Fate of Listeria monocytogenes During the Manufacture and Ripening of Blue Cheese

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ABSTRACT

The ability of Listeria monocytogenes to grow during the manufacture of blue cheese and to survive during its ripening was examined. Pasteurized skim milk was standardized to a milk fat content of 3.7% by addition of pasteurized homogenized cream (35% milk fat), was inoculated to contain ca. 1.0·2.0 x 10⁵ L. monocytogenes [strain Scott A or California (CA)] cfu/ml, and was made into blue cheese according to the modified Iowa method. Blue cheese was ripened at 9-12°C and a relative humidity of 90-98% for 84 d, and then cheese was stored at 4°C. Duplicate samples of milk, curd, whey, and cheese were tested for pH and for numbers of Listeria by surface plating of appropriate dilutions [made in Tryptose Broth (TB) with 2% sodium citrate] on McBride Listeria Agar (MLA). Initial TB dilutions were stored at 4°C and surface-plated on MLA after 2, 4, 6, and 8 weeks, if the pathogen was not quantitated in the original sample. Selected Listeria colonies were confirmed biochemically. L. monocytogenes was entrapped in curd during cheese-making with the population in curd before hooping being ca. 1.0 log₁₀ cfu/g greater than in the inoculated milk; whey contained an average of 3.6% of the cells in the initial inoculum. L. monocytogenes in cheese increased in numbers by 0.58 to 1.22 log₁₀ cfu/g during the first 24 h of the cheese-making process. Only modest growth (0.12 to 0.30 log₁₀ cfu/g) was noted in two lots with rapid acid production. Growth of L. monocytogenes ceased when the pH of cheese dropped below 5.0. Populations of both strains of the pathogen decreased significantly (P<0.005) during the first 50 d of ripening, by an average of 2.68 log₁₀ cfu/g compared to populations of 1-d-old cheese. From days 50 to 120 the environment of blue cheese became more favorable (pH of cheese increased because of growth by Penicillium roqueforti), and this resulted in improved survival but no growth of the pathogen. Strain Scott A survived without any more substantial decrease in numbers during days 50 to 120 of storage. Strain CA survived during days 50 to 80, and then populations of the pathogen decreased gradually so that direct plating at 110 d (one trial) and 120 d gave negative results, but the same samples gave positive results after cold enrichment.

Listeria monocytogenes is a ubiquitous gram-positive, nonsporeforming, short, psychrotrophic rod which is motile with peritrichous flagella when grown at 20-25°C, and is pathogenic for humans and animals. Clinical manifestations of illness caused by L. monocytogenes comprise mainly meningitis-encephalitis followed by septic infections and isolated organic involvement. In fetal infections acquired during pregnancy via the transplacental route, listeriosis takes the form of septic granulomatous disease which either kills the fetus in utero or leads to abortion or stillbirth. Most individuals who have contracted listeriosis have been pregnant women, neonates or immunocompromised adults (14,28,37). The pathogen can cause disease in such animals as cattle, sheep, goats, buffaloes, pigs, and chickens (12,14,28), and can be transmitted to humans from infected animals (13,17,25) or through consumption of Listeria-contaminated foods. The pathogen appears in leukocytes in the milk of animals suffering from Listeria-mastitis even 3 months after clinical symptoms have disappeared (12,39). Dairy products are the foods most often incriminated as responsible for human listeriosis (3,4,10,18,22,36).

L. monocytogenes appears to be more heat resistant than many vegetative microbes (5,7,19,40) and when in a sufficiently large number of leukocytes in milk can survive the minimum high-temperature short-time pasteurization process (7,11). Also, the ability of the pathogen to multiply at refrigeration temperatures (6,31), to survive in the environment (23) and its salt tolerance (26,38,40,41) serve to cause occasional enormous problems both for public health and the dairy industry. L. monocytogenes was incriminated in at least 1322 reported deaths in various countries, for the period 1949-1987 (3,10,18,29,36).

Recently four foodborne outbreaks of human listeriosis in North American and Europe were associated with consumption of (a) coleslaw in Nova Scotia in 1981, (b) contaminated pasteurized milk in Massachusetts in 1983, (c) Mexican-style cheese in southern California in 1985, and (d) a soft cheese of the Vacherin Mont d’Or type in Canton Vaud, Switzerland in 1987 (3,10,18,36). In recent studies, L. monocytogenes was isolated from raw milk (8,16,20), dairy factories (2), and from soft and semisoft cheeses (19).

Studies with cheeses made from Listeria-inoculated milk, indicated that L. monocytogenes behaves differently in different kinds of cheeses. This behavior varies from inhibi-
tion to survival to growth in cheese (27, 32-35, 39, 42), depending mainly on conditions during manufacture, ripening, and storage of cheese. Included are time-temperature conditions of manufacture, composition of cheese, ripening and storage temperature, lactic starters, pH of cheese, strain of the pathogens involved, the initial population of *Listeria* in milk, and possibly other unknown factors. This research was undertaken to determine the behavior of *L. monocytogenes* during the manufacture and ripening of blue cheese.

**MATERIALS AND METHODS**

*Cultures of L. monocytogenes*

Two strains of *L. monocytogenes* were used in this study, strain California (CA) (isolate obtained from Mexican-style cheese implicated in a listeriosis outbreak in California, serotype 4b), and strain Scott A (clinical isolate, serotype 4b). Stock cultures were maintained at 4°C on Tryptose Agar (TA) (Difco Laboratories, Detroit, MI) slants and were transferred monthly. *Listeria* cultures used to inoculate milk for manufacture of blue cheese were prepared as previously described (26,42). Working *Listeria* cultures were decimally diluted in sterile 2% citrate solution and a calculated volume of the second decimal dilution was added to 67 lb of pasteurized (standardized to a milk fat content of 3.7%) cow's milk, in a small cheese vat, so that the number of *L. monocytogenes* in the inoculated milk was ca. 1.0-2.0 x 10⁶ cfu/ml.

**Starter culture**

A combination of *Streptococcus lactis* and *Streptococcus cremoris* was used as the starter culture. *Streptococcus lactis* subsp. *diacetylactis* also was used (0.15% of the starter) to help create open-structured cheese. All commercial cultures were obtained from the Marschall Division of Miles Laboratories, Inc., Madison, WI. Frozen concentrated cultures were activated in sterile skim milk, in a small cheese vat, so that the number of *L. monocytogenes* used in this study, Two strains of *L. monocytogenes* were used in this study, strain California (CA) (isolate obtained from Mexican-style cheese implicated in a listeriosis outbreak in California, serotype 4b), and strain Scott A (clinical isolate, serotype 4b). Stock cultures were maintained at 4°C on Tryptose Agar (TA) (Difco Laboratories, Detroit, MI) slants and were transferred monthly. *Listeria* cultures used to inoculate milk for manufacture of blue cheese were prepared as previously described (26,42). Working *Listeria* cultures were decimally diluted in sterile 2% citrate solution and a calculated volume of the second decimal dilution was added to 67 lb of pasteurized (standardized to a milk fat content of 3.7%) cow's milk, in a small cheese vat, so that the number of *L. monocytogenes* in the inoculated milk was ca. 1.0-2.0 x 10⁶ cfu/ml.

**Manufacture and sampling of blue cheese**

Blue cheese was manufactured according to the modified Iowa method (24). Typical data obtained during manufacture are shown in Table 1. Sixty lb of pasteurized (75.0°C/16 s) skimmed cow's milk was standardized to a milk fat content of 3.7% by the addition of 7 lb of pasteurized (82.0°C/30 s) homogenized (2000 psi, single stage) cream (35% milk fat), and was placed in a stainless steel cheese vat resting in a larger pilot-plant-sized, steam-jacketed cheese vat filled with water. Milk was inoculated with *L. monocytogenes* as described earlier. After warming the milk to 31.0°C, 2% (v/v) starter culture described earlier and calcium chloride (2.5 g) were added. One hour after adding the starter, the pH of milk was reduced to ca. 6.45 and 6.5 ml of the clotting agent, Marzyme Single Strength Microbial Rennet (Marschall Division-Miles Laboratories, Inc., Madison, WI), diluted 1:40 in cold water, was added. After 40 min the curd was cut into 1/2 in. cubes and then was left undisturbed for 15 min. Contents of the vat were warmed gradually to 35-36°C in 30 min and cooking was continued at this temperature for about 45 more minutes. During cooking curd/whey were stirred gently to prevent matting. After cooking, the volume of curds and whey in the vat was reduced to 40% of the original milk volume by removal of whey. The water of the steam-jacketed cheese vat also was drained to lower the temperature of curd/whey in the other vat. Salt (0.6% of the original milk weight) diluted in 1 L of whey was added to the remaining curds and whey. The salted curds and whey were stirred gently for an additional 15 min when cubes of curd were removed and held for about 20 min on cheese cloth on a screen so curd was firm and relatively free from whey at the time of hooping, and so individual pieces barely adhered to each other. *P. roqueforti* spore powder (2.5 g) was added (dusted using a salt shaker) during placing of the curd from 67 lb of milk into two rectangular (26 x 13 x 13 cm) metal hoops. Hoops of curd were turned 10 min after filling, at 30-min intervals for the next 2 h and then every hour for 2 h. The temperature during drainage of whey was 22-25°C. Cheeses were removed from hoops on the next day and placed into a container with salt equivalent to 5% of the weight of the cheese. Dry salting of cheeses was done during the next 7 d according to the schedule in Table 1. After completion of the salting process, each cheese was manually skedowed 70-75 times, with a 3/32-in. diameter needle, to facilitate growth of mold on the interior of the cheese.

The pH of the 1-d-old cheese before salting was in the range of 4.65 and 4.95 (six lots). Cheeses were ripened at 9-12°C and a relative humidity of 90-98% for 84 d, and then cheese was stored at 4°C.

<table>
<thead>
<tr>
<th>Step</th>
<th>Time</th>
<th>Temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skim milk pasteurization</td>
<td>9:00 A.M.</td>
<td>75.0</td>
</tr>
<tr>
<td>Cream pasteurization</td>
<td>9:30</td>
<td>82.0</td>
</tr>
<tr>
<td>Milk standardization (3.7% fat)</td>
<td>(0.01% fat) + Homog. cream (35%)</td>
<td>10:30</td>
</tr>
<tr>
<td>Add <em>L. monocytogenes</em></td>
<td>11:00</td>
<td>8.0-10.0</td>
</tr>
<tr>
<td>Add starter culture</td>
<td>12:00 P.M.</td>
<td>31.0-32.0</td>
</tr>
<tr>
<td>Add calcium chloride</td>
<td>12:00</td>
<td>31.0-32.0</td>
</tr>
<tr>
<td>Add rennet</td>
<td>1:00</td>
<td>31.0-32.0</td>
</tr>
<tr>
<td>Cut curd</td>
<td>1:40</td>
<td>31.0-32.0</td>
</tr>
<tr>
<td>Heat curd/whey</td>
<td>1:55-2:25</td>
<td>31.0-36.5</td>
</tr>
<tr>
<td>Cook curd/whey</td>
<td>2:25-3:15</td>
<td>35.0-36.5</td>
</tr>
<tr>
<td>Remove part of whey (40%)</td>
<td>3.15</td>
<td>35.0-36.0</td>
</tr>
<tr>
<td>Add salt (0.6%)</td>
<td>3.20</td>
<td>---</td>
</tr>
<tr>
<td>Remove curd to screen</td>
<td>3.35</td>
<td>---</td>
</tr>
<tr>
<td>Add mold powder - hooping</td>
<td>4.00</td>
<td>---</td>
</tr>
<tr>
<td>1st turn</td>
<td>4:10</td>
<td>---</td>
</tr>
</tbody>
</table>

**Turn every 30 min for the next 2 h and then every 1 h for 2 h**

<table>
<thead>
<tr>
<th>Salting (Age of cheese)</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>First (1 d)</td>
<td>Second (3 d)</td>
</tr>
<tr>
<td>Amount of salt (% wt of cheese)</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*a*Cheese was ripened at 9-12°C and a relative humidity of 90-98% for 84 d, and then cheese was stored at 4°C.
Duplicate samples for enumeration of *L. monocytogenes* and determination of pH, were taken during manufacture and ripening of blue cheese according to the following schedule: (a) pasteurized milk, (b) inoculated milk, (c) inoculated milk before addition of rennet, (d) curd before cooking, (e) whey, (f) curd before hooping, and (g) curd after 2 and 4 h of draining. Samples to be tested for *L. monocytogenes* and pH were taken from cheeses that were 1.2, 4.6, 8, and 10 d old. Additional samples were taken at 10-d intervals during ripening until the cheese was 120 d old. Samples from the outer 1/2-3/4 in. of the cheese were also tested for *L. monocytogenes* after 70 d of ripening and at 10-d intervals until the cheese had aged for 120 d.

**Enumeration of L. monocytogenes**

Duplicate samples of undiluted pasteurized milk (0.25 ml), inoculated milk (0.1 ml), whey (0.25 ml), and/or appropriate dilutions (0.1 ml) of inoculated milk, curd and cheese, made in a warm (42 ±2°C) sterile solution of 2% sodium citrate (43), were used to enumerate *L. monocytogenes* by surface-plating on McBride Listeria Agar (MLA) (21). The initial (1:10) dilution of curd (10 g) or cheese (10 g) was made in warm (42 ±2°C) sterile Tryptose Broth with 2% sodium citrate contained in sterile Stomacher bags of ca. 500-ml capacity (Tekmar Co., Inc., Cincinnati, OH) (43). Samples were blended in a Stomacher 400 for 3 min. To increase the detection level from 100 to 10 cfu/g, 0.25 ml of the initial (1:10) dilution was surface-plated on each of four plates of MLA. The plates were dried enough to absorb the inoculum within 10-15 min. All samples initially diluted 1:10 in TB from which *L. monocytogenes* was not detected, were re-examined after 2, 4, 6, and 8 weeks of cold enrichment at 4°C. All plates (from direct plating and cold enrichment samples) were incubated at 35°C for 48 h in an atmosphere of 5% O2: 10% CO2: 85% N2. Colonies typical of those formed by *L. monocytogenes* were counted, and selected colonies were confirmed as *L. monocytogenes* according to the procedure of Ryser and Marth (32), which is based on a positive catalase reaction, tumbling motility, appearance of blue-green colonies on Tryptose Broth with 2% sodium citrate contained in sterile Stomacher bags (Analytab Products, Plainview, NY).

**Determination of pH and contents of moisture, milk fat, and salt**

The pH of milk, curd, whey, and cheese was determined at the time of testing for *L. monocytogenes*, using a pH-meter (Corning Model 10) equipped with a flatbottom standard combination electrode (30). Duplicate moisture and fat determinations were made as described in *Standard Methods for the Examination of Dairy Products* (30). Duplicate NaCl determinations were made on 10-g cheese samples using the Quantab chloride titrator test strip method (Miles Laboratory, Inc., Elkhart, IN). Salt in the free moisture of the cheese was calculated as described by Morris (24).

**Statistical methods**

For statistical analysis of results, the method of repeated measures analysis of variance was used. Computations were done using the SAS package. The level of significance used to compare differences commonly was 0.05.

**RESULTS AND DISCUSSION**

**Composition of cheese**

Results of determinations of moisture, fat in dry matter (FDM), salt content, and of calculations of salt in the water phase (SWP) are in Table 2. The standard of identity for blue cheese in the U.S. provides for a maximum moisture content of 46% and that one-half of the solids must be fat. Most commercial blue cheese in the U.S. contains 40 to 44% moisture and the salt content varies between 3 and 5%. Other blue-veined cheeses such as Gorgonzola and Roquefort cannot contain more than 42 or 45% moisture, respectively (24).

The average moisture content for blue cheese (40-d-old) from six trials was 38.90% (S.D. ± 0.90), and the average FDM was 52.52% (S.D. ± 1.82). The two criteria of the standard of identity were met in all our trials. The NaCl content of cheese in the six trials ranged between 4.26 and 4.85%, with an average of 4.51% (S.D. ± 0.22). The average calculated SWP was 11.52% (S.D. ± 0.55). In general, a SWP concentration of ca. 10% should be maintained in commercial blue cheese; this seems to be sufficient to discourage growth of most contaminants of the cheese. *P. roqueforti* can grow at a SWP of up to 16% (15.24).

**Behavior of L. monocytogenes during manufacture of blue cheese**

All six lots of pasteurized milk were free (direct plating and cold enrichment up to 8 weeks) of *L. monocytogenes* before they were inoculated with the pathogen. The behavior of *L. monocytogenes* strains Scott A and CA during manufacture of blue cheese is shown by data in Fig. 1 and 2, respectively. No growth of *L. monocytogenes* was observed during the 2 h before addition of rennet; this is consistent with results obtained during the manufacture of Feta cheese (27). Rosenow and Marth (31) also showed that the same strains of *L. monocytogenes* have a lag phase of approximately 2 h when grown in autoclaved whole milk incubated at 35°C. *L. monocytogenes* was entrapped in curd during cheesemaking, with the population in curd before cooking being an average of 0.62 (S.D. ± 0.07) log10 cfu/g greater than in the inoculated milk. The population in curd before hooping was an average of 1.00 (S.D. ± 0.20) log10 cfu/g greater than in the inoculated milk. Whey from all six trials contained less than 5.8% of the cells in the inoculum with an average of 3.6% (S.D. ± 1.4) (Table 3).

*L. monocytogenes* strain Scott A in cheese increased in numbers by ca. 1.2 log10 cfu/g during the first 24 h until the pH values of cheese decreased to 4.7 - 4.9. The same strain increased in numbers by only ca. 0.30 log10 cfu/g and growth of the pathogen ceased as the pH of the curd decreased to 4.85, 4 h after hooping a lot with rapid acid production (Table 3, Trial 1). *L. monocytogenes* strain CA, in cheese, increased in numbers by ca. 0.72 log10 cfu/g during the first 24 h until...
the pH values decreased to 4.90-4.95. The same strain, in a lot with rapid acid production (Trial 4) increased in numbers by only 0.12 log\textsubscript{10}cfu/g and growth of the pathogen ceased as the pH of the curd decreased to 5.0, 4 h after hooping.

Growth of the pathogen was calculated considering the yield of 24-h-old cheese, the total inoculum and the number of \textit{Listeria} cells that escaped in the whey. Because of the concentration of the pathogen in the curd and growth of \textit{L. monocytogenes} during the first 24 h (mainly the first 9 h of manufacture), the population was 0.97 to 2.30 log\textsubscript{10}cfu/g greater in the 1-d-old cheese than in the inoculated milk (Table 3), with a maximum number of 9.8 x 10\textsuperscript{4} cfu/g.

Statistical analysis of all data from the first 24 h indicated: (a) no difference between the two strains in their behavior during the first 9 h; (b) significantly (P ≤ 0.0006) different between the two strains and populations of strain Scott A in the 1-d-old cheese were significantly (P ≤ 0.002) higher than those of strain CA; (c) populations of trials 1 (strain Scott A) and 4 (strain CA), with rapid acid production, in the 24-h-old cheese were significantly (P ≤ 0.003) lower than those of the other four trials with normal acid development, regardless of strain; (e) changes in pH values of cheeses of trials 1 and 4 were significantly (P ≤ 0.01) different (lower) than those of cheese of the other four trials during the first 9 h. However, no significant difference was noted among the six trials for time 0 to 24 h, as the pH values in all of them decreased significantly (P ≤ 0.006); (f) the pH of the 1-d-old cheese before salting was in the range of 4.65 and 4.95 without any significant (P ≤ 0.05) difference between the six trials.

**TABLE 3. Fate of \textit{Listeria monocytogenes} during the first 24 h of blue cheese making.**

<table>
<thead>
<tr>
<th>Trial</th>
<th>Cells lost in whey (% of inoculum)</th>
<th>Increase from initial number in curd (Log\textsubscript{10})</th>
<th>Population before hooping (Log\textsubscript{10})</th>
<th>Actual growth (Log\textsubscript{10})</th>
<th>Total incr. after 24 h (Log\textsubscript{10})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.09</td>
<td>0.65</td>
<td>1.00</td>
<td>0.30</td>
<td>1.16</td>
</tr>
<tr>
<td>2</td>
<td>2.73</td>
<td>0.51</td>
<td>1.14</td>
<td>1.22</td>
<td>2.05</td>
</tr>
<tr>
<td>3</td>
<td>5.78</td>
<td>0.65</td>
<td>1.33</td>
<td>1.21</td>
<td>2.04</td>
</tr>
<tr>
<td>4</td>
<td>2.79</td>
<td>0.67</td>
<td>0.74</td>
<td>0.12</td>
<td>0.97</td>
</tr>
<tr>
<td>5</td>
<td>3.33</td>
<td>0.56</td>
<td>0.89</td>
<td>0.85</td>
<td>1.38</td>
</tr>
<tr>
<td>6</td>
<td>4.78</td>
<td>0.66</td>
<td>0.92</td>
<td>0.58</td>
<td>1.41</td>
</tr>
</tbody>
</table>
Results obtained during manufacture of blue cheese indicate the growth of *L. monocytogenes* during the first 24 h (mainly during the first 9 h) because of the high-moisture curd and high temperature, up to 22-25°C. However, growth of the pathogen ceased when the pH value of cheese decreased to 5.0 or less. Growth of *L. monocytogenes* also was observed during manufacture of Feta cheese (27), and modest growth occurred during manufacture of Cheddar and Camembert cheeses (32,33). Substantial growth of the pathogen occurred in Camembert cheese during the ripening process (33).

**Survival of *L. monocytogenes* during ripening of blue cheese (2-120 d)**

Blue cheese was ripened at 9-12°C and a relative humidity of 90-98% for 84 d and then cheese was stored at 4°C. Satisfactory mold growth occurred in 30 to 80 d, and the pH of the 80-d-old cheese was in the range of 5.70 to 6.20 (except for one lot which had a pH of 5.3, because of minimal mold development). Survival of *L. monocytogenes* strains Scott A and CA decreased significantly (*P* ≤ 0.005) during the first 50 df of ripening of the cheese, and did so by an average of 2.65 and 2.73 log<sub>10</sub> cfu/g, respectively, compared to populations of the 1-d-old cheese before salting. Strain Scott A survived without any further substantial decrease in numbers of the pathogen in cheese during days 50 to 120 (Fig. 3). Strain CA survived during days 50 to 80 (Fig. 4). Populations of the pathogen decreased gradually until they became undetectable by direct plating at 110 d (Trial 4) and 120 d (Trial 4, 5, and 6). The same samples gave positive results after cold enrichment (Table 4).

Acidification at the beginning of cheesemaking (pH 4.6 to 4.9 during the first 20 d) played a major role in reducing the population of the pathogen. The decrease (ca. 2.7 log<sub>10</sub> cfu/g) of *L. monocytogenes* during days 1-50 in blue cheese appears to be greater than occurred in Feta cheese having pH values as low as 4.3 (27,42). Apparently, *P. roqueforti* may have contributed to this inactivation of *L. monocytogenes* during the first 50 d of ripening through intense proteolysis and lipolysis and resultant production of free fatty acids in blue cheese (15,24). The increase in pH later during ripening (days 50-120, Fig. 3 and 4) modified the unfavorable environment of blue cheese and allowed *L. monocytogenes* strain Scott A to survive more successfully during the rest of the ripening period. Strain CA was less tolerant of the same conditions and was gradually inactivated, but viable cells remained in the 120-d-old cheese (positive cold enrichment samples). Recent studies also indicated that strain CA was less tolerant than were other strains of conditions in Feta, brick, and Colby cheese (27,34,42).

No growth of *L. monocytogenes* was observed in blue cheese during the ripening period even with the favorable conditions.
This amount of salt in fatty acids, methyl ketones and their corresponding secondary alcohols is the result of the high salt content (4.26 to 4.85%). This salt content, and about 33.54 to 35.62% free moisture, resulted in an effective brine concentration of 10.68 to 12.32% (Table 2). In commercial blue cheese the brine concentration is about 10% (24). This amount of salt in solution is too much to permit growth of *L. monocytogenes*. Other experiments have also shown that the same strains of the pathogen not only failed to grow but the population was gradually inactivated in whey or skim milk containing 12% NaCl (26). Substantial growth of *L. monocytogenes* in Camembert cheese (33) coincided with an increase in cheese pH; also, the moisture content (ca. 54%) was higher, and the salt content (ca. 2.4%) was lower than in blue cheese. Thus the high salt content in blue cheese likely was the main reason for lack of growth by *L. monocytogenes*. Also, lipolysis in blue cheese is more extensive than in Camembert cheese; use of homogenized milk (or homogenized cream) enhances this action. Furthermore, production free fatty acids and methyl ketones derived from fatty acids via the beta-oxidation pathway, and their corresponding secondary alcohols (15) may contribute to the unfavorable environment for *L. monocytogenes*.

**Survival of *L. monocytogenes* on the surface of blue cheese**

Results on survival of *L. monocytogenes* in the outer 1/2-3/4 in. of the cheese after 70 d of ripening and at 10-d intervals until the cheese had aged for 120 d are in Table 5. Strain Scott A survived in this surface layer although the population of the pathogen gradually decreased. The 120-d-old cheese had approximately the same population in the outer layer as did the interior of cheese. Strain CA was less tolerant to conditions in the surface layer of cheese than was strain Scott A; the 110-d and 120-d-old cheese in all three trials (inoculated with strain CA) gave us negative results on direct plating. These samples were all positive after cold enrichment (Table 6).

Results of this work indicate that, if present in milk, *L. monocytogenes* can grow during the initial stage of manufacture of blue cheese until the pH of the cheese is reduced to 5.0 or below. The rate of death of the pathogen during the first 50 d of ripening was substantial, but later, as the pH of cheese increased, the pathogen survived more successfully. These results emphasize the tenacity of *L. monocytogenes* during ripening of blue cheese even in unfavorable conditions (e.g., presence of high salt content, free fatty acids, methyl ketones, and their corresponding secondary alcohols). Consequently, one cannot depend on the environment of blue cheese to promptly inactivate the pathogen during the cheese ripening process. Thus to insure *Listeria*-free blue cheese, the pathogen must be kept out of cheese during cheese-making and subsequent handling of the product. Properly pasteurized milk and use of adequate hygienic practices are needed to accomplish this goal.

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REFERENCES