APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1981, p. 915-918
0099-2240/81/040915-04$02.00/0

Vol. 41, No. 4

# Bacterial Growth in Ground Beef Prepared from Electrically Stimulated and Nonstimulated Muscles†

J. L. BUTLER, G. C. SMITH, J. W. SAVELL, AND C. VANDERZANT*

*Department of Animal Science, Texas Agricultural Experiment Station, Texas A & M University, College Station, Texas 77843*

Received 11 September 1980/Accepted 26 January 1981

Ground beef samples prepared from electrically stimulated and nonstimulated biceps femoris and infraspinatus muscles were inoculated with *Lactobacillus* sp., *Pseudomonas* sp., *Acinetobacter* sp., or a mixture of *Lactobacillus* spp., *Pseudomonas* spp., *Acinetobacter* spp., *Moraxella* sp., *Microbacterium thermosphactum*, and *Erwinia herbicola*. There were no significant differences in growth of various bacteria in ground beef made from electrically stimulated and nonstimulated muscles.

Electrical stimulation of beef and lamb carcasses is now applied widely in the meat industry as a means for improving tenderness of meat (4, 6). Other benefits of electrical stimulation include increased firmness of lean, brighter lean color, and earlier development of marbling (5). Reports on the effect of electrical stimulation on the bacteriological condition of meat are not consistent (1-3). According to Raccach and Henrickson (3), electrical stimulation prolonged the lag phase of the bacterial population of ground beef, but enhanced its growth rate between days 3 and 5 of refrigerated storage. The shelf life of electrically stimulated ground beef at 5°C was extended by 3 days as compared with that of the nonstimulated control (3). Mrigadat et al. (2) reported that electrical stimulation of pork carcasses did not affect the aerobic plate count of the skin surface. Aerobic plate counts of cutaneous trunci muscles from electrically stimulated sides of beef and lamb carcasses were similar to those of muscles from nonstimulated sides of carcasses. Aerobic plate counts of ground beef and steaks (blade steaks, rib steaks, and T-bone steaks) from stimulated sides were often numerically lower than those of corresponding samples from nonstimulated sides (2); however, differences in aerobic plate counts between electrically stimulated and control samples usually were not significant ($P > 0.05$). Electrical stimulation did not cause any consistent, substantial changes in microbial types found in ground beef or steaks (2). Gill (1) reported on the development of spoilage bacteria on electrically stimulated and nonstimulated mutton legs and in minces prepared from either type of leg with either the natural microflora or after inoculation with *Pseudomonas fluorescens*. There was no difference in lag phase, growth rate, or maximum cell density of the bacteria between electrically stimulated and control samples (1). The present study compares the growth of various bacteria commonly isolated from fresh meats in ground beef from electrically stimulated and nonstimulated muscles.

† Technical article no. 16273 from the Texas Agricultural Experiment Station, College Station, Tex.

## MATERIALS AND METHODS

**Meat samples.** Ten cross-bred steers (279 to 378 kg) were processed by normal slaughter dressing procedures in the Texas A & M University Meats Laboratory. Immediately after splitting of the carcass, one side was electrically stimulated with 16 pulses (1.8 s duration each, with a 1.8-s interval between pulses) at 550 V, 5 A of alternating current. The other side of each carcass served as the nonstimulated control. The electrical stimulation source was an experimental "Lectro-Tender" unit manufactured by the LeFiell Co., San Francisco, Calif. After storage for 2 h in a cooler (1 to 3°C), the biceps femoris and infraspinatus muscles were removed from both the electrically stimulated and control (nonstimulated) sides of each carcass. The pH value of each muscle was determined with a pH meter (Corning model 12) by using a 3-g sample macerated with 25 ml of 0.005 M iodoacetate in a blender for 1 min. The exterior part of each muscle was burned thoroughly with a gas flame to destroy microorganisms on the surface of the samples. The burned exterior part of the sample was removed with sterile instruments. The interior portion of the sample was cut aseptically with sterile scalpels into pieces (5 by 5 by 5 cm) and ground in a sterile grinder (model H; General Co., Walden, N.Y.). Inoculated and noninoculated (control) portions (10 g) of the ground meat were placed on disposable micro-weigh boats (Vanlab; VWR Scientific Inc., San Francisco, Calif.) and were overwrapped with polyvinyl chloride film. Both inoculated and control samples were stored at 5°C for 0, 1, 3, 5, 7, or 9 days.

**Microbiological procedures.** Cultures used in this study were *Lactobacillus* spp. 49 and 642, *Pseudomonas* spp. 148, *Acinetobacter* sp. 4, and a mixture consisting of *Lactobacillus* spp. 49 and 642; *Pseudomonas* spp. 98, 102, and 148; *Acinetobacter* spp. 4 and 59; *Microbacterium thermosphactum* 78; *Erwinia herbicola* 139, and *Moraxella* sp. 65. These cultures were isolated from raw beef in a previous study (7). They were maintained on tryptic soy agar (Difco Laboratories, Detroit, Mich.) slants at 25°C. Inocula for the experiments were prepared by placing a loopful of the culture from a 24-h tryptic soy agar slant into a tube with 5 ml of sterile brain heart infusion broth (Difco). For the *Lactobacillus* sp., lactobacillus MRS broth (Difco) was used instead of brain heart infusion broth. Broth cultures were incubated overnight at 25°C.

Inocula representing individual species were added to the ground meat to give an initial concentration of approximately $10^4$ cells per g. The inoculum, consisting of a mixture of bacteria, was prepared by mixing broth cultures of individual species so that the concentrations of the individual species in the mixture were approximately the same, and the total cell concentration in the inoculated ground meat was approximately $10^4$ cells per g. At each sampling interval, a sample (10 g) was placed in a Stomacher bag with 90 ml of sterile 0.1% peptone broth (Difco) and macerated for 1 min in a Stomacher 400. Plating was done by spreading 0.1-ml portions of appropriate dilutions (0.1% peptone broth) onto prepoured plates of tryptic soy agar. Plates were incubated for 3 days at 25°C. Statistical analyses of the data were made by using two-way analysis of variance (8).

## RESULTS AND DISCUSSION

pH values of electrically stimulated muscles (2 h after stimulation) were significantly ($P < 0.05$) lower than those of nonstimulated muscles. Differences in pH value between stimulated and nonstimulated muscles ranged from 0.2 to 1.11 and from −0.06 to 0.63 for biceps femoris and infraspinatus muscles, respectively. Counts of the *Lactobacillus* spp. and *Pseudomonas* sp. in ground beef increased during storage under refrigeration (Table 1). Increases in count were somewhat larger with *Lactobacillus* sp. 642 and *Pseudomonas* sp. 148 than with *Lactobacillus* sp. 49. On the other hand, counts of *Acinetobacter* sp. 4 in minced beef decreased during refrigerated storage. Total counts of ground beef inoculated with a mixture of various bacteria increased during refrigerated storage. Examination of individual isolates from countable plates of ground beef inoculated with various bacteria showed that increases in count occurred because of growth of *Lactobacillus* spp. and *Pseudomonas* spp.

TABLE 1. *Mean log counts of ground beef from electrically stimulated (ES) and nonstimulated (Not-ES) muscles inoculated with various bacteria and stored under refrigeration for up to 9 days*

| Microorganism | Muscle[a] | Treatment | Mean log count per g on day: | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 0 | 1 | 3 | 5 | 7 | 9 |
| *Lactobacillus* sp. 642 | BF | ES | 4.22 | 4.61 | 5.85 | 7.22 | 7.96 | 8.22 |
| | BF | Not-ES | 4.37 | 4.65 | 5.81 | 7.24 | 7.86 | 8.23 |
| | IS | ES | 4.30 | 4.62 | 6.15 | 7.39 | 7.99 | 8.21 |
| | IS | Not-ES | 4.36 | 4.62 | 5.96 | 7.28 | 8.00 | 8.01 |
| *Lactobacillus* sp. 49 | BF | ES | 4.49 | 4.66 | 4.59 | 4.60 | 4.78 | 5.17 |
| | BF | Not-ES | 4.62 | 4.61 | 4.73 | 4.70 | 5.04 | 5.34 |
| | IS | ES | 4.27 | 4.76 | 5.31 | 6.01 | 6.71 | 6.96 |
| | IS | Not-ES | 4.53 | 4.62 | 5.25 | 6.13 | 6.60 | 6.91 |
| *Pseudomonas* sp. 148 | BF | ES | 4.08 | 4.16 | 5.74 | 7.90 | 8.45 | 9.17 |
| | BF | Not-ES | 4.25 | 4.23 | 5.96 | 8.01 | 9.09 | 8.89 |
| | IS | ES | 4.21 | 4.41 | 6.91 | 8.98 | 9.69 | 9.93 |
| | IS | Not-ES | 4.11 | 4.47 | 6.70 | 8.82 | 9.36 | 9.86 |
| *Acinetobacter* sp. 4 | BF | ES | 4.32 | 4.18 | 4.12 | 3.79 | 3.74 | 3.51 |
| | BF | Not-ES | 4.19 | 4.20 | 4.00 | 3.90 | 3.81 | 2.83 |
| | IS | ES | 4.49 | 4.33 | 4.25 | 4.11 | 4.00 | 3.05 |
| | IS | Not-ES | 4.34 | 4.27 | 4.31 | 4.23 | 4.12 | 3.31 |
| Mixture[b] | BF | ES | 4.59 | 4.72 | 5.74 | 7.46 | 8.49 | 8.77 |
| | BF | Not-ES | 4.69 | 4.71 | 5.57 | 7.03 | 8.52 | 8.78 |
| | IS | ES | 4.55 | 5.00 | 6.11 | 8.21 | 9.13 | 9.50 |
| | IS | Not-ES | 4.61 | 4.92 | 6.30 | 8.16 | 9.00 | 9.37 |

[a] BF, Biceps femoris; IS, infraspinatus.

[b] Mixture consisted of two *Lactobacillus* spp., three *Pseudomonas* spp., two *Acinetobacter* spp., one *Moraxella* sp., *M. thermosphactum*, and *E. herbicola*.

Analysis of the data (Table 2) showed that differences in counts of inoculated ground beef from electrically stimulated and nonstimulated muscles were not significant ($P > 0.05$) for any of the individual bacteria or for the mixture of bacterial species tested. The results of the analysis (Table 2) also support the data presented in Table 1 in which there were large increases in count during refrigerated storage of ground beef inoculated with *Lactobacillus* sp. 642 and *Pseudomonas* sp. 148, less extensive growth of *Lactobacillus* sp. 49, and no significant change in count of *Acinetobacter* sp. 4.

Since there were no significant differences in count between inoculated ground beef samples from electrically stimulated and nonstimulated muscles, a two-way analysis of variance was run to test for differences in bacterial growth between muscles (Table 3). Counts of *Lactobacillus* sp. 49, *Pseudomonas* sp. 148, and the mixture of bacterial species in ground beef prepared from the biceps femoris were significantly different from counts of comparable samples prepared from the infraspinatus muscle. In summary, the data of the present study show no differences in growth of various bacteria in ground beef from electrically stimulated and nonstimulated muscles. These results are in general agreement with those reported by Gill (1) and Mrigadat et al. (2).

TABLE 2. *Comparison of growth of various bacteria in electrically stimulated and nonstimulated ground beef stored at 5°C for up to 9 days*

| Microorganism | Muscle[a] | Two-way analysis of variance | | | | |
|---|---|---|---|---|---|---|
| | | Total df | Mean squares | | | Error |
| | | | Treatment[b] (df = 1) | Days[c] (df = 5) | Interaction (df = 5) | |
| *Lactobacillus* sp. 642 | BF | 119 | 0.005266 | 56.626023[d] | 0.033263 | 0.069733 |
| | IS | 119 | 0.155362 | 55.604116[d] | 0.062108 | 0.098607 |
| *Lactobacillus* sp. 49 | BF | 119 | 0.488236 | 1.384385 | 0.051533 | 0.621933 |
| | IS | 119 | 0.001051 | 21.858811[d] | 0.125319 | 0.551726 |
| *Pseudomonas* sp. 148 | BF | 119 | 0.738842 | 98.755282[d] | 0.442716 | 0.507266 |
| | IS | 119 | 0.533240 | 130.263846[d] | 0.089220 | 0.321668 |
| *Acinetobacter* sp. 4 | BF | 117 | 0.003845 | 1.481888 | 1.529813 | 1.851232 |
| | IS | 116 | 0.101783 | 0.495361 | 0.120662 | 2.273588 |
| Mixture[e] | BF | 119 | 0.174918 | 68.352925[d] | 0.184345 | 1.102676 |
| | IS | 119 | 0.017000 | 88.901191[d] | 0.076745 | 0.391700 |

[a] BF, Biceps femoris; IS, infraspinatus.
[b] Treatment reflects the difference between $\log_{10}$ bacterial counts for electrically stimulated ground beef and nonstimulated samples.
[c] Takes into account storage of ground beef for 0, 1, 3, 5, 7, or 9 days.
[d] Mean square value is significant at $P \leq 0.01$.
[e] The mixture of microorganisms consisted of two *Lactobacillus* spp., three *Pseudomonas* spp., two *Acinetobacter* spp., *M. thermosphactum*, *E. herbicola*, and one *Moraxella* sp.

TABLE 3. *Comparison of growth of various bacteria in ground beef prepared from different muscles and stored at 5°C for up to 9 days*

| Microorganism | Two-way analysis of variance | | | | |
|---|---|---|---|---|---|
| | Total df | Mean squares | | | Error |
| | | Muscle[a] (df = 1) | Days[b] (df = 5) | Interaction (df = 5) | |
| *Lactobacillus* sp. 642 | 239 | 0.175895 | 112.100084[c] | 0.130055 | 0.082536 |
| *Lactobacillus* sp. 49 | 239 | 48.053177[d] | 16.220516 | 7.022680[c] | 0.561968 |
| *Pseudomonas* sp. 148 | 239 | 23.384218[d] | 227.348885[c] | 1.670243[c] | 0.409898 |
| *Acinetobacter* sp. 4 | 233 | 3.917934 | 1.869697 | 0.107552 | 1.996916 |
| Mixture[e] | 239 | 13.985944[c] | 156.061428[c] | 1.192688 | 0.714429 |

[a] The muscles used to make the meat samples included the biceps femoris and infraspinatus.
[b] Takes into account storage of ground beef for 0, 1, 3, 5, 7, or 9 days.
[c] Mean square value is significant at $P \leq 0.01$.
[d] Mean square value is significant at $P \leq 0.05$.
[e] The mixture of microorganisms consisted of two *Lactobacillus* spp., three *Pseudomonas* spp., two *Acinetobacter* spp., *M. thermosphactum*, *E. herbicola*, and one *Moraxella* sp.

## ACKNOWLEDGMENTS

This research was supported in part by the LeFiell Company, San Francisco, Calif., the King Ranch, Kingsville, Tex., and the Oscar Schmidt Meat Research Development Fund.

## LITERATURE CITED

1. **Gill, C. O.** 1980. Effect of electrical stimulation on meat spoilage floras. J. Food Prot. **43**:190–194.
2. **Mrigadat, B., G. C. Smith, T. R. Dutson, L. C. Hall, M. O. Hanna, and C. Vanderzant.** 1980. Bacteriology of electrically stimulated and unstimulated rabbit, pork, lamb and beef carcasses. J. Food Prot. **43**:686–693.
3. **Raccach, M., and R. L. Henrickson.** 1978. Storage stability and bacteriological profile of refrigerated ground beef from electrically-stimulated hot-boned carcasses. J. Food Prot. **41**:957–960.
4. **Savell, J. W.** 1979. Industry acceptance of electrical stimulation, p. 113–117. *In* Proceedings of the 32nd Annual Reciprocal Meat Conference, vol. 32. National Livestock and Meat Board, Chicago.
5. **Savell, J. W., G. C. Smith, and Z. L. Carpenter.** 1978. Effect of electrical stimulation on quality and palatability of light-weight beef carcasses. J. Anim. Sci. **46**: 1221–1228.
6. **Savell, J. W., G. C. Smith, T. R. Dutson, Z. L. Carpenter, and D. A. Suter.** 1977. Effect of electrical stimulation on palatability of beef, lamb and goat meat. J. Food Sci. **42**:702–706.
7. **Seideman, S. C., C. Vanderzant, M. O. Hanna, Z. L. Carpenter, and G. C. Smith.** 1976. Effect of various types of vacuum packages and length of storage on the microbial flora of wholesale and retail cuts of beef. J. Milk Food Technol. **39**:745–753.
8. **Snedecor, G. W.** 1956. Statistical methods, 5th ed. The Iowa State College Press, Ames.