Growth and Enterotoxin A Production by *Staphylococcus aureus* in Fluid Dairy Products

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ABSTRACT

*Staphylococcus aureus* strain 100 grew better in skim milk and whole milk (3.5% fat) than in half and half (10.5%), and whipping cream (30% fat) at 37 C. Enterotoxin A production was 1.14, 0.88, 0.24, and 0.18 μg per 100 g of skim milk, whole milk, half and half, and cream, respectively. Sufficient cell numbers were not obtained for enterotoxin production after 16 h at 22 C in these same media.

Enterotoxin A has been implicated in more food poisoning cases than other enterotoxins of *Staphylococcus aureus*, but conditions necessary for enterotoxin A production have not been well defined. Tatini et al. (9) found that a population of 2 to 3 million cells per ml was associated with detectable enterotoxin A in milk relatively free from competing microorganisms. Donnelly et al. (3) observed that *S. aureus*, strain MF24, grew in both raw and pasteurized milk. Tatini et al. (8) and Donnelly et al. (3) further showed that production of enterotoxin A in milk was associated with an increase in cell numbers, but the quantity of enterotoxin produced was not determined. Minor and Marth (7) reported that both growth of *S. aureus* and enterotoxin A production can occur in cream at 37 C.

Since the concentration of milkfat varies greatly in different fluid dairy products, it is pertinent to determine enterotoxin A production in such products with different amounts of milk fat.

MATERIALS AND METHODS

Fluid dairy products

Pasteurized skim milk, whole milk (3.5%), and cream were obtained from the Washington State University Creamery. Whipping cream and half and half were standardized to 30.0 to 10.5% fat, respectively.

Source and preparation of cultures

*S. aureus* strain 100, known to produce enterotoxin A, was obtained from M. S. Bergdoll, Food Research Institute, University of Wisconsin, Madison, Wisconsin. The culture was carried on slants of Trypticase Soy Blood Agar Base and prepared by inoculating Trypticase Soy Broth (TSB) after touching a loop to a stock slant. After 18 h of incubation at 37 C, a loopful of inoculum was transferred to a second tube of TSB and incubated 18 h at 37 C before inoculating the product under test.

Product inoculation

In the growth studies, 1 ml of *S. aureus* culture was added to give a 10⁶ cell concentration per ml in each of the products tested. The inoculated products were incubated 24 h at 37 C. One-ml portions were removed and plated, using appropriate dilutions, with *Staphylococcus* 110 medium containing sodium azide and incubated at 37 C for 48 h.

Enterotoxin extraction

In the enterotoxin production studies, test products were inoculated as described above. After 16 h of incubation at 37 C, all samples were extracted according to the procedure described by Mahnke and Rathman (5) with the following modification of the initial step. One hundred ml of skim milk or milk were blended with 1.2 g of NaCl in a Waring Blender at high speed for 3 min. When cream was analyzed, 300 ml of 0.2 N NaOH was added before blending. The blended mixture was adjusted to pH 7.5 with 5 N NaOH and centrifuged at 36,400 × g for 10 min at 5 C. The sediment was re-extracted by adding 100 ml of 0.2 N NaCl, adjusted to pH 7.5, and mixed with a Sorvall omnimixer at 8000 rpm for 1 min. The slurry was centrifuged at 36,400 × g for 10 min at 5 C and the supernatant fluid from the two extractions was pooled. To the pooled supernatant fluid, in a separatory funnel, 10 ml of chloroform was added and shaken vigorously 10 times through an arc of 90°. This solution was centrifuged at 36,400 × g at 5 C for 10 min. The supernatant fluid was collected and concentrated by over-night dialysis against 50% polyethylene glycol at 5 C. The concentrate was removed and dialysis tubing was washed with 0.01 M phosphate buffer at pH 7.5. The concentrate was centrifuged at 48,200 × g for 10 min at 5 C. The supernatant fluid was re-extracted with 2 ml of chloroform and the mixture centrifuged at 48,200 × g for 10 min at 5 C. The supernatant fluid was clear to slightly cloudy. Occasionally the supernatant fluid was definitely cloudy with a definite separation between the chloroform and aqueous phases. When this occurred, extraction with chloroform was repeated to remove more of the solids that would interfere with absorption of the enterotoxin onto the carboxymethyl cellulose column.

Once a clear supernatant fluid was obtained, the pH was adjusted to 4.6 with 6 N HCl while mixing with a magnetic stirrer. This solution was then centrifuged at 5 C for 10 min at 48,200 × g. The pH of the supernatant fluid was adjusted to pH with 1 N NaOH while mixing with a magnetic stirrer. The remaining portion of the extraction procedure was the same as described by Mahnke and Rathman (5), starting with Step 11D. The extracted material was also tested for presence of enterotoxin by the procedure of Mahnke and Rathman (5).
Efficiency of enterotoxin A extraction.

Efficiency of extraction and assay by the slide immunodiffusion test was determined by adding 1 ml of saline solution containing 10 μg of enterotoxin A/ml to 100 ml of pasteurized skim milk, whole milk, half and half, and whipping cream. The “spiked” samples were extracted using the procedures of Casman and Bennett (2) and Mahnke and Rathman (5).

RESULTS AND DISCUSSION

Staphylococcus aureus growth in fluid dairy products

Figures 1 and 2 show the growth curves obtained at 37 and 22 C with S. aureus strain 100 in skim milk, whole milk, half and half, and whipping cream. Best growth was observed at 37 C in skim milk and whole milk. Maximum cell concentration was reached at 16 h of incubation at 37 C regardless of the medium employed; however, the cell concentration in the high-fat products was less than the level found in the low fat products (Fig. 1). Comparable results have been reported by Minor and Marth (7). According to Vadehra and Harmon (10), the lipolytic activity of the staphylococci may limit their growth in cream. They reported that the lipolytic enzyme system of S. aureus was very active on milkfat and the release of 0.05% capric acid and 0.1% caprylic acid completely inhibited growth of S. aureus.

Enterotoxin production in fluid dairy products

The mean values for enterotoxin produced in skim milk, whole milk, half and half, and whipping cream were 1.14, 0.88, 0.24, and 0.18 μg per 100 g, respectively. The mean values are the result of examining three samples per product and nine micro samples per product. The efficiency of the extraction procedure and assay by the slide immunodiffusion test was found to be 40 to 60% depending on the product analyzed. An analysis of variance by Duncan’s multiple range test indicated that the amount of enterotoxin produced between the low-fat and the high-fat products was statistically significant at the 5% level of probability. Markus and Silverman (6) reported that production of enterotoxin A occurred during the logarithmic growth phase and was correlated with cell counts (6). Since less growth occurred in the high-fat products, less entero­toxin was expected.

In any discussion on growth of staphylococci in food, the number of cells required to produce sufficient enterotoxin to cause a typical syndrome of food poisoning must be considered. Jones et al. (4) found staphylococcal counts in milk or cheese in proportion to the amount of inoculum, suggesting that staphylococci inoculated in low numbers would have less chance of reaching the cell density required to produce enterotoxin. An inoculum of 10^3 cells per ml was selected in this study to simulate counts approximating those frequently found in commercially produced raw milk (7). However, the S. aureus cells added were known to be enterotoxigenic, whereas this may not be true with commercially produced raw milk.

For food poisoning to occur in humans, approximately 1 μg of enterotoxin A must be consumed; however this will vary with individuals (7). The data from this study show that only 0.24 μg and 0.18 μg of enterotoxin were produced per 100 g of half and half and whipping cream, respectively.

Assuming an extraction efficiency of 50%, these quantities do not appear sufficient to cause food poisoning since approximately 200 to 300 g of product would have to be consumed to provide 1 μg of enterotoxin A. Normally 200 to 300 g of half and half or whipping cream are not consumed at one time. However, S. aureus growth in cream must be considered a potential health hazard if the cream is held at a suitable growth
temperature and then made into a manufactured product such as butter. One case of food poisoning has been traced to butter made from cream containing enterotoxin A. Milk could be consumed in quantities greater than 400 g and could provide sufficient toxin to cause food poisoning. However, milk has been involved in few outbreaks of staphylococcal food poisoning because sufficient cell concentrations to produce enterotoxins probably would occur only under prolonged storage conditions at elevated temperatures. Another safety factor is the inhibitory effect of other competing microbes against S. aureus (8).

Based on the cell populations attained at 22 C and previous reports (4, 7, 9), these products were not analyzed for enterotoxin. The results reported herein suggest that the low temperatures used to preserve fluid dairy products are sufficient to prevent staphyloccocal growth and enterotoxin production whenever the original count is $10^3$ cells per ml or less and the holding temperature is 22 C or less.

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REFERENCES