Identification of Coagulase-Positive *Staphylococcus intermedius* and *Staphylococcus hyicus* subsp. *hyicus* Isolates from Veterinary Clinical Specimens

WALTER E. PHILLIPS, JR.1 *AND WESLEY E. KLOOS*2

Mississippi Veterinary Diagnostic Laboratory, Jackson, Mississippi 39216,1 and Department of Genetics, North Carolina State University, Raleigh, North Carolina 276952

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Coagulase-positive isolates of *Staphylococcus intermedius* from dogs and coagulase-positive isolates of *Staphylococcus hyicus* subsp. *hyicus* from a pig and cows were identified initially by a simplified scheme which can be readily performed in a routine diagnostic laboratory. Characters tested in the scheme included coagulase activity, colony pigment, and aerobic acid production from maltose. The identity of these isolates was confirmed by deoxyribonucleic acid-deoxyribonucleic acid hybridization experiments. The strains of *S. hyicus* subsp. *hyicus* isolated from porcine sources were positive for protein A, whereas the strains recovered from bovine mastitic milk were negative for protein A.

A scheme for the biochemical differentiation of the coagulase-positive species *Staphylococcus aureus* and *Staphylococcus intermedius* was described after the investigation of coagulase-positive staphylococci isolated from pigeons, dogs, mink, and horses (5). Results from deoxyribonucleic acid (DNA)-DNA hybridization studies (9, 11), bacteriolytic activity patterns (14), and other cell wall and isoenzyme analyses (13) have provided additional evidence for the separation of *S. aureus* biotypes A to D into the species *S. aureus* and biotypes E and F into the species *S. intermedius*.

*Staphylococcus hyicus*, a species containing some coagulase-positive strains, has been divided into two distinct subspecies, *S. hyicus* subsp. *hyicus* and *S. hyicus* subsp. *chromogenes*, on the basis of biochemical characteristics and DNA-DNA relatedness (4). Coagulase-positive strains of *S. hyicus* subsp. *hyicus* have been isolated from: the skin of pigs with and without exudative epidermitis (2); the intact skin and mange lesions of cattle (3); and bovine udder lesions (1). The pathogenic significance of *S. hyicus* subsp. *chromogenes* is not yet clear.

In a recent publication (10), we described the isolation and identification of a coagulase-positive strain of *S. hyicus* subsp. *hyicus* (subsequently referred to as *S. hyicus*) from a pig with septic polyarthritis. The key phenotypic character utilized to differentiate *S. hyicus* from *S. intermedius*, which could be performed in a routine diagnostic laboratory, was aerobic acid production from maltose and, to a lesser extent, aerobic acid production from D-mannitol (4, 5).

This report describes the identification of coagulase-positive veterinary clinical isolates of *S. intermedius* and *S. hyicus* from animals by using a simplified scheme and subsequent confirmation by DNA-DNA hybridization. It also considers the use of protein A as a possible marker to distinguish ecotypes of *S. hyicus*.

**MATERIALS AND METHODS**

The bacteriological examination, staphylocoagulase assay, and DNA-DNA hybridization studies of the isolates have been previously described (7, 10). Plates of purple agar base medium (Difco Laboratories, Detroit, Mich.) supplemented with 1% maltose were prepared for the detection of aerobic acid production from this carbohydrate (6). Protein A determination was made by W. Schae and H. Blobel (Institut fur Bakteriologie und Immunologie, Justus Liebig-Universität, Giessen, West Germany) (12).

**RESULTS**

The origins of the coagulase-positive staphylococci investigated in this study are summarized in Table 1. Strain 393B is an isolate of *S. hyicus* cultured from the coxofemoral and shoulder joints of a pig with septic polyarthritis (10). Strain A4 was isolated by Brown et al. (1) from a bovine udder. Strains 451 and 3813 were recovered from milk samples of two cows showing clinical signs of mastitis. Five of the canine strains (4177, 5018, 5678, 6131, 6317) of *S. intermedius* were isolated from dogs with pyoderma, and strain 5066 was cultured from the infected milk of a female German shepherd.
TABLE 1. Origins of strains of coagulase-positive staphylococci investigated

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain no.</th>
<th>Animal species</th>
<th>Specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. hyicus</td>
<td>393B</td>
<td>Pig</td>
<td>Joints</td>
</tr>
<tr>
<td>subsp. hyicus</td>
<td>A₄</td>
<td>Cow</td>
<td>Milk</td>
</tr>
<tr>
<td></td>
<td>451</td>
<td>Cow</td>
<td>Milk</td>
</tr>
<tr>
<td></td>
<td>3813</td>
<td>Cow</td>
<td>Milk</td>
</tr>
<tr>
<td>S. intermedius</td>
<td>4177</td>
<td>Dog</td>
<td>Pyoderma</td>
</tr>
<tr>
<td></td>
<td>5018</td>
<td>Dog</td>
<td>Pyoderma</td>
</tr>
<tr>
<td></td>
<td>5066</td>
<td>Dog</td>
<td>Milk</td>
</tr>
<tr>
<td></td>
<td>5678</td>
<td>Dog</td>
<td>Pyoderma</td>
</tr>
<tr>
<td></td>
<td>6131</td>
<td>Dog</td>
<td>Pyoderma</td>
</tr>
<tr>
<td></td>
<td>6317</td>
<td>Dog</td>
<td>Pyoderma</td>
</tr>
</tbody>
</table>

S. hyicus (Table 3). At the stringent temperature of 70°C, the DNA from the clinical isolates of S. intermedius showed between 77 and 88% relative binding with the DNA from S. intermedius CFDD, but had very low relative binding (7 to 14%) with S. hyicus NCTC 10350. The DNA from strains 393B and 3813 had 79 and 82% relative binding, respectively, with DNA from S. hyicus NCTC 10350 at 70°C, and very low relative binding (16%) with DNA from S. intermedius CFDD. The DNA from bovine strains 451 and A₄ was 30 and 39% homologous, respectively, to the DNA from S. hyicus NCTC 10350 at the stringent temperature of 70°C.

DISCUSSION

The differentiation of S. intermedius from S. hyicus was successfully accomplished by determining the color of the culture and of the region of the purple agar base-maltose medium under the culture streak after incubation for 48 to 72 h at 37°C. Isolates of canine S. intermedius produced a slight yellowish to yellow-green color under the culture streak, whereas S. hyicus strains did not produce this slight acid reaction, but rather produced a diffuse purple alkaline reaction surrounding the culture streak. This marked reaction difference was also noted in earlier comparative studies with 24 canine S.

TABLE 2. Characteristics used in the differentiation of coagulase-positive Staphylococcus species

<table>
<thead>
<tr>
<th>Strain</th>
<th>Tube coagulaseb</th>
<th>Colony pigment</th>
<th>Acid aero-</th>
<th>Substreak color on 1% maltose PAB medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>Canine S. inter-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Yellowish, yellow-green</td>
</tr>
<tr>
<td>mediussubsp. hyicus</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Purple*</td>
</tr>
</tbody>
</table>

* Symbols: +, positive; -, weak to very weak; __, negative. NA, Not applicable.
* Coagulase plasma-ethylenediaminetetraacetic acid (Difco).
* Diffuse yellow color surrounding culture streak on purple agar base (PAB) medium containing 1% maltose.
* Some strains may not produce the yellow-orange pigment, and some positive strains may not produce pigment under certain culture conditions in 18 to 24 h.
* Most strains produce a diffuse alkaline color (deeper purple) surrounding the culture streak on PAB medium containing 1% maltose.

After an axenic culture of each isolate was obtained, a representative colony of each isolate, determined to be a catalase-positive, gram-positive coccus that produced staphylococagulase, was selected for further characterization. The characteristics utilized to identify coagulase-positive staphylococci are listed in Table 2. S. intermedius and S. hyicus strains were distinguished from S. aureus on the basis of being achromogenic and unable to produce rapid or significant quantities of acid from maltose under aerobic conditions.

To confirm the identity of these coagulase-positive Staphylococcus species by using the simplified scheme shown in Table 2, DNA-DNA hybridization experiments were performed with a canine reference strain (CFDD) of S. intermedius and the type strain (NCTC 10350) (4) of S. hyicus.
intermedius and 7 porcine S. hyicus strains isolated from clinical specimens at the Institut für Bakteriologie und Immunologie, Justus Liebig-Universität Giessen (Kloos and Blobel, unpublished data) and then later in our investigation of S. hyicus cultured from a pig with polyarthritis (10). It should be noted that reactions with maltose may differ somewhat from those indicated here for S. intermedius strains isolated from other animal sources, e.g., the pigeon type strain CCM5739 does not produce a yellowing of the culture streak, and some horse strains are maltose positive. In contrast to the other two coagulase-positive species, S. aureus produces a rapid acid reaction surrounding the culture streak within 12 to 24 h.

The results from the DNA-DNA hybridization experiments indicate that strains 4177, 5018, 5066, 5678, 6131, and 6317 are related to S. intermedius on a species level and confirm the biochemical identification of canine S. intermedius. The high relative binding at 70°C between the DNA from strains 393B and 3813 and NCTC 10350 suggests a relationship at the subspecies level and confirms the biochemical identification of S. hyicus for these two isolates. Furthermore, the 68% relative binding at 55°C and 39% relative binding at 70°C between the DNAs of strain A4 and S. hyicus NCTC 10350 are in general agreement with the results of Devriese et al. (4), which showed approximately a 50% DNA-DNA homology between the DNAs from strain A4 and S. hyicus NCTC 10350 at 60°C. These authors (4) proposed that strain A4 and similar strains provisionally be identified as S. hyicus. Since the DNA from bovine strain 451 showed 60% and 30% relative binding at 55 and 70°C, respectively, with the DNA from S. hyicus NCTC 10350, we propose that strain 451 also be identified as S. hyicus, although such reduced binding, especially at 70°C, suggests the identity of a separate subspecies status. Generally, organisms placed in a separate species demonstrate relative DNA-binding values of less than 60 to 70% at an optimal criterion and usually more than 50% reduction in binding from those values at a stringent criterion.

The porcine strains (NCTC 10350 and 393B) of S. hyicus were positive for protein A, but the bovine strains (3813, A4, 451) were negative for protein A. Lachica et al. (8) also assayed 32 strains of S. hyicus from cows and 2 strains from porcine and reported that all were devoid of this cell wall component. On the other hand, P. Muller, W. Schaeg, and H. Blobel (Kurzfassung der Vortrage der Arbeitstagung der Deutschen

Gessellschaft für Mikrobiologie, 25–26 September 1980, Mainz) have recently reported that 40 of 43 porcine S. hyicus strains contained protein A, though it was not identical to the protein A of S. aureus in that it has a lower molecular weight and a more acid isoelectric point. The presence of protein A in the porcine strains and the absence of it in the bovine strains might be a useful marker for distinguishing ecotypes within S. hyicus.

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LITERATURE CITED