Role of Lactic Acid Bacteria, Curing Salts, Spices and Temperature in Controlling the Growth of Yersinia enterocolitica

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(Received for publication July 15, 1983)

ABSTRACT

Growth of Yersinia enterocolitica 0.3 and 0.8 (10³ CFU/g) in cured meat at 35°C was controlled (inhibition of 3.9 to 4.0 \log_{10} CFU/g) by each one of the lactic acid bacteria (LAB) Pediococcus pentosaceus, Pedicoccus acidilactici and Lactobacillus plantarum. The pH of the meat was reduced by LAB to 4.9 to 5.1. At 27°C, growth of Y. enterocolitica 0.3 and 0.8 (10³ CFU/g) in cured meat was almost totally controlled with or without LAB. This inhibition of growth was observed with populations of Y. enterocolitica up to 10⁶ CFU/g of meat. In plain meat (devoid of any additive) at 27°C, LAB inhibited up to 10⁶ CFU/g of meat. Spices, garlic powder and white pepper did not control the growth of either serotype of Y. enterocolitica. The pH of the meat was reduced by LAB to 2.3 to 3.6 \log_{10} CFU/g of growth of Y. enterocolitica 0.3 and 0.8. Sodium nitrite (156 mg/kg), at a concentration about 200 times lower than that of sodium chloride (3.0%), was as efficient an inhibitor to Y. enterocolitica as sodium chloride. Dextrose was slightly inhibitory to Y. enterocolitica 0.3 only. Spices, garlic powder and white pepper did not control the growth of either serotype of Y. enterocolitica. A temperature of 27°C in combination with either curing salts or LAB played an important role in controlling the growth of Y. enterocolitica in meat thus contributing to the safety of the product.

Yersinia enterocolitica is widely distributed in the environment (25) and human infections have been reported worldwide (14). In the U.S., one outbreak of foodborne (chocolate milk) yersiniosis has been fully documented (3). In another outbreak, water used in the processing of tofu (soybean curd) was incriminated as the source of Y. enterocolitica infection associated with tofu consumption (1). Y. enterocolitica cells possess lipopolysaccharide O-antigens which are the basis for classification into serotypes (31). Y. enterocolitica serotype 0.3 predominates in eastern Canada, Europe, Japan and South Africa, whereas serotype 0.8 predominates in the USA and western Canada (4). Both serotypes (0.3 and 0.8) are predominant human isolates (7, 22, 28, 32). Swine has been implicated as a major reservoir of Y. enterocolitica serotypes involved in human infections, including 0.3 and 0.8 (7, 8, 30). Indeed, potentially pathogenic Y. enterocolitica have been isolated from retail pork products (10, 13, 20, 23). Pork alone or in combination with other muscle foods is a major ingredient in the formulation of sausage (11). Certain types of fermented sausage, such as some dry sausages, are not heat-treated (11). Thus, the bacteriological stability and safety of these products depends on antimicrobial agents added or formed in the product. The purpose of this study was to evaluate the role of lactic acid bacteria (LAB), curing salts, spices and temperature in controlling the growth of Y. enterocolitica in meat.

MATERIALS AND METHODS

Bacterial strains

The LAB used in this study were Pediococcus pentosaceus (ATCC 10791), Pedicoccus acidilactici (NRRL B5624) and Lactobacillus plantarum (NRRL B5632). The two strains of Y. enterocolitica used were serotype 0.3 (obtained from H. H. Mollaret, Pasteur Institute, Paris), a human isolate from Europe, and serotype 0.8 (ATCC 27729; P. B. Carter strain WA) isolated from the blood of a human patient (5, 6). Both strains autoagglutinated at 35°C but not at 25°C. The autoagglutination test was performed according to the method of Laird and Cavanaugh (12) as modified by Aulisio et al. (1). The results of the autoagglutination test were in agreement with those of Stern and Damarè (24).

Preparation of LAB frozen concentrates

Each LAB was subcultured (35°C, 18 h) three consecutive times in All Purpose Tween (APT) broth (Difco). The third transfer was used as inoculum (1% vol/vol) for the preparation of culture concentrates. After an 18 h of incubation, the cells were centrifuged (16,300 × g, 10 min, 4°C), collected, and resuspended to one-tenth the original volume using sterile APT broth. Sterile glycerol (1.1 M) was added to the suspending broth as a cryoprotective agent. The cells were dispensed in 2-ml portions and stored in screw-top glass vials (6.5 cm × 1.7 cm). The concentrated cultures were frozen and stored (−80°C) in a Rheem Ultra Low Temperature freezer (Rheem Inc., Ashville, NC). The frozen culture concentrates had the following counts (APT agar, 35°C, 48 h): P. pentosaceus, 1.6 × 10⁶ CFU/ml; P. acidilactici, 2.2 × 10⁶ CFU/ml; and L. plantarum 1.6 × 10⁶ colony-forming unit (CFU)/ml. When needed, the cultures were removed from the freezer and allowed to thaw in tap water (2 to 3 min). The cultures were then diluted (1:10) using peptone water (0.1% peptone wt/vol) and inoculated into the meat to obtain a final concentration of ca. 3.0 × 10³ CFU/g.
Preparation of Yersinia enterocolitica
The organism was maintained on brain heart infusion (BHI) agar (Difco) slants (4°C) and subcultured three consecutive times in BHI broth. The culture was incubated statically at 27°C for 12 to 16 h. Incubation (1% vol/vol) from the third transfer was used to prepare the culture for the experiment work. After 14 h at 27°C, the culture (ca. 10^6 CFU/ml) was added to the meat to provide final concentrations ranging from 10^3 to 10^6 CFU/g.

Preparation of the meat system
A fermented sausage-like product was prepared from pork and, in some experiments, was formulated with the following additives (% w/w): NaCl, 3.0; dextrose, 1.0; NaN_2, 0.0156; garlic powder, 0.016; and whole white pepper, 0.02. The meat was ground using an Integre Model C3 grinder (0.5-cm openings), and heated (121°C, 30 min) to avoid any effect of the meat indigenous flora on Y. enterocolitica. The additives were then mixed with the meat and portions (50 g) of the meat system (meat and additives) were placed into 100-ml sterile beakers covered tightly with aluminum foil.

Treatments
Each of the LAB was examined for its ability to control the growth of each of two strains of Y. enterocolitica in the meat system. The meat system was prepared with (35 and 27°C) and without (27°C) the additives. The meat system was prepared with Y. enterocolitica. Controls (Y. enterocolitica alone without LAB or additives) were used to assess the effect of each treatment. The treatments and controls were incubated at temperatures used in commerce 35°C (M. Raccach, Proc. 26th Eur. Meet. Res. Workers, Am. Meat Sci. Assoc. 1980, vol. 2, pp. 335-338), or 27°C (J8) for 12 h in a Forma Scientific incubator (water jacketed, model 3114).

Enumeration of Yersinia enterocolitica in meat
Meat samples (25 g) were taken for enumeration before and after the incubation period. The samples were blended for 2 min with 225 ml of sterile 0.1% peptone water. Further decimal dilutions, as required, were made with the same diluent. Portions (0.1 ml) of the appropriate dilution were surface spread, in duplicate, onto BHI plates and were incubated at 27°C for 48 h. Plates containing 30 to 300 colonies were counted. The LAB formed a "lawn" on BHI agar, whereas Y. enterocolitica formed distinct colonies. The LAB did not overgrow Y. enterocolitica.

Growth indicators
The colony counts of Y. enterocolitica were used to calculate the following growth indicators: (a) Population increase = log_{10} (final population/initial population) and (b) Inhibition = log_{10} (final population control/final population treatment).

pH measurements
The pH of the meat was measured at the beginning and at the end of the incubation period. The 1:10-slurry prepared for the enumeration of Y. enterocolitica was used for pH measurement after it was sampled for colony count.

RESULTS
Yersinia enterocolitica 0.3 and 0.8, when grown alone in cured meat at 35°C, increased in population from 10^3 CFU/g to 8.9 X 10^6 and 3.1 X 10^6 CFU/g, respectively (Table 1). This was a 3.9 and 3.3-log_{10} CFU/g increase for serotype 0.3 and 0.8, respectively. Each of the LAB controlled the growth of Y. enterocolitica. Growth inhibition of 3.9 to 4.0 log_{10} CFU/g was observed with Y. enterocolitica 0.3 as compared to 2.6 to 3.1 log_{10} CFU/g with serotype 0.8. The pH of the meat did not change when Y. enterocolitica grew alone; however, in the presence of LAB the pH decreased to 4.9 to 5.1 (Table 1). At 27°C, Y. enterocolitica 0.3 and 0.8 alone and in combination with the LAB in cured meat, increased little (up to 0.6 log_{10} CFU/g) in population (Table 2). LAB inhibited the growth of both serotypes up to 0.7 log_{10} CFU/g. The same phenomenon was observed with concentrations of Y. enterocolitica of 10^4, 10^5 and 10^6 CFU/g of meat. The pH of the meat changed only in the presence of LAB, but in all instances it was in the range of 5.1 to 5.7.

At 27°C, there was little growth of Y. enterocolitica in cured meat (with or without the LAB). However, Y. enterocolitica 0.3 and 0.8 alone in plain meat (27°C) in-

### TABLE 1. Control of Y. enterocolitica (10^3 CFU/g) in cured meat at 35°C by lactic acid bacteria.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Final population (CFU/g)</th>
<th>Population increase</th>
<th>Inhibition</th>
<th>pH values</th>
<th>Initial</th>
<th>Final</th>
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</thead>
<tbody>
<tr>
<td>Serotype 0.3</td>
<td></td>
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<td></td>
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<tr>
<td>Control (Yersinia enterocolitica alone)</td>
<td>8.9 X 10^6</td>
<td>3.9</td>
<td>0.0</td>
<td>5.9</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>Y. enterocolitica and P. pentosaceus</td>
<td>1.1 X 10^3</td>
<td>&lt;0.0</td>
<td>3.9</td>
<td>6.0</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>Y. enterocolitica and P. acidilactici</td>
<td>8.3 X 10^2</td>
<td>&lt;0.0</td>
<td>4.0</td>
<td>6.0</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Y. enterocolitica and L. plantarum</td>
<td>8.5 X 10^2</td>
<td>&lt;0.0</td>
<td>4.0</td>
<td>6.0</td>
<td>5.0</td>
<td></td>
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<tr>
<td>Serotype 0.8</td>
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<tr>
<td>Control (Yersinia enterocolitica alone)</td>
<td>3.1 X 10^6</td>
<td>3.3</td>
<td>0.0</td>
<td>6.1</td>
<td>6.1</td>
<td></td>
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<tr>
<td>Y. enterocolitica and P. pentosaceus</td>
<td>7.4 X 10^3</td>
<td>0.70</td>
<td>2.6</td>
<td>6.2</td>
<td>4.9</td>
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<td>3.1</td>
<td>6.2</td>
<td>4.9</td>
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<td>Y. enterocolitica and L. plantarum</td>
<td>2.4 X 10^3</td>
<td>0.50</td>
<td>3.1</td>
<td>6.1</td>
<td>5.0</td>
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</table>

*Population increase = log_{10} (final population/initial population).

**Inhibition = log_{10} (final population control/final population treatment).
increased from $10^3$ to $3.2 \times 10^7$ CFU/g and $3.0 \times 10^7$ CFU/g meat, respectively. This represented a $>4.0$ log$_{10}$ CFU/g increase for both serotypes (Table 3). Each serotype, in combination with LAB, increased up to $2.3 \log_{10}$ CFU/g. LAB inhibited growth of *Y. enterocolitica* 0:3 and 0:8 by 1.9 to 2.7 log$_{10}$ CFU/g. It is interesting to note that the pH values were in all instances within the range of 5.8 to 6.1 (Table 3), indicating little change (a reduction of 0.1 to 0.2 pH unit).

The next step was to identify the component(s) of the curing mixture that caused the inhibition of *Y. enterocolitica* in cured meat. At 27°C, each of the salts, i.e., sodium chloride and sodium nitrite, inhibited 3.6 log$_{10}$ CFU/g of serotype 0:3. Serotype 0:8 was inhibited 2.3 and 2.4 log$_{10}$ CFU/g by sodium nitrite and sodium chloride, respectively. Dextrose inhibited serotype 0:3 by 0.7 log$_{10}$ CFU/g but had less effect on serotype 0:8. Spices, garlic powder and white pepper were not effective at tested concentrations in controlling the growth of *Y. enterocolitica* 0:3 and 0:8 (Fig. 1).

**DISCUSSION**

LAB efficiently controlled the growth of *Y. enterocolitica* 0:3 and 0:8 at 35°C in cured meat (Table 1). The lactic acid produced by the LAB (pH 4.9 to 5.1) may have been the major factor contributing to the inhibition of growth of *Y. enterocolitica*. Grau (9) found that *Y. enterocolitica* did not grow under anaerobic conditions on beef muscle of pH 5.4 to 5.85. The presence of lactate was important to the inhibition. In addition to the

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Final population (CFU/g)</th>
<th>Population increase (log$_{10}$)</th>
<th>Inhibition (log$_{10}$)</th>
<th>pH value</th>
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<td><strong>Serotype 0:3</strong></td>
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<tr>
<td>Control (Yersinia enterocolitica alone)</td>
<td>4.4 x 10$^3$</td>
<td>0.6</td>
<td>0.0</td>
<td>6.0</td>
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<tr>
<td><em>Y. enterocolitica</em> and <em>P. pentosaceus</em></td>
<td>1.3 x 10$^3$</td>
<td>0.0</td>
<td>0.5</td>
<td>5.9</td>
</tr>
<tr>
<td><em>Y. enterocolitica</em> and <em>P. acidilactici</em></td>
<td>2.3 x 10$^3$</td>
<td>0.3</td>
<td>0.3</td>
<td>6.0</td>
</tr>
<tr>
<td><em>Y. enterocolitica</em> and <em>L. plantarum</em></td>
<td>9.3 x 10$^2$</td>
<td>&lt;0.0</td>
<td>0.7</td>
<td>6.0</td>
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<tr>
<td><strong>Serotype 0:8</strong></td>
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<td></td>
</tr>
<tr>
<td>Control (Yersinia enterocolitica alone)</td>
<td>5.0 x 10$^3$</td>
<td>0.4</td>
<td>0.0</td>
<td>6.1</td>
</tr>
<tr>
<td><em>Y. enterocolitica</em> and <em>P. pentosaceus</em></td>
<td>1.3 x 10$^3$</td>
<td>&lt;0.0</td>
<td>0.6</td>
<td>6.2</td>
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<tr>
<td><em>Y. enterocolitica</em> and <em>P. acidilactici</em></td>
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<td>0.3</td>
<td>0.0</td>
<td>6.2</td>
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<td><em>Y. enterocolitica</em> and <em>L. plantarum</em></td>
<td>2.0 x 10$^3$</td>
<td>0.4</td>
<td>0.40</td>
<td>6.2</td>
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</table>

<table>
<thead>
<tr>
<th>Treatment</th>
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<th>Population increase (log$_{10}$)</th>
<th>Inhibition (log$_{10}$)</th>
<th>pH values</th>
</tr>
</thead>
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<tr>
<td><strong>Serotype 0:3</strong></td>
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<td></td>
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<tr>
<td>Control (Yersinia enterocolitica alone)</td>
<td>3.2 x 10$^7$</td>
<td>4.1</td>
<td>0.0</td>
<td>6.0</td>
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<tr>
<td><em>Y. enterocolitica</em> and <em>P. pentosaceus</em></td>
<td>2.2 x 10$^5$</td>
<td>1.9</td>
<td>2.2</td>
<td>6.1</td>
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<td><em>Y. enterocolitica</em> and <em>P. acidilactici</em></td>
<td>3.7 x 10$^5$</td>
<td>2.3</td>
<td>1.9</td>
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<td><em>Y. enterocolitica</em> and <em>L. plantarum</em></td>
<td>8.0 x 10$^4$</td>
<td>1.6</td>
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<td>5.9</td>
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<tr>
<td><strong>Serotype 0:8</strong></td>
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<tr>
<td>Control (Yersinia enterocolitica alone)</td>
<td>3.0 x 10$^7$</td>
<td>4.2</td>
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<td><em>Y. enterocolitica</em> and <em>P. pentosaceus</em></td>
<td>1.2 x 10$^5$</td>
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<td>6.0</td>
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<td><em>Y. enterocolitica</em> and <em>P. acidilactici</em></td>
<td>4.1 x 10$^5$</td>
<td>2.3</td>
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<td>6.0</td>
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<td>6.1 x 10$^4$</td>
<td>1.6</td>
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</table>

JOURNAL OF FOOD PROTECTION, VOL. 47, MAY 1984
results may impact on the bacteriological stability and Y. enterocolitica having 0:3 increased 0.6 log10 CFU/g more than did serotype Y. enterocolitica, combination with the additives used brought about com­

cation obtained from this study is that lowering the en­

or without LAB (Table 2). An important industrial appli­

Y. inhibitory to serotype 0:3. This was not observed with

0:8.

reported that an initial pH value of 4.4 (adjusted with

HC1) inhibited the growth of a strain of Y. enterocolitica

Stern et al. (26) found that car­

inhibitors of either of the tested strains of Y. enterocolitica.

In summary, it was shown that P. pentosaceus, P. acidilactici and L. plantarum can be used to control the growth of Y. enterocolitica in meat. The control of Y. enterocolitica by LAB was substantial at 35°C. At 27°C, complete inhibition of Y. enterocolitica was observed with and without LAB. Sodium chloride and sodium nit­rite were found to be important inhibitors of Y. enterocolitica.

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