flaming on meat cutting knives. J. Rapid Methods Automat. Microbiol. 3:237–243.
12. Glass, K. A., and M. P. Doyle. 1989. Fate and thermal inactivation of *Listeria monocytogenes* in beaker sausage and pepperoni. J. Food Prot. 52:226–231.
13. Glass, K. A., J. M. Loeffelholz, J. P. Ford, and M. P. Doyle. 1992. Fate of *Escherichia coli* O157:H7 as affected by pH or sodium chloride and in fermented, dry sausage. Appl. Environ. Microbiol. 58: 2513–2516.
14. Johnson, J. L., M. P. Doyle, R. G. Cassens, and J. L. Schoeni. 1988. Fate of *Listeria monocytogenes* in tissues of experimentally infected cattle and in hard salami. Appl. Environ. Microbiol. 54:497–501.
15. Kang, D. H., and D. Y. C. Fung. 1996. Effect of $MnSO_4$ and oxyrase on bacteriocin production by *Pediococcus acidilactici. In* Proceedings of Food Safety Consortium Annual Meeting, Kansas City, Mo.
16. Luchansky, J. B., K. A. Glass, K. D. Harsono, A. J. Degnan, N. G. Faith, B. Cauvin, G. Baccus-Taylor, K. Arihara, B. Bater, A. J. Maurer, and R. G. Cassens. 1992. Genomic analysis of *Pediococcus* starter cultures used to control *Listeria monocytogenes* in turkey summer sausage. Appl. Environ. Microbiol. 58:3053–3059.
17. Nancy, G. F., N. Parniere, T. Larson, T. D. Lorang, C. W. Kaspar, and J. B. Luchansky. 1998. Viability of *Escherichia coli* O157:H7 in salami following conditioning of batter, fermentation and drying of sticks, and storage of slices. J. Food Prot. 61:377–382.
18. Nickelson II, R., C. W. Kaspar, E. A. Johnson, and J. B. Luchansky. 1996. Update on dry fermented sausage and *Escherichia coli* O157: H7 validation research: an executive summary update by the Blue Ribbon Task Force of the National Cattlemen's Beef Association with the Food Research Institute, University of Wisconsin-Madison. Research Report no. 11-316. National Cattlemen's Beef Association, Chicago, Ill.
19. Raccach, M. December 1, 1981. U.S. patent 4,303,679.
20. Raccach, M. October 4, 1983. U.S. patent 4,407,383.
21. Reed, C. A. 1995. Challenge study: *Escherichia coli* O157:H7 in fermented sausage. U.S. Department of Agriculture, Food Safety and Inspection Service, Washington, D.C.
22. Sable, D., A. E. Yousef, and E. H. Marth. 1991. Behavior of *Listeria monocytogenes* during fermentation of beaker sausage made with or without starter culture and antioxidant food additives. Lebensm.-Wiss. Technol. 24:252–255.
23. SAS Institute. 1985. SAS user's guide: statistics, 5th ed. SAS Institute, Cary, N.C.
24. Woolley, D. W. 1941. Manganese and the growth of lactic acid bacteria. J. Biol. Chem. 140:311–312.