Synthetic Enterotoxin B DNA Probes for Detection of Enterotoxigenic Staphylococcus aureus Strains

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DNA-DNA colony hybridization experiments with three different synthetic probes were carried out with 210 strains of Staphylococcus aureus. The synthetic probes encoded the amino acids 15 to 29 (probe 1), 179 to 192 (probe 2), and 207 to 219 (probe 3) of staphylococcal enterotoxin B (SEB). The amino acid sequences of these parts of SEB are identical to those of SEC1, All 21 SEB-producing strains tested reacted with each of the three probes. Of the 69 SEC-producing strains, 21 reacted with probe 1, none reacted with probe 2, and all 69 reacted with probe 3. With other strains no hybridization signals were obtained. The results presented here show that with a single synthetic DNA probe (probe 3) both SEB- and SEC-producing strains are detectable.

Staphylococcus aureus is a major cause of bacterial food poisoning. Staphylococcal food poisoning results from the ingestion of enterotoxins preformed in food by certain strains of S. aureus (2). A number of staphylococcal enterotoxins (SE) have been differentiated by the letter designations SEA through SEE (12). Three subtypes of SEC, numbered 1, 2, and 3, have been described (1, 4, 15). The immunological differences between these subtypes are small, and complete neutralization occurs with heterologous antibodies (15). SE-producing strains can be detected by several methods, mostly based on immunological detection of the toxin in culture fluid of the strains (5, 7, 16). A disadvantage of these methods is that single colonies have to be cultured and tested. To avoid this disadvantage, Peterkin and Sharpe (14) developed a method based on a direct demonstration of enterotoxigenic colonies on membrane filters by using the principles of the enzyme immunoassay. However, because of nonspecific reactions, unequivocal results were not obtained. A promising technique for direct detection of enterotoxigenic S. aureus strains is DNA-DNA hybridization. With this technique, the presence of DNA sequences encoding the amino acid sequences of the enterotoxin can be determined. The complete amino acid sequence of SEB and SEC have been determined (8, 17); an extensive homology exists between the amino acid sequences of both toxins. Recently the nucleotide sequence of the SEB gene from S. aureus was determined (9). We used three different synthetic DNA probes: a 42-mer DNA probe coding for amino acids 15 through 29 of the SEB molecule, a 39-mer DNA probe coding for amino acids 180 through 192 of the SEB molecule, and a 39-mer DNA probe coding for amino acids 207 through 219 of the SEB molecule. These probes were tested for hybridization with the DNA of a number of SEB-producing strains. Since amino acids 15 through 23, 180 through 192, and 207 through 219 of the SEB molecule are identical to those of SEC1 (8, 17), we also investigated hybridization with SEC-producing strains.

**MATERIALS AND METHODS**

*S. aureus strains.* The S. aureus strains used included reference strains (FRI 196E, S6, 14458, FRI 137, FRI 361, FRI 1230, FRI 1151M, FRI 326E, and FRI 1183 producing SEA, SEB and SEB, SEC1, SEC2, SEC3, and SEC4, and toxic shock syndrome toxin 1, respectively) and wild-type strains. The wild-type strains were obtained from A. Wieneke (Public Health Laboratory Service, London) and from C. Müller (Institut für Tierärztliche Lebensmittelhygiene, University of Zürich). They were tested for SE production by using the sac culture method of Donnelly et al. (6) and an enzyme-linked immunosorbent assay (13). For detection of SEC production, the antibodies used were prepared by imprinting sheep with SEC1 purified from culture fluid of strain FRI 137.

**DNA probes.** The synthesized DNA probes had the following nucleotide sequences: probe 1, 5' AGATAAT TACCTGGTTGATGAAATATGAAAGTTTGTAT 3'; probe 2, 5' TCACCTTATGAAACCGGATATATATAT TATAGAAAAT 3'; probe 3, 5' AAATGACCAAAT CAAATATTTAATGATGACAATGAC 3'. Probe 1 encoded amino acids 15 through 29, probe 2 encoded amino acids 180 through 192, and probe 3 encoded amino acids 207 through 219 of the SEB molecule. About 200 ng of DNA probe was labeled with 32P by the method of Maxam and Gilbert (11).

**Colony hybridization.** A colony hybridization procedure was used for testing the probes with S. aureus DNA. For this, S. aureus was grown on brain heart infusion-agarose plates for 12 h at 37°C. Colonies were transferred onto Gene-Screen Plus membranes (Du Pont Co.). The cells were lysed by an alkaline steaming procedure: the membranes were placed on filter paper soaked in 0.5 M NaOH and incubated for 5 min just above the water level in a boiling water bath. Then the Gene-Screen Plus membranes were placed on 1 ml of fresh lysis solution and neutralized with 1 ml of 1 M Tris (pH 7.5). This neutralization step was repeated once. The membranes where immersed in 100 ml of 5× SSC (1× SSC contains 0.15 M NaCl and 0.015 M sodium citrate). While immersed they were rubbed thoroughly with a tissue. To fix the DNA onto the membranes, the membranes were allowed to air dry. The membranes were prehybridized for 4 h at 40°C in 15 ml of a solution containing 50 mM Tris (pH 7.5), 10 mM EDTA, 1 M NaCl, 10× Denhardt solution (1× Denhardt solution contains 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin), 100 μg of denatured herring sperm DNA per ml, 0.1% sodium pyrophosphate, and 1% sodium dodecyl sul-
Each of the three DNA probes reacted with all SEB-producing strains of *S. aureus* (Table 1). This indicates that for the regions tested the gene encoding SEB is well conserved. On the other hand, all 74 wild-type strains producing no detectable quantities of SEB give no hybridization signals, indicating that if the gene is present production of SEB occurs. DNA probe 1, coding for the amino acids 15 through 29 of the SEB molecule, reacted with the SEC1-producing *S. aureus* strain FRI 137. In the course of our experiments the nucleotide sequence of the SEC1 gene has been determined as well (3), and there exists only one base mismatch with the nucleotide sequence of the 42-mer DNA probe 1. However, the probe did not react with strains FRI 361 and FRI 1230, which produce SEC2 and SEC3, respectively. Of 66 wild-type strains producing SEC only, 20 gave a signal with probe 1. DNA probe 2, coding for the amino acids 180 through 192 of the SEB molecule, did not show hybridization with SEC-producing *S. aureus* or with strain FRI 137. Since amino acid sequences 180 through 192 of SEC1, produced by strain FRI 137, are identical to those of SEC2 (8, 17), it is obvious that the codon usage differs from that of the SEC gene. Comparing the nucleotide sequence of the 39-mer DNA probe 2 with the nucleotide sequence of SEC1 indeed shows more mismatches (5). All SEC-producing strains reacted with DNA probe 3, which encodes amino acids 207 through 219 of the SEB molecule. The SEC1-, SEC2-, and SEC3-producing strains were also positive. This probe differs by only two bases from the nucleotide sequence of the SEC1 gene. The results demonstrate that with these DNA-DNA hybridization experiments the SEC1-producing strain (FRI 137) is distinguishable from the SEC2- and SEC3-producing strains (FRI 361 and FRI 1230, respectively). Enterotoxins SEC1, SEC2, and SEC3 have different patterns in isoelectric focusing (15). Furthermore SEC3 has serine as the N-terminal residue, whereas SEC1 and SEC2 have glutamic acid for their N-terminal residue (15, 17). Precipitation reactions with polyclonal antibodies are not suitable to determine these subtypes, since immunological differences are minor (10). Thompson et al. (18), however, produced

![FIG. 1. DNA-DNA hybridization results obtained by colony blotting of 88 different strains of *S. aureus*. Eight rows of 11 colonies each were inoculated. Hybridization experiments were carried out with a synthetic DNA probe encoding amino acids 180 through 192 of SEB. With the hybridization procedure presented in Materials and Methods only SEB-producing strains showed signals (A, strain 14458; B, strain S6; other positive signals are of wild-type strains producing SEB).](image)
monoclonal antibodies that reacted specifically with type SEC, and with both type SEC\(_2\) and SEC\(_4\), which allow discrimination of SEC\(_1\)-producing strains from SEC\(_2\) and SEC\(_3\)-producing strains as well.

Of the 14 strains producing both SEC and toxic shock syndrome toxin 1, 10 hybridized with probes 1 and 3. This reaction is similar to strain FRI 137, indicating similarities with SEC\(_1\)-producing strains. Of the 52 other wild-type strains producing SEC, only 10 hybridized with both probes. S. aureus strains without detectable SEB or SEC production did not give hybridization signals with any of the DNA probes applied.

The results described here clearly demonstrate that the gene encoding SEB is well conserved, whereas the gene encoding SEC is conserved to a lesser degree. The DNA-DNA hybridization technique for detection of SEB-producing strains can therefore be carried out with several different synthetic DNA probes. For detection of SEC-producing strains, selected DNA probes are needed. A DNA probe encoding amino acids 207 through 219 of the SEB molecule is suited for this purpose, enabling detection of both SEB- and SEC-producing strains. Furthermore, it has become clear that if the gene encoding either SEB or SEC is present production of the respective toxins occurs. This means that DNA hybridization is of advantage in detecting enterotoxigenic S. aureus above current methods. In contrast to the current methods, high numbers of strains can be tested simultaneously for their ability to produce enterotoxin.

LITERATURE CITED