Decarboxylase Broth Protocol

Created: Wednesday, 02 September 2015

Author

- Archana Lal
- Naowarat Cheeptham

Information

HISTORY

The decarboxylase media, used to identify bacteria's ability to decarboxylate amino acids, were first introduced by Moeller (12) for detecting lysine and ornithine decarboxylase and arginine dihydrolase among bacteria belonging to *Enterobacteriaceae*. The Moeller's basal media contained peptone, beef extract, bromocresol purple, cresol red, pyridoxal, and glucose. To test decarboxylase activity, 1% of the appropriate amino acid was added. The medium was poured in narrow tubes as a column of about 2 cm in height and autoclaved, after which a layer of about 5 mm sterile paraffin oil was poured in each tube. The decarboxylase activity was measured on the basis of a pH rise of the amino acid reagent that was made visible by an indicator. After incubation the color changes in the tubes were followed for up to 10 days (13).

Utilizing the lysine decarboxylase reaction, Calquist developed a medium to differentiate *Salmonella arizonae* from *Citrobacter* (4). Falkow developed a lysine decarboxylase medium for the identification and differentiation of *Salmonella* and *Shigella* (7). Falkow's medium was further extended to examine ornithine and arginine decarboxylase reactions (5).

Gilardi suggested that these tests may also be used in identification of various non-fermentative Gram-negative bacilli (10 & 11). All of the above methods for detecting ornithine decarboxylase activity required up to 4 days of incubation.

Fay & Barry (1972) modified the conventional Moeller decarboxylase medium by removing glucose, decreasing the pH to 5.5, and decreasing the test volume to 1 ml. They used this modified ornithine decarboxylase medium and obtained highly reliable results in only 2-4 hours of incubation at 35 – 37^{o}C (8) as compared with the 4-day incubation time required by Moeller (13). This rapid ornithine decarboxylase test differentiated *Klebsiella* from the *Enterobacter-Serratia* group of organisms (*K. pneumoniae* cells are non-motile and ornithine decarboxylase negative, *Enterobacter* species are motile and usually ornithine decarboxylase positive except for *Enterobacter agglomerans* (6)). It was also helpful in the separation of *Proteus* species (About 93% of *P. mirabilis* and 100% of *P. morganii* strains are ornithine decarboxylase positive, whereas *P.*

vulgaris and *P. rettgeri* are ornithine decarboxylase negative (6)).
With slight modification of Fay & Barry's medium, Brooker et al. detected lysine decarboxylase in various members of *Enterobacteriaceae* (2).
Similar to Fay & Barry's ornithine decarboxylase media, Brooks & Sodeman (1974) published
a technique to rapidly assay for lysine and arginine decarboxylase and arginine dihydrolase (3). They compared the rapid test of Fay & Barry with the Moeller method to test several species of the*Enterobacteriaceae* and Gram-negative, non-fermenting rod-shaped bacteria and found close agreement between the two methods.
Oberhofer et al. evaluated the rapid decarboxylase and dihydrolase test and reported them to be accurate, extremely sensitive, and specific for the differentiation of a wide range of non-fermentative bacteria (15).

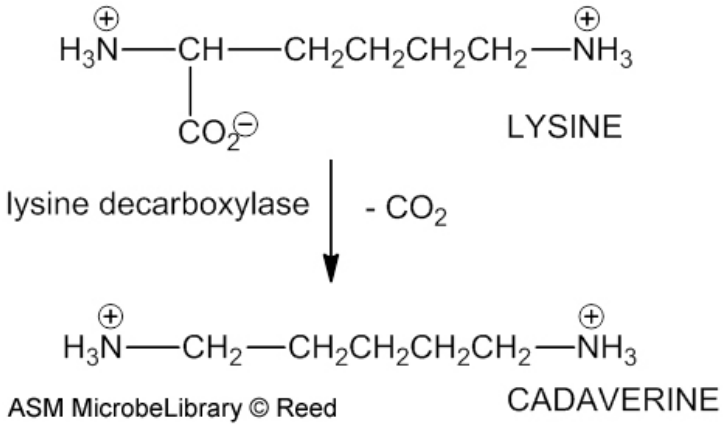
THEORY

The decarboxylase test has long been used to determine decarboxylase production and amino acid decarboxylation in bacteria particularly in the family of *Enterobacteriaceae* and *Aeromonas* species (1 & 3). This simple test is able to measure the ability of bacteria to produce decarboxylase or dihydrolase that are used to remove carboxyl group from amino acids resulting in the formation of amines. Decarboxylases remove the carboxyl group from amino acids like lysine and ornithine while arginine decarboxylation occurs with the help of dihydrolase in addition to decarboxylase (Figs 1, 2, and 3).

Decarboxylase medium contains beef extract and peptic digests of animal tissues that provide nitrogenous nutrients for the growth of the organisms. Glucose in the medium is a fermentable carbohydrate. Bromocresol purple and cresol red are the pH indicators that change colors under acidic and alkaline conditions and help in the detection of glucose fermentation and amino acid decarboxylation. Pyridoxal is the co-factor for the decarboxylase (9). Decarboxylation and dihydrolation are anaerobic reactions therefore a layer of sterile mineral oil is overlaid in each tube to separate the medium from external oxygen and promote fermentation.
Upon inoculation of medium with a bacterium capable of fermenting glucose, acid is produced resulting in a decrease in the pH and the pH indicator changes the color of the medium to yellow. The production of acid also stimulates the decarboxylase. Decarboxylase decarboxylates the added amino acid resulting in the formation of amines. Amine formation results in the increase of the pH of media causing the pH indicator to change color from yellow to purple (1).

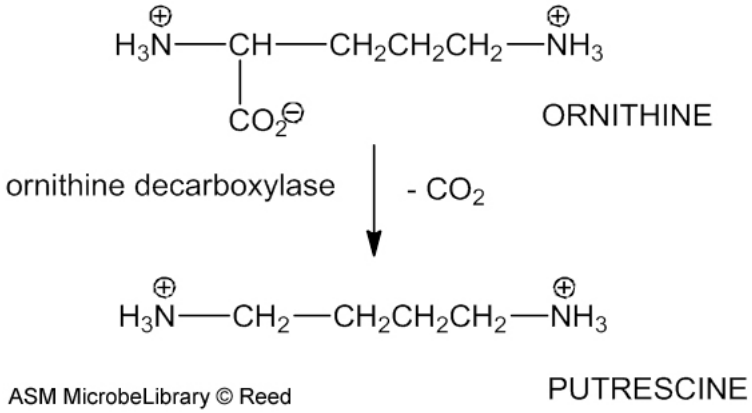
If the test organism ferments glucose but does not produce decarboxylase, the medium remains acidic or yellow in color.
Decaboxylation of lysine, ornithine, and arginine occur as follows:
1. The carboxyl group in lysine is removed by lysine decarboxylase yielding cadaverine (Fig.1)

FIG 1 Lysine Decarboxylation



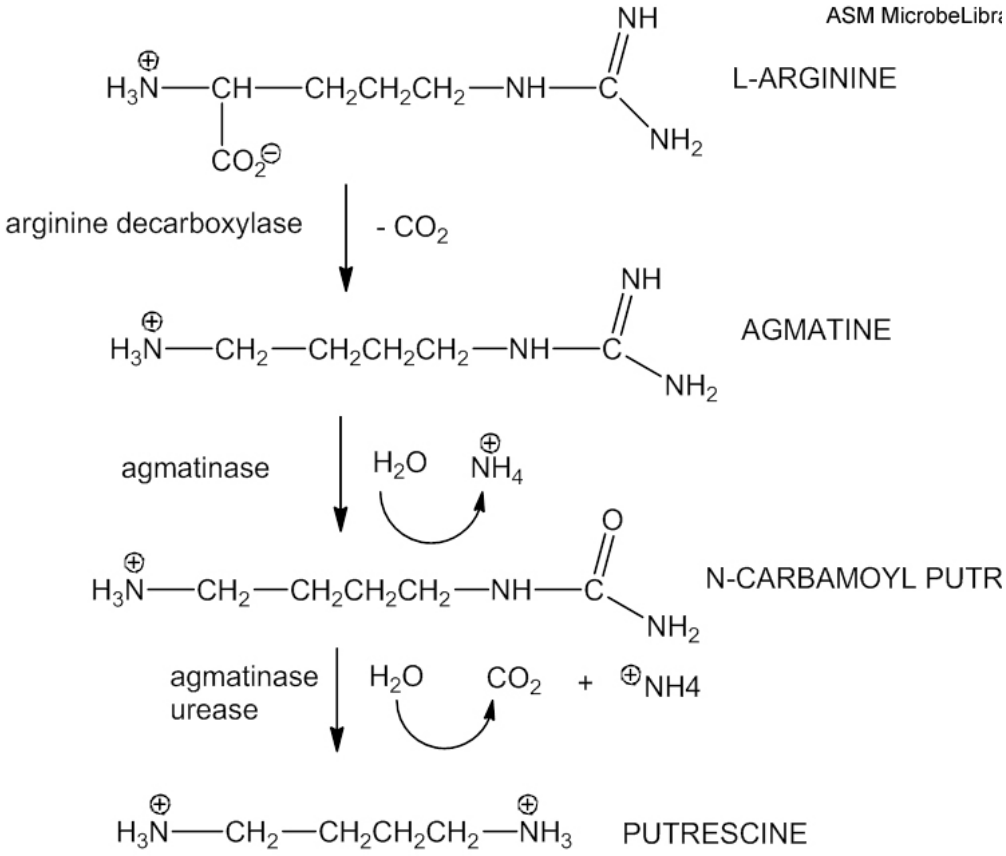
2. Ornithine is decarboxylated by ornithine decarboxylase giving rise to putrescine (Fig.2).

FIG 2 Ornithine Decarboxylation



3. Arginine decarboxylation requires a combination of decarboxylase and dihydrolase to achieve the complete decarboxylation of arginine to putrescine. Arginine decarboxylase decarboxylates the amino acid arginine into agmatine (Fig. 3). Some members of *Enterobacteriaceae* are capable of degrading agmatine into putrescine and urea by agmatinase. Bacterial strains with urease can further breakdown urea into ammonia and carbon dioxide.

FIG 3 Arginine Decarboxylation and Deamination



RECIPE

Moeller's Decarboxylase Broth Composition (g/liter) (14)

Base broth	
Peptone	5 g
Beef Extract	5 g
Glucose	0.5 g
Bromocresol Purple	0.01 g
Cresol Red	0.005 g
Pyridoxal	0.005 g
Distilled water	1 L

To prepare different decarboxylase broths with one of the following three L-amino acid (14)

Arginine	10 g (1%)
Lysine	20 g (2%)
Ornithine	10 g (1%)

Sterilized Mineral Oil for overlaying the inoculated broth 200 ml

First prepare the base broth by suspending the first six ingredients in 1 liter of distilled water. Heat the solution gently until all the ingredients are dissolved completely. Next, add either L-arginine, or L-lysine, or L-ornithine to the base broth to prepare different decarboxylase broths. Mix and heat with frequent agitation and carefully bring to

boil. When L- ornithine is added, re-adjust the pH. Final pH of the medium should be 6.0 ± 0.2 at 25°C.

Dispense in 5 ml quantities in screw-cap tubes (12.5 cm x 1.5 cm tubes for a column height of about 3.5 cm).

Autoclave the medium at 121°C for 15 min at 15 psi.
Prepared broth is light brown in color. It should be stored below 8°C away from the direct light.

Decarboxylase broth is also commercially available as pre-mixed dehydrated powder from biological supply companies. The manufacturer's instructions should be followed to prepare the broth. It can also be purchased as pre-made broth from biological supply companies. Sterilized mineral oil can also be purchased from biological supply companies. (Please see comments and tips)

PROTOCOL

Inoculation:

Use a fresh (18- to 24-hour) pure culture of test bacteria as an inoculation source. Use a single isolated colony as inoculum and aseptically transfer it to the decarboxylase broth tube with one of the amino acid. Inoculate a control tube of decarboxylase broth base (without amino acid) in parallel with the amino acid containing decarboxylase broth (as a control).
After inoculation, overlay 1 ml of sterile mineral oil to each tube to separate the medium from external oxygen and promote fermentation of glucose and decarboxylation reactions and tighten the caps on the test tubes.

Incubation:

Incubate tubes at 37°C in an incubator. Check the tubes every day for color changes for up to 4 days. Final readings should be made after 4 days of inoculation. For some microorganisms, increased incubation for up to 10 days may be required.

Decarboxylase Test Interpretations:

Check the color of the medium (every day for up to 10 days) for signs of fermentation and decarboxylation.

- Uninoculated Moeller's base media and media with an amino acid are light brown in color.
- If there is no change in the color of the medium it indicates that the organism does not ferment glucose and is decarboxylase negative (-) for that amino acid.
- If the medium changes to yellow color (but remains yellow and does not change to purple) it indicates that the organism ferments glucose and acidic by products are formed but the organism is decarboxylase negative (-) for that amino acid. Appearance of yellow broth is an indication of fermentation of glucose but not an indication of decarboxylation.

- If the color of the media changes to purple it indicates decarboxylation of amino acid and formation of amine (alkaline by-products) by the test organism meaning that the organism is decarboxylase positive (+) for that amino acid. The lack of a purple color indicates that amino acid has not been decarboxylated and the organism did not produce the decarboxylase enzyme.

Table 1: Summary of result interpretations

Media color	Bacterial reaction
No Change (Media remains light brown)	Decarboxylase negative (-)
Changes to yellow but does not change to purple	Decarboxylase negative (-)
Changes to purple	Decarboxylase positive (+)

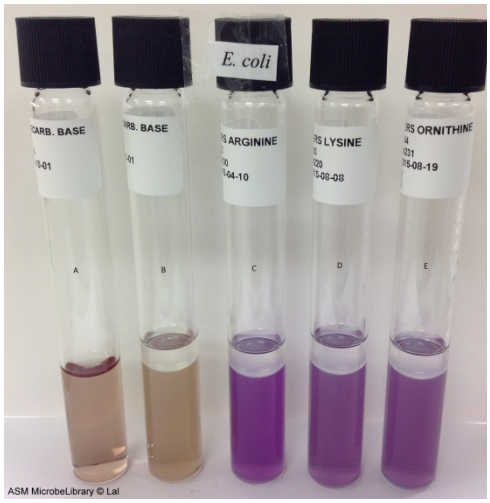


FIG 4 Moeller's Decarboxylase media. A: Uninoculated base, B: Base inoculated with *E. coli*, C: Arginine broth inoculated with *E. coli*, D: Lysine broth inoculated with *E. coli*, E: Ornithine broth inoculated with *E. coli*. Purple color in tubes C, D, and E indicate that this strain of *E. coli* is arginine, lysine, and ornithine decarboxylase positive.

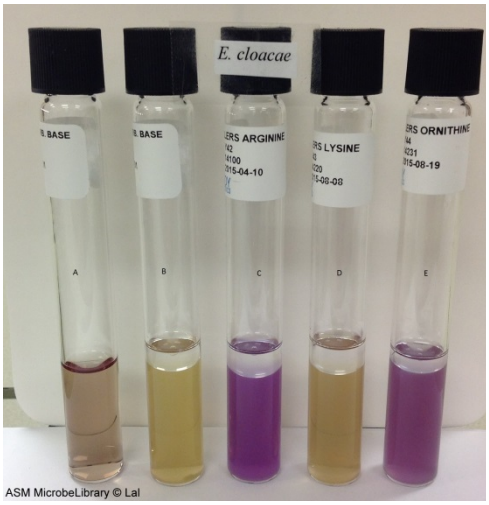


FIG 5 Moeller's Decarboxylase media. A: Uninoculated base, B: Base inoculated with*Enterobacter cloacae*, C: Arginine broth inoculated with *E. cloacae*, D: Lysine broth inoculated with *E. cloacae*, E: Ornithine broth inoculated with *E. cloacae*. Purple color in tubes C and E indicate that *E. cloacae* is arginine and ornithine decarboxylase positive

while yellow color in tube D (absence of purple color) indicates that it is lysine decarboxylase negative.

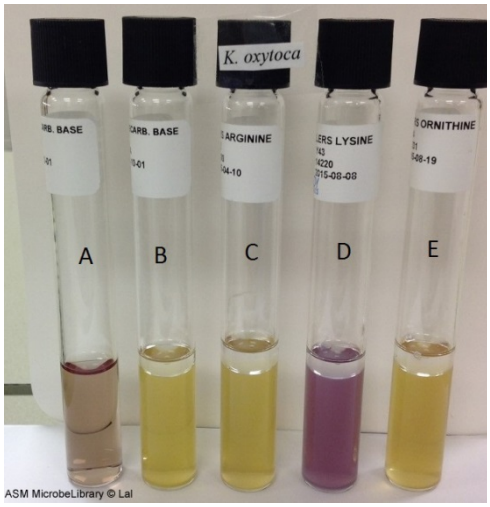


FIG 6 Moeller's Decarboxylase media. A: Uninoculated base, B: Base inoculated with*Klebsiella oxytoca,* C: Arginine broth inoculated with *K. oxytoca*, D: Lysine broth inoculated with *K. oxytoca*, E: Ornithine broth inoculated with *K. oxytoca*. Purple color in tube D indicates that *K. oxytoca* is lysine decarboxylase positive while yellow color in tubes C and E (absence of purple color) indicates that it is arginine and ornithine decarboxylase negative.

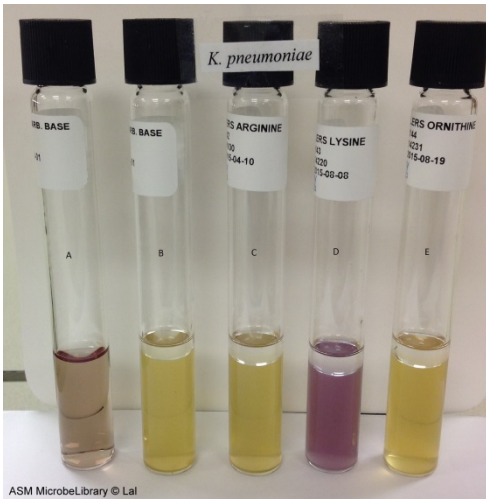


FIG 7 Moeller's Decarboxylase media. A: Uninoculated base, B: Base inoculated with*Klebsiella pneumoniae*, C: Arginine broth inoculated with *K. pneumoniae*, D: Lysine broth inoculated with *K. pneumoniae*, E: Ornithine broth inoculated with *K. pneumoniae*. Purple color in tube D indicates that *K. pneumoniae* is lysine decarboxylase positive while yellow color in tubes C and E (absence of purple color) indicates that it is arginine and ornithine decarboxylase negative.

Table 2: Summary of the results of the bacteria strain used in this protocol.

		Incubation time	Arginine decarboxylase	Lysine decarboxylase	Ornithine decarboxylase
1.	*Escherichia coli*	2 days	**+**	**+**	**+**
2.	*Enterobacter cloacae*	1 day	**+**	**-**	**+**

3.	*Klebsiella pneumoniae*	1 day	**-**	**+**	**-**
4.	*Klebsiella oxytoca*	1 day	**-**	**+**	**-**

ACKNOWLEDGEMENTS

Thanks to Dr. Norman Reed, Associate Professor of Organic Chemistry at Thompson Rivers University, for his time, knowledge, and assistance on making diagrams of the chemical reactions (Figures 1, 2, and 3) used in this article.

SAFETY

The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the ASM Curriculum Recommendations: Introductory Course in Microbiology and the Guidelines for Biosafety in Teaching Laboratories.

COMMENTS AND TIPS

1. Each isolate to be tested should be inoculated into a basal medium tube without the amino acid as a growth control and negative reference.
2. Inoculated broth should be protected from air by adding a layer of sterile mineral oil before incubation. Exposure to air may cause alkalization of the surface of the medium giving false positive.
3. The broths containing different amino acids look the same. Label them carefully as to which amino acid is present in which tube.
4. Test interpretation should only be made after at least 24 hours of incubation.
5. For some microorganisms, increased incubation of up to 10 days may be required.
6. Autoclaving mineral oil is not recommended as it is problematic due to incompletion of sterility. Sometimes water/condensation is introduced and the steam does not penetrate well, so sterility is rarely achieved. Many recommend dry oven sterilization of small aliquots or filtration of warmed mineral oil, but not autoclaving. Additionally, there are some reports of the steam/oil mixtures exploding.
7. There are two media that have been used in differentiating certain members of the*Enterobacteriaceae*. To differentiate *Salmonella* species, LIA (Lysine Iron Agar) is suggested, LIA distinguishes *Salmonella* by detecting hydrogen sulfide production and the decarboxylation or deamination of lysine. MIO (Motility, Indole Ornithine) medium has been recommended for use to test motility, indole production, and ornithine-decarboxylase activity of enteric bacilli.

3.

REFERENCES

1. **Atlas RM**. 1946. *Handbook of microbiological media for the examination of food.* pp 113
2. **Brooker DC, Lund ME, Blazevic DJ**. 1973. Rapid test for lysine decarboxylase activity in Enterobacteriaceae. *Appl. Microbiol.* 26: 622 - 623.
3. **Brooks K, Sodeman T**. 1974. A rapid method for determining decarboxylase and dihydrolase activity. *J Clin. Path.* 27: 148 - 152.
4. **Calquist PR. 1956**. A biochemical test for separating paracolon groups. *J. Bact.* 71: 339 - 341.
5. **Edwards D and Ewing. Pub. Health Lab**, 1960, 18: 77.
6. **Ewing WH**. 1968. *Differentiation of Enterobacteriaceae by biochemical reactions.* National Center for Disease Control, Atlanta, GA: US Department of Health, Education, and Welfare.
7. **Falkow S**. 1958. Activity of lysine decarboxylase as an aid in the identification of Salmonellae and Shigellae. *Am. J. Clin. Pathol.* 29: 598 - 600.
8. **Fay GD, Barry AL**. 1972. Rapid ornithine decarboxylase test for the identification of Enterobacteriaceae. *Appl. Microbiol* 23: 710 - 713.
9. **Fluka Analytical D2935 Decarboxylase broth base,** Moeller. www.sigma-aldrich.com
10. **Gilardi GL**. 1971. Characterization of Pseudomonas species isolated from clinical specimens. *Appl. Microbiol* 21: 414 - 419.
11. **Gilardi GL**. 1972. Practical scheme for the identification of non-fermentative gram negative bacteria encountered in medical bacteriology. *Amer. J. Med. Technol.* 38: 65 -72.
12. **Moeller V**. 1954. Activity determination of amino acid decarboxylases in Enterobacteriaceae. *Acta Pathologica et microbiologica Scandinavica* 34: 102 - 114.
13. **Moeller V**. 1955. Simplified tests for some amino acid decarboxylases and the arginine dihydrolase system. *Acta Pathol. Microbiol. Scand.* 36: 158 - 172.
14. **Moellers Decarboxylase Media – for identification of gram negative enteric bacteria**. Hardy Diagnostics. https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/MoellersDecarboxMed.htm. Accessed on Sept. 26, 2014
15. **Oberhofer TR, Rowen JW, Higbee JW, Johns RW**. 1976. Evaluation of the rapid decarboxylase and dihydrolase test for the differentiation of nonfermentative bacteria. *J. of Clin. Microbio.* 3: 137 - 142.

REVIEWERS

This resource was peer-reviewed. Participating reviewers:

Rebecca Buxton
University of Utah, Salt Lake City, Utah

Laura Cathcart
University of Maryland, College Park, MD

Brian M. Forster

Saint Joseph's University, Philadelphia, PA

Min-Ken Liao
Furman University, Greenville, SC

Patricia Shields
University of Maryland, College Park, MD

Erica Suchman
Colorado State University, Ft. Collins, CO