FLUORESCEIN AMINE MEDIA FOR RAPID DIFFERENTIATION OF STAPHYLOCOCCI

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ABSTRACT

KIMLER, ALEXANDER (National Institutes of Health, Bethesda, Md.). Fluorescein amine media for rapid differentiation of staphylococci. J. Bacteriol. **82**:106–109. 1961.—By modifying conventional media to include blood, mannitol, and fluorescein amine, staphylococci may be isolated and identified directly. *Staphylococcus aureus*, mannitol positive, fluoresces, whereas *Staphylococcus epidermidis*, mannitol negative, does not.

The coagulation of plasma by many strains of staphylococci was first observed by Loeb (1903). In the form of a tube coagulase test, it still remains the best single technique by which to evaluate the clinical significance of the organism (Elek, 1959). To the same end, the fermentation of mannitol has been considered both useful and reliable (Hallman, 1937; Chapman et al., 1938; Moss, Squires, and Pitts, 1941). *Bergey's Manual of Determinative Bacteriology* (Breed, Murray, and Smith, 1957) states that *Staphylococcus aureus* is coagulase positive and ferments mannitol, whereas *Staphylococcus epidermidis* (*Staphylococcus albus*) is coagulase negative and does not ferment mannitol. However, each of these tests can be applied only to pure cultures previously identified as Staphylococcus. Generally, a 48-hr lapse is necessary before the species is thus confirmed.

The primary isolation of potentially virulent staphylococci is currently performed on a number of media which find application in clinical bacteriology, in carrier surveys, and in investigating food-borne infections. A group of these media was modified so that each contained blood, mannitol, and fluorescein amine. After overnight incubation on these media, *Staphylococcus aureus* was seen to fluoresce under ultraviolet light but *Staphylococcus epidermidis* did not. This fluorescence was found to correlate with mannitol fermentation. The media then allowed rapid isolation and identification of staphylococci, based on the characteristics of colony morphology, pigment, hemolysis, and yellow fluorescence.

MATERIALS AND METHODS

Colonies were examined for fluorescence under ultraviolet irradiation of 3660 A (Wood's light).

Using stock cultures and direct clinical isolates, the characteristics delineating staphylococci, as noted in Bergey's Manual, were the bases for evaluating fluorescein amine agar.

The stock cultures of staphylococci were isolated from 216 human cases and carried on trypticase soy agar (BBL) slants for a maximum of 2 subcultures. The 200 clinical specimens, which served as the reservoir of direct isolates, were mostly nose and throat cultures from the employee Health Unit.

The basic blood agar medium used was trypticase soy agar (BBL) with sheep blood. To it were added D-mannitol (Difco) and fluorescein amine fraction I (Nutritional Biochemicals Corporation). The former was a 10% solution sterilized by Seitz filtration. Fluorescein amine was prepared as a 1:500 dilution by dissolving 0.2 g in 100 ml of 0.01 N sodium hydroxide and autoclaving at 121 C for 10 min.

Forty grams of the medium were dissolved with heat in 925 ml of distilled water and the pH adjusted to 7.0. After routine sterilization and cooling to 45 to 50 C, the following were added:

a) D-Mannitol (10%), 25 ml (final concentration 0.25%);

b) Defibrinated sheep blood, 50 ml (final concentration 5%);

c) Fluorescein amine, 25 ml (final concentration 1:20,000).

After mixing, pour plates were prepared. The stock cultures and clinical specimens were streaked and incubated overnight at 37 C.

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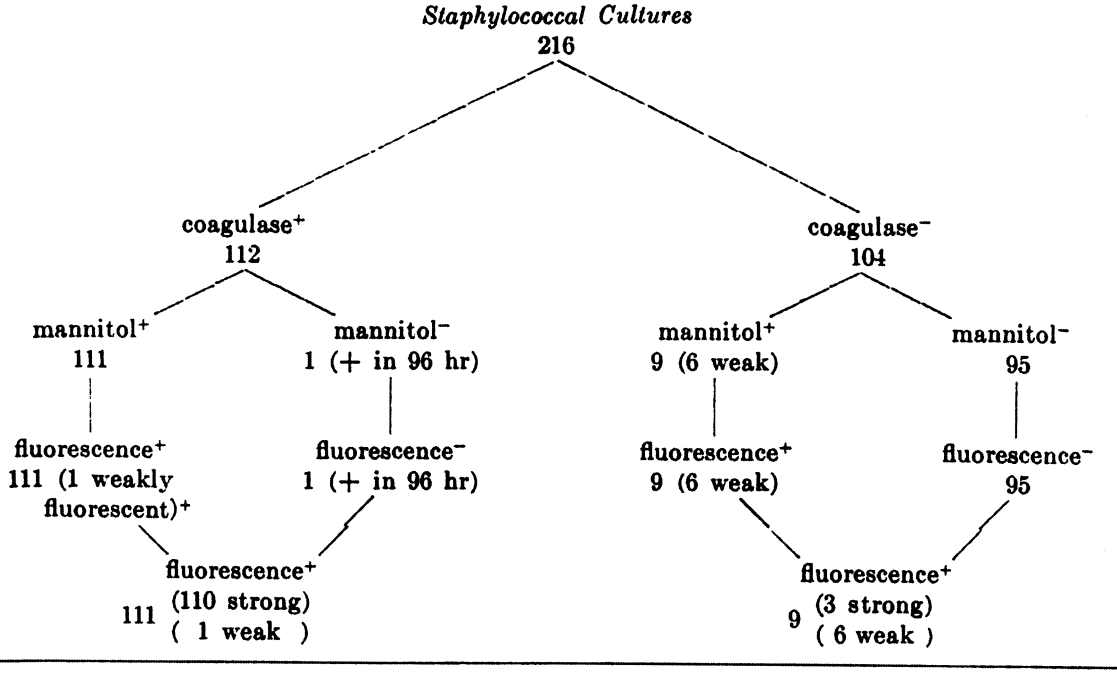
TABLE 1. *Coagulase production, mannitol fermentation, and colony fluorescence in a stock culture collection of staphylococci*



The other agar media used were sodium azide, phenylethyl alcohol, mannitol salt, and staphylococcus medium no. 110. These were prepared according to package instructions. After autoclaving and cooling to 45 to 50 C, they were modified by the addition of the following, to give the final concentrations indicated:

a) Sheep blood, 5%

b) Fluorescein amine, 1:20,000

c) Mannitol, 1% (where necessary).

The 216 stock cultures were subjected to the following:

a) A tube coagulase test using fresh rabbit plasma (Chapman, Berens, and Stiles 1941)

b) Mannitol fermentation in 0.5% broth

c) Fluorescence under ultraviolet irradiation on trypticase soy blood agar plates.

Clinical isolates were first studied under normal lighting conditions. Colonies resembling staphylococci, as verified by morphology, pigment and hemolysis, were next examined under ultraviolet for fluorescence and then evaluated by the same coagulase and mannitol fermentation studies as the stock cultures.

Twenty active mannitol-fermenting staphylococci and 20 nonmannitol fermenters were picked from the stock cultures and streaked on the sodium azide agar, phenylethyl alcohol agar, mannitol salt agar, and staphylococcus medium no. 110, as modified. These were incubated 24 hr at 37 C and examined under the Wood's light for fluorescence.

RESULTS

In organisms that were coagulase positive, there was virtually complete agreement with positive mannitol fermentation and fluorescence, in both the stock cultures (Table 1) and the primary isolates (Table 2). Analysis of the figures in both tables is shown in Table 3. Of all the coagulase-positive organisms studied, 98.7% were also mannitol positive and fluorescent. In this group, 1.3% were coagulase positive and mannitol negative. The incidence of coagulase-negative, mannitol-negative, and fluorescence-negative staphylococci was 92.0%. Here the coagulase-negative and mannitol-positive organisms totaled 8.0%.

Mannitol-fermenting organisms always fluoresced. In general, rapid sugar fermenters had maximal fluorescence, whereas those with weak fermenting activity had correspondingly weak fluorescence.

On each of the media streaked with the 20 mannitol-positive and 20 mannitol-negative stock cultures, yellow fluorescence was seen only with the former, whereas the latter were all nonfluorescent.

TABLE 2. *Coagulase production, mannitol fermentation, and colony fluorescence in staphylococci directly isolated from clinical specimens*

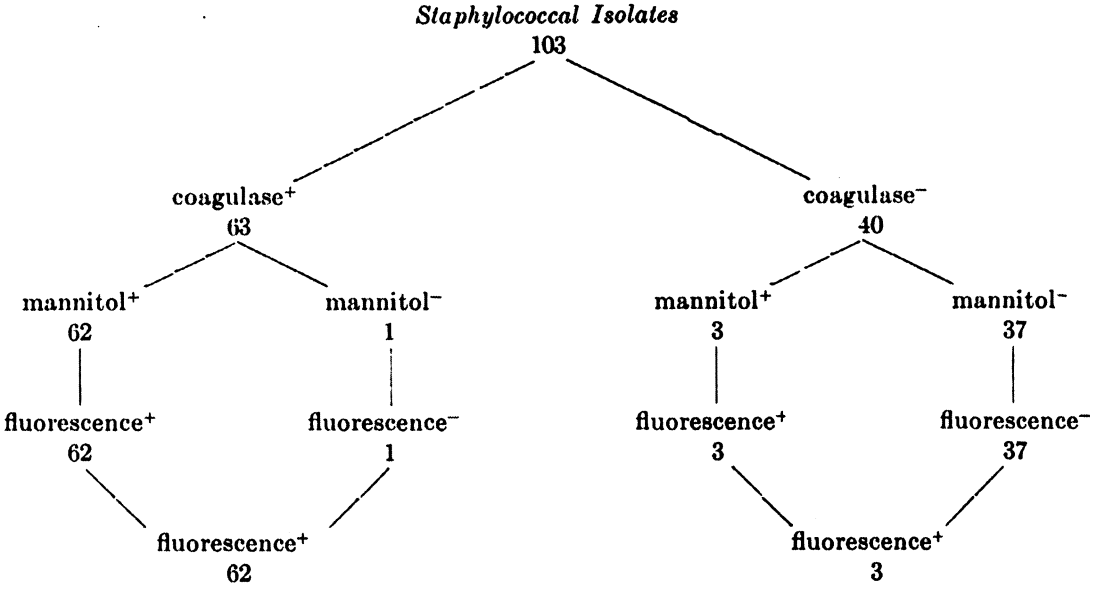


TABLE 3. *Analysis of coagulase production, mannitol fermentation, and fluorescence in both groups of staphylococci*

Coagulase+ group				Coagulase− group			
	Stock cultures	Clinical isolates	Combined		Stock cultures	Clinical isolates	Combined
	%	%			%	%	%
Coagulase+ and mannitol+	99.1	98.3	98.7	Coagulase− and mannitol−	91.4	92.5	92.0
Coagulase+ and fluorescence+	99.1	98.3	98.7	Coagulase− and fluorescence−	91.4	92.5	92.0
Fluorescence+ and mannitol+	100	100	100	Fluorescence+ and mannitol+	100	100	100
Coagulase+ and mannitol−	0.9	1.7	1.3	Coagulase− and mannitol+	8.6	7.5	8.0

When inoculated with clinical material, the fluorescein amine trypticase soy blood agar did not appear to inhibit the growth of *Diplococcus pneumoniae*, β, α and several group D streptococci, *Proteus*, *Pseudomonas*, *Escherichia*, *Klebsiella*, and *Aerobacter* species. With the exception of β Streptococcus, each of the gram-positive cocci also fluoresced, albeit weakly.

DISCUSSION

The figures in Table 3 raise the question of the interpretation of the coagulase-positive and mannitol-negative (1.3%) and coagulase-negative and mannitol-positive (8.0%) organisms. Clinically, this could best be resolved on the basis of the coagulase test alone.

Fluorescein amine blood agar does not substitute for the coagulase test but it does offer distinct advantages over a number of other media in current use. These are: (i) provides primary isolation and identification; (ii) does not change characteristics of colony morphology, pigment or hemolysis; (iii) does not inhibit other pathogens in clinical specimens.

After growth on the modified media, the pigment, hemolysis, and viability of the staphylococci appeared to be unchanged when subcultured to conventional blood agar plates.

Formerly fluorescent colonies showed no fluorescence after such transfer.

Butter yellow fluorescence was characteristic for most of the mannitol-fermenting staphylocci. However, some variation in the color was noted. This was due to the natural pigment, characteristic of each strain, when merged with the yellow of fluorescence. Whatever the nature of the phenomenon of this fluorescence, it is correlated with the fermentation of mannitol.

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