Effect of Milk on Fibronectin and Collagen Type I Binding to *Staphylococcus aureus* and Coagulase-Negative Staphylococci Isolated from Bovine Mastitis

J. Miedzobrodzki  
*University of Lund, Lund, Sweden*

A. S. Naidu  
*University of Lund, Lund, Sweden*

J. L. Watts  
*Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Homer, Louisiana*

Pawel Ciborowski  
*University of Nebraska Medical Center, pciborowski@unmc.edu*

K. Palm  
*University of Lund, Lund, Sweden*

Follow this and additional works at: [http://digitalcommons.unl.edu/virologypub](http://digitalcommons.unl.edu/virologypub)
Effect of Milk on Fibronectin and Collagen Type I Binding to Staphylococcus aureus and Coagulase-Negative Staphylococci Isolated from Bovine Mastitis

J. MIEDZOBRODZKI,† A. S. NAIDU, J. L. WATTS,‡ P. CIBOROWSKI,‡ K. PALM, and T. WADSTRÖM

Department of Medical Microbiology, University of Lund, S-223 62 Lund, Sweden, and Hill Farm Research Station, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Homer, Louisiana 71040

Received 8 August 1988/Accepted 8 December 1988

Tryptic soy broth (TSB)-grown cells of Staphylococcus aureus isolated from acute and chronic bovine mastitis bound mainly $^{125}$I-fibronectin ($^{125}$I-Fn), whereas strains of nine species of coagulase-negative staphylococci showed a predominant interaction with $^{125}$I-collagen ($^{125}$I-Cn) type I. A particle agglutination assay (PAA) was used to examine the interaction of coagulase-negative staphylococci with $^{125}$I-Fn and $^{125}$I-Cn immobilized on latex. All 368 coagulase-negative staphylococci demonstrated high $^{125}$I-Cn and moderate to low $^{125}$I-Fn interactions in the PAA. Cn-PAA reactivity was high among strains of Staphylococcus xylosus (84.2%), Staphylococcus simulans (77.8%), Staphylococcus epidermidis (76.7%), and Staphylococcus hyicus (74.3%), whereas all six Staphylococcus capitis strains clumped Cn-PAA reagent. Incubating TSB-grown cells in 10% skim milk for 1 h decreased the $^{125}$I-Fn- and $^{125}$I-Cn-binding affinity in most of the S. aureus and coagulase-negative staphylococci, while growth in 10% skim milk for 18 h resulted in more than 90% decrease or complete loss of interaction with these proteins. Decreased $^{125}$I-Fn binding in the presence of milk was correlated with protease production but not with $^{125}$I-Cn binding.

Staphylococcus aureus and coagulase-negative staphylococci are the most important udder pathogens causing bovine mastitis (1, 23). Earlier studies suggest that the proximity of staphylococci to the test cistern and contact with milk is the critical factor in the development of intramammary infections (1, 17, 26). Furthermore, S. aureus strains isolated from bovine mastitis and grown in raw milk or carbohydrate-salt-rich medium produce extracellular factors which cause toxic reactions in bovine skin (3).

Milk, although rich in essential nutrients, is regarded to be bacteriostatic as a result of the presence of immunoglobulins, plasminogen, complement components, lysozyme, lactoferrin, and the lactoperoxidase system (20, 21). However, S. aureus strains isolated from bovine mastitis demonstrate enhanced growth in whey prepared from the same milk the organism was isolated from (14). It has also been shown that staphylococci grown in milk show a decline in or complete loss of charge and hydrophobic cell surface properties (12).

S. aureus and Streptococcus agalactiae strains adhere to ductal epithelial cells (7). Hibbitt and Benians demonstrated that keratin, a cationic basic protein in the test duct, attaches to staphylococcal mastitis strains (9). Fibronectin (Fn), a glycoprotein, exists in a soluble form in serum and biological fluids and in an insoluble form in tissue matrices (15). S. aureus and coagulase-negative staphylococci isolated from various human infections bind Fn and collagen (Cn) (10, 25). Such interactions with the host mucosal surface at the molecular level may be important in the pathogenesis of staphylococcal intramammary infections (28). The Fn-binding receptor of S. aureus is a protein, susceptible to a wide range of proteases (22). However, the role of proteolytic enzymes produced during growth in milk is not known. In this study, we have explored the influence of skim milk on $^{125}$I-Fn and $^{125}$I-Cn binding to S. aureus and various species of coagulase-negative staphylococci isolated from acute and chronic bovine mastitis.

MATERIALS AND METHODS

Bacterial strains. A total of 80 S. aureus and 100 coagulase-negative staphylococcal strains, including 23 Staphylococcus epidermidis, 18 Staphylococcus simulans, 19 Staphylococcus xylosus, 16 Staphylococcus hominis, 14 Staphylococcus cohnii, 6 Staphylococcus capitis, and 4 Staphylococcus warneri, isolated from chronic and acute bovine mastitis were kindly supplied by P. Jonsson, National Veterinary Institute, Uppsala, Sweden. Additionally, 171 Staphylococcus hyicus, 50 Staphylococcus epidermidis, and 47 Staphylococcus chromogenes strains were isolated from bovine mastitis at the Mastitis Research Laboratory, Hill Farm Research Station, Homer, La. S. aureus was identified by standard procedures (4). Coagulase-negative staphylococci were identified according to the Schleifer-Kloos classification scheme and by Staph-Trac system (Analytab Products, Plainview, N.Y.) (24, 29). The accuracy of Staph-Trac system has been established previously and isolates yielding atypical results were identified by a previously described conventional method (29).

Chemicals. Fn, purified from porcine plasma by the method described by Vuento and Vaheri (27), was a kind gift from BioInvent AB International, Lund, Sweden. Vitrogen 100 collagen (containing 95% type I and 5% type III collagens, lot 87H18.3) was purchased from Collagen Corporation, Palo Alto, Calif. $^{125}$Iodine was purchased from Amersham Sweden AB, Solna, Sweden. Iodo-beads (N-chloro-benzenesulfonamide-derivatized, uniform nonporous polystyrene beads, lot 28666) were obtained from Pierce Chemicals Company, Rockford, Ill., and column PD-10 Sephadex G-25M was obtained.
from Sigma Chemical Co., St. Louis, Mo. Latex beads with a diameter of 0.8 μm (control 174660) were obtained from Difco Laboratories, Detroit, Mich., and Merthiolate was from KEBO Lab AB, Stockholm, Sweden. All chemicals used for the preparation of buffer solutions were of analytical grade.

**Bacteriological media.** Blood agar base was from Lab M, Salford, England. Tryptic soy broth (TSB) was from Difco. Standard methods caseinate agar was prepared as described previously (13). Skin milk was kindly supplied by the Swedish Dairies Association, Malmö, Sweden.

**Growth conditions.** Overnight cultures of staphylococci grown on blood agar were inoculated in TSB. TSB supplemented with 10% skim milk, or 10% skim milk at 37°C for 18 h with constant shaking. Cells were harvested, washed once in 0.02 M potassium phosphate (Pp) buffer (pH 6.8), resuspended in the same buffer to a density of 10^10 cells per ml, and stored at −80°C until tested.

**Proteolytic activity.** Proteolytic activity was determined on standard methods caseinate agar (13). The size and type of zone formed as a result of proteolysis were examined after 48 h of incubation.

**125I-Fn and 125I-Cn type 1 binding assay.** Fn and Cn were labeled with 125I by the chloramine-T method with Iodobeads (8). Binding of 125I-Fn and 125I-Cn to bacteria was quantitated by the method described by Fröman et al. (6). 125I-labeled portions were diluted to 13,000 to 25,000 cpm in phosphate-buffered saline (pH 7.2) containing 0.05% sodium azide and 0.1% bovine serum albumin. Labeled protein (50 μl) was mixed with 100 μl of staphylococcal cell suspension (10^10/ml) in a polystyrene centrifuge tube and kept at room temperature for 1 h. After adding 2 ml of ice-cold phosphate-buffered saline containing 0.05% azide and 0.1% Tween 20, tubes were centrifuged at 4,500 x g for 10 min and the supernatant was aspirated. Bound radioactivity of the bacterial pellet was measured in a 1260-Multigamma (Wallac, Turku, Finland). Laboratory strains of S. aureus Cowan 1 and S. aureus Newman served as positive controls, and two Micrococcus luteus dairy isolates served as negative controls in the binding assay.

**Fn and Cn particle agglutination assay (PAA).** Fn-PAA and Cn-PAA reagents were prepared by a method described by Naidu et al. (16) for the rapid screening of Fn and Cn receptors on staphylococcal cell surfaces. The agglutination reaction was performed on glass slides by mixing 20 μl of staphylococcal cell suspensions (10^9 cells per ml of Pp buffer) with an equal volume of Fn- or Cn-PAA reagent. The clumping was scored after 2 min as 1+, 2+, or 3+ reactions and recorded as positive, whereas +/− and − reactions were considered negative. Strains were checked for autoaggregation by mixing a drop of cell suspension with a drop of Pp buffer.

**RESULTS**

TSB-grown cells of S. aureus strains isolated from bovine mastitis showed high 125I-Fn and low 125I-Cn binding. TSB-grown S. aureus cells, when washed and incubated for 1 h in skim milk, showed certain changes in 125I-Fn-binding properties (Fig. 1). 125I-Fn binding was suppressed in 87.5% of S. aureus, while the remaining strains demonstrated an increased binding tendency. Similarly, cultivation of S. aureus strains in skim milk at 37°C for 18 h resulted in a sizeable decrease in 125I-Fn binding (Fig. 1). However, a few S. aureus strains expressed increased 125I-Fn (3.8%) and 125I-Cn (5.0%) binding.

All nine coagulase-negative staphylococcal species included in the present study showed high 125I-Cn and low 125I-Fn binding. The frequency of 125I-Fn and 125I-Cn binding among 368 coagulase-negative staphylococci isolated from bovine mastitis was tested in a PAA (Table 1). All nine species of coagulase-negative staphylococci grown on blood agar showed a high Cn-PAA and a low Fn-PAA reactivity comparable with that of the 125I-protein binding. S. hominis (62.5%) and S. capitis (50%) strains showed higher Fn-PAA reactivities. Cn-PAA reactivity was high among S. xylosus (84.2%), S. simulans (77.8%), S. epidermidis (76.7%), and S. hyicus (74.3%) strains. All six S. capitis strains agglutinated...
TABLE 1. Fn and Cn type I particle agglutination of coagulase-negative staphylococci isolated from bovine mastitis

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of isolates tested</th>
<th>No. positive (%)</th>
<th>Cn-PAA</th>
<th>AA&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. hyicus</td>
<td>171</td>
<td>127 (74.3)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>73</td>
<td>56 (76.7)</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>S. chromogenes</td>
<td>47</td>
<td>32 (68.1)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>S. simulans</td>
<td>18</td>
<td>14 (77.8)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. capitis</td>
<td>6</td>
<td>6 (100.0)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. hominis</td>
<td>16</td>
<td>10 (62.5)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>S. warneri</td>
<td>4</td>
<td>2 (50.0)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. xylosus</td>
<td>19</td>
<td>16 (84.2)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. cohnii</td>
<td>14</td>
<td>9 (64.3)</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Strains were grown on blood agar at 37°C for 18 h. Cells were washed once in Phosphate buffered saline and resuspended in the same buffer to a cell density of 10<sup>9</sup>/ml. AA, Autoaggregating strains.

Cn-PAA reagent. Coagulase-negative staphylococcal cells showed no high tendency to autoaggregate when harvested from blood agar; however, cells of a few isolates of S. hominis (18.8%) and S. epidermidis (5.5%) autoaggregated. 

<sup>125</sup>I-Cn binding of all coagulase-negative staphylococcal species, except S. hyicus, was suppressed after 1 h of incubation of TSB-grown cells in skim milk (Fig. 2). Skim milk-grown cells of coagulase-negative staphylococci demonstrated a considerable decrease in <sup>125</sup>I-Cn binding.

The proteolytic activity of S. aureus and coagulase-negative staphylococci was compared with the <sup>125</sup>I-Fn and <sup>125</sup>I-Cn-binding ability. Proteolytic strains of S. aureus showed a decrease in <sup>125</sup>I-Fn binding when grown in skim milk, whereas the <sup>125</sup>I-Fn binding to nonproteolytic strains was not affected by growth conditions in skim milk (Table 2). This phenomenon was observed mostly among S. aureus strains with greater than 10% binding of <sup>125</sup>I-Fn.

Of 70 coagulase-negative staphylococcal strains from bovine mastitis, only five strains bound <sup>125</sup>I-Fn to a degree greater than 10% or more. These isolates produced extracellular proteases and lost their ability to bind <sup>125</sup>I-Fn when grown in skim milk. However, protease production did not show any correlation to <sup>125</sup>I-Cn binding by S. aureus and coagulase-negative staphylococci in any of the growth conditions tested.

DISCUSSION

The adherence, survival, and multiplication of staphylococci on the udder epithelium are the decisive events in the pathogenesis of bovine mastitis (1). Frost et al. (7) demonstrated the adhesion of staphylococci to the ductular epithelium of the mammary gland in vivo. The effect of milk on <sup>125</sup>I-Cn type I binding to different species of coagulase-negative staphylococci isolated from acute and chronic bovine mastitis is shown in Fig. 2. In general, milk-grown cells of coagulase-negative staphylococcal species showed a decrease in <sup>125</sup>I-Cn type I binding after incubation in skim milk for 1 h. Except for strains of S. simulans and S. chromogenes, the decrease in binding was more prominent when cells were grown in skim milk at 37°C for 18 h.

![Fig. 2](image-url)
EFFECT OF MILK ON Fn AND Cn BINDING TO STAPHYLOCOCCI

This work was supported by grants from the Swedish Medical Research Council (B88-16X-04723-12C and B88-16X-08294-01), Industriesfonden, and Alfa Laval.

LITERATURE CITED


