APPLIED MICROBIOLOGY, Nov. 1969, p. 948-949
Vol. 18, No. 5

Printed in U.S.A.

Atypical Reactions of *Escherichia coli* on Eosin Methylene Blue Agar

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Received for publication 30 June 1969

Evidence is presented that atypical reactions of *Escherichia coli* on eosin methylene blue agar are due to variations in *p*H in localized areas of the medium.

The use of eosin methylene blue (EMB) agar as a medium for the differentiation of *Escherichia coli* and other coliforms from salmonellae and shigellae has been recommended by numerous sources (1, 2). Typical colonies of *E. coli* on EMB agar are usually described as blue-black or having a dark center with a colorless periphery and, when viewed with reflected light, a green metallic sheen. In this note, we describe some unusual reactions of *E. coli* on EMB agar.

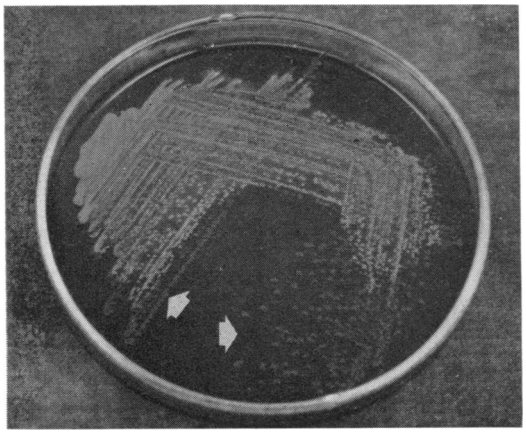

FIG. 1. *Absence of metallic sheen over isolated areas of EMB Agar.*

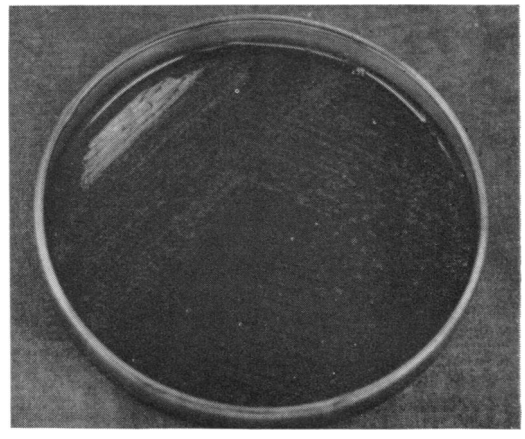

FIG. 2. *Atypical reaction over almost entire EMB Agar plate.*

During the course of other studies with this organism, EMB Agar (Difco) was selected as the medium to determine the purity of cultures. This medium was prepared as recommended by the manufacturer and autoclaved at 121 C for 15 min. Plates were dried at 37 C for 24 hr before use. Cultures were streaked for isolation and incubated at 37 C for 18 to 24 hr. Many atypical areas of growth, some so pronounced as to make the preliminary identification of *E. coli* impossible, were observed. In some cases, the metallic sheen was not evident over isolated areas of the plate (Fig. 1). Of even greater significance, however, were the plates covered almost entirely with gray opaque growth (Fig. 2). Not all plates of medium prepared at the same time and inoculated with the same or different strains of *E. coli* contained such atypical areas of growth. Subcultures from such areas were either typical or atypical. To determine whether these peculiarities of growth were caused by a particular manufacturer's lot of medium, different lots of media from the same manufacturer (Difco) were inoculated and atypical areas of growth were similarly observed. When Levine EMB Agars from different sources (BBL or Fisher Scientific) were inoculated, atypical areas of growth could still be observed. The substitution of plastic petri dishes for glass ones did not obviate these reactions.

To determine whether variations in *p*H could account for the unusual reactions with EMB Agar, either 0.5% KH_2PO_4 or K_2HPO_4 was added to this medium before autoclaving. After sterilization, the *p*H was either 6.5 or 7.2, respectively,

whereas that of the control medium (Difco EMB Agar) was 7.0. After growth at 37 C for 18 to 24 hr, medium adjusted to *p*H 6.5 contained typical growth, whereas medium adjusted to *p*H 7.2 contained growth in which both the dark coloration and metallic sheen of colonies were absent (Fig. 3). When filter paper discs saturated in 5% K_2HPO_4 were placed on freshly inoculated EMB Agar, the result shown in Fig. 4 was produced.

To account for atypical areas of growth in medium prepared normally, the occurrence of variations in *p*H in localized areas of EMB agar remains suspect. Uneven cooling of autoclaved media could result in an uneven distribution of ions throughout the colloidal medium. The mixing which results from the normal swirling of media before pouring into petri dishes may not be sufficient to disperse these ions. As a result, fluctuations in *p*H may be present throughout the medium upon solidification. It is not the intent of this note to describe the physico-chemical forces presumably responsible for atypical reactions observed with EMB agar, but rather to alert investigators of the possible errors in the interpretation of results when growing *E. coli* on EMB agar.

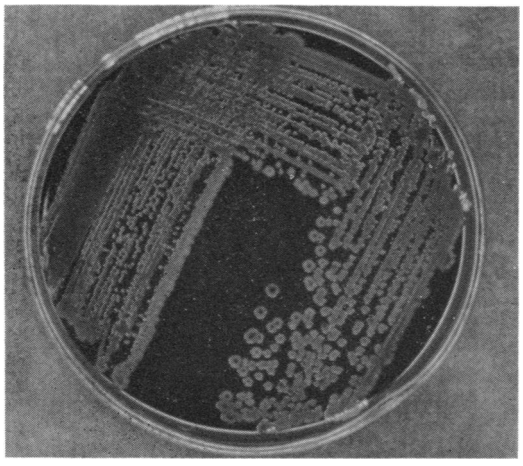

FIG. 3. *Atypical reaction produced in EMB Agar adjusted to pH 7.2.*

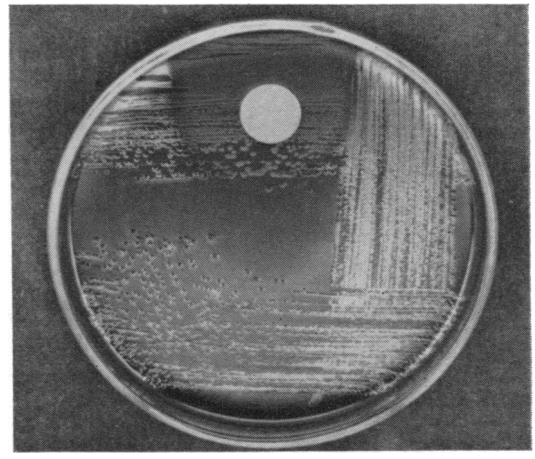

FIG. 4. *Atypical reaction in EMB Agar produced by diffusion of K_2HPO_4.*

This investigation was supported by Public Health Service grant AI-07637 from the National Institute of Allergy and Infectious Diseases.

We thank W. H. Krass for the photographs. Colored composite slides are available from the authors upon request.

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