BIO 201 Lab 12
Experiment 15 Results

Professor Diane Hilker

Overview

I. **Exp. 15: Physiology of Bacteria**

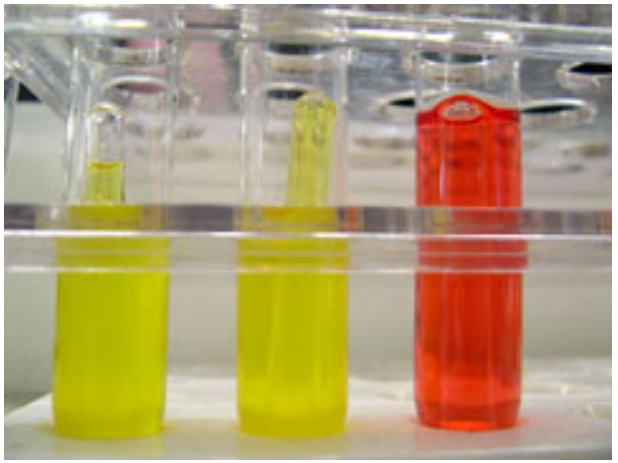
I. Exp. 15: Physiology of Bacteria

- **Purpose:** To examine specific enzymatic activities of microbes that are frequently used to identify bacterial species.

- Refer to Exp. 21, Table 9 & Figure 8, for biochemical reactions of various Gram neg. rods
- Inoculated last lab:
  - Gram Neg. rods: *E.coli, Enterobacter, Proteus*
  - Gram Pos. rod: *Bacillus stearothermophilus*

I. Exp. 15: Physiology of Bacteria

- **Phenol Red Dextrose Broth (PRDB):** does the microbe ferment glucose or dextrose?
  - Yellow with gas: +
  - Yellow without gas: –
  - Red with or without gas: –

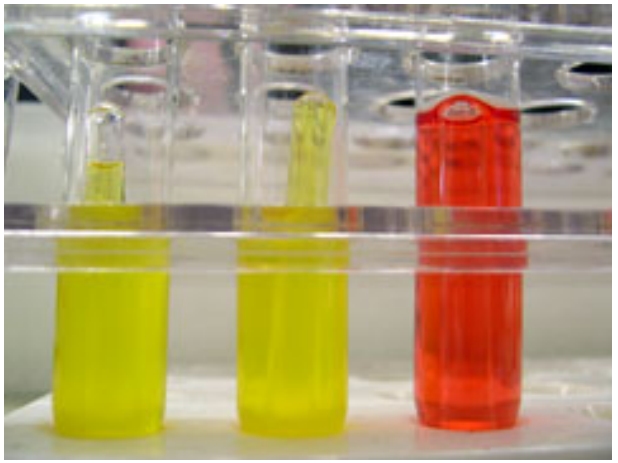


\+ – –

  - Yellow/red with or without gas: +/–

I. Exp. 15: Physiology of Bacteria

- **Phenol Red Lactose Broth (PRLB):** does the microbe ferment lactose?
  - Yellow with gas: +
  - Yellow without gas: –
  - Red with or without gas: –
  - Yellow/red with or without gas: +/–



\+ – –

I. Exp. 15: Physiology of Bacteria

- **Nitrate Broth:** Does the microbe produce an enzyme called nitratase?

ADD: 2-3 drops Nitrate A
2-3 drops Nitrate B
} Mix; look for color development in 30sec.

I. Exp. 15: Physiology of Bacteria

- **Nitrate Broth**

RESULTS: Peach/pink: +
Not peach/pink: -

\- +

I. Exp. 15: Physiology of Bacteria

- **Tryptone Broth:** Does the microbe produce an enzyme called tryptophanase?

Tryptophan  Indole

Tryptophanase

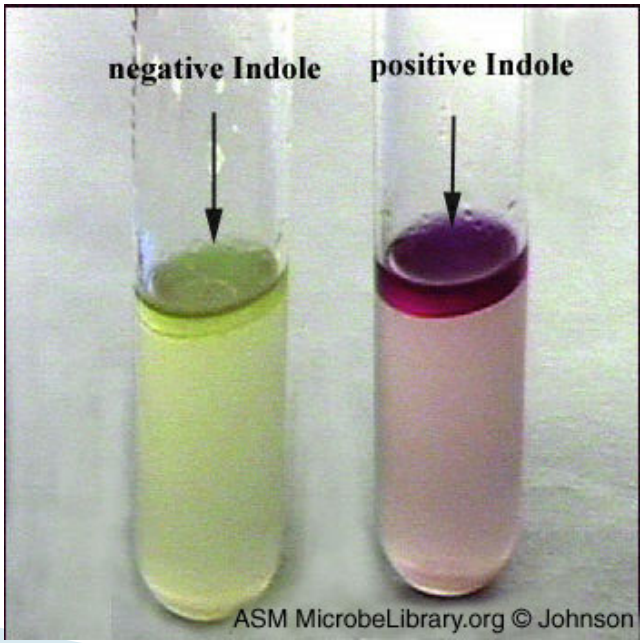
ADD: 10-12 drops of Kovacs Reagent

Mix; look **immediately** for the reaction

I. Exp. 15: Physiology of Bacteria

- Tryptone Broth

RESULTS: Maroon top layer: +
No maroon top layer: -



I. Exp. 15: Physiology of Bacteria

- **Methyl Red- Voges Proskauer Broth (MRVP)**

First divide the tube in half using a Pasteur pipette. Transfer ½ of broth to a 2nd empty glass tube. Cap both tubes. One tube you will perform the Methyl Red Test and the 2nd the Voges Proskauer Test

I. Exp. 15: Physiology of Bacteria

- **Methyl Red Test:** Does the microbe produce a large amount of acid end product from glucose fermentation?

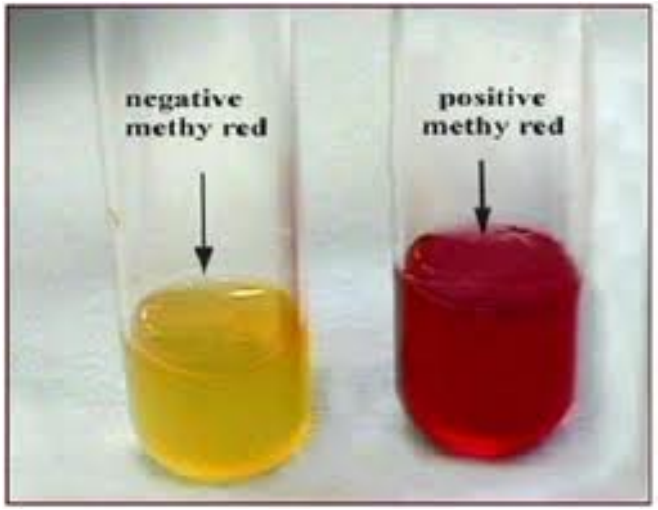
Glucose  pH below 4.4

ADD: 4 drops of Methyl Red Reagent
Mix; look **immediately** for the reaction

I. Exp. 15: Physiology of Bacteria

- **Methyl Red Test**

RESULTS: Pink: +
Not Pink: –



I. Exp. 15: Physiology of Bacteria

- **Voges Proskauer Test:** Does the microbe produce a compound called acetoin during glucose fermentation?

Glucose  Acetoin

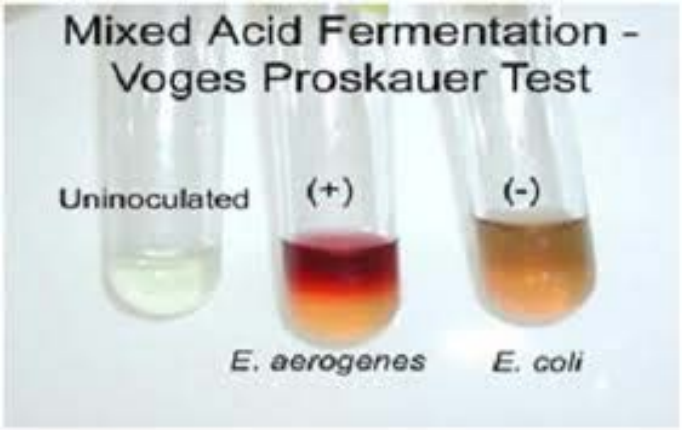
ADD: 18 drops of Barritts A Reagent and
18 drops of Barritts B Reagent

Mix tube well & let stand for 10 minutes. If neg., return tube to rack and look periodically over the next 50 minutes for a pos. reaction. **Do not reshake tube.**

I. Exp. 15: Physiology of Bacteria

- **Voges Proskauer Test**

RESULTS: Top maroon/pink **film**: +
Film NOT maroon/pink: –



I. Exp. 15: Physiology of Bacteria

- **Simmon's Citrate Test:** Does the microbe utilize citrate as a source of carbon?

- The Simmons Citrate slant contains citric acid and bromothymol blue (green when acidic). When citrate is utilized, the pH increases to 7.6 and bromothymol blue turns blue.

I. Exp. 15: Physiology of Bacteria

- **Simmon's Citrate Test**

RESULTS: Observe for color change on the **surface** of the slant

Blue slant: +

Green slant: -

\- +

I. Exp. 15: Physiology of Bacteria

- **Hydrolysis of Urea:** Does the microbe produce an enyzme called urease that breaks down urea?

- Urea Broth tube contains urea and phenol red. When urea is broken down to CO_2 & ammonia, the pH increases to 8.4 and phenol red turns to a magenta/purple color.

Urea  Ammonia (pH 8.4)

I. Exp. 15: Physiology of Bacteria

- **Hydrolysis of Urea**

RESULTS: Observe for a color change

Magenta/purple: +

Not magenta/purple: –

– +

I. Exp. 15: Physiology of Bacteria

- **Production of Hydrogen Sulfide (H_2S):** Does the microbe produce H_2S when decomposing proteins?

- **TSI Tube** must be stabbed. The tube contains three sugars and iron. Only analyzing the tube for H_2S production which is indicated by a black coloration (any amount) in the butt or bottom of the tube.

I. Exp. 15: Physiology of Bacteria

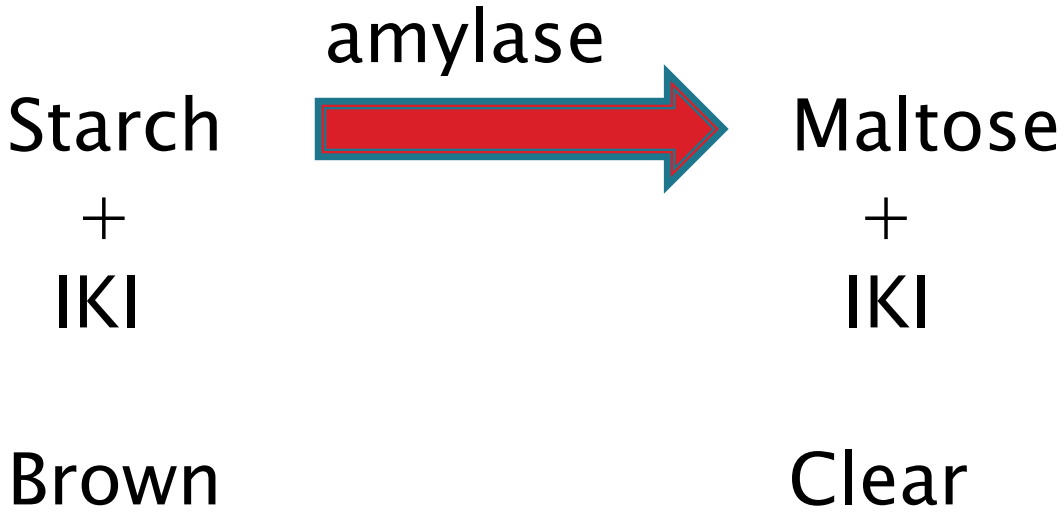
- **Hydrogen Sulfide (H_2S) Test**

RESULTS: Black color in butt/bottom of tube: +
No back color in butt/bottom of tube: –

– – + –

I. Exp. 15: Physiology of Bacteria

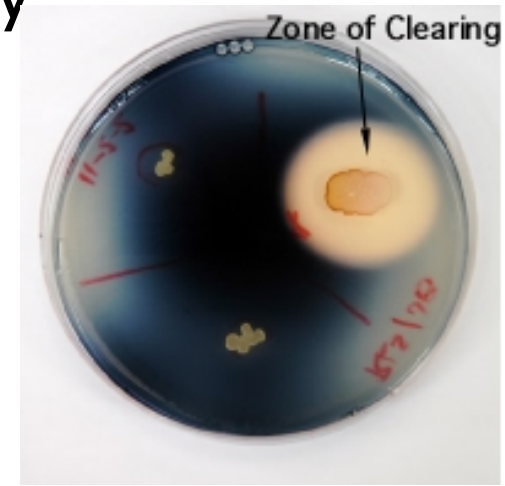
- **Hydrolysis of Starch:** Does the microbe produce an exoenzyme called amylase that breaks starch into maltose?



I. Exp. 15: Physiology of Bacteria

- **Hydrolysis of Starch**

ADD: Flood the plate with iodine or IKI.
Observe for a clearing around colony.
Clear halo surrounding colony: +
Brown color surronding colony: –



BIO 201 Lab 12
Exp. 16: Week 1 Unknowns

Professor Diane Hilker

Overview

I. **Exp. 16: Identification of Unknown Microorganisms**

I. Exp. 16: Identification of Unknown Microorganisms

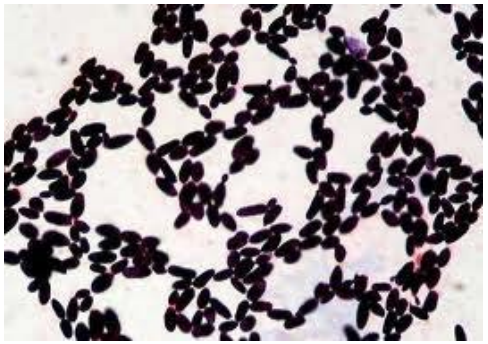
- **Purpose:** To determine the identity of unknown microorganisms by using various test methodologies.

- TODAY:
  - **Set up Temperature Study (Exp. 7):** 5, 25, 37, 60°C
  - **Set up Colony Morphology Plate**
  - **Do a Wet Mount:** motility & size
  - **Do a Simple Stain:** cocci, rod or large ovoid cells
  - **Do a Gram Stain:** Gram + or −; shape

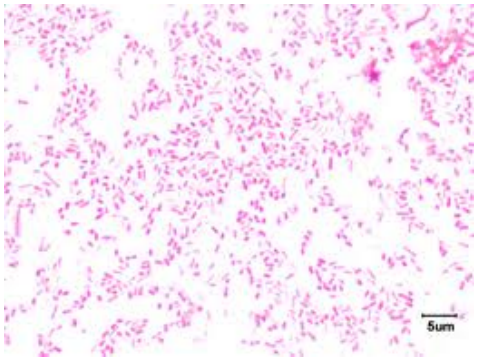
I. Exp. 16: Identification of Unknown Microorganisms

- Gram Stain

Yeast



Gram Neg.Rod



Gram Pos. Cocci