Detection of Toxie Shock Syndrome Toxin-1 Gene in Staphylococcus aureus Bovine Isolates and Bulk Milk by the Polymerase Chain Reaction

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(Received 3 June 1996/Accepted 10 July 1996)

ABSTRACT. Staphylococcus aureus isolates from mastitic cow's milk and farm bulk milk were examined for toxic shock syndrome toxin-1 (TSST-1) gene (tsl gene) by the polymerase chain reaction (PCR). The 179 bp band of tsl gene was observed in almost all the bovine isolates which showed TSST positive in a latex agglutination, as well as in human strain FRI 1169, but was not observed in bovine isolates of TSST negative. The lowest detectable threshold of the PCR for tsl gene was 1.2 x 10^6 cells/ml. When 125 bovine milk samples were cultured selectively for staphylococci and examined by PCR, the tsl gene was detected in 10 of the 35 culture fluids, in which staphylococci were recognized by Gram's staining.__KEY WORDS: polymerase chain reaction, Staphylococcus aureus, toxic shock syndrome toxin 1.


Toxic shock syndrome toxin-1 (TSST-1), produced by Staphylococcus aureus, has been recognized as the major cause of toxic shock syndrome (TSS) characterized by fever, hypotension, congestion in multiple organs and lethal shock in humans [1-3]. Some experimenters [4, 6, 11, 15], have found that TSST, identified by specific antibodies against TSST-1, is produced along with enterotoxins by some isolates of S. aureus from domestic animals such as cows, sheep, goats and horses. In particular, S. aureus isolates from cows, goats and sheep produced TSST at a relatively high frequency [13, 14, 16].

The TSST-1 purified from human-associated TSS isolates is a single-chain polypeptide with a molecular weight of 22,000 and an isoelectric point (pI) of 7.0 to 7.2 [9]. On the other hand, Ho et al. [7, 8] reported that all S. aureus isolates from sheep and goats produced TSST variant (TSST-ovine) with a pI of 8.6. Only 1 out of 10 bovine isolates produced the variant, whereas the other isolates produced TSST-1 (pI between 7.0 and 7.2). Takeuchi et al. [16] also reported that the TSST produced by bovine isolates of S. aureus was identical to TSST-1 from human isolates, with regard to properties of molecular size (22 kDa) and pI (7.2). Moreover, Lee et al. [12] demonstrated by nucletide sequence analysis that TSST from bovine isolates was identical to TSST-1 from human isolates while TSST-ovine had 14 nucleotide differences changing resulting in 9 amino acid residues.

The polymerase chain reaction (PCR) is powerful in vitro technique to detect various pathogenic organisms. PCR can produce a number of copies of a specific nucleic acid sequence in only a few hours. Recently, with the development of this technique, a number of infectious organisms have been detected directly from infected tissues or clinical specimens. Johnson et al. [6] and Jaulhac et al. [5] developed PCR procedures which will rapidly and specifically detect gene (tsl) for TSST-1 of S. aureus. Using PCR procedures, they examined the tsl gene of clinical isolates from human, isolates from foods, and reference strains, but did not test the tsp gene of isolates from domestic animals. The present study was thus conducted to examine the tsp gene in S. aureus isolates from clinical and subclinical mastitic cow's milk, and farm bulk milk by the PCR procedure. In addition, we tried to detect tsp gene of S. aureus from farm bulk milk of cows.

A total of 31 isolates of S. aureus were used in the present experiment. Of these isolates, 12, 10 and 9 were isolated from clinical and subclinical mastitic cow's milk, and farm bulk milk, respectively. S. aureus FRI 1169, a human reference strain, was used as tsp gene positive control. The reference strain was kindly provided by Dr. Igarashi of the Department of Microbiology, Tokyo Metropolitan Research Laboratory of Public Health.

The organisms were inoculated into 5 ml of brain heart infusion broth (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) in a test tube at 37°C for 18 hr with a Recipro shaker (Teitco Co., Ltd., Saitama, Japan) at 100 rpm. The cultures were centrifuged at 12,000 x g at 4°C for 10 min and then the resulting culture supernatants were tested for the presence of TSST by a semiquantitative reverse passive latex agglutination (RPLA) test using TSST-RPLA kit (Denka Seiken Co., Ltd., Tokyo, Japan).

The bacterial cells were suspended in 0.5 ml TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) with lysozyme (Sigma, 100 units/ml) and incubated at 37°C for 30 min or until viscous. After centrifugation, nucleic acids in the supernatants were extracted twice with phenol and once with phenol-chloroform-isomyl-alcohol (50-48-2) and then precipitated with ethanol. The DNA samples were dissolved in TE buffer and used in PCR amplification.

PCR amplification was performed in a total volume of 100 µl containing about 15 ng of the bacterial DNA samples, 20 pmol of each primer, 2.5 mM of each of the four deoxynucleotides, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl2, 50 mM KCl, and 2.5 units of Taq polymerase (Takara Shuzou Co., Ltd.). The sequence of synthesized primer 1 was 5'-TTTACATTGTGAAGATGTCAAGACC CACT-3' and primer 2 was 5'-TACTAACATGATT TTTTATCGAAGCCCTT-3' [5]. The reaction mixtures were overlaid with mineral oil and subjected to 35 cycles of amplification in a PCR thermal cycler (Takara Shuzou Co.,
Fig. 1. Agarose gel electrophoresis of the PCR products. Lane 1: molecular size marker, Lane 2: TSST-1 human strain, Lanes 3–6: TSST-positive bovine isolates, Lanes, 7–8: TSST-negative bovine isolates.

Fig. 2. Sensitivity of the PCR. Lane 1: molecular size marker, Lane 2: 1.2 × 10^6 cells, Lane 3: 1.2 × 10^5 cells, Lane 4: 1.2 × 10^4 cells, Lane 5: 1.2 × 10^3 cells, Lane 6: 1.2 × 10^2 cells, Lane 7: 1.2 × 10^1 cells, Lane 8: 1.2 × 10^0 cells/10 μl.

Table 1. Relation between TSST titers and \( t \)st gene detection

<table>
<thead>
<tr>
<th>TSST titers</th>
<th>( t )st gene detection</th>
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<tr>
<td>2,560&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3/3 (100%)</td>
</tr>
<tr>
<td>1,280</td>
<td>3/3 (100%)</td>
</tr>
<tr>
<td>640</td>
<td>3/3 (100%)</td>
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<tr>
<td>320</td>
<td>3/3 (100%)</td>
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<tr>
<td>160</td>
<td>2/2 (100%)</td>
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<tr>
<td>80</td>
<td>2/3 (66%)</td>
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<tr>
<td>40</td>
<td>1/2 (50%)</td>
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<tr>
<td>20</td>
<td>0/2 (0%)</td>
</tr>
<tr>
<td>10</td>
<td>0/2 (0%)</td>
</tr>
<tr>
<td>5</td>
<td>0/2 (0%)</td>
</tr>
<tr>
<td>&lt; 5</td>
<td>0/2 (0%)</td>
</tr>
</tbody>
</table>

a) Reversed passive latex agglutination titer.

b) No. of positive isolates/No. of tested isolates.

Table 1 shows the relation between the RPLA titer of TSST and the detection of \( t \)st gene by PCR amplification. The \( t \)st gene was detected in all isolates, showing RPLA titers of 160 to 2,560, but not in any isolates showing RPLA titers of 20 or less. From this result, it seems that the PRLA reaction, showing titers of 5 to 20, is due to the non-specific reaction between sensitized latex particles and culture supernatants of the isolates.

The lowest detectable threshold of the PCR for the \( t \)st gene was examined as follows. An overnight culture of \( S. aureus \) FRI 1169 was serially 10-fold diluted in 0.85% saline solution and inoculated into tryptose-agar (Eiken Chemical Co., Ltd., Tokyo, Japan) to determine viable cell numbers. In another test, the diluted samples were heated at 95°C for 5 min and then centrifuged at 12,000 × g for 10 min. The resulting supernatants (crude DNA samples) were used in the PCR amplification as template DNA. As shown in Fig. 2, the 179 bp band of \( t \)st gene was observed in the PCR products ranging from 1.2 × 10^1 to 1.2 × 10^6 viable cells, but not from the 1.2 × 10^0 per 10 μl sample. This indicates that the lowest detectable threshold is 1.2 × 10^1 viable cells per 10 μl (1.2 × 10^2/ml). The same result was obtained in 2 bovine isolates, showing RPLA titers of 1,280 and 2,560 (data not shown).

A total of 125 bovine milk samples were collected from 30 dairy farms and examined for \( t \)st gene. That is, one hundred μl of the milk samples were inoculated into tryptose broh (Eiken), to which NaCl was added at a final concentration of 7.5% for selective growth of staphylococci. After incubation for 18 hr at 37°C, each culture was examined for staphylococci by Gram’s staining and then heated at 95°C for 5 min. After centrifugation, the resulting supernatants were used in the PCR amplification for the detection of the \( t \)st gene of \( S. aureus \). Staphylococci were observed in the culture fluids of 35 (28%) of the milk samples. In the remaining culture fluids, streptococci and bacilli were observed. When the culture fluids of the samples containing staphylococci were examined by the PCR amplification, \( t \)st gene was detected in 10 (28.6%) of 35 culture fluids. That is, \( t \)st gene was demonstrated in 10
(8%) of 125 farm bulk milk samples by the PCR amplification. From these result, it seems that the PCR amplification is useful for the detection of S. aureus carrying \( \text{tst} \) gene in bovine milk and for the diagnosis of bovine mastitis, which is caused by TSST positive isolates.

REFERENCES