Relationships Among Coagulase, Enterotoxin, and Heat-stable Deoxyribonuclease Production by *Staphylococcus aureus*¹

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The relationship between heat-stable deoxyribonuclease and coagulase production was investigated in the interest of developing more rapid diagnostic and quantitative procedures for distinguishing toxigenic and pathogenic staphylococci from closely related saprophytic organisms.

The most widely used criterion for distinguishing toxigenic and pathogenic staphylococci (*Staphylococcus aureus*) from closely related saprophytic organisms is the coagulase test. Although it is recognized that on rare occasions *S. aureus* may lose this characteristic and still retain toxigenicity and pathogenicity, many laboratories still rely solely on the coagulase test. Attempts to utilize this characteristic by the addition of plasma to plating media have been proposed. However, in some instances the plate and tube reactions did not correlate (2, 3) and, in addition, the cost of adding plasma to large volumes of routine testing media is considerable.

Generally, colony isolation and coagulate testing require an additional 24 hr in most diagnostic schemes. Efforts to develop more rapid methods as well as to correlate coagulate production with other physiological characteristics have been undertaken with the hope that properties other than coagulate could be detected more rapidly. However, investigations relating lysozyme, lipase, phosphatase, fibrinolysin, or hemolysin have failed to demonstrate a high correlation with coagulate production. Hyaluronidase does correlate favorably, but assay procedures are comparatively involved and costly.

Our interest in rapid diagnostic and quantitative procedures prompted an investigation of the relationship between deoxyribonuclease and coagulate production. In addition, some importance was attached to determining the relationship of heat-stable deoxyribonuclease and coagulate production. Previous investigators have observed the marked heat resistance of deoxyribonuclease produced by *S. aureus*; however, data regarding its correlation with coagulate production and enterotoxin are not available.

The coagulate test was performed by using ethylenediaminetetraacetate-treated plasma (Difco) and 24-hr cultures grown in Brain Heart Infusion (BHI). The production of deoxyribonuclease was detected by the acridine-orange overlay procedure (Lachica and Deibel, *submitted for publication*) using streak plate cultures grown for 24 hr on BHI containing 1.5% agar. For heat-stable deoxyribonuclease determinations, BHI broth cultures were incubated overnight and heated in a boiling-water bath for 15 min before testing by the acridine-orange-disc procedure (Lachica and Deibel, *submitted for publication*). Enterotoxin was determined either by monkey feeding tests (4) or serological procedures (1). The enterotoxin-producing potential of many strains had been determined previously and the information was given with the cultures. Most of the cultures used in this study were supplied by M. S. Bergdoll, Food Research Institute, Madison, Wis., and constitute a variety of strains collected over a period of 20 years from foods and clinical sources. The collection did not reflect a random choice of cultures, as 82% of them produced enterotoxin (Table 1). This is somewhat higher than the approximate 50% figure associated with a random distribution of coagulate-producing *S. aureus* strains (M. S. Bergdoll, *personal communication*).

Of the coagulate-positive strains, 95% also produced a heat-stable deoxyribonuclease (Table 1). Although 10 of the 41 coagulate-negative strains

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The excellent relationship of heat-stable deoxyribonuclease to deoxyribonuclease production (271/283 or 96%) suggests the possibility of employing the heat stability of the enzyme in the description of the organism as well as in diagnostic and quantitative procedures. Among the enterotoxin-producing strains in Table 1, 93% produce coagulase and 95% produce heat-stable deoxyribonuclease. It would appear that these characteristics correlate equally well with enterotoxin production.

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LITERATURE CITED


Table 1. Comparison of nuclease and enterotoxin with coagulase production by Staphylococcus aureus

<table>
<thead>
<tr>
<th>Coagulase production</th>
<th>Deoxyribonuclease production*</th>
<th>Enterotoxin production (no. of strains)</th>
<th>Total no. of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unheated culture</td>
<td>Heated culture</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>+</td>
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<td>228</td>
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</tr>
<tr>
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<td>0</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>

* The acridine-orange overlay procedures were used (Lachica and Deibel, submitted for publication). For the heated test, the broth culture was placed in a boiling-water bath for 15 min.

produced a heat-stable deoxyribonuclease, 9 of the 10 strains also produced enterotoxin. These strains probably represent mutants that have lost the ability to produce coagulase.