DECARBOXYLATION TEST

Principle and Purpose

Bacteria possess a variety of enzymatic processes to metabolize different compounds. Among these compounds are amino acids which, when metabolized, are converted to other substances including precursor molecules and different amino acids. Such reactions include the removal of carboxyl or amino groups from amino acids. These metabolic processes can be used to help identify different species within the enteric bacilli (Enterobacteriaceae) and other bacteria.

Decarboxylases and dihydrolases are a group of substrate-specific enzymes that respectively remove the carboxyl (COOH) or amino (NH_2) portions of amino acids. The production of many of these enzymes is induced only in an acidic environment. However, the metabolic reactions of these enzymes result in the formation alkaline-reacting amines and carbon dioxide (Fig. 1). The result is that the pH of the local environment increases. This property is exploited in the exercise described below in determining if a microbe possesses decarboxylase or dihydrolase activity.

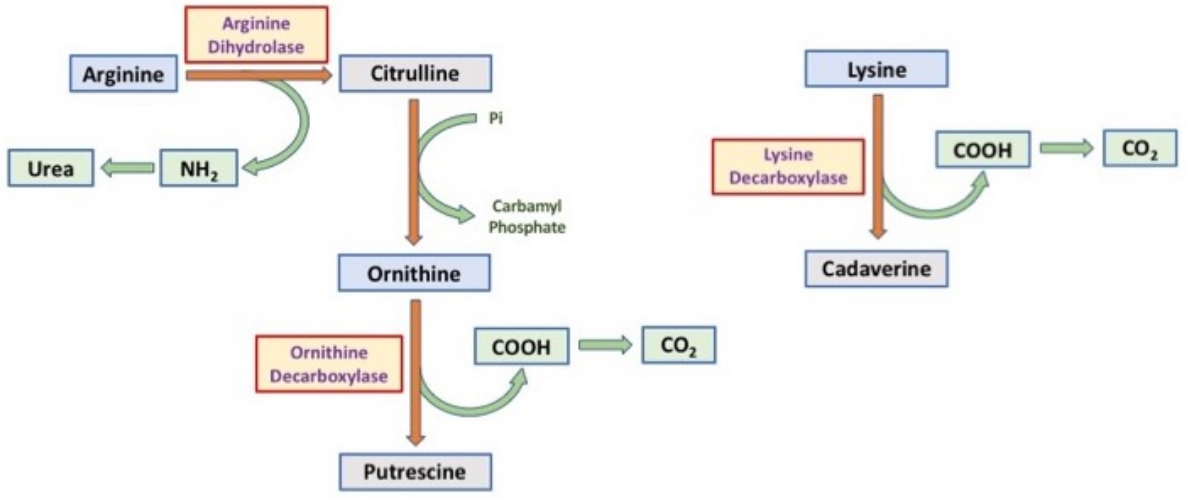

Figure 1. Overview of Decarboxylation Reactions of Arginine, Ornithine, and Lysine.

Moeller Decarboxylase Media is used in this exercise. Among other ingredients, the basal media contains pyridoxal (which is an essential co-factor for decarboxylase activity), glucose, and the pH indicators bromcresol purple and cresol red. Arginine, lysine, or ornithine are added to the basal medium to detect the production of specific amino acid decarboxylase and dihydrolase enzymes. The alkaline nature of the medium causes the pH indicators to appear purple in color.

When the medium is inoculated, an overlay of sterile mineral oil is added to each tube and the lids of the tubes should be tightened. These actions promote fermentation by locking out oxygen and helps prevent false alkalinization at the surface of the medium. If glucose is fermented by the microbe, an acidic state shall develop. This change in pH results in a color shift from purple to yellow. If the microbe is genetically predisposed, the acidic environment induces expression of the specific decarboxylase and dihydrolase enzymes for the amino acid in the medium (either arginine, lysine, or ornithine). These enzymes then degrade the amino acid to yield various amine by-products as well as carbon dioxide (Fig. 1). The formation of these reaction products helps to re-establish an alkaline environment. As such, this increased pH results in a color shift from yellow back to a purple or gray-purple color (Fig. 2). If the glucose-fermenting microbe

does not produce the appropriate enzyme, then the medium will remain yellow in color (Fig. 2). In contrast, non-glucose-fermenters that possess decarboxylase and dihydrolase enzymes may display weak decarboxylase activity to produce little or no color change as compared to an uninoculated tube of basal medium. Moreover, non-glucose-fermenters that do not possess the genes for decarboxylase and dihydrolase enzymes should remain nearly the same color as the basal medium, but results from such microbes may be unreliable.

In the following exercise, students will examine the decarboxylation reactions of different microbes using media containing either arginine, lysine, or ornithine. To obtain valid results, students need to note the following: 1) reaction tube lids need to be tightened; 2) a mineral oil overlay is essential; and 3) tests must be read no earlier than 18 hours after inoculation, but negative tests should be observed for changes occurring up to 7 days later.

Figure 2. Decarboxylase Reactions Using Moellers Decarboxylae Medium. The left image depicts the results of decarboxylase media inoculated with *Enterobacter aerogenes*. The left tube is basal medium (no amino acids), whereas the remaining tubes contain lysine, arginine, and ornithine, respectively. Note that the basal medium indicates that *E. aerogenes* ferments glucose. Also, this microbe possesses lysine and ornithine decarboxylases, but no reaction occurs in the presence of arginine. The right image is uninoculated media.

Learning Objectives

Upon completion of this exercise, a student should be able to:

- Understand the biochemical basis of the decarboxylation reaction;
- Properly perform the decarboxylase test;
- Analyze data to determine the decarboxylation potential of a given microbe; and
- Discern how this information can be used to differentiate and identify microbial species.

Materials Required

The following materials are necessary to successfully conduct this exercise:

Organisms – these bacterial cultures should be 18-24 hours old

- TSA slant culture of *Citrobacter fruendii* (ATCC 8090) [abbreviated as *C. fruendii*]
- TSA slant culture of *Proteus hauseri* (ATCC 13315) [abbreviated as *P. hauseri*]
- TSA slant culture of *Enterobacter aerogenes* (ATCC 13048) [abbreviated as *Ent. aerogenes*]
- TSA slant culture of *Klebsiella pneumoniae* (ATCC 13883) [abbreviated as *K. pneumoniae*]

Materials

- Moellers Decarboxylase Base (Cat. No. Y41; Hardy Diagnostics, Santa Maria, CA)*
- Moellers Arginine Decarboxylase (Cat. No. Y42; Hardy Diagnostics, Santa Maria, CA)*
- Moellers Lysine Decarboxylase (Cat. No. Y43; Hardy Diagnostics, Santa Maria, CA)*
- Moellers Ornithine Decarboxylase (Cat. No. Y44; Hardy Diagnostics, Santa Maria, CA)*
- Mineral oil
- Sterile, plastic bulb pipets

*Information regarding the various forms of Moellers Decarboxylase media is described at https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/MoellersDecarboxMed.htm.

Procedures

Note: To conserve resources, the laboratory instructor may assign different groups one or more specific microbes to use in this exercise. If this is implemented, then the data obtained by all groups should be shared with all students in the laboratory.

1) For each organism to be inoculated, obtain one tube each of arginine decarboxylase broth, lysine decarboxylase broth, and ornithine decarboxylase broth. Using a Sharpie marker (NOT tape), label each tube with the microbe to be used and other relevant information.
2) For each organism to be inoculated, obtain one tube of basal medium (Moellers Decarboxylase Base). Using a Sharpie marker (NOT tape), label this tube with the microbe to be used and other relevant information.
3) Obtain a second tube of basal medium (Moellers Decarboxylase Base). Using a Sharpie marker (NOT tape), label this second tube basal medium as "Control" and set it aside. The "Control" tube shall NOT be inoculated.

Note: Regardless of the number of organisms being tested by a student group, only one "Control" tube is needed. This tube shall be used for comparing all color reactions of the other inoculated tubes (see step 7). The use of multiple "Control" tubes wastes resources.

4) Using a microbiological loop, use aseptic technique to inoculate each tube of broth (except the "Control") with the appropriate microbe (preferably from an 18-24-hour culture).
5) Use a sterile plastic bulb pipet to carefully overlay each inoculated tube with 1 ml of sterile mineral oil.

Note: Be careful not to contaminate the pipet with media containing any microbes. If this occurs, discard the contaminated pipet and use a new one.

6) Snugly tighten the caps (do not leave them loose) on the inoculated tubes and incubate them at 37°C for 18-96 hours.
7) Remove the tubes daily from the incubator and observe for color reactions by comparison with the un-inoculated "Control" tube.

   Record any observations on the data report sheet attached to this document.

Note: Observations should **NOT** be made prior to 18 hours of incubation. The induction of decarboxylase and dihydrolase enzymes will not take place until the acidic state of the medium has been established.

Interpretation of Results

- A positive decarboxylase result is indicated by the development of a purple to pale yellow-purple color.
- A negative decarboxylase result is indicated by the development of a bright yellow color for glucose-fermenting microorganisms.
- Organisms that do not produce the appropriate enzyme, but do utilize glucose, will result in a yellow color development in the medium.
- If a tube is yellow at one observation time, but turns purple subsequently, consider this a positive decarboxylase test.
- Non-glucose-fermenters will result in little or no color change as compared to an uninoculated tube.
- For some organisms other than those used in this exercise, increased incubation for up to 10 days may be necessary.
- Development of a purple (alkaline) color in the uninoculated control tube of basal medium invalidates all test results, and test interpretation should not be made.

Disposal/Recycling of Materials

Materials used in this exercise should be disposed or recycled as indicated below:

- All used decarboxylation test broth tubes, regardless of inoculation status, should be placed in the proper disposal rack for sterilization. All tubes should have lids put in place, though they should not be tightly secured.
- All used plastic bulb pipettes should be placed in the disposal bin on the bench top.

Staple Here

Student Name: _____

COMPLETE THE FOLLOWING TABLE BASED UPON YOUR VISUAL OBSERVATIONS

Organism/Observation		Medium				
		"Control"	Basal Medium	Lysine	Arginine	Ornithine
Citrobacter fruendii	Color					
	Pos./Neg.?					
Proteus hauseri	Color					
	Pos./Neg.?					
Enterobacter aerogenes	Color					
	Pos./Neg.?					
Klebsiella pneumoniae	Color					
	Pos./Neg.?					

Discussion Questions:

1. If an organism does not ferment glucose, what modification to the decarboxylation test could be made to determine if the organism produced decarboxylases or dihydrolases?

2. The addition of mineral oil prevents the medium from absorbing oxygen. Why does this promote fermentation?