Rapid Methods for Determining Decarboxylase Activity: Ornithine and Lysine Decarboxylases

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A rapid biochemical method for the determination of ornithine and lysine decarboxylase (EC 4.1.1.18) activity has been developed for use in the routine clinical laboratory. It is based on the detection of the amine end product produced in response to the single key amino acid added to a synthetic medium. A modified ninhydrin reagent is used to detect the amine after a chloroform extraction. This procedure can be used with a 1- to 4-hr incubation period (utilizing an initial concentrated inoculum) or with an overnight culture. Thus, measurements based on the alkalization of the medium after a lengthy incubation period are avoided. Optimal parameters for enzyme activity are discussed.

MATERIALS AND METHODS

Media. Several media were used in these experiments. The basal synthetic medium contained (per liter of distilled water): K2HPO4, 7 g; KH2PO4, 3 g; (NH4)2SO4, 1 g; NaCl, 2 g; glucose, 15 g; pyridoxal phosphate, 0.005 g; niacin, 0.01 g, and 10 ml of the mineral stock solution. This mineral stock solution contained (per liter of distilled water): MgSO4, 10 g; CaCl2, 1 g; and FeSO4, 0.5 g. L-Tyrosine (0.5 g/liter) and L-methionine (0.25 g/liter) were added to this medium later to standardize it for all three decarboxylase systems. The medium was usually sterilized by membrane filtration but could be autoclaved for 15 min at 121 C without the glucose (which was sterilized separately and later added aseptically to the cooled medium). The final pH and amino acid concentrations are discussed later in this paper.

Decarboxylase medium (Difco 0872), Falkow lysine decarboxylase medium (Difco 0215), and Carlquist lysine decarboxylase medium (Difco 0740) were all prepared and sterilized as directed by the manufacturer. Unless otherwise mentioned, 2 ml of media was dispensed in screw-cap test tubes (125 by 16 mm) for all experiments. Antibiotic Medium 3 (Difco 0243) was used for overnight cultures and for stock maintenance with 2.5% agar added to solidify the medium. All amino acids were purchased from Nutritional Biochemicals Corp. Only the t. forms were used.

Organisms. The organisms used in these studies were both clinical isolates and stock cultures, including several from the American Type Culture Collection (ATCC). All organisms were grown on slants of Antibiotic Medium 3 for 24 hr, refrigerated, and transferred to fresh slants every 14 days.

Preparation of the inoculum. Organisms were transferred from 24-hr slants to 250-ml Erlenmeyer flasks

One of the common tests used in differentiating the Enterobacteriaceae and other gram-negative organisms is based on their ability to decarboxylate ornithine, lysine, and arginine (3-5, 14, 15). Usually based on a change in the color of a pH-sensitive indicator (6, 8, 11, 12), the tests take from 1 to 4 days. If the organisms happen to attack the indicator (11) or the solution is not sharply acidic or alkaline, decarboxylase activity cannot be measured with any certainty. Gale (7) measured CO2 evolution manometrically, and Leclerc (9) used a CO2-phenolphthalein system in an automated procedure.

Several authors (1, 2, 11) devised a biochemical test for cadaverine and putrescine, the respective end products of lysine and ornithine decarboxylase activity. They extracted the amines with ninhydrin. Although Möllner (11) first suggested this procedure, he used a lengthy incubation period and a reaction procedure which involved boiling the chloroform extract with the ninhydrin reagent. No one has reported a similar method for arginine decarboxylase or adapted these procedures for routine laboratory tests. In this paper, we present a rapid method for the determination of ornithine and lysine decarboxylase based on the chloroform extraction procedure and describe some of the parameters for optimal activity. A companion paper, which immediately follows in this journal, discusses similar studies with the arginine decarboxylase (EC 4.1.1.19) and dihydrolase systems.

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containing 30 ml of medium and were grown overnight at either 27 or 37 °C on a gyratory New Brunswick controlled-environment incubator (model G-26) set at a speed of 200 rev/min. The cells were harvested and washed twice by centrifugation in sterile distilled water. After the last wash, the inoculum was divided and resuspended in 2 ml of experimental medium at approximately the original concentration.

**Incubation.** The tubes were incubated without shaking at 27 or 37 °C for the desired time intervals. When Møller’s procedure was followed, the tubes were incubated for 4 days under a layer of sterile mineral oil. No mineral oil was added to tubes of the synthetic medium.

**Ninhydrin reagent.** A 4% (w/v) stock solution of ninhydrin was prepared by dissolving the ninhydrin in concentrated dimethylsulfoxide (DMSO). Amber glass bottles were used for storage. This solution remained stable indefinitely in a refrigerator. The ninhydrin reagent consisted of 1.5 ml of ninhydrin stock solution, 1 ml of concentrated acetic acid, and enough chloroform to make a final volume of 100 ml. This was also refrigerated in an amber container and prepared on a bimonthly basis.

**Extraction and detection procedures.** After the desired incubation period, 1 ml of 40% KOH was added to the cultures in their test tubes, and a Vortex mixer was used to agitate the tubes. Then 2 ml of chloroform was added and the cultures were again agitated for 30 to 60 sec. The chloroform layer settled to the bottom of the tubes, and 0.5 ml of the ninhydrin reagent was pipetted directly into this layer. The presence of a blue-purple color within 2 min was recorded as a positive result.

**Chromatography.** Precast silica gel thin-layer chromatography plates (without fluorescent indicator) manufactured by E. Merck AG, Darmstadt, Germany (no. 5610-0050) were purchased from Brinkmann Instruments, Inc. They were preactivated by heating for 30 min at 100 to 110 °C and could be stored for several months in a desiccator chamber without further reactivation. The plates were spotted with 20 to 40 μl of the chloroform extracts from cultures as well as known solutions and mixtures of amino acids and amines. Ascending one-dimensional chromatograms were run with a solvent system of cresol-acetic acid-water (2:1:2). After drying at room temperature, the chromatograms were developed by spraying with a ninhydrin spray reagent and heating to 100 to 110 °C, just until the purple color appeared. The ninhydrin spray reagent was prepared as follows: 0.1 g of ninhydrin was dissolved in a few milliliters of 95% ethanol and brought to a final volume of 100 ml with chloroform. This was placed in a 125-ml chromatography sprayer (Scientific Products Co.).

**RESULTS AND DISCUSSION**

The presently used methods for determining ornithine and lysine decarboxylase activity take from 1 to 4 days and depend on a color change in the medium (6, 11). Several rapid methods which have been reported for lysine decarboxylase are not very suitable for routine laboratory procedures (1, 2, 8). No rapid tests for ornithine or arginine decarboxylase activity have been reported. We have developed a rapid method, easily adapted to the clinical microbiology laboratory, in which the biochemical determination for the presence of the amines can be made in the same test tubes initially used to culture the organisms. Decarboxylase activity can be determined either within 4 hr (by using a large inoculum) or after an overnight incubation period.

**Media.** The ornithine and lysine decarboxylase enzyme systems are inducible in microorganisms (7). Thus, no enzyme activity is found in the absence of the particular amino acid substrate. When we began these studies by using a large inoculum in a complex medium containing beef or yeast extract and peptone (such as Møller’s Carquist’s or Falkow’s) we observed several false-positive results, both in the basal medium and in the medium containing the amino acid. For example, *Escherichia coli* ATCC 11246 was negative on the synthetic medium with or without ornithine but was positive in the complex medium in both instances. Since this strain is lysine decarboxylase-positive, enough lysine was present in the complex medium to stimulate this enzyme and produce a positive result, even though cadaverine rather than putrescine was formed. Other authors have reported similar results (1, 2, 13). Perhaps this is the reason for many discrepancies noted in the literature.

Thus, we decided to use a synthetic medium in these studies so that amine production would be directly related to the single “parent” amino acid present in the medium and thus the results could be interpreted with greater confidence. The use of a washed culture which was initially grown on a complex medium for an inoculum gave the same results as were obtained with cells grown on the synthetic medium. This is of value in the diagnostic laboratory, where a culture is often initially isolated on a complex medium. In addition, the use of a large inoculum and a 4-hr incubation period gave positive results with some of the fastidious organisms, as well as with *Shigella* and *Proteus* which also grew slowly at first in the synthetic medium. This procedure provided enough cells for detectable enzyme induction in the absence of rapid growth. Sher and Mallette (13) indicated that enzyme formation could occur within 1 hr under these conditions in *E. coli*.

We have combined several features from previously published media for various decarboxylase systems (2, 7, 10). Gale (7), for example, stated that pyridoxine (the coenzyme for several decarboxylases) and niacin were necessary for
optimal activity. We found that niacin also promoted better growth of the Proteus strains but did not have much effect on enzyme activity under our conditions. Melnykovych and Snell (10) reported that several amino acids such as tyrosine and methionine stimulated arginine decarboxylase activity. The data reported in this paper (Tables 1–4) were obtained with organisms grown in the synthetic medium without tyrosine and methionine. To derive a single basal medium which could be used for all three decarboxylase systems, the effect of adding these two amino acids to the lysine and ornithine decarboxylase medium was investigated. No stimulatory or inhibitory effects were observed when several of the parameters such as pH or amino acid concentration were retested on the modified medium. Thus, a single medium was derived which could be used interchangeably for the three enzyme systems. The mineral salts in the synthetic medium were adapted from synthetic media used by several authors (2, 10, 13).

Effect of amino acid concentration. When cells were grown on the basal synthetic medium in the absence of ornithine or lysine, no amines were detected in the chloroform extracts. Several concentrations of lysine and ornithine were tested for optimal enzyme activity within a 4-hr period. Representative organisms were used for these studies. As can be seen in Table 1, 1.0% gave the best results for both enzyme systems and was slightly better than 0.5%. Activity appeared within 2 to 4 hr with the organisms containing the enzyme. A concentration of 5% gave the poorest results. These results were similar at different pH and temperature ranges. Therefore, an amino acid concentration of 1.0% was used for both enzyme systems in the remainder of these experiments.

**Effect of pH.** According to Gale (7), an acid growth medium stimulates decarboxylase activity and an alkaline medium stimulates deaminase activity. The initial pH of the complex media usually ranged from 6 to 7.5. Therefore, both an acid (5.5) and a neutral (7.0) pH were tested. The initial experiments were run at 37°C, but data for 27°C were also obtained. Table 2 shows that an initial pH of 7.0 was more effective in stimulating putrescine formation with most of the representative organisms screened for this purpose. In the case of lysine decarboxylase, however, poising the medium at pH 5.5 resulted in more rapid amine production. In fact, positive results were noted with most strains in about half the time required at the higher pH (Table 3). Positive results were routinely obtained within 2 hr at this pH.

**Effect of temperature.** Gale (7) reported an optimal temperature of 26°C for these enzyme systems, whereas others (2, 4, 9) used 37°C. We therefore used similar temperatures. Since an initial difference in the pH had been observed for these two decarboxylases, the temperature experiments were done at different pH levels as well, to detect the most favorable combination of temperature and pH. In general, the higher temperature of 37°C resulted in earlier amine production in both enzyme systems, regardless of initial pH of the medium. These data are presented in Table 2 for ornithine decarboxylase and in Table 3 for lysine decarboxylase. With most of the organisms tested, cadaverine was routinely detected by 1 hr at 37°C, whereas the average time for putrescine production at 37°C was 3 hr. At a temperature of 27°C, longer incubation periods were necessary for both enzymes. In fact, several strains of Salmonella were still negative for

### Table 1. Effect of amino acid concentration on decarboxylase activity*

| Organism                        | Amino acid concn |
|---------------------------------|------------------|
|                                 | 0.5%             |
|                                 | 1.0%             |
|                                 | 2.5%             |
|                                 | 5.0%             |
|                                 | O    | L    | O    | L    | O    | L    | O    | L    |
| Klebsiella pneumoniae           | 2    | 2    | 2    | 2    | 2    | 2    | 2    | 2    |
| Escherichia coli 11246 (ATCC)  | 2    | 2    | 2    | 2    | 2    | 2    | 2    | 2    |
| Salmonella typhimurium          | 2    | 2    | 2    | 2    | 2    | 2    | 2    | 2    |
| Pseudomonas maltophilia        | 2    | 2    | 2    | 2    | 2    | 2    | 2    | 2    |
| Serratia marcescens            | 2    | 2    | 2    | 2    | 2    | 2    | 2    | 2    |
|                                 | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |

* Results show the time required (hours) for a positive reaction; W = weak reaction followed by a stronger reaction within the next hour; — = negative at 24 hr (organism lacking the enzyme); + = positive at 24 hr. Overnight cultures grown in the basal synthetic medium were washed and resuspended in several concentrations of the amino acids (O = ornithine; L = lysine), which were added to the synthetic medium. Cultures were incubated 1 to 24 hr at 37°C at an initial pH of 7.0.
putrescine production at 4 hr, whereas duplicate cultures were positive much earlier at the higher temperature. This temperature effect was only observed with the rapid method involving 1- to 4-hr incubation periods, as overnight cultures were positive regardless of the incubation temperature.

**Extraction and detection methods.** Putrescine and cadaverine, the respective amine end products of ornithine and lysine decarboxylase, respectively, were soluble in chloroform, whereas the two amino acids were not. Taking advantage of this differential solubility, Møller (11) and others (1, 2) used a reaction of the chloroform extract with ninhydrin to detect the presence of the amine. All of these authors removed the chloroform layer from the reaction tubes before adding the ninhydrin. Møller, in fact, boiled his chloroform extract for 3 to 4 min. In an attempt to adapt these procedures for the routine laboratory, we have tried to limit the amount of glassware and manipulation involved in this procedure. The presence of DMSO in the ninhydrin reagent sharpened the sensitivity of the reaction and allowed the detection of the amine without removal of the chloroform layer from the tube. The ninhydrin reagent was merely pipetted into the chloroform layer at the bottom and was followed by a gentle agitation. The appearance of a blue-purple color was recorded as a positive result. After 0.5 to 1 hr, some mixing occurred, and the upper layers of the tube (containing the amino acids and cellular debris) also turned purple.

Thus, growth (either an overnight or a 1- to 4-hr culture), extraction, and detection of the amines can take place in the same tube. The alkali, chloroform, and ninhydrin reagent can be added automatically to the banks of test tubes with various commercial pipettors.

**Chromatography.** Ascending one-dimensional thin-layer chromatograms on silica gel plates were used to determine more accurately the presence and number of amines produced in the synthetic culture medium in response to the amino acid substrates. Known samples of cadaverine and putrescine were run as controls. The procedure is described in Materials and Methods.

These experiments provided definitive data which supported that observed in the culture tubes. No amines were detected in the chloroform extracts when organisms were grown in the basal

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**Table 2. Effect of pH and temperature on putrescine formation**

| Organism                  | Time (hr) for positive reaction* |
|---------------------------|----------------------------------|
|                           | 27 C | 37 C |
|                           | pH 5.5 | pH 7.0 | pH 5.5 | pH 7.0 |
| *S. marcescens*           | 3W   | 2W   | 3W   | 1W   |
| *Citrobacter* B090 (ATCC)| 4W   | 4W   | 3     | 2    |
| *Enterobacter* hafniae    | -    | 4W   | 4W   | 3W   |
| *E. cloacaee*             | -    | 4W   | 3W   | 2W   |
| *Shigella sonnei*         | 2    | 2W   | 2W   | 1W   |
| *Arizona*                 | 3W   | 4W   | 2W   | 2W   |
| *Proteus mirabilis*       | 4W   | 3W   | 3     |      |
| *P. vulgaris*             | -    | 4W   | 2W   | 3W   |
| *P. morganii*             | 3W   | 2    | 2W   | 1    |
| *Salmonella saphra*       | -    | 4    | 3W   | 3W   |
| *S. paratyphi*            | -    | 2W   | -    | 3    |
| *S. typhimurium*          | -    | 2W   | 4W   | 2    |
| *S. newport*              | -    | 2W   | -    | 1W   |
| *S. anatum*               | -    | -    | -    | 3    |
| *S. gaminara*             | -    | 3W   | 3    |      |
| *S. oranienburg*          | -    | 2W   | -    | 1W   |

* W = weak reaction followed by a strong reaction within the next hour; = negative within 4 hr but positive after overnight incubation. Overnight cultures grown in the basal synthetic medium were washed and resuspended in the synthetic medium plus 1% ornithine.

**Table 3. Effect of pH and temperature on cadaverine formation**

| Organism                       | Time (hr) for positive reaction* |
|--------------------------------|----------------------------------|
|                                | 27 C | 37 C |
|                                | pH 5.5 | pH 7.0 | pH 5.5 | pH 7.0 |
| *Klebsiella pneumoniae*        | 1    | 3W   | 1     | 3    |
| *S. marcescens*               | 1W   | 4W   | 1W    | 3W   |
| *Pseudomonas maltophilia*      | 1    | 3W   | 1     | 2W   |
| *Arizona*                     | 2W   | 4W   | 1W    |      |
| *Enterobacter hafniae*         | 1    | 2W   | 1     | 2W   |
| *Salmonella typhi*             | 1W   | 4W   | 1     | 3W   |
| *S. paratyphi*                 | 1    | 3W   | 1     | 3    |
| *S. typhimurium*               | 1    | 4W   | 1     | 3    |
| *S. gaminara*                  | 1    | 4W   | 1     | 2W   |
| *S. saphra*                    | 1W   | 4W   | 1     | 3    |
| *S. oranienburg*               | 1    | 3    | 1     | 2    |
| *S. anatum*                    | 1    | 3W   | 1     | 2W   |
| *S. newport*                   | 1    | 3    | 1     | 2W   |
| *Escherichia coli* K-12        | 3W   | 4W   | 1W    | 3W   |
| *E. coli* B                    | 2W   | 3    | 1W    | 3    |
| *E. coli* 11246 (ATCC)         | 2W   | 3W   | 1     | 2W   |
| *E. coli* clinical isolate      | 1W   | 3W   | 1     | 1    |

* W = weak reaction followed by a strong reaction within the next hour; = negative within 4 hr but positive after overnight incubation. Overnight cultures grown in the basal synthetic medium were washed and resuspended in the synthetic medium plus 1% lysine.
The addition of ornithine to the medium resulted in putrescine production in organisms containing the enzyme. Similarly, only cadaverine was produced when lysine decarboxylase-positive organisms were cultured on the synthetic medium containing lysine. No other amines or ninhydrin-reacting compounds were detected. A representative chromatogram of three organisms is shown in Fig. 1. As can be seen, a strain of *E. coli* which was lysine decarboxylase-positive and ornithine decarboxylase-negative produced only cadaverine in response to lysine, and no amines were produced on the medium containing ornithine. *Enterobacter cloacae*, ornithine decarboxylase-positive and lysine decarboxylase-negative, had a positive spot only for putrescine. *Arizona*, which had both decarboxylases, produced both amines. However, each was an individual response to the specific amino acid present in the medium. It is important to note here that, in every case, only the single related decarboxylase was induced by an amino acid, regardless of the genetic potential of the organisms. No other ninhydrin-positive compounds were found in the chloroform extracts of organisms grown on the amino acids.

**Comparison of procedures.** We have compared many other laboratory strains as well as several hundred clinical isolates with the standard methods and found a good correlation with results reported in the literature and with those obtained with Möller's medium when Möller's test itself is not ambiguous (decolorization of the indicator or uninterpretable changes compared with the controls). Some data obtained with Carliquist's medium varied with the *Salmonella* organisms, which were routinely positive on synthetic medium plus lysine (Table 4). As can be seen in Table 4, there were some differences when Fal-

![](image)

**Fig. 1. Thin-layer one-dimensional chromatogram of the chloroform extracts from various organisms grown in the synthetic medium containing either ornithine or lysine. The chromatogram was developed by spraying with a ninhydrin reagent; 4-hr cells were used. C = cadaverine; P = putrescine; L = lysine decarboxylase; O = ornithine decarboxylase; + = positive reaction; − = negative reaction.**

| Organism                  | Synthetic | Möller | Fal- | Literature |
|---------------------------|-----------|--------|-----|------------|
|                           | O L       | O L    | O L |            |
| *Escherichia coli* K-12   | − + − + +  | + + + ± | ±  | ±          |
| *E. coli* 11246 (ATCC)    | + − − + +  | − − − − | −  | −          |
| *Proteus vulgaris*        | + − − − −  | + − − − | −  | −          |
| *P. mirabilis* 9240 (ATCC)| + − − + +  | − − − − | +  | −          |
| *P. morganti*             | + − − − +  | − − − − | +  | −          |
| *Pseudomonas aeruginosa*  | + − − + +  | − − − − | +  | −          |
| *P. maltophilia*          | + − − + +  | − − − − | +  | −          |
| *Klebsiella pneumoniae*   | + − − − +  | − − − − | +  | −          |
| *Shigella sonnet* 9290    | + − − − +  | − − − − | +  | −          |
| *Citrobacter* 8090 (ATCC)| + − − − −  | − − − − | −  | −          |
| *Arizona*                 | + − − + +  | − − − − | −  | −          |
| *Salmonella typhi*        | + − − + +  | − − − − | −  | −          |
| *S. typhimurium*          | + − − + +  | − − − − | −  | −          |
| *S. paratyphi*            | + − − + +  | − − − − | −  | −          |
| *Serratia marcescens*     | + − − + +  | − − − − | −  | −          |
| *Enterobacter cloacae*    | + − − + +  | − − − − | −  | −          |
| *E. liquefaciens*         | + − − + +  | − − − − | −  | −          |
| *E. hafniae*              | + − − + +  | − − − − | −  | −          |

*S Symbols: + = positive for the decarboxylase; − = negative for the decarboxylase. Cultures were harvested and observed for amine production after 4 hr on the synthetic medium, 24 hr on Falkow's medium, and 4 days on Möller's medium. A 24 hr culture grown on the basal synthetic medium was used as an inoculum.*
kow's medium was used. Enterobacter and Pseudomonas aeruginosa were negative on our medium and on Møller's but positive on Falkow's. Further studies along these lines with a greater selection of organisms and strains are contemplated. When Møller's medium was similarly extracted with chloroform and the ninhydrin reagent was added, the tubes that were strongly positive after 4 days also gave a positive reaction. Results obtained from negative or uninterpretable Møller's tubes were variable in their response to the ninhydrin reagent. Some negative tubes which had changed toward the end of the 4-day period were negative for the amines whereas others were positive.

Thus, on the basis of the data presented in this paper, it would appear that a synthetic medium for determining decarboxylase activity which contains only the key amino acid can be used for rapidly and accurately assessing the biochemical potential of various members of the Enterobacteriaceae and related organisms.

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