Lysine Decarboxylase Activity in Broth and Agar Media

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Received for publication 4 August 1971

Four lysine decarboxylase media were studied by testing them with 305 Enterobacteriaceae and 42 nonfermenting bacilli. A comparison was made between lysine decarboxylase broth medium (Moeller base) and Johnson's semisolid agar without lactose and Bachrach's broth medium and lysine-agar slants which contain lactose. The nonlactose media, lysine decarboxylase broth and the semisolid medium of Johnson, were the best media for use with all of the bacteria studied. The exclusion of lactose from lysine decarboxylase medium seems desirable to extend the usefulness of this medium among members of the Enterobacteriaceae. When the results with lysine decarboxylase broth and Johnson's semisolid medium without lactose were compared, a 6% difference existed between the results obtained with lysine decarboxylase broth and Johnson's semisolid agar. When the results with Bachrach's broth and lysine-agar slants with lactose were compared, a 1% difference existed between Bachrach's broth and the agar slant method. At times, reading and interpretation were difficult because of intermediate degrees of color change. The inability of Pseudomonas aeruginosa or Herellea to utilize glucose under the anaerobic condition of the medium makes the lysine decarboxylase test an undesirable procedure for these organisms. Of the four test media used, the lysine-lactose-agar slants seemed to be the least desirable because of the more frequent occurrence of indistinct color reactions and shifts in color.

Amino acid decarboxylase activities are useful tests in determinative microbiology. Since Moeller (7) in 1955 described practical tests for determining decarboxylases among the Enterobacteriaceae, microbiologist have used Moeller's methods, or modifications, with increasing frequency. Today, these are standard tests in clinical and public health microbiology laboratories. Unfortunately, some of the modifications that were aimed at making the test easier and more convenient are not as accurate as Moeller's methods. This is true whether one is attempting to demonstrate lysine, ornithine, or arginine decarboxylase. The modified Moeller medium as described by Difco was simplified by having one indicator, and the technique required no extraction or addition of reagents, in contrast to some other methods. Johnson's (6) semisolid medium (Moeller base) was modified by the addition of 0.3% agar, which avoids the necessity of the oil overlay. Bachrach (1) incorporated lactose into the Moeller base medium to permit differentiation of the Salmonella-Arizona strains from Escherichia, Klebsiella, and certain Enterobacter strains, which also decarboxylate lysine. The lysine-lactose-agar medium was a modification of the medium of Edwards and Fife (3) because of the addition of lactose and the omission of iron salts. It was useful in differentiating most Salmonella, nonlactose-fermenting Arizona, Edwardsiella, Serratia, and certain Enterobacter from Shigella, Escherichia, Klebsiella, Enterobacter aerogenes, and Citrobacter.

In the course of identifying a number of microorganisms isolated from biological specimens in the clinical laboratory, procedures for determining lysine decarboxylase activity were evaluated. The problem of interpreting results developed when inconsistent readings from lysine decarboxylase broth medium were obtained. Sometimes these results were positive, and at other times they were negative. At times, no change in indicator (purple color) can be seen. This can be confused with a positive result (purple color), when a change of indicator from yellow to purple is not noticed. This is partially due to the rapid pH shift resulting from the breakdown of glucose substrate and low hydrogen ion concentration. Also, a change of indicator from purple to yellow in the fermentation of glucose may be missed during the overnight incubation period. The biggest problem seems to involve nonlactose-
fermenting gram-negative bacilli that fail to ferment glucose. Although published reports indicate the advantage of using a lysine-agar medium (3, 6), decarboxylase broth is more commonly used. It therefore seemed worth while to examine four lysine decarboxylase methods in the hope of arriving at some conclusion regarding a method of choice.

MATERIALS AND METHODS

The bacterial strains included recent isolates from 149 urines, 104 sputa, 25 wounds, 6 body fluids, 4 ear swabs, and 2 fecal specimens as well as strains taken from our culture library. Original isolations from biological specimens were made on human blood-agar and MacConkey agar. An individual isolate was planted on Kliger iron agar (KIA). When a question of purity arose, one half of the colony was transferred to MacConkey agar for purification. Tests for hydrogen sulfide; urease; phenylalanine deaminase production; indole formation; citrate utilization; lysine decarboxylase activity; glucose, dulcitol, and lactose fermentation; gelatinase; and motility were made from KIA as described by Edwards and Ewing (2). With nonfermenters, the carbohydrate oxidative-fermentative (O-F) test medium of Hugh and Leifson (5), the nitrate test, Kovacs oxidase test (4), and the gluconate test as described by Haynes (4) were added. These biochemical tests were used for identification of the wild strains. Occasionally, a more elaborate series of tests was necessary to identify wild strains. Identified cultures were inoculated into four lysine decarboxylase test media.

The four test media employed here were as follows. Lysine decarboxylase medium (Moeller base). This medium contained decarboxylase basal medium (Difco) with 1% L-lysine and bromocresol purple indicator. An inoculated control made up of the above ingredients minus L-lysine was included with each strain tested. The results were read after incubation for 24 hr and for as long as 4 days.

Johnson's semisolid medium. This medium consisted of decarboxylase basal medium (Difco) with 1% L-lysine. Agar (0.3%) was added as indicated by Johnson (6) in the ornithine decarboxylase semisolid medium. The indicator was bromocresol purple. The semisolid medium was inoculated by a single stab with a straight wire to the bottom of the tube. The inoculated control was made up of decarboxylase basal medium in 0.3% agar without L-lysine and was included with each strain tested. Results were read after 24 hr of incubation.

Bachrach's lysine-lactose broth. This medium was that described by Bachrach (1) with L-lysine made up to 1%. The indicator used was bromothymol blue. The inoculated control consisted of decarboxylase basal medium and lactose without L-lysine and was included with each strain tested. Results were read after 16 to 24 hr of incubation.

Lysine-lactose-agar slants. The test medium contained lysine decarboxylase basal medium (Difco) with 1% L-lysine, 1% lactose, 1.5% agar, and bromocresol purple as the indicator. An inoculated control made up of the above ingredients minus L-lysine was included with each strain tested. Results were read at 24 and 48 hr.

These media were dispensed in 5-ml samples in sterile screw cap tubes (125 by 16 mm) and were sterilized in the autoclave for 15 min at 15 lb of pressure (121 C).

RESULTS

Initial studies were done with a series of 305 Enterobacteriaceae and 42 nonfermenters. The latter group included 40 Pseudomonas aeruginosa organisms and 2 Herellea strains (Table 1). The two Alkalascens-Dispar strains were positive in Bachrach's broth after the prescribed 24-hr incubation period. Whether this may represent a very weak decarboxylase manifesting itself late is open to question. In Table 3, these results were recorded as negative. These two strains were positive with all of the other methods except Johnson's semisolid medium. Repeated tests produced the same results. One of the atypical Escherichia strains was negative in Johnson's semisolid agar but positive in lysine decarboxylase broth. Of the seven atypical Escherichia strains, six were positive by the lysine decarboxylase broth and Johnson's semisolid method, although all were negative by the lysine-lactose media. As expected, Bachrach's broth with lactose and the lysine-lactose-agar slants gave negative reactions with most of the Escherichia and Klebsiella strains. Poor results were obtained with Enterobacter aerogenes with the nonlactose medium of Johnson. Of 14 E. hafniae strains which were positive in lysine decarboxylase broth, 7 were positive in Bachrach's broth, 8 in Johnson's semisolid medium, and five in the lysine-lactose-agar slants. When the results of lysine decarboxylase broth and Johnson's semisolid medium without lactose were compared, 8 of the 16 (50%) strains tested in the latter medium were in agreement with the same strains tested in the former medium. When the results of Bachrach's broth and lysine slants with lactose were compared, 5 of the 16 (31%) strains tested in the latter medium were in agreement with the same strains tested in the former medium. The results of the four lysine decarboxylase methods with the Proteus-Providence group were good except for two P. rettgeri strains which gave false-positives in the lysine-lactose-agar slants. The Salmonella test results with the four methods correlated very well. Two of the Citrobacter strains negative in lysine decarboxylase broth were not tested with the other test media. With the 20 remaining Citrobacter strains, six discrepancies were found between the results obtained in lysine decarboxylase broth and the test medium of Bachrach and Johnson, whereas five were found
between lysine carboxylase broth and the lysine-agar slants. These discrepancies represent six of the nine *Citrobacter* strains positive in lysine carboxylase broth. One of the six strains was positive in lysine carboxylase broth in 24 hr, five were positive only after 48 to 72 hr of incubation, whereas all six were negative in the media of Bachrach and Johnson in the prescribed incubation time of 24 hr. One of the latter strains produced a weak reaction in a lysine-lactose-agar slant in 24 hr.

Of 40 *Pseudomonas aeruginosa* strains tested, 7 failed to grow in the lysine media, whereas 31 of 33 strains produced no change in the indicator by all methods used. One of the two remaining strains changed the indicator to yellow (acid) only in Bachrach's broth, whereas the other strain produced an acid reaction in both lysine decarboxylase and Bachrach's broth media. In these two instances, it is probable that the rate of metabolism was increased to such a point that the acid formed changed the color of the media to yellow. Both of these strains produced acid in conventional glucose fermentation tubes. Both of the *Herellea* strains utilized glucose on O-F medium and in conventional broth but failed to use it in any of the lysine carboxylase media.

**DISCUSSION**

All four methods were reasonably reliable when dealing with nonlactose fermenters, whereas the lysine carboxylase broth method and the semisolild method of Johnson were better for all of the types of bacteria studied. A major explanation for the great difference in the test results with lactose fermenters such as *Escherichia* and *Klebsiella* has been the incorporation of lactose into the medium (Bachrach's broth and lysine-lactose-agar). The high acidity produced from lactic acid usually cannot be overcome by lysine carboxylase activity in this type of medium. Lysine-agar slants as originally designed by Edwards and Fife (3) are used mainly to separate *Salmonella* and *Arizona* strains from the *Citrobacter* group. The exclusion of lactose from lysine decarboxylase medium seems desirable to extend further the range of usefulness of this medium to other members of the enteric family. For example, this would in-

| Biochemical types              | No. of strains | Broth methods* | Agar methods* |
|--------------------------------|---------------|----------------|---------------|
|                                |               | Lysine decarboxylase | Bachrach | Johnson | Lysine-lactose slant |
| *Shigella*                     | 3             | 0/3 | 0/3 | 0/3 | 0/3 |
| *Alkalescens-Dispar*           | 2             | 2/2 | 0/2 | 0/2 | 2/2 |
| *Escherichia*                  | 80            | 77/80 | 1/80 | 73/80 | 2/80 |
| *Klebsiella*                   | 82            | 77/82 | 4/82 | 76/82 | 4/82 |
| *Enterobacter aerogenes*       | 9             | 7/9 | 2/6 | 2/6 | 2/6 |
| *E. cloacae*                   | 4             | 0/4 | 0/4 | 0/4 | 0/4 |
| *E. hafniae*                   | 16            | 14/16 | 7/16 | 8/16 | 5/16 |
| *Serratia*                     | 10            | 10/10 | 7/7 | 7/7 | 7/7 |
| *Proteus mirabilis*            | 37            | 0/37 | 0/37 | 0/37 | 0/37 |
| *P. rettgeri*                  | 17            | 0/17 | 0/17 | 0/17 | 0/17 |
| *P. morganii*                  | 6             | 0/6 | 0/6 | 0/6 | 0/6 |
| *P. vulgaris*                  | 2             | 0/2 | 0/2 | 0/2 | 0/2 |
| *Providence*                   | 5             | 0/5 | 0/5 | 0/5 | 0/5 |
| *Salmonella typhimurium*       | 2             | 2/2 | 2/2 | 2/2 | 2/2 |
| *S. paratyphi B*               | 1             | 1/1 | 1/1 | 1/1 | 1/1 |
| *S. typhi*                     | 3             | 3/3 | 3/3 | 3/3 | 3/3 |
| *S. gallinarum*                | 1             | 1/1 | 1/1 | 1/1 | 1/1 |
| *Salmonella group E*           | 1             | 1/1 | 1/1 | 1/1 | 1/1 |
| *Citrobacter*                  | 22            | 9/22 | 3/20 | 3/20 | 4/20 |
| *Arizona*                      | 2             | 2/2 | 2/2 | 2/2 | 2/2 |
| *Pseudomonas aeruginosa*       | 40            | 0/40 | 0/40 | 0/40 | 0/40 |
| *Herellea*                     | 2             | 0/2 | 0/2 | 0/2 | 0/2 |

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

b Includes intermediate and atypical strains.

**TABLE 1. Results comparing two broth and two agar methods in determining lysine carboxylase activity**
increase the positive results with lactose-positive *Arizona* strains. When the results of lysine decarboxylase broth and Johnson's semisolid medium without lactose were compared, the latter gave 6% fewer positives (Table 2). The poor results that were obtained with *E. aerogenes* strains in Johnson's semisolid medium could not be explained. The results with lysine decarboxylase broth and Johnson's semisolid medium were identical with *Klebsiella*, except for one strain which produced a positive reaction in lysine decarboxylase broth. False-positives produced by Voges-Proskauer-positive *Klebsiella* did not occur with our strains after 24 hr of incubation. The addition of agar to Moeller's basal medium did not seem to affect our results, even though the addition was suggested by Johnson et al. (6) to decrease the chances for false-positives among these strains. The difference in results between lysine decarboxylase broth and Johnson's medium with *Citrobacter* strains might be accounted for by the longer incubation period afforded the decarboxylase broth method. An advantage to Johnson's semisolid medium is that it does not require a paraffin oil seal added to the medium in the tube; however, a tightly secured screw cap tube works equally well. A 1% difference existed between the results obtained with Bachrach's broth and the lysine-agar slant with lactose (Table 3). Most lactose fermenters will give a negative reaction in lysine decarboxylase medium containing lactose even in the presence of enzyme activity.

The physical state of the media (broth versus agar) seems to have no effect on decarboxylase production. In the lysine decarboxylase media with bromocresol purple indicator, the color range extends from a deep purple to yellow to purple. At times, reading was found to be difficult with intermediate degrees of color change, especially with the *Proteus* group; similar color-changing problems were also encountered to some extent with our controls. Nonetheless, the controls were generally useful in reading and interpreting results.

Occasionally, the change from purple (alkaline) to yellow (acid) was not discernible. This is mainly due to the fact that *Enterobacteriaceae* fermented glucose during the overnight growth period, since these bacteria usually utilize glucose in the first 6 to 8 hr of incubation. On occasion, we have experienced negative reactions involving glucose in which the yellow (acid) color was absent or faint and the medium had a colorless appearance (reduction of the indicator). Sometimes this happened in the bottom two-thirds of the tube. In some cases, decoloration changed very little from 6 to 24 hr. This occurred mostly in Johnson's semisolid medium. An inexperienced individual could fail to recognize the first color change brought about by the utilization of glucose, especially when weak reactions occur. Determining the pH of the colorless medium with phHydron (Micro Essential Laboratory, Brooklyn, N.Y.) paper usually revealed a change to acid.

One of the discrepancies of Bachrach's broth with bromothymol indicator was that quite often the lysine decarboxylase-positive bacteria changed the color of the indicator from yellow to green rather than blue.

Another problem has been the shift in color from pale yellow to purple in the lysine-agar slants from 6 to 48 hr of incubation which may result in erroneous positives. Sometimes this was evident in the lysine-containing medium but not in the controls.

When tests depend on pH changes as a result.

### Table 2. Comparison of lysine decarboxylase test media without lactose

| Method                              | Results          | Positive* | Negative* | Per cent positive | Per cent negative |
|-------------------------------------|------------------|-----------|-----------|-------------------|-------------------|
| Lysine decarboxylase broth (24 hr to 4 days at 37 C)… | 206/347          | 141/347   | 59        | 41                |                   |
| Johnson's semisolid agar (24 hr at 37 C)… | 179/339          | 160/339   | 53        | 47                |                   |

* Data are recorded as the number of positive and negative tests/number of strains tested.

### Table 3. Comparison of lysine decarboxylase test media with lactose

| Method                              | Results          | Positive* | Negative* | Per cent positive | Per cent negative |
|-------------------------------------|------------------|-----------|-----------|-------------------|-------------------|
| Bachrach's lysine-lactose broth (16 to 24 hr at 37 C)… | 34/339           | 305/339   | 10        | 90                |                   |
| Lysine-lactose-agar slant (24 to 48 hr at 37 C)… | 38/339           | 301/339   | 11        | 89                |                   |

* Data are recorded as the number of positive and negative tests/number of strains tested.
of the activity of bacterial enzymes on a substrate, a high percentage of discrepancies occur. With any of the methods, a positive reaction usually is reliable although false-negatives can occur. A pH change was not seen in the lysine decarboxylase media with the two Herellea strains. The pH of the broth media did not fall appreciably over a 10-hr period. Repeated tests showed the concentration (milligrams/100 ml) of glucose in lysine decarboxylase broth before inoculation and during and after a 24-hr incubation period to be about the same. Several P. aeruginosa strains were checked in the same manner and gave similar results. The inability of P. aeruginosa or Herellea to utilize glucose under the anaerobic conditions of the medium is a feature which makes the lysine decarboxylase test an undesirable procedure for these organisms.

In conclusion, of the four test media used, the lysine-lactose-agar slants seemed to be the least desirable because of the more frequent occurrence of indistinct color reactions and shifts in color. A test method the results of which can be interpreted in 24 hr would be a more suitable one for diagnostic work.

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