Comparative Study of Media for Determination of Lysine Decarboxylase Activity

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Received for publication 27 April 1973

Three lysine decarboxylase media were studied with 2,764 Enterobacteriaceae. This comparison was made with Möller, Falkow, and Taylor decarboxylase broths. Taylor broth proved to be the most suitable for routine diagnostic work.

The lysine decarboxylase test is one of the common tests used in differentiating Enterobacteriaceae (1, 3, 7, 9, 10, 12, 14, 16, 18–21, 23, 25, 26, 28–32). Depending on the method by which the products of the lysine decarboxylase activity are established, the reaction may be determined by: (i) measuring CO₂ formation (Gale [23] performs this manometrically, whereas Leclerc [27] employs a CO₂-phenolphthalein system in an automated procedure); (ii) determining the presence of cadaverine, the end product of lysine decarboxylase. The presence of this amine can be established (i) by extraction—cadaverine is soluble in chloroform, whereas lysine is not (Carlquist [4] uses this differential solubility in a reaction of the chloroform extract with ninhydrin [1, 2, 3-triketohydrindene] to detect the presence of this amine); (ii) by detection of pH changes in the media with a pH-sensitive indicator. This permits direct reading of the reaction after incubation. During the initial stage of incubation, the fermentation of dextrose with the production of acid by the respective organism leads to changes in the color of the indicator. On further incubation, if lysine is decarboxylated to cadaverine, there will be an alkaline reaction, and the color will revert to that initially present. This method is widely used as a routine test.

Möller (28–30) can perhaps be credited with the first practical application of the amino acid decarboxylase test for distinguishing between various microorganisms. Impressed with the work of Gale (23) on bacterial amino acid decarboxylases, Möller studied this enzyme system to determine its usefulness for differentiating the Enterobacteriaceae. He observed that the production of lysine, ornithine, arginine, and glutamic acid decarboxylases by various members of Enterobacteriaceae afforded a valuable adjunct to other biochemical tests in the differentiation of bacteria with other closely related physiological characteristics.

Since then, there have been several modifications of Möller medium (2, 6, 8, 11, 21, 24, 31), which make the test easier and more convenient to use. Unfortunately, not all of them are sufficiently precise in comparison with Möller original medium.

We compared the media of Falkow (21) and Taylor (31) with that of Möller (accepted as the standard method) in an attempt to determine a precise and convenient medium for the differentiation of the Salmonella and Arizona genera from the rest of the Enterobacteriaceae.

MATERIALS AND METHODS

Media for testing. The lysine decarboxylase media used in our experiments were prepared in the Laboratory of Ekariage as directed by the authors (Table 1). In the first stage of this study, 5 ml of each medium was dispensed in test tubes (12 by 120 mm). Later this amount was reduced to 0.5 ml. The media were then sterilized for 15 min at 121 C.

Nutrient agar (NA), Kligler iron agar with 2% urea (KIA), MacConkey agar (MCA), deoxycholate citrate agar (DCA), brilliant green agar (BGA), and bismuth sulfite agar (BSA) were used. All media except BSA, which was an Oxoid product, were prepared as dehydrated media by the Research Institute for Epidemiology and Microbiology. The media were rehydrated, sterilized, and poured as directed by the manufacturers.

Test strains. A total of 2,764 cultures of Enterobacteriaceae were used as test strains in this study. Included were both freshly isolated and stock cultures. All organisms were grown on slants of egg yolk agar. A list of the number and origin of these strains is given in Table 2. There were 1,404 Salmonella strains from 45 serotypes and 158 Arizona strains from 117 serotypes.

Taxonomy and nomenclature. The taxonomy and nomenclature used in this study are those proposed by Edwards and Ewing (10) and Ewing (13, 15, 17).

Inoculation of lysine broths. The inoculum was...
obtained by a slight touch of the needle to a slant of NA or KIA and to the top center of a single, well-isolated colony grown on the respective selective media.

Ewing et al. (19) employed an inoculated control without added amino acid for comparison and suggested that this practice was essential for accurate determinations. We also used an inoculated control without the addition of amino acid to each medium.

Incubation and reading of the results. After inoculation, the broths were sealed with a layer about 1 cm thick of sterile mineral oil and incubated at 37°C. The results were read after 24 h of incubation. It is important to note that more than 24 h of incubation in Falkow and Taylor broths led to false-positive results. When Möller medium was used, the test tubes showing negative results were reincubated up to 4 days and read daily.

RESULTS

The present study was carried out in two stages. (i) During stage I, the results obtained in the three media with different Enterobacteriaceae were compared (Tables 3 and 4). Since the results showed that Taylor broth was more efficient than Falkow broth, the former was chosen for further study. (ii) During stage II, the possible influence of various factors on the results obtained with Taylor medium was investigated.

Effect of the amount of lysine broth. Each test strain was inoculated into three test tubes containing 5.0 and 0.5 ml, respectively, of Taylor broth. The results show that the amount of medium, within these limits, did not affect the test.

Effect of media on which strains were cultivated. The possibility of direct inoculation of lysine decarboxylase broth with colonies from selective plating media, such as MCA, DCA, BGA, and BSA, and from differential tube media, such as KIA, is very tempting. This would not only enable us to use the same materials, but it would also give us the opportunity to test a large number of suspected colonies simultaneously and in parallel with KIA and henceforth to achieve rapid biochemical differentiation within the genus Enterobacteriaceae.

We explored this possibility by using Taylor broth with 109 Salmonella strains from 25 serotypes and with 158 Arizona strains from 117 serotypes. The results obtained by these test strains, grown on their respective plating media, showed that all could decarboxylate lysine in Taylor broth.

Effect of antibiotic resistance. The problem of acquired antibiotic resistance and the eventual changes occurring in the properties of bacteria are extremely important. There are existing data (22) to show that Salmonella...
Table 3. Comparison of the results obtained in three media

| Organism*         | Möller | Control | Falkow | Control | Taylor | Control |
|-------------------|--------|---------|--------|---------|--------|---------|
| *Escherichia coli*| 246/246| 0/246   | 246/246| 0/246   | 246/246| 0/246   |
| Biotype alcaligenes-dispar | 0/2 | 0/2 | 1/2 | 1/2 | 0/2 | 0/2 |
| *Shigella* sp.    | 0/229  | 0/229   | 0/229  | 0/229   | 0/229  | 0/229   |
| *Proteus* sp.     | 0/295  | 0/295   | 0/295  | 0/295   | 0/295  | 0/295   |
| *Klebsiella pneumonia* | 112/112 | 0/112 | 94/112 | 3/112 | 100/112 | 0/112 |
| *Enterobacter cloacae* | 0/33 | 0/33 | 2/33 | 2/33 | 0/33 | 0/33 |
| *E. aerogenes*    | 12/12  | 0/12    | 10/12  | 0/12    | 12/12  | 0/12    |
| *Salmonella* sp.  | 1,496/1,496 | 0/1,496 | 1,496/1,496 | 0/1,496 | 1,496/1,496 | 0/1,496 |
| *Arizona* sp.     | 158/158| 0/158   | 158/158| 0/158   | 158/158| 0/158   |
| *Citrobacter* sp. | 0/364  | 0/364   | 47/364 | 47/364  | 0/364  | 0/364   |
| *Providencia* sp. | 0/2    | 0/2     | 0/2    | 0/2     | 0/2    | 0/2     |
| *Klebsiella ozenae* | 1/1 | 0/1    | 1/1    | 0/1     | 1/1    | 0/1     |
| *K. rhinoscleromatis* | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 | 0/1 |
| *Serratia* sp.    | 2/2    | 0/2     | 2/2    | 0/2     | 2/2    | 0/2     |
| *Bioserotype Salmonella-paratyphi A* | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 |

*The data obtained with *Providencia, Klebsiella ozenae, K. rhinoscleromatis, and Salmonella paratyphi A* are grouped together because the number of positive results tested is insignificant. Repeat tests produced the same results.

*Data are recorded as the number of positive results/number of the strains tested.

Table 4. Discrepancies between the results obtained in three media

| Organism discrepancies obtained with various media* | Möller | Falkow | Taylor |
|---------------------------------------------------|--------|--------|--------|
| +, F+                                              | 112    | 0      | 18     |
| -                                                  | 0      | 0      | 0      |
|                                                    | 94     | 3      | 0      |
|                                                    | 4      | 3      | 3      |
|                                                    | 18     | 0      | 0      |
|                                                    | 100    | 0      | 0      |
|                                                    | 0      | 12     | 12     |
|                                                    | 0      | 33     | 33     |
|                                                    | 12     | 0      | 0      |
|                                                    | 0      | 0      | 0      |
|                                                    | 364    | 0      | 364    |

* +, Positive results; F+, false-positive results; -, negative results.

strains that acquired antibiotic resistance are lysine decarboxylase negative. At present, when antibiotic resistance and the selection of strains on this basis are gaining ground, this problem is of paramount importance. That is our reason for checking the reliability of Taylor broth by using freshly isolated *Salmonella* strains from humans and animals (domestic and wild); these strains have naturally acquired resistance to many antibiotics. By using this medium, with freshly isolated *Salmonella* and *Arizona* strains, it was possible to carry out unobstructed observations with regard to the precision of the test. Eighty-six percent of the *Salmonella* strains proved to be polyresistant to the most frequently used antibiotics. Our results point out that this increased resistance does not affect the precision of the test.

DISCUSSION

The precision of Möller medium is universally acknowledged, and the decarboxylase tests carried out in this medium are useful in the biochemical differentiation and classification studies of enteric bacteria and of many other gram-negative bacteria, i.e., *Pseudomonas* species. They are accepted as standard tests in determinative microbiology. Although Falkow lysine broth is convenient for routine use in the identification of enteric bacilli, Cowan and Steel (7) found it unsatisfactory in differentiating the *Enterobacter* and *Klebsiella* genera. Douglas and Washington (9) suggest that Falkow's modification is unsatisfactory for Voges-Proskauer-positive organisms. It is Taylor's opinion (31) that this medium is of inferior precision in comparison with the ninhydrin test. Taylor suggests that the peptone in Falkow broth is a potential source for the release of NH₃, which alkalizes the media, i.e., it becomes a factor in false-positive results. Taylor omitted the peptone from Falkow broth and obtained a
medium that approaches the precision of the ninhydrin test.

Our study confirms the good results obtained with Taylor broth. In precision, this medium is close to that of Möller. We obtained analogous results with all of the three media for the Salmonella, Arizona, Escherichia, Shigella, and Proteus genera. Differences were noted, however, for the most part with members of the Klebsiella and Citrobacter genera. The results obtained with Taylor medium were somewhat closer to those with Möller medium than they were to Falkow medium.

The possibility of using small quantities (0.5 ml) of Taylor broth makes this medium suitable for wide application; and a lysine decarboxylase test broth with low price cost, the precision of which is not affected by the medium on which the test strains are cultivated, is highly advantageous. Our results show that the bacteriostatic agents in the four selective media most commonly employed for the isolation of enteric pathogens do not inhibit Salmonella and Arizona decarboxylase activity in Taylor broth.

This gave us grounds to include this test in our scheme for the rapid biochemical differentiation of Enterobacteriaceae (3).

Our results clearly point out that the Taylor lysine decarboxylase test does not decrease in diagnostic value with increased antibiotic resistance.

ACKNOWLEDGMENTS

The authors thank P. A. M. Guinee, The National Institute of Public Health, Utrecht; L. LeMinor, Institute Pasteur, Paris; A. B. Paterson and H. N. Spears, Central Veterinary Laboratory, Weybridge; J. Sourek, Czechoslovak State Collection of Type Cultures; J. Sedlak, Microbiological Institute, Prague; G. H. Snoeyenbos, University of Massachusetts; and R. Sakazaki, National Institute of Public Health, Tokyo, for kindly providing the stock cultures. We are greatly indebted to T. Vassileva for technical assistance and to J. Janev for translation of the manuscript. We are very much obliged to Martinov for correction of the text. We also thank I. Bojkov, Director of Veterinary Medicine, Ministry of Agriculture, Sofia, for permission to publish.

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