Destruction of *Yersinia enterocolitica* by *Lactobacillus sake* and *Pediococcus acidilactici* During Low-temperature Fermentation of Turkish Dry Sausage (sucuk)

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**ABSTRACT:** Effects of starter cultures, *Lactobacillus sake* and *Pediococcus acidilactici*, on the survival of *Yersinia enterocolitica* were studied in Turkish dry sausage (sucuk). Inoculated *Y. enterocolitica* (approximately 5.0 log CFU/g) was eliminated completely in sausages by *L. sake* (about 7.0 log CFU/g) and *P. acidilactici* (about 7.0 log CFU/g) by the 3rd day of fermentation but was not totally eliminated in natural fermentation without starter(s) after completion of 4 d of fermentation and 12 d drying (*p* < 0.05). pH decreased from 6.3 to about 4.7 in starter culture treatments and from 6.3 to 5.6 in natural fermentation after completion fermentation and drying (*p* < 0.05).

Key words: *Lactobacillus sake*, *Pediococcus acidilactici*, *Yersinia enterocolitica*, Turkish dry sausage

**Introduction**

Epidemiological studies on *Y. enterocolitica*, an environmental organism, have shown that its transmission is associated mostly with foods of animal origin, such as milk, beef, lamb, pork, chicken, and products treated with contaminated water (Lee 1977; Stern and Pierson 1979; Swaminathan and others 1982; Zink and others 1982). In addition, *Y. enterocolitica* can multiply at refrigeration temperatures; thus, it can occur in foods produced or stored at these temperatures (Tacket and others 1984 and 1985).

Meat fermentation research is based mainly on starter cultures of rapid acid-producing lactic acid bacteria. Addition of selected strains of starter cultures produce substances, such as lactic acid (Lucke 1994), bacteriocins (Tagg and others 1976), and hydrogen peroxide (Raccach and Baker 1978), which are antagonistic towards spoilage and pathogenic organisms (Lucke 1994). The use of starter cultures ensures numerical dominance of desired microorganisms over the natural flora (Bacus and Brown 1981) and also inhibits the growth of the undesirable organisms by converting fermentable sugars into lactic acid (Daeschel 1989).

*Lactobacillus sake*, a psychrotrophic bacterium, is an important starter culture in fermented sausages, and many strains have been isolated from naturally fermented sausages (Schillinger and Lucke 1987; Sanz and others 1988; Ray 1996). Because *L. sake* can tolerate a high salt concentration (8%) and ferment various carbohydrates, such as glucose, fructose, and sucrose (Champomier and others 1987), it can be used in European style summer sausage, which requires a maximum fermentation temperature of 25°C (Bacus 1984).

*Pediococcus acidilactici*, having an optimum growth temperature of 40°C, grows over a wide range of pH values. It is used commonly in meat, cereal, vegetables, and the other types of fermented foods as well as in secondary cultures for ripening and flavor production of some cheeses. For high temperature and low pH fermentations, the strains of *P. acidilactici* are preferred (Ray 1996; Jay 1992; Smith and Palumbo 1981).

Turkish dry sausage (sucuk) is a traditional type of dry sausage commonly fermented without starter culture. It is made from beef and/or mutton with salt, sugar, and various seasonings (Table 1). It does not undergo any heat treatment during processing (Unluturk and Turantas 1991; Turantas and Unluturk 1993).

To date no study has been reported on the use of these starter cultures in the production of sucuk and related control of *Y. enterocolitica*. In order to improve fermentation and control of foodborne pathogen in sucuk, effects of starter cultures, *L. sake* and *P. acidilactici*, on fermentation and control of inoculated *Y. enterocolitica* during the sausage manufacturing process were studied.

**Results and Discussion**

**Water activity profile of sausage during fermentation and drying**

During the 4-d fermentation period, water activity in natural, *L. sake*, and *P. acidilactici* fermentations decreased from 0.97 to 0.94. After 12 d of drying, it was about 0.89 to 0.90, a level that allows most bacteria to survive. No significant differences (*p* > 0.05) in water activity were observed among the treatments (data not shown).

**pH profile of sausage during fermentation and drying**

Natural fermentation lowered pH from 6.3 to 5.6 after 4 d of fermentation and 12 d of drying (Table 2). However, pH values of sausages fermented by *L. sake* and *P. acidilactici* were reduced to

<table>
<thead>
<tr>
<th>Table 1—Composition of Turkish dry sausage (sucuk)*</th>
<th>Amount (kg)</th>
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</thead>
<tbody>
<tr>
<td>Ground beef (80% lean)</td>
<td>1.0</td>
</tr>
<tr>
<td>Salt</td>
<td>0.025</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.015</td>
</tr>
<tr>
<td>Sodium nitrite</td>
<td>0.0002</td>
</tr>
<tr>
<td>Seasoning</td>
<td>0.034</td>
</tr>
<tr>
<td>Cumin</td>
<td>0.014</td>
</tr>
<tr>
<td>Black pepper</td>
<td>0.004</td>
</tr>
<tr>
<td>Red pepper</td>
<td>0.004</td>
</tr>
<tr>
<td>Allspice</td>
<td>0.004</td>
</tr>
<tr>
<td>Garlic</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*Source: Unluturk and Turantas (1991).*
5.5 and 5.6, respectively, after 1 d of fermentation and to 4.8 and 4.6, respectively, after completion of drying. These sizeable decreases in pH were achieved by the production of lactic acid as an end-product of glucose metabolized by lactic acid bacteria. The pH reduction in lactic acid bacteria fermentations was significantly greater \( (p < 0.05) \) than that in natural fermentation.

**Proximate analysis and total titratable acidity**

Total titratable acidity increased from 0% to 0.6%, 0.9%, and 1.1% with natural, \( L. \) *sake*, and \( P. \) *acidilactici* fermentations, respectively, after completion of fermentation and drying. Dry sausages contain 30% to 40% moisture content (Jay 1992). After drying, the moisture content of each sausage sample was reduced from 59.9% to 36.4%, 35.7%, and 37.8% with natural, \( L. \) *sake*, and \( P. \) *acidilactici* fermentations, respectively. These reductions in moisture content resulted in relative increases of ash, protein, and fat contents in sausages (Table 3). Proximate analysis results of this study are in agreement with the results of previous studies. The average composition of a fermented sucuk was found to be 23.0% protein, 32.3% moisture, 28.0% fat, and 4.16% salt, and others (Ömurtag and others 1968).

**Growth of lactic acid bacteria in sausages during fermentation and drying**

Growth data of \( L. \) *sake* and \( P. \) *acidilactici* in sausages with inoculated \( Y. \) *enterocolitica* are presented in Table 4. \( Lactobacillus \) *sake* increased from 6.2 to 7.7 log CFU/g during fermentation, but the cell counts were reduced to 6.1 log CFU/g during drying. \( Pediococcus \) *acidilactici* had a 0.4 log CFU/g increase during fermentation, but counts dropped to 6.5 log CFU/g during drying. Growth of these lactic acid bacteria in the sausages was not high in terms of cell numbers, but the resultant acid and other antimicrobial products might have been sufficient to destroy the inoculated \( Y. \) *enterocolitica*.

** Destruction of \( Yersinia \) enterocolitica in sausages during fermentation and drying**

In natural fermentation without addition of starter cultures, the cell count of \( Y. \) *enterocolitica* decreased from about 5.0 to 1.8 log CFU/g during 4 d of fermentation and to 0.5 log CFU/g after 12 d of drying (Table 5). In both \( L. \) *sake* and \( P. \) *acidilactici* fermentations, the pathogen was reduced from about 5.0 to 0.5 log CFU/g in 3 d of fermentation (Table 5). In addition, after the 4th d of fermentation, no \( Y. \) *enterocolitica* colonies were detected on either CIN or KV-202 media by both direct plating and by the more stringent and sensitive enrichment procedures. The number of \( Y. \) *enterocolitica* detected using either CIN and KV-202 agar in \( L. \) *sake* and \( P. \) *acidilactici* fermentation was significantly lower than natural fermentation during fermentation and drying \( (p < 0.05) \).

The KV-202 medium was designed to differentiate \( Y. \) *entero-
was a major factor. Glucose (1%) reduced \( Y.\ enterocolitica \) O:3 by 0.7 log CFU/g but had no significant effect on \( Y.\ enterocolitica \) O:8. The antimicrobial effectiveness of garlic against foodborne pathogens, such as \( E.\ coli \), \( L.\ monocytogenes \), \( S.\ aureus \), \( Salmonella\ typhi \), \( Salmonella\ typhimurium \), and \( Bacillus\ cereus \), has been reported (Shelef 1983; Kumar and Berwal 1998). Zaika (1988) stated that cumin and allspice have medium effect, and red pepper and black pepper have weak antimicrobial effect. In natural fermentation, therefore, the major inhibition factors that reduced the number of \( Y.\ enterocolitica \) O:3 were sodium chloride, sodium nitrite, garlic powder, and lactic acid from glucose.

### Materials and Methods

#### Cultures preparation

A freeze-dried form of \( L.\ sake\) ATCC 15521 was obtained from the American Type Culture Collection (Rockville, Mass., U.S.A.). \( Pediococcus\ acidilactici\) was obtained from the Food Microbiology Culture Collection (Kansas State University, Manhattan, Kan., U.S.A.), and \( Y.\ enterocolitica\) O:3 (ATCC 24839) was obtained from the Centers for Disease Control and Prevention (Atlanta, Ga., U.S.A.).

Lactic acid bacteria, \( L.\ sake\) and \( P.\ acidilactici\), were grown in MRS broth (Difco, Detroit, Mich., U.S.A.) at 30 °C and 37 °C for 24 h, respectively. \( Y.\ enterocolitica\) was grown in brain heart infusion broth (Difco, Detroit, Mich., U.S.A.) at 37 °C for 24 h. The cultures were centrifuged at 9000 × g for 25 min. The supernatant was discarded, and cells were resuspended with 0.1% peptone water (Difco, Detroit, Mich., U.S.A.). The final volume of each culture was calculated to be 30 ml for 10 kg of meat mix.

#### Sausage preparation and culture inoculation

Ground beef was obtained from the meats laboratory at Kansas State University (Manhattan, Kan., U.S.A.) and ground first through a 3/8 inch (9.5 mm) and then a 1/8 inch (3.2 mm) plate. The other ingredients were added to the ground meat in the order listed and mixed thoroughly for 2 min according to a commercial sucuk recipe (Turantas and Unluturk 1993).

After mixing, the beef batter was divided into 3 equal portions of 10 kg each. The 1st portion was inoculated with \( Y.\ enterocolitica\), the 2nd portion was inoculated with \( Y.\ enterocolitica\) and \( L.\ sake\), and the 3rd portion was inoculated with \( Y.\ enterocolitica\) and \( P.\ acidilactici\). Lactic acid bacteria, \( L.\ sake\) and \( P.\ acidilactici\), were added to the mix at a level of about 6.0 to 7.0 log CFU/g, and \( Y.\ enterocolitica\) was added at about 5.0 log CFU/g. Each of the sausage batters was mixed for 5 min after inoculation and then stuffed into cellulose casings (Viskase Corp., Chicago, Ill., U.S.A.) to make links 5.0 cm in diameter and 15.0 cm in length.

#### Fermentation, drying, and sampling of sausage

The sausage links were placed into a U.S. Department of Agriculture-approved Alkar fermentation chamber (Alkar, Dec Int., Lodi, Wis., U.S.A.) and fermented at 24 °C (> 90% RH) for 2 d and then at 22 °C (80% to 90% RH) for an additional 2 d. Drying was completed in a Travaglini drying chamber (Milano, Italy) at 18 °C (70% to 80% RH) for 12 d. Sampling was performed by randomly selecting 2 links of each sausage preparation every 24 h during the 4 d of fermentation and at the 3rd, 7th, and 12th days of drying.

#### Analysis of sausage

Water activity of each sample was monitored on a similar schedule using an AQUA LAB water activity meter according to the manufacturer’s instructions (Decagon Devises Inc., Pullman, Wash., U.S.A.). Proximate analysis and titratable acidity (lactic acid %) of sausages were determined before and after fermentation and after completion of drying (AOAC 1990).

For pH measurements, 10 g of each sample were stomached in 100 ml of distilled water for 2 min (Acton and others 1977). The pH of the filtered solution was measured using a Beckman F 45 pH meter (Beckman Instruments, Fullerton, Calif., U.S.A.). Measurements were made immediately after addition of bacteria to sausage mixes and at each sampling time during fermentation and drying.

For bacterial analysis at each sampling time, 25 g of sausage were weighed and homogenized in 225 ml of 0.1% peptone water solution for 2 min, using a stomacher Lab-Blender 400 (Seward Medical Inc., London, U.K.). Serial dilutions were made using 9.0 ml of 0.1% peptone water solution. Lactic acid bacteria counts were determined on prepoured MRS medium (Difco, Detroit, Mich., U.S.A.) incubated in an anaerobic incubator at 35 °C for 2 d. For enumeration of \( Y.\ enterocolitica\), besides the commercially
available CIN medium (Oxoid, Unipath Ltd., Hampshire, England), a new KV-202 medium recently developed at Kansas State University (Vichienroj and Fung 1997) was used for comparison. Both media were incubated at 37 °C for 24 h.

**Recovery of injured *Yersinia enterocolitica* from sausage**

Direct plating sometimes may not recover small numbers of injured *Yersinia enterocolitica* cells from sausages. To better recover these cells, a 2-step selective enrichment procedure also was employed. Ten grams of ground sausage sample were placed into selective sorbitol bile broth (SBB) and incubated at 25 °C for 24 h (Vichienroj 1997). After incubation, liquid culture from SBB was streak plated onto CIN and KV-202 media, and the plates were incubated at 37 °C for 24 h. Typical colonies on each medium indicating the presence of *Y. enterocolitica* were recorded.

**Statistical analysis**

A split-plot repeated experimental design was adopted and a general linear model (GLM) procedure (SAS Inst. Inc., Cary, N.C., U.S.A.) was used for analysis of data. Least significant differences (LSD) were used with significance established at the 0.05 probability level. All experiments were repeated 3 times.

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**References**


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