

**Final Report:**  
**Anti-Listeria Action of Levulinate**  
Carpenter and Broadbent, Co-PIs  
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**Overview of project**

Levulinic acid (4-oxopentanoic acid) is a commercially available 5-carbon organic acid that has GRAS (Generally Recognized as Safe) status for direct addition to food as a flavoring agent or adjunct (21 CFR, 172.515). Our previous research established that addition of 1.4% sodium levulinate in fresh sausage inhibited growth of aerobic bacteria to the same degree as 2.7% sodium lactate (Vasavada et al. 2003. J. Muscle Foods 14:119-129). This study validated the use of levulinate as an antilisterial additive for ready to eat meat products.

The specific objectives of this study were to:

**Objective 1.** Evaluate the extent to which sodium levulinate inhibits growth of *Listeria monocytogenes* in ready-to-eat meat products as compared to the current industry standards of sodium lactate and sodium lactate/diacetate mixture.

**Objective 2.** Establish the impact of sodium levulinate on the sensory acceptability of ready-to-eat meat products as compared to the current industry standards of sodium lactate and sodium lactate/diacetate mixture.

**Results**

***Listeria monocytogenes.*** The following strains were obtained from ILSI North American Database housed at Cornell University with the approval of Dr. Martin Wiedmann and AMIF.

- **FSL J1-177**; ribotype DUP-1051D; lineage I; serotype 1/2b; isolated from human sporadic case.
- **FSL C1-056**; ribotype DUP-1030A; lineage II; serotype 1/2a; isolated from human sporadic case.
- **FSL N3-013**; ribotype DUP-1042B; lineage I; serotype 4b; food isolate associated with human listeriosis epidemic in the UK (1988-1990)
- **FSL R2-499**; ribotype DUP-1053A; lineage II; serotype 1/2a; human isolate associated with US outbreak linked to sliced turkey (2000).
- **FSL N1-227**; ribotype DUP-1044A; lineage I; serotype 4b; food isolate associated with US outbreak (1998-1999).

The identity of the bacterial strains was confirmed by Gram staining and plating on Bio-Rad RAPID'L Mono selective agar. Also, we isolated template DNA from each strain and performed PCR with universal primers for 16S ribosomal DNA. PCR products were purified using Amicon Micron PCR centrifugal filter devices, then sequenced. The

strains were identified according to their 16S ribosomal DNA sequence (performed by MC Labs, South San Francisco, CA). The results from each of these tests confirmed the expected identity all strains.

**Objective 1.** Turkey breast roll and bologna were prepared to contain (v/wt) sodium lactate (2%); sodium lactate in combination with sodium diacetate (1.875% sodium lactate, 0.125% sodium diacetate); sodium levulinate (1, 2, or 3%); or no antilisterial additive. Samples were sliced, inoculated with a 5-strain cocktail ( $10^2$  to  $10^3$  CFU/cm<sup>2</sup>) of *L. monocytogenes*, vacuum packaged, and stored at 2°C for 0-12 weeks. Two trials were performed for each turkey roll and bologna. Results of these experiments are reported in Figures 1 and 2, and in Tables 1 and 2, respectively.

Bacterial counts recovered from turkey controls exceeded  $10^8$  CFU/cm<sup>2</sup> after eight weeks, while in the bologna controls they exceeded  $10^7$  CFU/cm<sup>2</sup> in 12 weeks. Sodium lactate prevented growth of *L. monocytogenes* above inoculation levels for four weeks in turkey, and for 12 weeks in bologna. Sodium lactate combined with sodium diacetate prevented growth for eight weeks in turkey, and for 12 weeks in bologna. In turkey breast, 1% sodium levulinate prevented growth up to six weeks, while 2 and 3% prevented growth for 12 weeks. In bologna, sodium levulinate (1, 2, and 3%) completely inhibited growth of *L. monocytogenes* for 12 weeks.

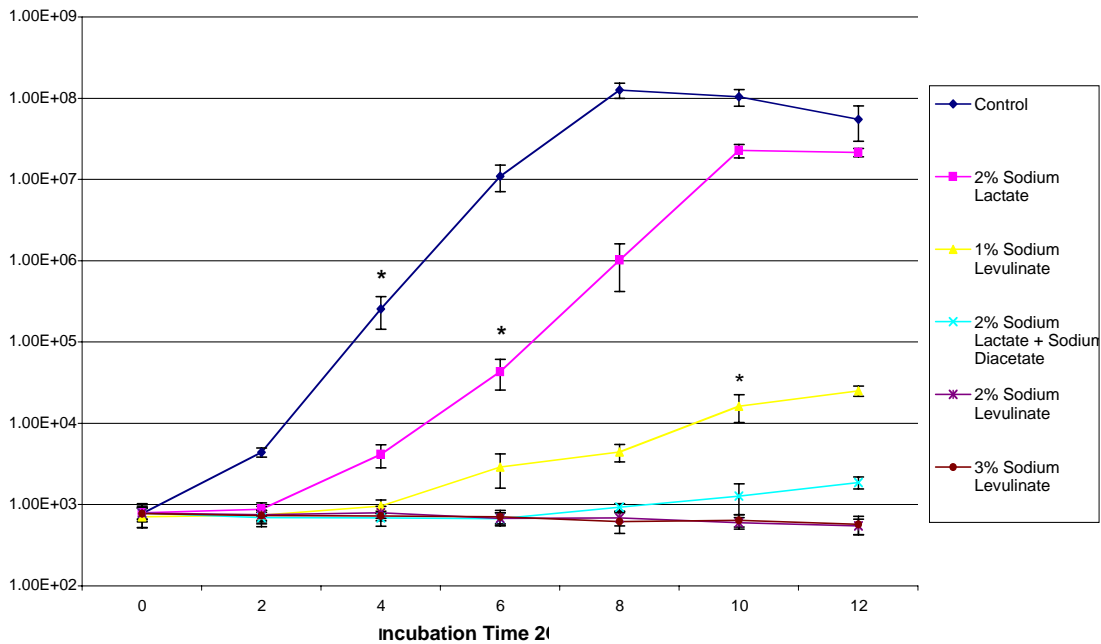


Figure 1. Growth of *L. monocytogenes* on turkey breast roll incubated for up to 12 weeks at 2°C. \* Indicates the first time when growth was significantly higher than original inoculation levels.

| Treatment Type             | First growth (weeks) | Mean Recovery (log) |
|----------------------------|----------------------|---------------------|
| No Antimicrobial           | 4                    | 7.63 <sup>a</sup>   |
| 2% Sodium Lactate          | 6                    | 6.81 <sup>b</sup>   |
| Sodium Lactate + Diacetate | 12 +                 | 2.99 <sup>cd</sup>  |
| 1% Sodium Levulinate       | 10                   | 3.86 <sup>c</sup>   |
| 2% Sodium Levulinate       | 12 +                 | 2.84 <sup>d</sup>   |
| 3% Sodium Levulinate       | 12 +                 | 2.83 <sup>d</sup>   |

Table 1. Growth of *L. monocytogenes* on turkey breast roll incubated for up to 12 weeks at 2°C. First growth is storage time at which *L. monocytogenes* was recovered at levels greater than inoculated ( $p < 0.01$ ). Mean recoveries are over the entire 12 wks of storage, and values sharing the same superscript letters are not different ( $p > 0.01$ ).

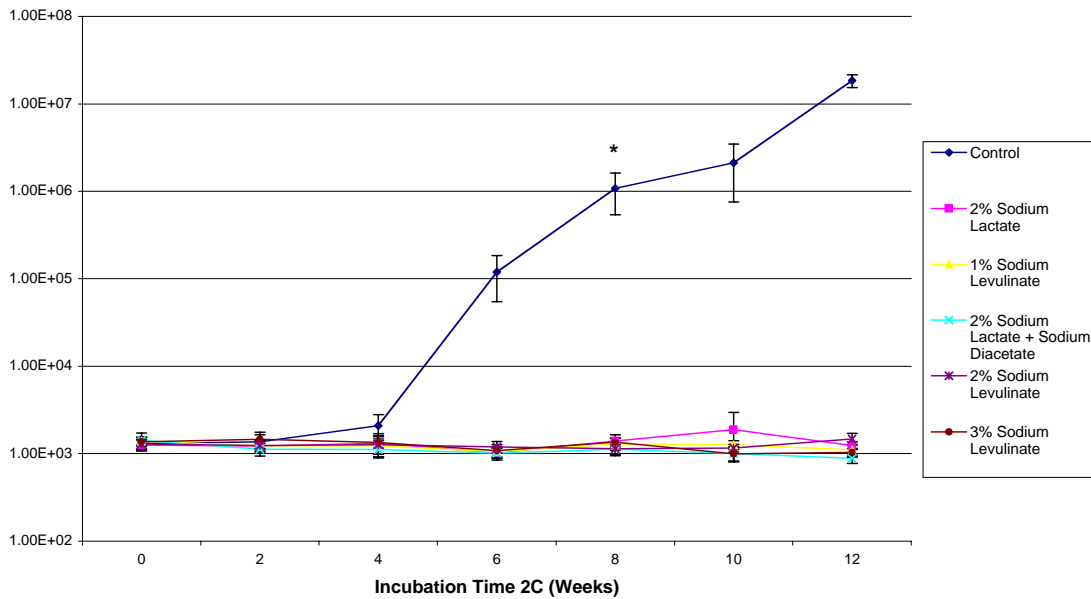


Figure 2. Growth of *L. monocytogenes* on bologna incubated for up to 12 weeks at 2°C. \* Indicates the time when growth was significantly higher than inoculation levels.

| <b>Treatment Type</b>      | <b>First growth (weeks)</b> | <b>Mean Recovery (log)</b> |
|----------------------------|-----------------------------|----------------------------|
| No Antimicrobial           | 8                           | 3.12E+06 <sup>a</sup>      |
| 2% Sodium Lactate          | 12 +                        | 1.33E+03 <sup>b</sup>      |
| Sodium Lactate + Diacetate | 12 +                        | 1.10E+03 <sup>b</sup>      |
| 1% Sodium Levulinate       | 12 +                        | 1.22E+03 <sup>b</sup>      |
| 2% Sodium Levulinate       | 12 +                        | 1.25E+03 <sup>b</sup>      |
| 3% Sodium Levulinate       | 12 +                        | 1.23E+03 <sup>b</sup>      |

Table 2. Growth of *L. monocytogenes* on bologna incubated for up to 12 weeks at 2°C. First growth is storage time at which *L. monocytogenes* was recovered at levels greater than inoculated ( $p < 0.01$ ). Mean recoveries are over the entire 12 wks of storage, and values sharing the same superscript letters are not different ( $p > 0.01$ ).

**Objective 2.** A sensory panel consisting of anonymous consumers rated turkey and bologna samples for their overall liking of the products. Samples were scored on a scale of 1 to 9, with 1 = strongly dislike, 5 = neither like nor dislike, and 9 = strongly like. In the turkey roll panel, judges were given five samples including no antimicrobial, 2% sodium lactate, 2% sodium lactate plus sodium diacetate, 2% sodium levulinate, and 3% sodium levulinate. They were not given a sample containing 1% sodium levulinate because it had not inhibited growth of *L. monocytogenes* for the twelve week challenge study. In the bologna analysis, the consumers were also given 5 samples to taste and score according to overall liking of the product: control containing no antimicrobial, 2% sodium lactate, the 2% combination of sodium lactate and sodium diacetate, 1% sodium levulinate and 2% sodium levulinate. A product containing 3% sodium levulinate was not analyzed because it was no more inhibitory to growth *L. monocytogenes* than 1% and 2% sodium levulinate.

There were 132 consumers that participated in the sensory panel for the turkey breast roll and 112 for the bologna. Of those who participated in the turkey breast panel, 66 were female and 66 were male. There was a wide range of age among the participants, with the majority being in the 18 to 25 age group). Similar demographic data was found among the participants of the bologna sensory panel. There were 58 females and 54 males, most of who were in the age group 18 to 25.

Results of the sensory studies are given in Table 3. There were no differences in overall liking among the preparations of turkey breast roll or bologna.

|                | Control | 2% Sodium Lactate | 2% combined Sodium Lactate/Diacetate | 1% Sodium Levulinate | 2% Sodium Levulinate | 3% Sodium Levulinate | p-Value |
|----------------|---------|-------------------|--------------------------------------|----------------------|----------------------|----------------------|---------|
| <b>Turkey</b>  | 6.8     | 6.9               | 6.8                                  | NA                   | 6.6                  | 6.6                  | 0.19    |
| <b>Bologna</b> | 6.2     | 6.2               | 6.2                                  | 6.0                  | 6.0                  | NA                   | 0.42    |

Table 3. Mean scores for overall liking of turkey breast roll and bologna (1 = strongly dislike, 5 = neither like nor dislike, and 9 = strongly like.)

### Impact of research

Verification measures used by FSIS to determine the effectiveness of antimicrobial agents are based on the suppression of *L. monocytogenes* growth during the refrigerated shelf life of the product. Agents that allow more than 2 log growth are generally not eligible as antimicrobials, while agents that allow  $\leq 1$  log growth are classified as antimicrobial. Products containing antimicrobials may be considered for the least sampling regimens as stipulated by FSIS. Our study employed an 84 day storage at 2°C for vacuum packaged slices of turkey roll and bologna. Under these conditions, sodium levulinate and the mixture of sodium lactate and diacetate met FSIS expectations for an antimicrobial additives in both bologna and turkey roll. Sodium lactate met antimicrobial expectations only in bologna, but not in the turkey roll. Addition of antimicrobials (sodium lactate, sodium lactate plus diacetate, or sodium levulinate) were not detrimental to the sensory acceptability of turkey roll or bologna.

### Dissemination of information

Thompson, R. L., J. Broadbent, C. Carpenter, and S. Martini. 2007. *Listeria monocytogenes* control in ready-to-eat meats. Proc. IFT annual Meeting (Abstract O48-02)