

NOTES

DETERMINATION OF THE CHROMOGENIC PROPERTY OF STAPHYLOCOCCI¹

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Much has been written on the difficulties and irregularities encountered in studying the chromogenic properties of staphylococci. Based upon the work of Fujita and Yoshioka (1923) several recent authors proposed that chromogenesis be tested on a medium containing 10 per cent of evaporated milk. Little has been written about the influence of temperature and no reports have shown a comparison of different methods. Coagulation of plasma was used in the experiments to be discussed, to compare tests of chromogenesis.

A series of 18 cultures was tested on (1) Bacto proteose no. 3 agar to which had been added when almost cool 10 per cent of evaporated milk and on (2) Bacto proteose lactose agar, which contained 1 per cent of lactose. The plates were incubated at 37°C. and at "room" temperature (which was from 20 to 23°C.). Only 8 were chromogenic on lactose agar at 37°C., 9 on lactose agar at room temperature and 10 on milk agar at 37°C., but 17 of the 18 were chromogenic on milk agar at room temperature.

Because room temperature gave a higher proportion of positive results, 42 coagulase-negative and 108 coagulase-positive staphylococci were tested on milk and lactose agars at room temperature. There was agreement with coagulase in 91 per cent of milk agar but in only 79 per cent of lactose agar cultures. The average positive reaction appeared in 1.9 and 2.1 days, respectively, and reached a maximum intensity of color in 4.5 and 5.5 days, respectively, although some cultures did not develop pigment until 10 days. The average final intensity of color was similar on both media but, because of the white background, the milk agar cultures were easier to observe.

A series of 448 routine cultures was plated on lactose agar and incubated "overnight" (the customary method). Those that gave white or doubtful colored growths were retested on milk agar at room temperature. There was agreement with coagulase in only 80.1 per cent of those on lactose agar at 37°C. but when the cultures were confirmed on milk agar at room temperature, the agreement was increased to 93.3 per cent.

These results suggest that "*Staphylococcus albus*" cultures should be retested by plating the cultures on 10 per cent evaporated milk agar, sealing the plates with "Parafilm," leaving them on the laboratory table up to 10 days and then

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observing the colors. Results are less satisfactory when the room temperature rises above 22°C.

REFERENCE

- FUJITA, A., AND YOSHIOKA, S. 1923 A new medium favorable for pigment production by staphylococcus, also a contribution to the knowledge of pigment production. Japan Med. World, 3, 47-51.

A MEDIUM ADAPTED TO THE BACTERIOPHAGE OF RHIZOBIUM LEGUMINOSARUM¹

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In a series of studies on soil bacteriophage, progress was found to depend upon the development of better technic in connection with the test-tube method of detecting bacteriophage. The medium M₁ of Laird (1932), for example, although generally regarded as satisfactory for the bacteriophage of *Rhizobium spp.*, was found to have certain disadvantages: It does not allow sufficient growth of *R. leguminosarum*, the pea nodule organism; the CaCO₃, added to favor development of lysis, causes a turbidity due to precipitated calcium salts on the wall of the tube, which sometimes makes it difficult to determine whether lysis has occurred; the light brown color of the yeast extract is sometimes a disadvantage for the same reason.

The first change made in this medium has been to follow the suggestion of Albrecht and McCalla (1937) to supply the needed growth factors in the form of sauerkraut juice instead of yeast extract. This furnishes a colorless medium, allowing even better growth of the bacteria in question than when yeast extract is employed.

The second change has been to replace the CaCO₃ and K₂HPO₄ of Laird's medium with calcium glycerophosphate. The latter contains both calcium and phosphorus, but unlike the simple calcium phosphates, is soluble at neutrality and at weakly alkaline reactions; its use, therefore, eliminates the objectionable haziness. It has the further advantage of supplying a small amount of available carbon; this promotes growth without the formation of sufficient gummy mate-

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