Research Note

Characterization of Staphylococcal Bovine Mastitis Isolates Using the Polymerase Chain Reaction

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ABSTRACT

A polymerase chain reaction (PCR) assay was adapted to detect toxin genes of staphylococcal isolates from cases of bovine mastitis. Samples were obtained from three geographical areas: Korea and Idaho and Washington in the northwest United States. Samples from Korea and Washington were randomly chosen. Idaho samples were from a prospective study of mastitis etiology. Forty-one milk samples from 25 commercial farms in south-central Idaho were collected from cows with symptoms of mastitis. Although Staphylococcus aureus constituted 37.5% of mastitis isolates, these isolates lacked genes for staphylococcal enterotoxins (SEs), toxic shock syndrome toxin, and exfoliative toxins. In contrast, 4 of 13 isolates from Washington and 6 of 20 isolates from South Korea expressed SEs. These results suggest that PCR may be an effective means of screening bovine isolates for toxins. They also emphasize the potential for significant geographic differences in mastitis etiology.

Materials and Methods

Nine S. aureus samples were obtained from a prospective study of mastitis etiology in south-central Idaho. Samples were collected at 25 dairies from cows with clinical signs of mastitis or from cows with elevated milk somatic cell counts. The samples were collected using aseptic techniques and cultured using standard protocols (17). Microbial cultures were identified as described previously (16). In addition, 13 strains from Washington state and 20 strains from South Korea were randomly selected from stock cultures at Washington State University, Pullman, and the National Veterinary Research Institute at Anyang, Korea, respectively. Staphylococcal genomic DNA was isolated from lysostaphin-treated cells and processed as described previously (2). The DNA was extracted with phenol-chloroform (1/1 vol/vol) and chloroform, then precipitated with ethanol using standard techniques. The DNA was digested with HindIII, agarose gel (1%) stained with ethidium bromide, and visualized as previously described (18).

Although Staphylococcus aureus may produce a cadre of exoproteins, the staphylococcal enterotoxins (SEs) types A through E (SEA, SEB, SEC,SED, and SEE), toxic shock syndrome toxin (TSST), and exfoliative toxins (ETs) A and B (ETA and ETB) have the most widespread potential significance for human and animal health and dairy product safety. Studies to estimate the fraction of S. aureus bovine mastitis isolates that produce these toxins have produced estimates ranging from less than 5% to more than 30% (1, 7, 9, 11, 14). These studies relied on measurement of the phenotypic expression of the toxin proteins using immunological techniques. Phenotypic expression of toxin production by S. aureus is dependent upon culture conditions (15). Moreover, detection of toxins by methods using antisera can be inaccurate when expression is very low (2) or below the threshold of detection. In addition, problems with nonspecificity may arise as a result of protein A or cross-reactive staphylococcal proteins. Thus, detection of specific gene sequences coding for S. aureus exotoxins could provide a more sensitive estimate of the potential for S. aureus mastitis isolates to produce important toxins. The purpose of this study was to develop a polymerase chain reaction (PCR) procedure that would detect toxin genes in the genome of bovine S. aureus isolates and assess PCR’s feasibility for use in studying the role of toxins in clinical diseases and their epidemiology.

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### TABLE 1. Properties of primers used for PCR in this study

<table>
<thead>
<tr>
<th>Primer designation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Nucleotide sequences of primers</th>
<th>Location in structural gene&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE(ADE)-forward</td>
<td>5'-GAI'TTG CII AAA AAI TCT GA-3'</td>
<td>sea&lt;sup&gt;d&lt;/sup&gt; 103–122</td>
</tr>
<tr>
<td>SE(BC)-forward</td>
<td>5'-TTA GCA GAG AGI CAA CCA GA-3'</td>
<td>sed&lt;sup&gt;e&lt;/sup&gt; 397–416</td>
</tr>
<tr>
<td>SE(ABCE)-reverse</td>
<td>5'-GTT All CCI CCI TAC ATA CA-3'</td>
<td>see&lt;sup&gt;f&lt;/sup&gt; 103–122</td>
</tr>
<tr>
<td>SE(D)-forward</td>
<td>5'-ATT CTT GlA IIG TIA CATTT-3'</td>
<td>seb&lt;sup&gt;g&lt;/sup&gt; 319–338</td>
</tr>
<tr>
<td>TSST-forward</td>
<td>5'-GCAAAA GCA TCT ACAAAC GA-3'</td>
<td>sec&lt;sup&gt;h&lt;/sup&gt; 388–407</td>
</tr>
<tr>
<td>ET(AB)-forward</td>
<td>5'-CAT CAA TATTTATAG GTG GT-3'</td>
<td>se&lt;sup&gt;i&lt;/sup&gt; 661–680</td>
</tr>
<tr>
<td>ET(AB)-reverse</td>
<td>5'-GGT All GTI TTT GTI AAA GG-3'</td>
<td>et&lt;sup&gt;j&lt;/sup&gt; 526–545</td>
</tr>
<tr>
<td></td>
<td>5'-ATT AAATAT ACC TGATCCAG-3'</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5'-ATT AAA TAT ACC TGA TCC AG-3'</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Letters in parenthesis refer to serogroups of toxin homologous to each primer.

<sup>b</sup> Based on sequences deposited in GenBank (accession numbers below) at National Center for Biotechnology Information, Bethesda, Md., USA.

<sup>c</sup> Deoxyinosine.

<sup>d</sup> Gene encoding SEA, accession number M18970.

<sup>e</sup> Gene encoding SED, accession number M28521.

<sup>f</sup> Gene encoding SEE, accession number M21319.

<sup>g</sup> Gene encoding SEB, accession number M11118.

<sup>h</sup> Gene encoding SEC, accession number X05815.

<sup>i</sup> Gene encoding TSST-1, accession number J02615.

<sup>j</sup> Gene encoding ETA, accession number M17347.

<sup>k</sup> Gene encoding ETB, accession numbers M17348 and M13775.

### RESULTS AND DISCUSSION

To minimize the number of reactions necessary for toxin screening, some primers were designed based on sequences conserved between two or more homologous genes. Although this approach facilitates screening, some toxin pairs (i.e., SEB and SEC, SEA and SEE, ETA and ETB) could be detected but not distinguished from each other. Figure 1 shows that each primer pair successfully amplified its target gene in DNA from well-characterized positive controls but did not generate nonspecific amplification of DNA from an non-toxigenic S. aureus strain. None of nine strains of S. aureus isolated from Idaho possessed gene sequences coding for SEs, TSST, or ETs. However, among 13 isolates from Washington state, two strains had either the seb or sec genes and two more strains harbored the sed gene. Among 20 isolates from South Korea, four strains had either the sea or see genes, and two strains had seb or sec genes (Table 3).

The results of the isolates from Washington state and South Korea were similar to those reported by Olsvik et al. (18) in Scandinavia, Kenny et al. (9) in New York, Matsunaga et al. (14) in Japan, and Orden et al. (19) in Spain. These combined reports showed that more than 19% of the S. aureus isolated from cows with mastitis produced SEs or other exotoxins. In contrast, but similar to the analysis of isolates from Idaho state, Aarestrup et al. (1) in Denmark found that none of 106 mastitis-related S. aureus isolates produced SEs. The findings from this present study suggest that production of potentially significant SEs and other staphylococcal toxins is variable, depending on geographical and possibly temporal factors.

Toxigenic strains of S. aureus are a concern for several reasons (4). First, many of the toxins produced by this organism are superantigens that modulate the immune system and affect the health of the animal (5, 6). Another concern is that S. aureus toxins are associated with foodborne and other diseases in humans (4). The European Union has issued a directive that stipulates the threshold of S. aureus colony counts acceptable in raw milk (3). The disparate results found in this study demonstrate a large...
variability in the risk of staphylococcal toxin production and stress the need for a reliable, rapid means of assessing that risk to animals, humans, and dairy products.

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REFERENCES


