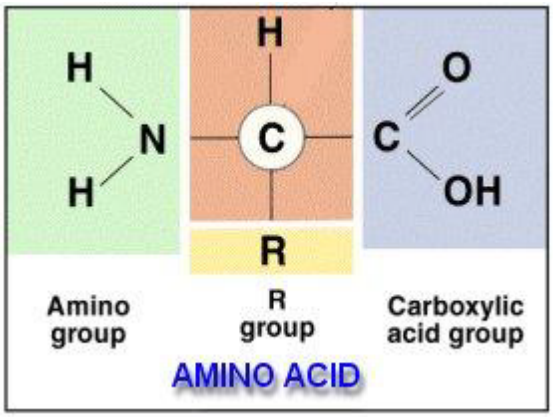
DECARBOXYLATION & DEAMINATION OF Amino Acids



These are about 20 amino acids, and most of them can be used one bacterium or another. Many of the biochemical tests are based on protein and amino acid use. In this lab you will look at 2 different amino acid tests, plus I have added a 3rd that you may want to run at a later time.

There are 3 decarboxylase enzymes we can test for---arginine decarboxylase, ornithine decarboxylase, and lysine decarboxylase. These enzymes break the bond holding the carboxylic (-COOH) group to the rest of the amino acid. As a result, the end product is a basic chemical which causes the pH to go up, changing the indicator brom cresol purple to turn purple.

The deaminases do the opposite, knocking off the amino groups, and producing chemicals which are acidic. We run one deaminase test---phenylalanine deaminase---which uses $FeCl_3$ as the reagent, reacting with the phenylpyruvic acid that results from the breakdown of phenylalanine.

NOTE: The decarboxylase test needs to be anaerobic (assuming your unknown is NOT a strict aerobe), so you overlay the broths with a layer of sterile mineral oil.

A base broth without amino acid is run on each organism as an inoculated control. Since the medium has sugar in it, you are making sure that the organism uses the sugar, turning the indicator yellow. This is NOT a reaction that you record.

OBJECTIVES:
Learn the various methods for determining deamination and decarboxylation.

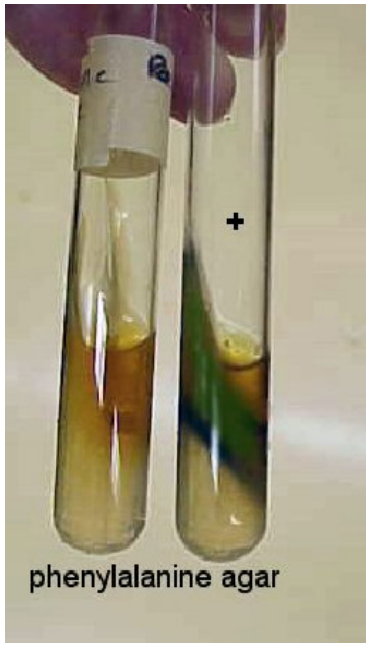
MATERIALS NEEDED: each pair of students will run the unknown

Moeller base broth, no amino acid added
Moeller decarboxylase broths—ornithine, lysine, arginin
phenylalanine agar slant
mineral oil
pipette and pi-pump
AFTER INCUBATION: $FeCl_3$ reagent

THE PROCEDURE:

1. Inoculate the decarboxylase broth with the bacterium and **overlay** with a layer of **mineral oil** (NOT immersion oil). The layer should be 1/4-1/2 inch deep.

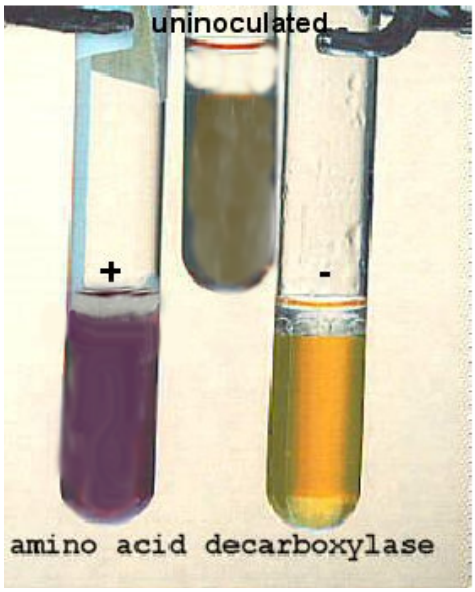
2. Inoculate the phenylalanine deaminase slant as you would a normal slant.
3. Incubate at organism's optimal temperature, 25 or 37 degrees C, for a couple of days.
4. AFTER INCUBATION:
    - The decarboxylase broths are read as is, no reagent added.
    - 6-8 drops of ferric chloride are added to the phenylalanine agar slant, washing down the slant. Read immediately.



INTERPRETATION:

ARGININE, LYSINE, ORNITHINE DECARBOXYLASE BROTHS: In a basic pH, as a result of the decarboxylation process, the brom cresol purple will be a **purple** or **purple-gray.**

PHENYLALANINE DEAMINASE: The $FeCl_3$ reacts with the acid produced as a result of deamination, turning the slant an **avocado green.**



QUESTIONS:

1. In the presence of the enzyme decarboxylase, the amine side chain of the amino acid molecule will be chopped off---TRUE **or** FALSE?.

2. Why add mineral oil to the decarboxylase amino acid broths?

3. What is the amino acid in the deamination agar?
