Fate of \textit{Staphylococcus aureus} in Whey, Whey Cream, and Whey Cream Butter

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ABSTRACT

Fresh Cheddar cheese whey was inoculated with ca. $10^6$ \textit{Staphylococcus aureus}/ml and held at 4, 25, and 37°C for 48 h. Numbers of staphylococci decreased in whey at 25 and 37°C and decreased or remained constant in whey at 4°C. When Cheddar cheese whey was neutralized with sodium hydroxide before inoculation with ca. $10^2$ or $10^6$ \textit{S. aureus}/ml, numbers of the bacterium increased at all incubation temperatures. Viability of \textit{S. aureus} strains in whey butter made from inoculated whey cream (from Cheddar cheese whey) was determined. Whey cream was either neutralized to a titratable acidity of .15% or untreated before inoculation with ca. $10^4$ \textit{S. aureus}/ml. Butter churned from the whey cream was held at 4, 25, and 30°C for up to 4 wk. Viability of \textit{S. aureus} was enhanced in lightly salted (1%) whey cream butter and in butter made from neutralized whey cream. Strains of \textit{S. aureus} did not survive in unsalted or in salted (1.5%) butter made from untreated whey cream.

INTRODUCTION

Cheese and cheese foods are important to the dairy industry and account for much of the milk used in dairy manufacturing. During cheese making, whey is separated from cheese curd. Whey is largely water but also contains lactose, some milk fat, ash and minerals, and whey protein. Because of disposal problems associated with the large quantities of whey produced, and the loss of potentially valuable food components in the whey (such as milk fat and whey protein), interest has increased in the use of whey and whey by-products (9).

Fresh whey recovered from cheese making is usually of excellent microbial quality. However, outbreaks of staphylococcal food poisoning have been associated with consumption of cheeses in which \textit{Staphylococcus aureus} has grown and produced enterotoxin (4, 15, 16, 17). Use of whey from cheese making operations is of concern because of the potential for staphylococcal contamination and the presence of the water-soluble enterotoxins.

Studies have determined the fate of \textit{S. aureus} in cheese whey (1, 9, 13, 18). In whey held at ≥25°C, staphylococcal counts decreased (1, 9, 18). Filtration, pasteurization, and presence of salt in whey enhanced the ability of \textit{S. aureus} to grow or survive and to produce enterotoxin (1, 9, 13). Westhoff and Engler (18) observed that storage of cottage cheese whey at 5°C permitted survival of \textit{S. aureus} for 9 d if the inoculum was at least 200 cells/ml.

It is often the practice to save whey from cheese making and to pass it through a cream separator to recover the milk fat. Cream that is recovered, called whey cream, is churned into butter as in conventional operations. Butter that results is called whey cream butter and often is indistinguishable from butter made from sweet cream (7). Since the potential exists for \textit{S. aureus} to be present in whey, we determined behavior of the pathogen in Cheddar cheese whey and in whey cream butter made from inoculated whey cream.

MATERIALS AND METHODS

Cultures

\textit{Staphylococcus aureus} strains 100-A, 196-E (both from M. S. Bergdoll, Food Research Institute, University of Wisconsin-Madison), 254, 473, 505, and 521 (from R. W. Bennett, Food and Drug Administration, Washington, DC) were used in this study. All strains were pure, coagulase-positive cultures of \textit{S. aureus}.
Cultures were stocked on brain-heart infusion (BHI) agar (Difco, Detroit, MI) slants, which were refrigerated. Before use for experiments, strains of *S. aureus* were transferred to BHI broth (Difco), which was incubated for 24 h at 37°C, and then transferred to BHI broth again, which was incubated for 18 h at 37°C. For each culture, cells were harvested by centrifugation at 6000 × g for 20 min at 4°C, washed twice in cool sterilized sodium phosphate buffer (10°C, pH 7.4), and resuspended in 10 ml of the sterilized phosphate buffer. Dilutions were prepared, if necessary, to achieve the appropriate inoculum.

**Whey and Whey Cream**

Fresh Cheddar cheese whey was obtained from the University of Wisconsin dairy factory. Whey was held at 4°C for a maximum of 24 h before use. When appropriate, fresh whey was neutralized to pH 6.8 with NaOH according to the procedure of Westhoff and Engler (18). Whey was processed with a hand-operated cream separator (Subitas, Istanbul) at 30 to 40°C to recover the milk fat. Whey cream was neutralized with sodium bicarbonate or remained untreated. Tables developed by Hunziker (5) were consulted when neutralizing whey cream to a titratable acidity (TA) of .15%. Neutralizer was dissolved in sterilized distilled water at the rate of .08 g/ml. Whey cream was warmed to 25°C, and the neutralizer solution was added while the cream was stirred. Neutralized whey cream was stirred for an additional 5 min to distribute the solution. Neutralized and untreated whey cream were inoculated with *S. aureus* and churned to obtain whey cream butter.

**Inoculation and Incubation of Whey and Whey Cream**

A 300-ml sample of untreated or neutralized whey was placed in a 500-ml Erlenmeyer flask and inoculated. Untreated whey was inoculated with ca. 10⁶ *S. aureus*/ml. Neutralized whey was inoculated with ca. 10² or 10⁵ *S. aureus*/ml. Inoculations with ca. 10² or 10⁶ *S. aureus*/ml were used to simulate extremes in contamination of whey. Westhoff and Engler (18) noted that a population of 10⁶ *S. aureus*/ml of whey would never be encountered in practice, but that an inoculum of 10² *S. aureus*/ml was probable. Flasks containing inoculated whey were shaken to distribute the inoculum, and then plugged. Inoculated whey was held at 4, 25, and 37°C for 48 h. All trials were done in duplicate.

A 2-L volume of neutralized or untreated whey cream was inoculated to contain ca. 10⁴ *S. aureus*/ml and churned into whey cream butter. These trials were done in duplicate.

**Production of Whey Cream Butter**

Before churning, whey cream was held overnight at 4°C and then was brought to 11 to 13°C. A 2-L volume of whey cream was churned in an electric churn (Gem Dandy Jr. Electric Churn, NASCO, Ft. Atkinson, WI). After the cream broke, buttermilk was removed. Whey cream butter was washed with 1 L of sterilized cool water (8 to 10°C), a second 1 L of wash water was added, the churn was operated for 1 min, and the wash water was again drained. Butter made from untreated whey cream was either not salted or was salted to contain 1 or 1.5% (wt/wt) sodium chloride. Butter made from neutralized whey cream was not salted. Whey cream butter was worked, then 200-g portions were transferred to 228-g (8-oz) yogurt cups obtained from the University of Wisconsin dairy factory and held at 4, 25, and 30°C for up to 4 wk.

**Analyses**

Aerobic plate count, staphylococcal count, and pH were determined at designated times. Determinations of pH were made in duplicate. All platings were in triplicate. Whey samples were analyzed after 6 h, and at 12-h intervals up to 48 h of incubation. Whey cream butter was analyzed after 4, 8, 14, 20, and 28 d of storage. Procedures in *Standard Methods for the Examination of Dairy Products* were followed in sampling whey, whey cream, and whey cream butter and in making pH and TA determinations (8). Standard methods agar was used to determine aerobic plate count (8).

Presence and numbers of *S. aureus* were determined using Baird-Parker agar (Difco) and Vogel-Johnson agar (Difco). Plates were incubated at 37°C for 48 h and colonies were counted. Counts of *S. aureus* were confirmed by selecting colonies presumed to be *S. aureus* for determination of gram reaction and coagu-
lase production by isolates derived from such colonies. Values reported for numbers of *S. aureus* are means obtained from counts of colonies on Baird-Parker and Vogel-Johnson agars. Values reported as non-staphylococcal aerobic bacteria were obtained by subtracting mean staphylococcal counts from mean aerobic plate count values.

**RESULTS**

**Inoculated Untreated Whey**

Untreated Cheddar cheese whey inoculated with ca. 10⁶ *S. aureus/ml* did not support growth of any strain at 4, 25, or 37°C. Data for strains 100-A (Figure 1) and 254 (Figure 2) are representative of those for all strains of *S. aureus* used in this study. We noted a rapid decrease in staphylococcal count in whey held at 4 and 25°C. At 37°C, numbers of *S. aureus* in untreated whey decreased approximately 1000-fold during 48 h of incubation (Table 1). *Staphylococcus aureus* survived in greater numbers at 4°C than at either 25 or 37°C. The population of non-staphylococcal aerobic bacteria in inoculated untreated whey held at 4°C increased or decreased slightly, or remained constant, for both sizes of inoculum (data not shown). When neutralized whey was held at 25°C, the population of *S. aureus* increased ca. 10-fold, depending on the size of the inoculum and *S. aureus* strain (data not shown). At 37°C, the staphylococcal count increased to approximately 10⁵/ml after 48 h, and non-staphylococcal aerobic plate count values increased to >10⁶/ml (Table 1).

**Inoculated Neutralized Whey**

When Cheddar cheese whey was neutralized to pH 6.8 before inoculation, viability of *S. aureus* strains was enhanced, independent of size of inoculum. Staphylococcal counts increased ca. 100-fold in neutralized whey held at 37°C for 48 h as indicated by data for strain 100-A (Figure 3) and strain 254 (Figure 4). At 4°C, the number of *S. aureus/ml* increased or decreased slightly, or remained constant, for both sizes of inoculum (data not shown). When neutralized whey was held at 25°C, the population of *S. aureus* increased ca. 10-fold, depending on the size of the inoculum and *S. aureus* strain (data not shown). At 37°C, the staphylococcal count increased to approximately 10⁵/ml after 48 h, and non-staphylococcal aerobic plate count values increased to >10⁶/ml (Table 1).

**Treated Versus Untreated Whey**

Data for strains 100-A and 254 are representative of those for all strains of *S. aureus* used in this study and indicate growth occurred in neutralized cheese whey at 37°C, but there...
TABLE 1. Fate of Staphylococcus aureus (SA) strains in untreated and neutralized Cheddar cheese whey (initial inoculum ca. 10⁶/ml) after 48 h at 37°C.

<table>
<thead>
<tr>
<th>SA strain</th>
<th>Untreated whey</th>
<th>Neutralized whey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SA count/ml</td>
<td>Non-SA APC/ml</td>
</tr>
<tr>
<td>100-A</td>
<td>9.6 x 10²</td>
<td>1.3 x 10⁶</td>
</tr>
<tr>
<td>196-E</td>
<td>8.7 x 10²</td>
<td>3.4 x 10⁶</td>
</tr>
<tr>
<td>254</td>
<td>9.3 x 10²</td>
<td>1.9 x 10⁶</td>
</tr>
<tr>
<td>473</td>
<td>9.2 x 10²</td>
<td>2.5 x 10⁶</td>
</tr>
<tr>
<td>505</td>
<td>9.2 x 10²</td>
<td>1.8 x 10⁶</td>
</tr>
<tr>
<td>521</td>
<td>9.5 x 10²</td>
<td>1.6 x 10⁶</td>
</tr>
</tbody>
</table>

¹Mean counts of colony-forming units on Baird-Parker and Vogel-Johnson agars.
²Nonstaphylococcal aerobic plate count (APC) minus mean SA counts = non-SA).
³From an initial pH (average) of 6.1.
⁴From an initial pH after neutralization (average) of 6.8.

was a loss of viability in untreated whey at 37°C, independent of the level of inoculum (Figures 3 and 4). Behavior of S. aureus strains in treated and untreated whey at 25°C was similar (data not shown).

Milk Fat Separation from Cheese Whey

Use of the hand-operated cream separator to process fresh Cheddar cheese whey yielded whey cream with a fat content of 23 to 31%. When fresh whey was inoculated with ca. 10⁶ S. aureus/ml, resulting whey cream had a population of S. aureus ranging from <10² to 10⁴/ml (data not shown).

Whey Cream Butter

Results obtained for S. aureus strain 521 were representative of the behavior of all strains in whey cream butter (Figures 5 and 6). Unsalted butter and butter with 1.5% (wt/wt) salt both made from untreated whey cream did not support growth of S. aureus at any incubation temperature. Lightly salted (1% salt) butter made from untreated whey cream supported growth of S. aureus strains at 25 and 30°C (Figure 5). Populations of S. aureus increased 10- to 100-fold, depending on strain and incubation temperature. At 4°C, numbers of S. aureus remained relatively constant or decreased.

Figure 3. Behavior of Staphylococcus aureus 100-A in Cheddar cheese whey (O) and in neutralized whey (●) held at 37°C, and behavior of nonstaphylococcal aerobic bacteria in whey (△) and neutralized whey (▲) held at 37°C.

Figure 4. Behavior of Staphylococcus aureus 254 in Cheddar cheese whey inoculated with a small (△) or large (●) inoculum and in neutralized cheese whey inoculated with a small (▲) or large (●) inoculum when whey samples were held at 37°C.
slightly. Similar results were obtained for all strains of *S. aureus* when butter was made from neutralized whey cream, as represented by results for strain 521 (Figure 6).

**pH and Titratable Acidity**

Values for pH decreased in inoculated neutralized and untreated cheese whey at all incubation temperatures. At 37°C, pH of inoculated untreated whey decreased from an initial average of 6.1 to an average of 4.9 (Table 1). For inoculated neutralized whey held at 37°C, the pH decreased from an initial average value of 6.8 to an average 5.6 (Table 1).

Whey cream from fresh cheese whey had a serum pH of 5.9 to 6.1 before inoculation and churning. Butter churned from untreated whey cream had an initial serum pH of 6.0, and an initial TA of .03%, which increased to .12% during storage at 30°C. Neutralization of whey cream to a TA of .15% gave a serum pH in the neutralized whey cream of 6.5 to 6.7 before inoculation and churning. The average serum pH in butter churned from the neutralized whey cream was 6.6. Acidity of the butter increased from .02 to >.07% TA during storage at 30°C.

**DISCUSSION**

Loss of viability by *S. aureus* in untreated Cheddar cheese whey was related, at least in part, to incubation temperature. Presence and growth of nonstaphylococcal aerobic bacteria in the normal flora of cheese whey, and increased acidity of the environment were inhibitory to growth of *S. aureus*. Similar results were obtained by Westhoff and Engler (18) and Miller and Ledford (9). The inhibitory effect of various acids, particularly lactic acid, on *S. aureus* has been reported (10). It also has been shown that many lactic acid bacteria can inhibit growth of various pathogens by producing lactic acid (14).

Miller and Ledford (9) noted that substances formed by lactic acid bacteria in addition to acid were present in Cheddar cheese whey and inhibited growth of *S. aureus*. Minor and Marth (11) obtained similar inhibition of *S. aureus* in acidified media. They concluded that the length of exposure in an acidified medium, in addition to the inhibitory activity of acids, contributed to inactivation of *S. aureus*.

*Staphylococcus aureus* lost viability in fresh Cheddar cheese whey but grew in neutralized whey. Beside neutralization of acid, heat treatment and filter sterilization also reduce the inhibitory properties of whey, allowing for growth of *S. aureus* (1, 9, 18). Recovery of whey cream from heavily inoculated cheese whey resulted in a 100- to 100-fold decrease in numbers of *S. aureus* in cream compared with those in whey.

Staphylococcal enterotoxins are water-soluble proteins. However, studies by Minor and
Marth (12) showed that during the manufacture of butter from cream in which S. aureus had grown and produced enterotoxin, the enterotoxin appeared in the butter as well as in the buttermilk and wash water. If cheese whey is contaminated with S. aureus and conditions favor growth and toxin production in the whey, the process of separating milk fat from whey could result in presence of enterotoxin in whey cream. In addition, S. aureus present in whey cream after separation of milk fat from whey could grow and produce enterotoxin, given the appropriate environmental conditions.

Behavior of strains of S. aureus in whey cream butter was influenced by neutralization of cream before churning and by presence of salt. Butter made from neutralized whey cream initially supported growth of S. aureus, followed by loss of viability by the pathogen as the length of storage increased. Lightly salting (1%, wt/wt) whey cream butter made from untreated whey cream enhanced the ability of S. aureus to grow at 25 and 30°C. Presence of 1.5% (wt/wt) salt in butter made from untreated whey cream and a high TA (12%) could have combined to inhibit growth of S. aureus. Salting the butter to 1.5% resulted in an average salt concentration of 8.7% in the water portion of the butter. Although S. aureus can tolerate relatively high salt concentrations, Helmy et al. (3) observed that salt concentrations of 7.5 and 10% in milk repressed growth of S. aureus. Ahmed et al. (1) studied the influence of salt on the ability of S. aureus to grow in raw and pasteurized Domiati cheese whey. In raw whey, salt enhanced survival of S. aureus because of its inhibitory effect on the other types of bacteria present in the whey. They also noted that salt seemed to be detrimental to growth of S. aureus in pasteurized whey (1). Minor and Marth (11) demonstrated that salt was more inhibitory to growth of S. aureus as the pH was lowered. The high salt concentration, therefore, was destined to inhibit rather than enhance growth of S. aureus in butter made from untreated whey cream.

Whey cream and whey cream butter can be made from cheese whey, or the whey can be dried. Dry whey is used by bakers in making bread, rolls, and pie crusts. It also is used in ice cream and frozen desserts and in canned soups, sauces, gravies, and cheese foods (7). Concern arises about the potential of S. aureus to grow to high levels in whey that could be used for production of dry whey and whey cream butter. Nonfat dry milk has been implicated in staphylococcal food poisoning, and it seems likely that whey contaminated with high levels of S. aureus also could serve as a vehicle for food poisoning outbreaks. Staphylococcus aureus is relatively resistant to drying and to heat, and growth of S. aureus to greater than 10^6/ml in whey can increase the resistance of staphylococci to thermal treatment (6).

It is important to prevent enterotoxigenic strains of S. aureus from contaminating cheese whey. The potential exists for staphylococcal food poisoning from products manufactured from whey contaminated with S. aureus and held at the wrong temperature (2). Presence of staphylococcal enterotoxins that are more resistant to adverse environmental conditions than cells of S. aureus also must be considered in assuring the safety of whey and whey products.

ACKNOWLEDGMENTS

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