PHENYLALANINE DEAMINATION TEST

Principle and Purpose

Phenylalanine deaminase (PAD) is an enzyme that removes an amino group from phenylalanine generating phenylpyruvic acid, water, and ammonium ions. This enzyme is used by some bacteria to exploit this amino acid as a carbon and energy source. Enteric bacteria tend not to produce this enzyme, whereas species of *Morganella*, *Providencia*, and *Proteus* do. Following growth in media containing phenylalanine, bacterial produced PAD is detected by the addition ferric chloride. Ferric chloride combines with phenylpyruvic acid to from a green complex, which indicates a positive reaction (Fig. 1). In this exercise, students will conduct PDA tests using commercially available (Key Scientific).

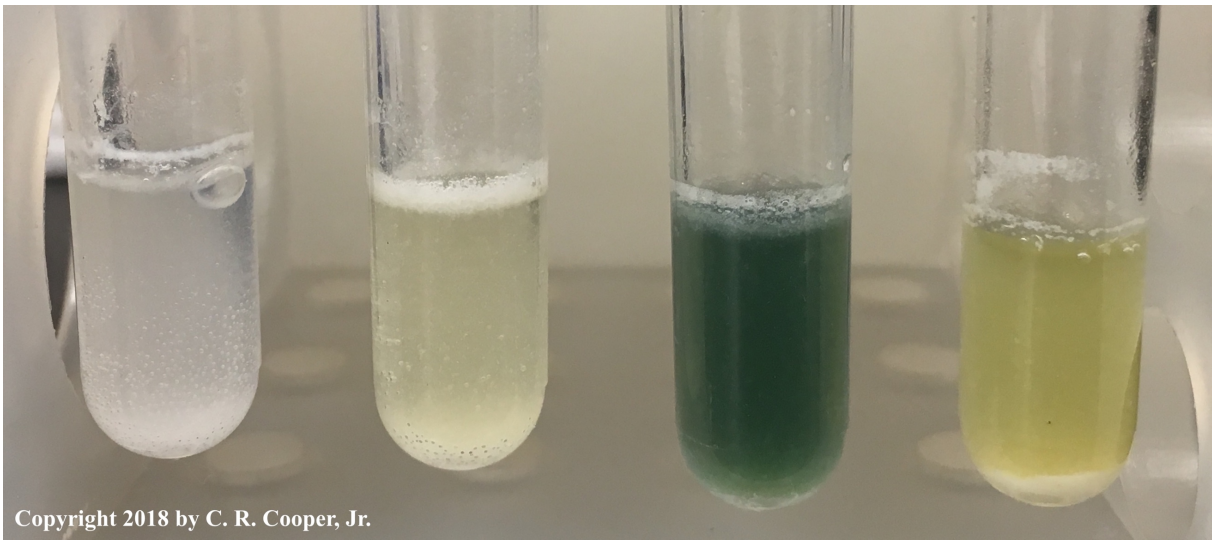

Figure 1. Phenylalanine Deaminase Test. These tubes contain a rehydrated PDA tablet and were incubated for 24 hours at 37°C. The left two tubes are uninoculated media and serve as experimental controls. The two right most tubes were inoculated with *Proteus hauseri* and *Escherichia coli*, respectively. Ferric chloride was added to all but the first tube on the left. A positive PDA reaction is exhibited by *P. hauseri*, but not *E. coli*. No reaction is observed in the controls with and without added ferric chloride.

Learning Objectives

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

- Understand the biochemical basis of the phenylalanine deaminase test;
- Properly perform the phenylalanine deaminase test;
- Distinguish between positive and negative reactions; 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- The following organisms should be provided as 18-24 hour TSA slant or plate cultures:

- *Escherichia coli* (ATCC 25922) [abbreviated as *E. coli*]
- *Proteus hauseri* (ATCC 13315) [abbreviated as *P. hauseri*]
- *Klebsiella pneumoniae* (ATCC 13883) [abbreviated as *K. pnuemoniae*]
- *Morganella morganii* (ATCC 25830) [abbreviated as *M. morganii*]

Materials

- Phenylalanine Deaminase Tablets (Cat. No. K530; Key Scientific, Stamford, TX; https://www.keyscientific.com/files/New%20Website%20Files/Phenylalanine%20Deaminase/K530-0805.PDF)
- Sterile water
- Alcohol wipes
- Sterile plastic bulb pipettes
- Sterile test tubes (12 x 75 mm; glass or polystyrene)
- 10% ferric chloride solution

Procedures

Students shall review and use the BIOL 3702L Standard Practices regarding the labeling, incubation, and disposal of materials.

1) Obtain two (4) sterile 12 x 75 mm test tubes. Label each tube with the microbe to be tested and other relevant information.
2) Use an alcohol wipe to sterilize a set of forceps by thoroughly wiping the outside and inside tip area of the instrument.
3) Using sterile forceps, add a single Phenylalanine Deaminase Tablet to each labeled tube.

Note: Be sure to retrieve tablets from the small white bottle marked "Phenylalanine Deaminase Tablet K530". Several other products used in this laboratory course come in similar containers.

4) Using a sterile plastic bulb pipet, aseptically add 1 ml of sterile water to each labeled tube.
5) Using a microbiological loop, *heavily* inoculate each tube with the appropriate bacterial species.
6) Incubate all the tubes at 37°C for 18-24 hours.
7) Remove the tubes from the incubator. To each tube, add 1-2 drops of ferric chloride reagent.

Interpretation of Results: A green color appearing within 1-5 minutes is a positive result for the production of phenylalanine deaminase. No color change indicates a negative result.

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

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Student Name: _____

COMPLETE THE FOLLOWING TABLE BASED UPON YOUR VISUAL OBSERVATIONS

Organism	Phenylalanine Deaminase (PDA) Activity
Escherichia coli	**Color After $FeCl_3$?**
	PDA Pos./Neg.?
Proteus hauseri	**Color After $FeCl_3$?**
	PDA Pos./Neg.?