Lysine-Iron Agar in the Detection of Arizona Cultures

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Abstract

Edwards, P. R. (Communicable Disease Center, Atlanta), and Mary A. Fife. Lysine-iron agar in the detection of Arizona cultures. Appl. Microbiol. 9:478-480.—A lysine-iron agar is described and recommended for the detection of Arizona strains which ferment lactose rapidly. Black colonies which appear on bismuth sulfite agar should be transferred to the medium. Salmonellae and Arizona cultures produce a distinctive reaction since they are the only recognized groups of enteric bacteria which regularly produce lysine decarboxylase rapidly and form large amounts of hydrogen sulfide. Use of the medium is particularly recommended in the examination of specimens from enteric infections in which shigellae and salmonellae are not detected.

When the Arizona group first was delineated 20 years ago (Peluffo, Edwards, and Bruner, 1942) it was thought to be made up exclusively of organisms which fermented lactose slowly. It later became apparent that some members of the group fermented lactose rapidly but it was not until LeMinor, Fife, and Edwards (1958) examined 400 Arizona cultures isolated from snakes that it became evident that active fermenters of lactose occurred very frequently. The cultures of LeMinor et al. (1958) were selected without regard to action on lactose and, of 400 cultures isolated from snakes, 64% fermented lactose actively on overnight incubation. Many of these cultures produced red or pink colonies on MacConkey's agar and deoxycholate-citrate agar and yellow colonies on brilliant green agar so that, in the usual examination for enteric pathogens, their presence would have been overlooked. Further, many of these cultures, when transferred to triple sugar iron (TSI) agar slants, produced a pronounced acidity throughout the medium and failed to blacken it, although large amounts of hydrogen sulfide were formed as demonstrated by their action in peptone-iron agar which exhibited typical blackening.

Recently, through the courtesy of A. A. Hajna and A. S. Browne, Arizona cultures from outbreaks of food infection in man were received. Lactose was fermented rapidly by these cultures. The cultures from A. A. Hajna were of particular interest since they produced acid colonies on isolation media, and completely acidified TSI agar slants with a suppression of blackening. They belonged to the same biotype and serotype as certain cultures found by LeMinor et al. (1958) in snakes captured in France.

Since it now is apparent that Arizona types which ferment lactose rapidly are found occasionally in outbreaks of food infection, it becomes important to determine the frequency with which they occur in outbreaks and sporadic cases of enteric disease and whether they may be etiological agents in certain incidents, the cause of which now is unknown.

Materials and Methods

It is obvious that if the incidence of such organisms is to be determined, a method of selection of cultures by means other than failure to ferment lactose and sucrose must be used. From the work of Moeller (1954) and of Ewing, Davis, and Edwards (1960), it is known that the only recognized groups of Enterobacteriaceae, which regularly decarboxylate lysine rapidly and which produce hydrogen sulfide in sufficient amount to blacken peptone-iron-agar, are members of the Salmonella and Arizona groups. Further, as a rule, Salmonella and Arizona strains produce typical black colonies on bismuth sulfite agar. By taking advantage of these facts it is possible to use isolation plates and differential media which contain no lactose or sucrose. One could, of course, pick black colonies from bismuth sulfite agar and inoculate each one into tubes of lysine decarboxylase broth and peptone-iron agar and thus determine whether each colony produced lysine decarboxylase and hydrogen sulfide. Since this method is inconvenient, a lysine-iron agar was prepared so that both reactions were determined in the same tube. After a number of formulas had been tested, a medium with the composition as listed in Table 1 was adopted.

<table>
<thead>
<tr>
<th>Table 1. Composition of growth medium</th>
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<tbody>
<tr>
<td>Bacto-yeast extract*</td>
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<tr>
<td>Bacto-peptone*</td>
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<tr>
<td>Glucose</td>
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<tr>
<td>L-Lysine</td>
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<tr>
<td>Ferric ammonium citrate</td>
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<tr>
<td>Sodium thiosulfate</td>
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<tr>
<td>Bromoresol purple</td>
</tr>
<tr>
<td>Agar</td>
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<tr>
<td>Distilled water</td>
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<td>pH adjusted to 6.7</td>
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* Difco Laboratories, Inc., Detroit, Mich.
The medium was tubed in amounts of 4 ml in screw-capped tubes (100 × 13 mm), sterilized at 121 C for 12 min, and slanted so as to provide a short slant and a deep butt. After cooling, the caps were closed tightly to prevent evaporation. The medium was inoculated with a straight needle by stabbing to the base of the butt and streaking the slant, just as TSI agar is inoculated. The caps were replaced loosely and the tubes incubated at 37 C overnight.

Results

In all, 1,247 cultures of the various groups of enteric bacteria were inoculated into the medium. Organisms which produced lysine decarboxylase rapidly in fluid medium invariably produced an alkaline reaction throughout the agar medium, whereas those which did not do so produced an alkaline slant and a distinctly acid butt. Cultures which blackened TSI agar also blackened the lysine-iron agar. Due to deamination of lysine, Proteus and Providence cultures produced a distinctive appearance, a red slant over an acid butt. If the indicator were omitted from the medium, Proteus and Providence cultures produced a distinct orange color throughout the slant and apparently it was the combination of this color and that of the indicator that was responsible for their unique appearance.

Citrobacter (Escherichia freundii) cultures produced an alkaline slant and an acid butt with blackening and gas production. Escherichia cultures, including the Alkalescens-Dispar group, produced alkaline slants and acid or neutral butts, depending upon the speed of decarboxylation of lysine. No blackening of the medium was observed and evidence of gas production appeared irregularly. Shigella strains, like Alkalescens-Dispar cultures, produced alkaline slants and acid butts without gas production or blackening.

Klebsiella cultures produced alkalinity throughout the medium due to decarboxylation of lysine but blackening was not observed. Gas production was irregular. Aerobacter aerogenes strains which were lysine positive [Cloaca B of Hormaeche and Munilla (1957)] produced alkalinity throughout the medium, whereas lysine-negative strains of Aerobacter cloacae (Cloaca A of Hormaeche and Munilla) produced alkaline slants and acid butts. Gas production by both was irregular but neither blackened the medium. Hafnia cultures resembled A. aerogenes in their action on the medium, as did also Serratia cultures, except that they uniformly failed to show evidence of gas production.

The action of 100 cultures of Salmonella typhi, 132 other Salmonella serotypes, and 163 Arizona types was examined. The S. typhi cultures regularly produced alkalinity throughout the medium and all but one, which also failed to show evidence of hydrogen sulfide production in other media, blackened the medium to a greater or lesser extent. With few exceptions, other Salmonella serotypes and Arizona types gave similar reactions but, as would be expected, produced more intense blackening of the medium. Gas production was suppressed and only rarely was gas formation evident. Very rarely, Salmonella and Arizona cultures failed to produce hydrogen sulfide and even more rarely failed to give a prompt positive test for lysine decarboxylase (Edwards, Fife, and Ewing, 1956). Such cultures gave atypical reactions in lysine-iron agar. However, it should be emphasized that atypical cultures are the rare exception and that none of the other groups tested gave reactions resembling those of salmonellae and Arizona types, except very rare cultures of Citrobacter which possessed an active lysine decarboxylase. It is true that Salmonella paratyphi A is hydrogen sulfide negative and does not decarboxylate lysine rapidly, but one would hardly expect to isolate that type from bismuth sulfite agar plates, inoculated with stools of patients in outbreaks of acute food-borne infection, nor would shigellae be expected to develop on the medium. Further, bismuth sulfite agar plates are incubated for a longer period than such media as MacConkey, deoxycholate-citrate, and brilliant green agars. By the time the bismuth sulfite agar plates are examined, some evidence regarding the presence of shigellae, salmonellae, or staphylococci in a given episode should have been obtained and one should be able to judge whether a search for lactose-fermenting incitants was indicated.

To recapitulate, it is known that Arizona strains, which ferment lactose rapidly, sometimes are found in food and patients in outbreaks of food-borne infection. If the importance of such organisms in outbreaks and sporadic cases of diarrheal disease is to be assessed, it is essential to employ a method of isolation and preliminary screening in which lactose fermentation is not involved. Since Arizona strains produce salmonella-like colonies on bismuth sulfite agar, and since salmonellae and Arizona cultures are the only delineated groups of Enterobacteriaceae which produce H2S vigorously and produce lysine decarboxylase rapidly, it is suggested that black colonies on bismuth sulfite agar plates, inoculated directly from specimens and from enrichment mediums, be picked to lysine-iron agar. In this way the presence of Salmonella and Arizona strains, including cultures which ferment lactose rapidly, can be detected, since they produce an alkaline reaction throughout the tube and blacken the medium. It is not meant to infer that other isolation media and TSI agar should not be used in the usual manner, because lysine-iron agar fails to distinguish between shigellae and many cultures of E. coli. However, if used as suggested, it should be useful in determining the role played by Arizona strains in the production of diarrheal disease.
LITERATURE CITED


A Bacteriophage-lysing Strain of Staphylococcus Employed in the Manufacture of Dry Sausage

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ABSTRACT

Gyllenberg, H. G. (University of Helsinki, Helsinki, Finland), and C. R. Hackman. A bacteriophage-lysing strain of Staphylococcus employed in the manufacture of dry sausage. Appl. Microbiol. 9:480-483. 1961.—A bacteriophage of a certain Staphylococcus (a strain of Staphylococcus lactis) employed in the manufacture of dry sausage has been characterized. The host range of this bacteriophage is wide. In addition to the original host, 15 other strains (out of 40 strains tested) were found to support reproduction of the phage. The sensitive strains represented Staphylococcus saprophyticus and different types of S. lactis.

The growth rate of the bacterial host did not influence the rates of phage adsorption, nor the maximal reproduction rate of new particles. With increasing bacterial growth rate, the "lag" observed before phage reproduction started was distinctly decreased. This phase was shorter with the original host strain than with other sensitive strains.

Resistant cultures of the original host strain were easily obtained. These cultures grew as rapidly and gave as good yields of cell mass as the original phage-sensitive host. However, phage resistance was frequently lost.

Although rather extensive information is available concerning the bacteriophages of medically important staphylococci, very little is known about the phages of those staphylococci which are specifically connected with food technology. Certain strains of Staphylococcus lactis [the nomenclature proposed by Shaw, Stitt and Cowan (1951) is used in this report] are particularly important in the processing of dry sausage varieties that are dependent upon nitrate reduction for color development (Pohja, 1960b), and may thus be added at high levels to sausage. In experiments on large-scale culture of a suitable strain of S. lactis by continuous methods, sudden lysis of the cultures was frequently observed. It was realized that this phenomenon was due to the occurrence of a specific bacteriophage, the general characteristics of which will be described in this report.

MATERIALS AND METHODS

The lytic effect was first observed in experiments carried out by R. Müller, Rudolph Müller and Company, Hamburg, Germany, and the active phage was isolated from a sample of lysed culture obtained from Mr. Müller. The original host strain (strain 132) together with 40 other strains representing different types of staphylococci of meat origin were received from M.S. Pohja, Research Laboratory of the Farmers' Cooperative Packinghouses, Hämeenlinna, Finland.

For cultivation of the staphylococci, the following substrate was employed: Yeast extract (Difco), 3 g; glucose, 1 g; sodium citrate, 3 g; sodium chloride, 25 g (in some experiments 50 g); potassium nitrate, 1 g; in 1,000 ml of tap water (final pH 7.2). To give a solid medium, this substrate was supplemented with 15 g of agar. Continuous cultivation was carried out employing the equipment of Gyllenberg and Hackman (1960). For ordinary batch cultures, a 1-liter fermentor was used. In both cases, intense aeration and agitation of the culture was applied.

The bacteriophage was regenerated in cultures of