Survival of *Listeria monocytogenes* During the Manufacture and Ripening of Swiss Cheese

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ABSTRACT

Rindless Swiss cheese was made from a mixture of pasteurized whole and skim milk that was inoculated to contain $10^4$ to $10^5$ cfu of *Listeria monocytogenes* (strain Ohio, California, or V7)/ml. During clotting of milk, numbers of *L. monocytogenes* remained nearly unchanged. When the curd was heated gradually to attain the cooking temperature (50°C), numbers of *L. monocytogenes* increased by approximately 40 to 45% over those in inoculated milk. Cooking curd at 50°C (122°F) for 30 to 40 min resulted in resilient curd having a pH of 6.40 to 6.45 and decreased *L. monocytogenes* by 48% compared with numbers of the pathogen in inoculated milk. After curd was pressed under whey, numbers of *L. monocytogenes* increased by approximately 52% over those in inoculated milk and reached their maxima at the end of this stage. A sharp decrease in numbers of *L. monocytogenes* occurred during brining of cheese blocks (7°C for 30 h). The population of *L. monocytogenes* continued to decrease during cheese ripening. Average D values for strains California, Ohio, and V7 were 29.2, 24, and 22.5 d, respectively. *Listeria* was not detected (direct plating, and cold enrichment) after 80, 77, and 66 d of ripening of Swiss cheese made from milk inoculated with strains California, Ohio, and V7, respectively. Thus, Swiss cheese made in this study did not permit extended survival of *L. monocytogenes*.

(Key words: *Listeria monocytogenes*, Swiss cheese, foodborne illness)

Abbreviation key: MLA = McBride Listeria agar, TA = tryptose agar, TB = tryptose broth.

INTRODUCTION

Fatal foodborne listeriosis has been linked to consumption of contaminated pasteurized milk (7) and cheese (9). These outbreaks of illness prompted work in our laboratory to determine the fate of *Listeria monocytogenes* in a variety of cheeses and to examine the behavior of *L. monocytogenes* during the manufacture and storage of other fermented dairy products. After the outbreaks just mentioned, *L. monocytogenes* was found by regulatory agencies or others in Cheddar, Ricotta, Feta, Brie, and other semi-soft cheeses (13). Seventy-five percent of these infected cheeses were made from pasteurized milk (25).

Analysis of raw milk from the Midwest showed that 13% of the samples contained *Listeria spp.;* 92% of the isolates obtained from the milk samples were pathogenic for mice (11). Inspection by the FDA of factories making soft cheese uncovered many deficiencies that could cause contamination with or permit survival of *L. monocytogenes* (2). When present in many kinds of cheeses, *L. monocytogenes* can survive longer than the minimum of 60 d at ≥1.7°C required to ripen cheese made from raw or heat-treated milk (13).

Different types of cheeses have been prepared using milk intentionally inoculated with *L. monocytogenes* to study behavior of the pathogen during making and ripening of the cheese. *Listeria monocytogenes* survived >434 d in Cheddar cheese at pH 5.0 (17), >180 d in cold-pack cheese food at pH 5.21 (19), >115 d in Colby cheese at pH 5.00 (26), >115 d in Blue cheese at pH 6.2 (14), >90 d in Feta cheese at pH 4.30 (15), and >28 d in cottage cheese.
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MATERIALS AND METHODS

Experimental Design

Nine lots of Swiss cheese were manufactured with each of three strains of L. monocytogenes: Ohio (trials 1, 2, and 3), California (trials 4, 5, and 6), and V7 (trials 7, 8, and 9). Each lot yielded two 4.5-kg (10-lb) blocks of cheese. Core samples of these cheeses were analyzed for numbers of L. monocytogenes, pH, and content of moisture, salt, and fat.

Bacterial Cultures

Stock cultures of the three strains of L. monocytogenes were maintained on tryptose agar (TA) slants stored at 4°C and transferred monthly. Intermediate cultures were prepared by transferring stock cultures into tryptose broth (TB) that was incubated for 24 h at 35°C. This TB culture was transferred to a second tube of TB that was similarly incubated for 24 h. Working cultures were prepared by transferring the second intermediate culture to 100 ml of sterile skim milk, which was incubated for 24 h at 35°C. This milk culture was then transferred to a second flask, containing 100 ml of sterile skim milk, and incubated for 48 h at 35°C. A sufficient volume of this culture was dispersed in 50 ml of pasteurized milk to yield 1 x 10^4 to 1 x 10^5 cfu L. monocytogenes/ml when added to 136 kg (300 lb) of pasteurized milk in the cheese vat.

Commercial starter cultures used for cheese making were Streptococcus salivarius ssp. thermophilus, Lactobacillus helveticus, and Propionibacterium shermanii (Marschall Division, Rhône Poulenc, Inc., Madison, WI). Starter cultures were cultivated separately and incubated 16 to 18 h at 35°C in sterile reconstituted NDM (10% total solids).

Cheese Manufacture

Swiss cheese was made according to the procedure described by Reinbold (16) with some modifications recommended by the personnel of the Center for Dairy Research, University of Wisconsin, Madison. A mixture of pasteurized whole [113 kg (249 lb)] and skim milk [27.8 kg (61 lb)] was warmed to 32°C (90°F). The inoculum of L. monocytogenes was added shortly before addition of starter cultures, S. salivarius ssp. thermophilus (.80%), Lb. helveticus (.05%), and P. shermanii (12.5 ml). Initial numbers of L. monocytogenes were set at 10^4 to 10^5/ml of milk because preliminary trials using 10^2 to 10^3/ml of milk resulted in inability to detect the pathogen in cheese after only a few days of warm ripening. Milk was allowed to ripen for 10 min before addition of calcium chloride (9.0 ml) (Marschall Division, Rhône Poulenc, Inc.). Five minutes later, single-strength rennet extract (27.0 ml) (Marschall Division, Rhône Poulenc, Inc.) was added to cheese milk. After appropriate ripening (usually 30 min after rennet addition), curd was cut using .64-cm (.25 in) cheese knives. The curd was stirred gently for about 5 min. The temperature of the vat contents was raised gradually to 50°C (122°F) within 30 min and was maintained at 50°C for 30 to 40 min until the curd was resilient and the pH was 6.40 to 6.45. The pH of curd
samples was determined using a pH meter (model 10, Coming Glass Works, Medfield, MA) equipped with a flat-bottomed standard combination electrode (12).

Curd was allowed to settle, and half the whey was drained from the vat. The curd was packed into two 4.5-kg (10-lb) hoops under the whey. A light stainless steel press plate was placed gently on top of the curd and depressed by hand to initiate leveling of the curd under the whey so that curd fusion was not disrupted. Whey was drained, and hoops of cheese were pressed with a small hydraulic cheese press at 1.8 kg/cm$^2$ (10 psi) for 16 h. Cheese blocks were stored in a saturated salt solution at 7°C (45°F) until the cheese pH reached 5.2 (time required was about 30 h). Excess salt solution was brushed off the cheese surface, cheese blocks were dried for 6 h at 7°C (45°F), and then cheese blocks were wrapped in polyvinyl acetate bags (Baxter, Inc., Chicago, IL). A vacuum was drawn, and the bags were heat sealed. The packaged cheese was stored in a cold room at 7°C (45°F) for 10 d and turned daily. Then cheese was held at 24°C (75°F) for up to 80 d (warm room treatment). Safety precautions exercised during cheese making were as described by Ryser et al. (21).

Duplicate samples for enumeration of $L.\ monocyctogenes$ were taken from milk, curd, whey, and cheese according to the following scheme: 1) pasteurized milk, 2) inoculated milk, 3) milk before ripening, 4) curd and whey after cutting, 5) curd and whey at the end of cooking, and 6) curd and whey after pressing in the vat. Cheese samples were taken after 16 h of pressing, after cold cure, and then weekly during the period of warm cure.

Enumeration

Samples of pasteurized milk (.2 ml), whey (.2 ml), and inoculated milk (.1 ml of undiluted and of $10^{-1}$ and $10^{-2}$ dilutions) were surface-plated onto McBride Listeria agar (MLA). Curd or cheese samples (10 g each) were blended for 2 min with 90 ml of a warm (40°C) sterile solution of 2% sodium citrate. Portions (.1 ml each) of undiluted and of $10^{-1}$ and $10^{-2}$ dilutions of curd or cheese samples were surface-plated in duplicate onto MLA. All plates were incubated at 35°C for 48 h. Cheese samples in which $L.\ monocyctogenes$ was not detected by direct plating on MLA were homogenized in TB and reexamined after 2, 4, 6, and 8 wk of cold enrichment (4°C). Colonies typical of those formed by $L.\ monocyctogenes$ (smooth, bluish gray, slightly raised, translucent, watery consistency, .5 to 1.5 mm in diameter, and weakly β-hemolytic) were counted, and several colonies from plates inoculated with the most diluted samples were transferred to TA slants, incubated 24 h at 35°C, and stored at 3°C for confirmation.

Confirmatory tests done on isolates thought to be $L.\ monocyctogenes$ included catalase reaction, observance of tumbling motility in TB-grown cultures incubated 24 h at 21°C, and presence of distinct blue-green colonies on TA when observed under obliquely transmitted light. Serological slide agglutination tests with commercially prepared antiserum (Difco Laboratories, Detroit, MI) were conducted according to the manufacturer's instructions on all isolates thought to be $L.\ monocyctogenes$ to confirm that the isolates were of serotypes 1 or 4.

Cheese Analysis

After approximately 60 d of storage, cheeses were sampled for determination of pH and content of moisture and fat as described in Standard Methods for the Examination of Dairy Products (12). The pH of cheese samples was determined at the time of bacteriological analysis, using a pH meter (model 10, Coming Glass Works, Medfield, MA) equipped with a flat-bottomed standard combination electrode (12). Sodium chloride in cheese was measured using the Volhard procedure as described in Official Methods of Analysis (3).

RESULTS AND DISCUSSION

Composition of Cheese

Federal standards (4) specify that Swiss cheese made from raw or heat-treated milk must be at least 60 d old, cannot contain more than 41% moisture, and must contain a minimum of 43% fat in DM. The composition of Swiss cheese made in this study (Table 1) conformed with the legal standards.
TABLE 1. Composition of Swiss cheese after 2 mo of warm ripening.

<table>
<thead>
<tr>
<th>Lot</th>
<th>Strain of Listeria monocytogenes</th>
<th>Moisture (%)</th>
<th>FDM (F)</th>
<th>Salt</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ohio</td>
<td>35.0</td>
<td>45.0</td>
<td>1.0</td>
<td>5.3</td>
</tr>
<tr>
<td>2</td>
<td>Ohio</td>
<td>36.5</td>
<td>44.5</td>
<td>1.2</td>
<td>5.3</td>
</tr>
<tr>
<td>3</td>
<td>Ohio</td>
<td>38.0</td>
<td>44.0</td>
<td>1.2</td>
<td>5.3</td>
</tr>
<tr>
<td>4</td>
<td>California</td>
<td>36.5</td>
<td>44.0</td>
<td>1.0</td>
<td>5.3</td>
</tr>
<tr>
<td>5</td>
<td>California</td>
<td>37.0</td>
<td>43.5</td>
<td>1.3</td>
<td>5.2</td>
</tr>
<tr>
<td>6</td>
<td>California</td>
<td>35.8</td>
<td>44.5</td>
<td>1.4</td>
<td>5.3</td>
</tr>
<tr>
<td>7</td>
<td>V7</td>
<td>37.0</td>
<td>44.5</td>
<td>1.4</td>
<td>5.3</td>
</tr>
<tr>
<td>8</td>
<td>V7</td>
<td>39.0</td>
<td>44.5</td>
<td>1.0</td>
<td>5.2</td>
</tr>
<tr>
<td>9</td>
<td>V7</td>
<td>39.0</td>
<td>44.2</td>
<td>1.0</td>
<td>5.4</td>
</tr>
</tbody>
</table>

1 Fat in DM.

Behavior In Curd

Populations of *L. monocytogenes* changed at different rates during the various phases of making Swiss cheese (Figures 1, 2, and 3). During the milk-clotting step, numbers of *L. monocytogenes* remained nearly unchanged. When heating of the curd had begun, numbers of *L. monocytogenes* had increased by approximately 43% over those of inoculated milk shortly before cooking. Most of that increase in population can be attributed to formation and shrinkage of curd, which entrapped cells of *L. monocytogenes*.

Cooking the curd at 50°C for 30 to 40 min decreased pathogens approximately 57% compared with numbers in the curd at the start of cooking. This decrease probably resulted because the heat treatment (cooking) was lethal to *L. monocytogenes*. Although an increase in numbers of *Listeria* cells would be expected as the curd shrinks from being heated, the lethal effect of the heat treatment apparently predominated. Because heat-injured cells may not form colonies on the selective MLA, the actual numbers of survivors may have been somewhat greater than those observed. The yield of Swiss cheese was approximately 8.0% after...
pressing the curd of Parmesan cheese (27), a decrease in population after pressing of curd was not detected in other cheese varieties (17, 26).

Inability of *L. monocytogenes* to grow during the manufacture of Swiss cheese and other cheese varieties (14, 15, 26, 27) may be related to the short time, 3.5 to 4 h, needed to make the cheese after addition of *L. monocytogenes* to milk in the vat. The pathogen had a lag phase of about 3 h when grown in TB at pH 5.6 and 35°C (95°F) (6). Even though cultures of *L. monocytogenes* were activated before inoculation of milk in the vat, cooking of curd and acid production by starters may have inhibited proliferation of or inactivated the pathogen. Schaack and Marth (22) found that presence of *S. salivarius* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* in milk permitted limited growth of *L. monocytogenes* during the initial 6 h of incubation in skim milk at 30°C (86°F).

**Behavior In Whey**

During the early stages of curd formation, numbers of *L. monocytogenes* were about 10-fold greater in curd than in whey. Numbers of *Listeria* in the whey continuously decreased during further stages of cheese manufacture (Table 2). Numbers in whey decreased slightly when the vat contents were heated to attain the cooking temperature. Cooking increased that ratio to an average of 101, and pressing the curd under whey extended the ratio to an aver-

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**TABLE 2. Distribution of Listeria monocytogenes** strains Ohio, California, and V7 in curd and whey at different stages in the manufacture of Swiss cheese.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Lot</th>
<th>Whey (cfu/ml)</th>
<th>Curd: whey&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Lot</th>
<th>Whey (cfu/ml)</th>
<th>Curd: whey&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Lot</th>
<th>Whey (cfu/ml)</th>
<th>Curd: whey&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forework</td>
<td>1</td>
<td>850</td>
<td>21</td>
<td>4</td>
<td>1068</td>
<td>32</td>
<td>7</td>
<td>1361</td>
<td>26</td>
</tr>
<tr>
<td>Steam off</td>
<td>81</td>
<td>99</td>
<td></td>
<td>113</td>
<td>107</td>
<td></td>
<td>166</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>Press in vat</td>
<td>94</td>
<td>226</td>
<td></td>
<td>212</td>
<td>180</td>
<td></td>
<td>241</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Forework</td>
<td>2</td>
<td>960</td>
<td>19</td>
<td>5</td>
<td>1122</td>
<td>27</td>
<td>8</td>
<td>1563</td>
<td>25</td>
</tr>
<tr>
<td>Steam off</td>
<td>70</td>
<td>114</td>
<td></td>
<td>110</td>
<td>112</td>
<td></td>
<td>147</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>Press in vat</td>
<td>116</td>
<td>224</td>
<td></td>
<td>192</td>
<td>163</td>
<td></td>
<td>246</td>
<td>181</td>
<td></td>
</tr>
<tr>
<td>Forework</td>
<td>3</td>
<td>920</td>
<td>20</td>
<td>6</td>
<td>1110</td>
<td>28</td>
<td>9</td>
<td>1576</td>
<td>24</td>
</tr>
<tr>
<td>Steam off</td>
<td>92</td>
<td>93</td>
<td></td>
<td>182</td>
<td>93</td>
<td></td>
<td>167</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>Press in vat</td>
<td>90</td>
<td>242</td>
<td></td>
<td>346</td>
<td>161</td>
<td></td>
<td>293</td>
<td>180</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Ratio of colony-forming units per gram in curd over colony-forming units per millimeter in whey.

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age of 195. Although cooking should have released extra amounts of whey from the curd, *L. monocytogenes* numbers in whey continued to decline. This reduction may have resulted from entrapment of the pathogen in curd as a result of curd formation and shrinkage or to greater sensitivity of the pathogen to heat when present in whey than in curd. Pressing of the curd under whey may have caused further concentration of the pathogen through expulsion of whey.

Behavior During Cold Ripening

The sharpest decrease in numbers of *L. monocytogenes* in Swiss cheese occurred during brining of cheese blocks at 7°C for 30 h. Further storage at 7°C for 10 d continuously reduced the population of *L. monocytogenes* (Figures 1, 2, and 3).

Behavior During Warm Ripening

The population of *L. monocytogenes* continued to decrease during cheese ripening (Figures 1, 2, and 3). Average D values for strains California, Ohio, and V7 were 29.2, 24, and 22.5 d, respectively. *Listeria* was not detected (direct plating and cold enrichment) after 80, 77, or 66 d of ripening of Swiss cheese made from milk inoculated with strain California, Ohio, or V7, respectively. In contrast, strain V7 survived longer than strain California in Cheddar (17), Colby (26), and Parmesan (27) cheeses. After ripening of Swiss cheese for 80 d, *L. monocytogenes* was not detected by either surface-plating or cold enrichment.

Results of cold enrichment were negative for all samples of cheese in which *L. monocytogenes* was not detected by surface-plating.

The combined effects of cheese pH, starter metabolites, fermentation by-products, water activity, temperature of storage, and salt content may impose an additive detrimental effect on foodborne pathogens during manufacturing and ripening of cheese. Culture extracts of several strains of lactic acid bacteria (8) demonstrated a bactericidal action without causing lysis of cells of pathogenic organisms. Such biologically active compounds were defined as bacteriocin-like substances. *Listeria monocytogenes* also was inhibited or inactivated by metabolites of lactic acid bacteria in cultured dairy products in earlier studies in the authors' laboratory (22, 23).

Production of propionate by eye-forming bacteria may have contributed to the demise of *L. monocytogenes* in Swiss cheese. In other work, less than 2000 ppm of sodium propionate inhibited growth of *L. monocytogenes* in TB at pH 5.0 (6). When TB was at pH 5.0 and contained 3000 ppm of sodium propionate, the *Listeria* population decreased 1000-fold during 67 d of incubation at 35°C and disappeared after 78 d. A 60-d-old Swiss cheese typically contains 3750 ppm of propionic acid (10).

Typical flavor of Swiss cheese is correlated with its content of acetic and propionic acids and with the extent of lipolysis (24). More lactate is fermented to acetate and CO₂ than to propionate by *P. shermanii* (5). Acetate may have played a major role in inactivating *L. monocytogenes* in Swiss cheese. Ahamed and Marth (1) examined the ability of different concentrations of acetic, citric, and lactic acid to prevent growth of *L. monocytogenes* in TB during extended incubation at 7 to 35°C. As little as 0.05% (500 ppm) acetic acid (pH 5.8) in TB caused noticeable inhibition of the pathogen. A 60-d-old Swiss cheese typically contains 2000 ppm of acetic acid (10). Although these acids were tested separately (1), they exist together with lactic acid, and, thus, the observations made with Swiss cheese partially reflect the combined effects of all the acids.

In conclusion, Swiss cheese made according to our procedure was quite unfavorable for extended survival of *L. monocytogenes*. Ripening of this cheese for 60 d seems more than likely to free the product from *L. monocytogenes* if the initial contamination is low, e.g., ≤10² cfu/ml of cheese milk. Finally, the fate of *L. monocytogenes* after postprocessing contamination of Swiss cheese remains to be investigated.

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