FIBRINOGEN MEDIA FOR STUDIES ON STAPHYLOCOCCI

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ABSTRACT

DENEKE, ANNELIESE (University of Wisconsin, Madison) AND HANS BLOBEL. Fibrinogen media for studies on staphylococci. J. Bacteriol. 83: 533-537. 1962—Fibrinogen media were prepared simply by spreading rabbit plasma or a bovine fibrinogen solution over the surface of certain solidified agar media, thus eliminating the necessity of incorporation of fibrinogen into liquefied agar media at a critical temperature. With agar media composed of heart infusion broth, blood agar base, or Trypticase soy broth, distinct coagulase reactions were observed which corresponded closely to those obtained with the tube test.

A bovine fibrinogen solution, applied to the surface of some of the commonly used selective media for staphylococci, provided an additional and more specific test system for coagulase production of individual colonies, and consequently aided in the presumptive identification of pathogenic staphylococci.

The ability of certain staphylococci to produce coagulase is used as an important criterion for estimating their pathogenicity. The enzymelike substance can be demonstrated by the coagulase reaction, which results in the conversion of fibrinogen into fibrin. A plasma factor participates in this reaction (Tager, 1956). In agar media containing fibrinogen, coagulase-mediated conversion into fibrin becomes evident by areas of opacity around staphylococcal growth. Thus, coagulase formation by individual colonies can be studied.

Various fibrinogen or plasma media have been described (Duthie and Lorenz, 1952; Klemperer and Haughton, 1957; Esber and Faulconer, 1959; Blobel, Berman, and Simon, 1960). All of these require incorporation of fibrinogen or plasma solutions into the respective liquefied agar media at temperatures close to 45 C, to prevent denaturation of fibrinogen by heat as well as hardening of the agar before complete mixing has been achieved. This is somewhat difficult and time consuming and may have hindered the use of fibrinogen media for routine diagnostic examinations.

In the course of studies on coagulase reactions in agar media, it has been found that the intricate step of incorporating fibrinogen, before the agar solidifies, can be eliminated. Instead, fibrinogen or plasma solutions simply applied onto the surface of solidified agar media are readily absorbed and give the same results. Further evaluation of such media has finally led to the incorporation of fibrinogen into some of the commonly used selective media.

MATERIALS AND METHODS

Heart infusion broth (Difco), Trypticase soy broth (Baltimore Biological Laboratory), nutrient broth (Difco), blood agar base (Difco), phenol red broth base containing 1% manniitol (Difco), manniitol salt agar (Difco), and tellurite-glycine agar (Difco) were prepared following the recommendations of the manufacturers. The polymyxin staphylococcus medium of Finegold and Sweeney (1961) was modified by substituting heart infusion broth (Difco) for its nutrient component, to obtain more distinct coagulase reactions. Agar concentrations of all of these media were adjusted to 1.75%. The respective media were poured into petri dishes and stored for several days, to allow gradual drying of the agar surface. Then, samples of 0.3 ml per petri dish of either sterile citrated rabbit plasma or of a bovine fibrinogen solution were spread uniformly over the surface of the respective agar medium by means of a sterile glass rod bent in the shape of a hockey stick. The fibrinogen solution of 3% (w/v) bovine fibrinogen (Armour Laboratories, Kankakee, Ill.) in physiological saline, with 3% (v/v) of rabbit plasma added as cofactor, had been previously sterilized by Seitz filtration.

Coagulase activity of individual staphylococcal cultures was determined by a modification of the
method of Duthie and Lorenz (1952). Usually, 0.2 ml of an 18-hr broth culture was added to 0.5 ml of a clotting mixture contained in tubes measuring 1.3 by 8 cm. The clotting mixture was composed of 1.5% (w/v) bovine fibrinogen and 1.5% rabbit plasma in physiological saline. Formation of a solid clot after incubation for 3 hr at 37 C indicated coagulase production.

RESULTS

Differential fibrinogen media. A solution of bovine fibrinogen or undiluted rabbit plasma applied onto the surface of certain solidified agar media provided an effective assay system for coagulase production of individual staphylococcal colonies. Surface incorporation of either of the
TABLE 1. Comparison of coagulase reactions of staphylococcal cultures in the tube test and on fibrinogen media

<table>
<thead>
<tr>
<th>Medium</th>
<th>Fibronogen test +*</th>
<th>Fibronogen test -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart infusion broth</td>
<td>Tube test + 377</td>
<td>Tube test - 3</td>
</tr>
<tr>
<td></td>
<td>Tube test + 1</td>
<td>Tube test - 91</td>
</tr>
<tr>
<td>Tryptase soy broth</td>
<td>Tube test + 375</td>
<td>Tube test - 0</td>
</tr>
<tr>
<td>Blood agar base</td>
<td>Tube test + 365</td>
<td>Tube test - 3</td>
</tr>
<tr>
<td>Nutrient broth agar</td>
<td>Tube test + 350</td>
<td>Tube test - 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tube test - 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tube test - 81</td>
</tr>
</tbody>
</table>

* Plus means “halo”; minus means no “halo.”

above-mentioned fibrinogen solutions gave consistently interpretable results with agar media containing heart infusion broth, Tryptase soy broth, or blood agar base. The media remained clear after absorption of the fibrinogen or plasma solutions. This was not always the case with the nutrient agar. Thus, on the first three media, presence or absence of opaque zones surrounding staphylococcal colonies were distinctly visible, particularly under indirect illumination. A typical reaction of coagulase-positive staphylococci on such a medium is shown in Fig. 1.

A close correspondence between the coagulase tube test and the reactions in fibrinogen agar media containing heart infusion broth, Tryptase soy broth, or blood agar base was observed with a number of staphylococcal cultures isolated from mammary secretions of cows (Table 1). This correlation was less favorable when fibrinogen medium containing nutrient agar was used, mainly because “halo” formation was not as distinct as on the other three media.

Plasma or fibrinogen solution applied to the surface of solidified phenol red broth base agar containing 1% mannitol permitted simultaneous screening of colonies for coagulase production and mannitol fermentation of staphylococci.

Selective fibrinogen media. Attempts were made to incorporate fibrinogen into some of the commonly used selective media, to increase their specificity. Satisfactory results were obtained with the tellurite-glycine agar of Zebovitz, Evans, and Niven (1955). The bovine fibrinogen solution, merely applied onto the surface of the solidified agar, constituted an effective means of determining coagulase reactions of individual colonies (Fig. 2) with a high degree of certainty (Table 2). It proved particularly valuable for detection of coagulase-positive staphylococci, which did not produce the typically black colonies on tellurite-glycine agar, and aided in the presumptive identification of staphylococcal colonies with a dubious appearance. Surface incorporation of rabbit plasma into tellurite-glycine agar, however, gave less distinct coagulase reactions.

A limited survey was then conducted to determine the effectiveness of the fibrinogen-tellurite-glycine agar for isolation and identification of coagulase-positive staphylococci under conditions where they may be greatly outnumbered by a wide variety of other microorganisms. Specimens of human origin and bovine milk samples were studied. The fibrinogen-tellurite-glycine agar yielded Staphylococcus aureus from all of 53 mixed cultures of human origin (courtesy of V. Allen, State Hygiene Laboratory, Madison, Wis.) containing this organism. In the case of the bovine milk samples, pathogenic staphylococci were demonstrated in a number of samples on the tellurite-glycine medium, but were overgrown occasionally by contaminating microorganisms on duplicate sheep-blood agar to such an extent that they could not be discovered. In one such test series, milk samples from 204 mammary quarters were examined bacteriologically. Of these samples, 32 yielded coagulase-positive staphylococci on the fibrinogen-tellurite-glycine medium and 24 on sheep-blood agar.

Distinct coagulase reactions were also observed when bovine fibrinogen was incorporated into the modified polymyxin staphylococcus medium of Finegold and Sweeney (1961). The latter medium, as well as the fibrinogen-tellurite-glycine agar, yielded practically quantitative recovery after inoculation with a strain of S. aureus which had been previously isolated from a cow with mastitis. When varying quantities of staphylococci of the

TABLE 2. Growth and coagulase reactions of staphylococcal cultures on fibrinogen-tellurite-glycine agar

<table>
<thead>
<tr>
<th>Coagulase reaction (tube test)</th>
<th>No. of cultures</th>
<th>Growth</th>
<th>No growth</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Halos</td>
<td>No halos</td>
</tr>
<tr>
<td>+</td>
<td>423</td>
<td>407</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>55</td>
<td>1</td>
<td>28</td>
</tr>
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<td></td>
<td></td>
<td>16</td>
<td>26</td>
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bovine strain were plated with a mixture of bacterial “contaminants” consisting of approximately equal numbers (10⁸ cells total per ml) of coagulase-negative staphylococci, *Escherichia coli*, *Aerobacter*, and *Pseudomonas*, both media were decisively selective for the coagulase-positive staphylococci. However, recovery of *S. aureus* cultivated with such a mixture was not quantitative (Table 3). Nevertheless, the coagulase-positive staphylococci, in certain concentrations, were demonstrable on both selective fibrinogen media; they were overgrown by the “contaminants” on the control nutrient agar, rendering them undetectable. *Proteus* was not included in the mixture of “contaminants,” since inhibition of its growth on neither the fibrinogen-polymyxin staphylococcus medium nor on the fibrinogen-tellurite-glycine agar was sufficient when large numbers were inoculated. Particularly when *Proteus* outnumbered the bovine strain of *S. aureus*, growth of the latter organism on both selective fibrinogen media was obscured.

Mannitol-salt agar (Chapman, 1945), staphylococcus medium #110 (Chapman, 1946), and Chapman-Stone medium (Chapman, 1948), whose selective principle is a high sodium chloride content, did not provide suitable fibrinogen media.

**DISCUSSION**

The simplified preparation of fibrinogen-agar media is believed to increase their usefulness for research and for diagnostic studies of staphylococcal infections. Fibrinogen solutions incorporated into certain media by surface application provide a convenient and reliable means of determining the coagulase production of individual colonies. In some of the widely used selective media, fibrinogen constitutes an additional and more specific system for the rapid presumptive identification of pathogenic staphylococci. Such an assay system practically eliminates the necessity of further coagulase tube testing; isolation and tentative identification of staphylococci can be accomplished in one step. Furthermore, particularly under conditions where pathogenic staphylococci are greatly outnumbered by other microorganisms, their detection becomes more reliable on the selective media. For these reasons, selective fibrinogen media constitute valuable tools in the laboratory diagnosis of staphylococcal infections.

Although only rabbit plasma and bovine fibrinogen solution, because of their availability, have been incorporated into the various media used in these studies, it is conceivable that plasmas of human or other origin, containing the coagulase-reacting factor (Tager, 1956), may give similarly distinct coagulase reactions. Fibrinogen solutions may also provide additional specific indicators for coagulase production in some of the recently proposed selective media for staphylococci, such as that described by Richardson (1961).

**LITERATURE CITED**


