Genetic Relatedness between the Type Strain of *Streptococcus anginosus* and Minute-Colony-Forming Beta-Hemolytic Streptococci Carrying Different Lancefield Grouping Antigens

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The taxonomic relationships among *Streptococcus anginosus* type strain NCTC 10713, beta-hemolytic streptococci belonging to serological groups A, C, G, and F, and related organisms were determined by using deoxyribonucleic acid deoxyribonucleic acid hybridization (S1 nuclease method), deoxyribonucleic acid base composition, and conventional biochemical tests. Minute-colony-forming beta-hemolytic streptococci which carry various Lancefield grouping antigens (antigens A, C, F, and G) or none of the known group antigens were closely related genetically and biochemically to the type strain of the *S. anginosus*, which carries group G antigen.

Minute-colony-forming strains of beta-hemolytic streptococci may carry various Lancefield grouping antigens, such as antigens A, C, G, and F, and some strains do not possess any identifiable antigen. The biochemical characteristics of these cocci closely resemble those of the type strain of *Streptococcus anginosus*, which carries Lancefield group G antigen. Facklam proposed that collectively these minute-colony-forming strains of beta-hemolytic streptococci should be called *S. anginosus* (3), although genetic relationships among them had not been studied.

Minute-colony-forming strains of beta-hemolytic streptococci and representative strains belonging to each Lancefield serologic group (groups A, B, C, G, and F) are listed in Table 1. The method used to determine deoxyribonucleic acid (DNA) base composition has been described previously (2). Free solution DNA-DNA hybridization was performed in 2.5X SSC buffer (1X SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 55°C for 20 h and was followed by digestion with S1 nuclease (1).

The levels of DNA-DNA homology between the type strain of *S. anginosus* and 14 strains of minute-colony-forming beta-hemolytic streptococci ranged from 74 to 85% (Table 1). The minute-colony-forming beta-hemolytic streptococci that carried a group A, C, G, or F antigen or were ungroupable were identical to *S. anginosus*, both biochemically and genetically. The type strain of *S. anginosus* carries a group G antigen; however, this organism is apparently different from large-colony-forming typical group G streptococci. Large-colony-forming group G streptococci resemble *Streptococcus dysgalactiae* ATCC 27957, which carries a group C antigen. The levels of DNA-DNA homology between large-colony-forming group G streptococci and *S. dysgalactiae* ranged from 71 to 81%. These data support recent proposals that large-colony-forming group G streptococci and "Streptococcus equisimilis" are genetically identical to *S. dysgalactiae* (4, 5), although our homology values (Table 1) were lower than the values reported by other investigators.

Minute-colony-forming group A beta-hemolytic streptococci are distinct both genetically and biochemically from typical group A streptococci (*Streptococcus pyogenes*). The levels of DNA-DNA homology between the type strain of *S. pyogenes* and the minute-colony-forming beta-hemolytic streptococci were less than 25%.

The levels of DNA-DNA homology between minute-colony-forming group C streptococci and typical group C organisms (*Streptococcus equi* or *S. dysgalactiae*) were also very low (12 to 36%).

These data confirm that some Lancefield group antigens are carried by organisms belonging to different streptococcal species. Also, a single species may possess different Lancefield antigens. Identification of the Lancefield antigens by clinical microbiologists, although very convenient for identifying streptococci from clinical samples, is not in every case accurate for taxonomic identification of bacterial species.

In the past, the hemolytic activities of streptococci have been considered to be an important factor in identifying streptococci; however, more recent reports (4, 5) and our data indicate that alpha-hemolytic *S. dysgalactiae*, beta-hemolytic "*S. equisimilis," and group G streptococci are closely related genetically. Furthermore, recent reports (4, 6) have indicated that beta-hemolytic *S. anginosus* and some strains of nonhemolytic *Streptococcus constellatus* and *Streptococcus intermedius* are closely related genetically. From these DNA homology data we conclude that the beta-hemolytic activities of streptococci cannot be uniformly used for differentiating species.

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<table>
<thead>
<tr>
<th>Taxon</th>
<th>Strain</th>
<th>Sero-group</th>
<th>Guanine-plus-cytosine content (mol %)</th>
<th>S. anginosus NCTC 10713&lt;sup&gt;T&lt;/sup&gt;</th>
<th>S. anginosus NCTC 5389</th>
<th>S. pyogenes ATCC 12344</th>
<th>&quot;S. equisimilis&quot;&lt;sup&gt;T&lt;/sup&gt;</th>
<th>Voges-Proskauer test</th>
<th>Arginine dehydrogenase</th>
<th>Esculin hydrolysis</th>
<th>Lactose fermentation</th>
<th>Pyrrolidone arylamidase</th>
<th>Hippurate hydrolysis</th>
<th>Trehalose fermentation</th>
<th>SXT&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Bacitracin</th>
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<tr>
<td>S. anginosus</td>
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<td>S</td>
<td>R</td>
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<sup>a</sup> Determined by the thermal denaturation method.
<sup>b</sup> SXT, Sulfamethoxazole and trimethoprim.
<sup>c</sup> T = type strain.
<sup>d</sup> +, Positive; -, negative; w, weakly positive; S, susceptible; R, resistant.
<sup>e</sup> This species is alpha-hemolytic.
LITERATURE CITED