AN AGAR MEDIUM INDICATING ACID PRODUCTION[1]

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Received for publication April 21, 1958

In order to undertake some genetic studies of micrococci, it was necessary to devise a medium clearly indicating the fermentation characteristics of these microorganisms. The medium devised has proved useful for studying 10 strains of micrococci, is also suitable for studying *Escherichia coli*, and may prove useful in studying other microorganisms.

EXPERIMENTAL METHODS AND RESULTS

The medium is a modification of MacConkey's agar and is based on the observations of Stacy and Webb (1947) that dehydrocholic acid, one of the bile acids, is nontoxic for staphylococci. After some trials, a medium of the following composition (in g per L) was devised[2]: Sigma pH 7 to 9 tris(hydroxymethyl)aminomethane buffer, 1.3; dehydrocholic acid, 1.5; yeast extract (Difco), 1.0; proteose peptone (Difco), 10; neutral red, 0.075; agar, 12. The pH of this medium with our reagents is 7.6. To this basic medium is added the desired carbohydrate at a concentration of 1 per cent. Since dehydrocholic acid is insoluble at pH 6.8 or less, the reagents should be dissolved in the order listed, except neutral red which is best dissolved in a small volume of water and added to the other ingredients. The completed medium is pale red in color. On plates, colonies of fermenters are dark red, whereas nonfermenters are white. The appearance of mannitol positive and negative mutants of *Staphylococcus aureus* and *Escherichia coli* on this medium is shown in figure 1.

In preparing this medium tests were made of the ingredients in the following concentration ranges (per L): dehydrocholic acid, 1.0 to 2.5 g; neutral red 3.0 to 75 mg. Proteose peptone was selected as most satisfactory over the following proteinaceous substrates: tryptose, neopeptone, peptone, tryptone, proteose peptone No. 3, (all Difco products) and Sheffield N-Z-Amine Type B. The complete medium listed above was tested at pH of 6.8, 7.0, 7.2, 7.4, and 7.6. Growth and colony differentiation was found clearest at pH 7.6. Sigma 7-9 buffer seems to sharpen the differentiation between "+" and "−" colonies, while substitution of phosphate buffers destroyed the usefulness of the medium. In the case of *E. coli*, standardization of the medium was achieved by means of known mutants, and in the case of *S. aureus* by comparison with phenol red broth cultures containing the appropriate carbohydrate.

The efficacy of this medium in studying *S. aureus* is illustrated by the fact that we have obtained lactose, maltose, sucrose, glucose, and mannitol negative mutants of our strains, a process which requires the selection of negative colonies in the presence of large numbers of positives. In transduction experiments employing staphylococcal phage, we have tested, for linkage between fermentation and drug resistance, genes using this medium; for example, S^rLac^+ (donor) $— \times$ S^sLac^- (recipient) $\rightarrow$ S^rLac^+ transformed cells in the case of linkage.

In studies with *E. coli* of transduction by phage λ (Morse, 1957) we have replaced the eosin methylene blue agar used previously with the above medium with satisfactory results. Phenotypes of galactose fermenters, nonfermenters, heterogenotes, and position effect heterogenotes were clearly distinguishable. A minimal medium with the dehydrocholic acid-neutral red indicator system has also been employed successfully in bacterial crosses.

In devising this medium some tests were made using other microorganisms. The medium as given above seemed satisfactory for a strain of

[1] Aided in part by grants from the United States Public Health Service (E1345), and the continuing Research Fund, University of Colorado School of Medicine. Contribution No. 71 from the Department of Biophysics.

[2] Source of the special ingredients: Sigma 7-9 buffer, Sigma Chemical Co., St. Louis 18, Missouri; Dehydrocholic acid, California Foundation for Biochemical Research, Los Angeles 65, California; neutral red, Matheson, Coleman Bell Division, Norwood, Ohio.

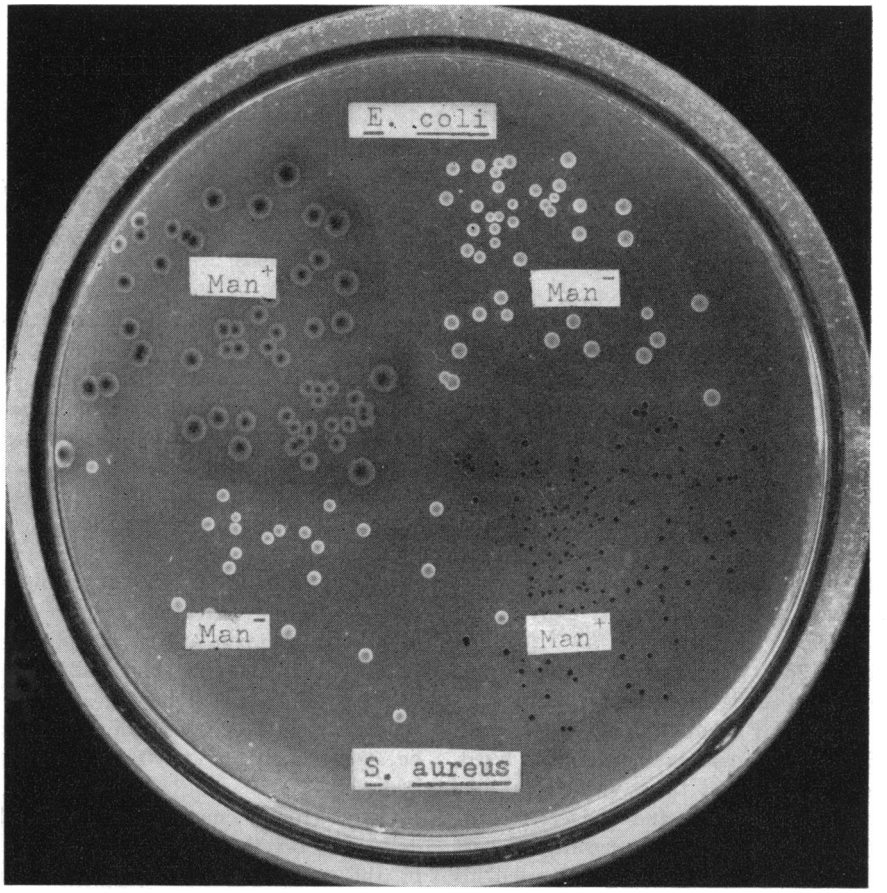

Figure 1. The appearance of mannitol positive (Man+) and mannitol negative (Man−) colonies of *Escherichia coli* and *Staphylococcus aureus* on dehydrocholic acid-neutral red agar.

Streptococcus faecalis. With lower concentrations of neutral red and dehydrocholic acid, the medium appeared satisfactory for a strain of pneumococcus, and omitting the proteose peptone suggested that the medium might work for some strains of bacilli. We suggest that if the medium as given is not ideal for particular species or strains, that minor modifications in the amounts of its components be made.

SUMMARY

An indicator agar employing dehydrocholic acid and neutral red has been devised. It has been used in the study of the fermentative activities of *Escherichia coli* and *Staphylococcus aureus*.

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