# Use of Gas Chromatography for Detecting Ornithine and Lysine Decarboxylase Activity in Bacteria

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A gas-liquid chromatography (GLC) procedure for the detection of L-ornithine and L-lysine decarboxylase (EC 4.1.1.17 and EC 4.1.1.18, respectively) activities of bacteria was developed and evaluated against Møller's method, a conventional biochemical test. Cultures were incubated for 2 to 4 h in a simple growth medium and tested by GLC for putrescine and cadaverine, the direct decarboxylation products of ornithine and lysine, respectively. Results obtained with various Enterobacteriaceae, pseudomonads, and vibrios showed that the GLC procedure was superior to the conventional test; clear, well-defined results were obtained within 3 to 5 h, even with cultures which gave weak, delayed, or variable reactions by Møller's method. This GLC procedure for the determination of decarboxylase reactions would be useful in microbiological laboratories for culture identification and for various other enzymatic studies.

Ornithine decarboxylase (EC 4.1.1.17) and lysine decarboxylase (EC 4.1.1.18) reactions are used by many laboratories to aid in the identification and characterization of microorganisms. These reactions are important in differentiating many members of the Enterobacteriaceae (2, 3, 7). They are also useful in identifying species of Pseudomonas, Vibrio, Aeromonas, Pasteurella, and Yersinia (1, 3, 5, 16). Bacterial decarboxylase activity is usually determined by the appearance of a violet color in Møller decarboxylase medium (14). Positive reactions in this medium are dependent upon a color change of a pH-sensitive indicator and are often difficult to interpret. This is especially evident if the test organism does not grow readily in Møller broth or if it attacks the indicator (14). Even with extended incubation periods (5 to 7 days), the reactions may still be ambiguous.

Several attempts have been made to develop more rapid and sensitive tests for detecting ornithine and lysine decarboxylase activities in bacteria (4, 8, 9, 12, 13). Goldschmidt and her co-workers (12) tested basic chloroform extracts of bacterial cultures with ninhydrin for the presence of putrescine and cadaverine, the direct decarboxylation products of ornithine and lysine, respectively. In a more recent report, Fay and Barry (9) detected ornithine decarboxylase activity by incubating bacteria for 2 to 4 h and observing the color change of a pH indicator in the media. Results from these rapid tests correlate well with conventional tests. However, they often have limitations in their practicality or specificity. Therefore, a gas-liquid chromatography (GLC) procedure for detecting putrescine and cadaverine was developed and evaluated with 57 bacterial cultures.

## MATERIALS AND METHODS

The cultures used in this study were from the stock strains of the Center for Disease Control (CDC). Their identity was confirmed by established cultural and biochemical procedures (2, 3, 5-7). The cultures were grown on Trypticase soy agar (TSA, BBL) slants for 16 to 24 h at 37 C. Growth from the top half of the slants was used to inoculate all media. Each culture was tested for ornithine and lysine decarboxylase activity in Møller media, as described by Edwards and Ewing (7), and in the special medium used for the GLC studies.

The special medium contained 5 g of peptone (Difco) and 3 g of yeast extract (Difco) per liter. L-Ornithine and L-lysine (Sigma Chemical Co., St. Louis, Mo.) were added to 100-ml samples of the medium so that the final concentration of each amino acid was 0.25%, 0.5%, and 1%. Media containing only one amino acid (ornithine or lysine) and a control medium containing no added amino acids were also prepared. The pH of all media, after sterilization at 121 C for 15 min, was approximately 6.

One milliliter of each medium was dispensed asep-

tically to small screwcap test tubes and refrigerated until used. The tubes of media were allowed to equilibrate to room temperature just before inoculation with growth from the TSA slants. The turbid suspensions were mixed well and incubated at 37 C for 2 to 4 h. Each culture was then saturated with sodium chloride crystals, made basic with 2 N NaOH (pH 10), and extracted with 2 ml of chloroform. If necessary, the tubes were centrifuged briefly to break any emulsion which formed during extraction. The lower chloroform layer, which contained the amines, was transferred to a test tube and concentrated to 0.2 ml under a gentle stream of dry nitrogen. Then, 0.1 ml of heptafluorobutyric anhydride (HFBA, Pierce Chemical Co., Rockford, Ill.) was added to the chloroform extract. The tube was tightly sealed with a Teflon-lined cap, and the mixture was heated in a steam jacket for 5 min. The contents of the tube were cooled to room temperature and evaporated just to dryness under nitrogen. The N-heptafluoryl-butyl (N-HFB) derivatives of the amines were dissolved in 100 μliters of ethyl acetate, and 3 μliters were injected into the gas chromatograph.

Standards of putrescine (1,4-diaminobutane) and cadaverine (1,5-diaminopentane; Aldrich Chemical Co., Milwaukee, Wis.) were prepared in chloroform. Fifty microliters of a 1:200 dilution of each amine were derivatized, as previously described, and 1 μliter was injected into a gas chromatograph.

A Perkin-Elmer model 990 instrument (Perkin-Elmer, Norwalk, Conn.) equipped with flame ionization detectors and a disk integrator recorder was used. Samples were analyzed on two coiled-glass columns 3.66 m by 0.635 cm outer diameter. One column was packed with 3% OV-1 (methylsilicone polymer; Applied Science Laboratories, State College, Pa.) and the other was packed with 15% Dexsil 300GC (Analabs, New Haven, Conn.). Both liquid phases were coated on 80 to 100 mesh, acid-washed, dimethyl-chlorosilane-treated, high-performance Chromosorb W. For analysis of the samples, the OV-1 column was temperature programmed from 150 to 210 C at 6 C/min. The Dexsil column was temperature programmed from 170 to 230 C at 6 C/min. The injection port temperature was 240 C, and the detector temperature was 280 C. The carrier gas was prepurified N₂, with a flow rate of approximately 50 ml/min. The electrometer range was 10, with an attenuation of 32.

## RESULTS AND DISCUSSION

Chromatograms of three culture extracts which were analyzed by GLC for decarboxylation products of L-ornithine and L-lysine are shown in Fig. 1. Chromatogram A, obtained from a culture of *Pseudomonas multivorans* (*cepacia*), has relatively large peaks for putrescine and cadaverine which clearly show that this culture contains an active decarboxylase for both ornithine and lysine. Only lysine was decarboxylated by *P. maltophilia*, as shown by the presence of a single, large peak for cadaverine in chromatogram B. Chromatogram C ob-
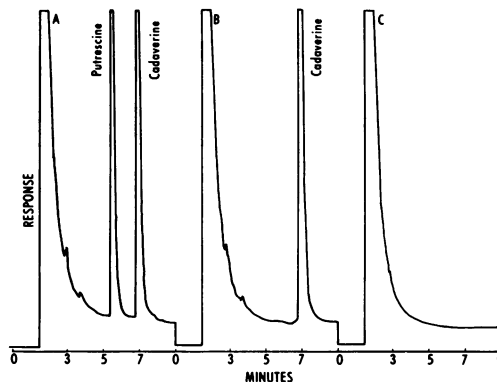


FIG. 1. *Gas chromatograms of the decarboxylation products of L-ornithine and L-lysine. The N-heptafluoryl-butyl derivatives of putrescine and cadaverine were run on a 3% OV-1 column. The cultures used were Pseudomonas multivorans (A), P. maltophilia (B), and P. aeruginosa (C).*

tained from *P. aeruginosa* contained no peaks, which indicated that this culture did not contain an active decarboxylase for either amino acid. The identities of putrescine and cadaverine were verified by comparing GLC retention times of known standards with bacterial decarboxylation products on both the OV-1 column and the Dexsil column.

A comparison of the decarboxylase activities of 57 cultures by the Møller method and by the GLC method is shown in Table 1. Results obtained by GLC agreed with those found by the conventional test. The distinct violet color, indicative of positive reactions in Møller media, was not readily apparent in some strains of *P. maltophilia* and *P. multivorans* (*cepacia*) even after 1 week of incubation in Møller broth. The color change in the test broth of these cultures was barely discernible when compared with color in the controls. In most cases, the results would have been recorded as questionable or negative by routine testing (5 days). However, positive decarboxylase reactions with these cultures were found by GLC after only 3 to 4 h of incubation. All decarboxylase-positive cultures produced chromatograms similar to those shown in A or B of Fig. 1. These data indicate that the GLC procedure is extremely valuable for reliable results even with slow or weakly positive cultures.

The results of the decarboxylase activities for the 57 cultures generally agreed with those reported in the literature (1-3, 5-7, 16). However, two strains of *Salmonella typhi* and one strain each of *P. maltophilia* and *Vibrio cholerae* failed to decarboxylate lysine. Eight of

TABLE 1. *Results of decarboxylase activities by the Møller method and by gas chromatography (GLC)*

| Organism | No. of strains | Ornithine + | | Lysine + | |
|---|---|---|---|---|---|
| | | Møller | GLC | Møller | GLC |
| *Pseudomonas maltophilia* | 6 | 0 | 0 | 5 | 5 |
| *Pseudomonas multivorans*[a] | 18 | 10 | 10 | 18 | 18 |
| *Pseudomonas*[b] species | 8 | 0 | 0 | 0 | 0 |
| *Escherichia coli* | 2 | 2 | 2 | 2 | 2 |
| *Salmonella typhi* | 8 | 0 | 0 | 6 | 6 |
| *Arizona hinshawii* | 1 | 1 | 1 | 1 | 1 |
| *Citrobacter freundii* | 1 | 0 | 0 | 0 | 0 |
| *Salmonella enteritidis* ser. anatum | 1 | 1 | 1 | 1 | 1 |
| *S. enteritidis* bioser. paratyphi A | 1 | 1 | 1 | 0 | 0 |
| *S. enteritidis* ser. typhimurium | 1 | 1 | 1 | 1 | 1 |
| *Vibrio cholerae* | 8 | 8 | 8 | 7 | 7 |
| *V. cholerae* biotype El Tor | 2 | 2 | 2 | 2 | 2 |

[a] Includes cultures known as *P. cepacia*, *P. kingii*, and EO-1.

[b] Includes *P. aeruginosa* (two strains), *P. putida* (two strains), and *P. stutzeri* (four strains).

the 18 cultures of *P. multivorans (cepacia)* failed to decarboxylate ornithine. All 18 cultures had been identified as *P. multivorans*, *P. cepacia*, *P. kingii*, and EO-1. Recent reports have suggested that these cultures are the same and should be classified as *P. capacia* (1, 17, 20). The production of ornithine decarboxylase has been reported as a variable biochemical characteristic of *P. cepacia* (6); the results of this study support this observation. Cultures which did not give expected decarboxylase reactions initially were retested by both methods. Even though larger inocula were used and the incubation times were extended, results were identical to those of the initial tests.

Decarboxylase-positive cultures were grown in media which contained each amino acid separately and in control media. GLC analysis of ornithine-positive cultures showed a single, large peak for putrescine only when ornithine was present in the medium. There was no evidence of putrescine when these cultures were grown in medium which contained only lysine or in control medium. GLC analysis of lysine-positive cultures showed a single, large peak for cadaverine when lysine was present in the medium. However, a small peak with a retention time identical to that of cadaverine was also present when the lysine-positive cultures were grown in medium containing only orni-

thine or in control medium. These data indicate that the medium contained a small amount of lysine which was decarboxylated to cadaverine. Thus, false-positive reactions can be recorded when ornithine-negative, lysine-positive cultures are tested by conventional procedures, because the color change can result from the production of cadaverine and not putrescine. The results with the GLC procedure were not affected by the presence of small amounts of lysine in the medium. These results further illustrate the need for adequate controls for each new or different lot of media when conventional procedures are used. Uninoculated media and media which were incubated with killed cells as controls were extracted and derivatized. Neither putrescine nor cadaverine was detected in the controls, and the chromatograms were similar to chromatogram C (Fig. 1).

Production of bacterial decarboxylases is quite dependent on environmental conditions, such as pH and temperature. Gale (10, 11), who has done extensive studies on the optimal conditions for decarboxylation in the *Enterobacteriaceae*, found that an acidic pH (below 5.5) and a temperature of 20 to 26 C was best for enzymatic activity. Several others (15, 18) have also concluded that an acidic pH is optimal for decarboxylation. Goldschmidt et al. (12) studied a wide variety of gram-negative rods and found that pH 5.5 enhanced cadaverine formation; however, they found that pH 7.0 enhanced putrescine formation. Both Goldschmidt et al. (12) and Møller (14) concluded that a temperature of 37 C was best for decarboxylation, rather than the lower temperature suggested by Gale (10, 11). The decarboxylase reactions in *Pseudomonas* species have not been studied as extensively as in the *Enterobacteriaceae*. Seaman (19), however, found that the enzymes of *Pseudomonas reptilivora* were most active at pH 5.8. The optimal conditions for decarboxylase activity apparently are different for each enzyme and perhaps also for each culture. Therefore, pH 6 and a temperature of 37 C were used throughout this study after preliminary tests with selected strains showed that these conditions were satisfactory for decarboxylase activity in both the *Enterobacteriaceae* and the pseudomonads.

The composition of the growth substrate reportedly can affect the enzymatic activity of microorganisms (10, 11, 15, 18). The peptone-yeast extract medium used in this study contains sufficient nutrients, vitamins, and cofactors for growth and metabolism of many different bacterial species. This medium also con-

tains pyridoxal phosphate which serves as the prosthetic group for decarboxylases (10, 15, 18). The components of the GLC medium are common, dehydrated media products which require minimal preparation. In contrast, synthetic medium, which has been used in some studies (12), is somewhat difficult and tedious to prepare and often does not contain all the factors necessary for the growth and metabolism of some microorganisms.

An additional factor influencing the enzyme activity could be the concentration of the amino acids in the medium. Goldschmidt et al. (12) found that a concentration of 1% was best for optimal enzyme activity when representative cultures were tested after growth in four concentrations of amino acids. Several cultures used in this study were incubated for 3 h in the GLC medium with 0.25, 0.5, and 1% ornithine and lysine concentrations. GLC analysis showed only minor differences in the relative amounts of putrescine and cadaverine. Therefore, all other determinations were done in media in which the final concentration of each amino acid was 0.25%.

The speed and sensitivity of the GLC procedure for detecting ornithine and lysine decarboxylase activity indicates that it is superior to the Møller method in several respects. Both decarboxylase reactions can be determined in one tube containing a small volume of a simple growth medium. The culture does not have to be overlaid with sterile mineral oil or paraffin. The GLC procedure also measures specific end products and gives clear well-defined results in 3 to 5 h for all cultures tested. After the cultures were extracted and derivatized, we were able to analyze five to six samples per hour. Because numerous cultures can be incubated and then extracted simultaneously, the number of samples processed each day would be dependent upon the length of time for GLC analysis. With this procedure, we were able to detect 8 to 10 μg putrescine and 6 to 8 μg of cadaverine. If desired, the sensitivity of the GLC procedure could be increased to detect nanogram or picogram amounts of the amines by adjusting the sensitivity setting of the gas chromatograph or by using an instrument equipped with an electron capture detector. In contrast, reactions in Møller media are much less sensitive, are nonspecific, and are often questionable even after 4 to 7 days of incubation.

Much of the information about the activity of ornithine, lysine, and other bacterial decarboxylases has been obtained by manometric techniques (11, 15, 18). This technique provides useful and precise information, but it is time consuming. Precautions must be taken to insure

that the evolved $CO_2$ is measured properly. Measurement of specific end products of decarboxylation rather than evolved $CO_2$ could be preferable in some enzyme studies. In these instances, the GLC technique described would be a preferred and excellent analytical tool.

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