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GENERAL SECTION

A SIMPLE STAIN FOR MOTILE BACTERIA

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ABSTRACT:

A simple method of staining living bacteria for motility or other studies is presented. The method is relevant to routine use in the microbiology laboratory.

INTRODUCTION:

It is customary in microbiology laboratories to observe various bacterial characteristics, one of importance being the presence or absence of bacterial cell motility. This is done by the preparation of hanging-drop or wet-mount preparations of bacterial colony suspensions, which are then viewed by enhancing contrast through manipulations of the condenser lens and light diaphragm.[2,3] This is usually enough to show dim outlines of bacteria as well as their motility, but clear outlines of bacteria are not visible. One of us (S.R.) thought of staining living motile bacteria to see them better, while the other did the laboratory work required to develop the procedure.

MATERIALS AND METHODS:

A-Motile Bacteria: Samples of bacteria were obtained from bacterial colonies cultured on routine and selective laboratory media in the microbiology laboratory. These samples were taken directly from culture media through rutine methods[2,3] and hanging drop or wet-mount preparations made in normal saline. Bacterial colonies were also incubated in liquid broth media and then direct wet-mount or hanging drop preparations were made for motility testing. Motile bacteria were identified through contrast enhancing and their colonies or broth cultures used for preparing the staining technique.

B — Staining: A number of commonly used laboratory stains were tested for their ability to stain bacteria. With most stains, whether in aqueous or alcoholic solutions, bacteria would stain, but would die out and motility could not be observed. Thus it was decided to use a vital dye, and neutral red was tested,[1] as it was easily available in the laboratory. An 0.5-1.0% aqueous solution was found adequate for both staining as well as maintaining bacterial viability and motility. A small drop of stain was added to a wet-mount or hanging drop preparation, allowed to stain for 1-2 mins and examined microscopically. Unstained and stained preparations were assessed for bacterial numbers, numbers of dead and living bacteria, motility and any changes in the pattern of motility. The stain was also put directly in liquid broth to see if bacteria would stain by this method (i.e. in culture).

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RESULTS:

Staining with 0.5-1.0% aqueous Neutral Red did not in any significant way alter the death rate, motility or patterns of motility. Bacteria took up the stain quickly (1-2 mins) and were clearly seen as strongly stained red cocci or bacilli moving about in their original fashion. It was easy and amusing to see them carry on their activities, including cell division unhindered. Both wet-mount and hanging drop methods gave good results, the former being easier to prepare. It was observed that fresh cultures and broths gave good results, while older cultures, particularly broths tended to stain poorly and had a higher death rate, with or without staining. This is attributed to a change in the cell wall of a mucoid, waxy nature that resists staining. Broths also showed the presence of similar substances in the medium. This change in bacteria is perhaps due to aging, overcrowding and decrease in nutrients, a kind of early encystment. Stain put directly in broth did not fare well, perhaps due to degradation by bacterial products in the medium.

DISCUSSION:

It is possible to devise a method to stain living bacteria easily and observe them in their natural live form microscopically. It is important though to use fresh culture or broth media, as well as to make wet-mount or hanging drop preparations in normal saline, which dilutes bacterial products and enhances staining. The method can be used not only to observe bacterial motility, but allows further prolonged studies of bacteria, as long as they are kept alive by warm stage techniques. Bacterial cell division, metabolic chemistry and the effects of drugs and bacteriophages are areas of possible study using this method.

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