A METHOD OF STAINING BACTERIAL FLAGELLA

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It has been thought useful to give the details of a modification of Muir's method of demonstrating the flagella of bacteria, recently developed in this laboratory. The method has proved successful in staining the flagella of about one hundred strains of soil bacteria and has been tested on other common types such as *B. coli*, *B. fluorescens*, and *B. mycoides*. The process has given much more certain results than any of the standard methods tested and possesses the advantage that very little precipitate remains on the film surrounding the bacteria.

CULTURES

The best results are obtained with twenty-four-hour to three-day old slope cultures on nutrient agar or some other medium suited to active growth. (Nutrient agar in the case of the soil organisms upon which the stain was successful is composed of pepton 5 gr., Lemco 3 gr., and agar 15 gr. per litre of water; pH adjusted to 7.3.) Suspensions are made in sterile distilled water in a watch glass and left for twenty minutes to half an hour at room temperature, during which time the organisms if active will be washed free of slime. They should be examined for motility in the fresh state, immediately before the film is made.

SLIDES

Clean slides are kept in ammonia-alcohol; for use they are dried with a clean duster and flamed by passing 24 times through the Bunsen at the level of the top of the inner cone. They are then put into an oven at 45° to 50°C. to cool, flamed side uppermost.

THE FILM

One large loopful of the suspension is placed near one end of the slide. A strip of unsized paper (e.g., typing paper) of a width less than that of the slide is lowered on to the loopful and drawn gently down the slide and off towards the operator. The slide is then put into the oven to dry. The paper strip is convenient in that it allows of a very thin film which dries off quickly.

THE MORDANT

This is made up of the following solutions which when stored separately have kept without deterioration for a year in this laboratory.

Solution 1

Potash Alum—Saturated aqueous sol..... 5 cc.
Tannic acid—20 per cent aqueous solution..... 2 cc.
(A few drops of $CHCl_3$ must be added to this if a large quantity is made up)
Mercuric chloride—Saturated aqueous solution..... 2 cc.

Solution 2

Basic fuchsin—Saturated alcoholic solution

For use 0.4 cc. of the fuchsin solution is added to solution 1; the solutions are mixed by rapid rotation of the test tube, when a precipitate will form. The solutions must be freshly mixed for each batch of slides as the mixture deteriorates after twenty-four hours.

About 0.5 cc. is allowed to act on each slide for ten minutes at room temperature.

STAIN

Wash off the mordant with a gentle stream of distilled water. When no more fine precipitate is removable apply a few drops of Ziehl's carbol fuchsin and leave for five or ten minutes. Wash off with tap water.