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.

- Inoculated last lab:
  - *E.coli, Enterobacter, Proteus*: Gram Neg rods
  - *Bacillus sp.:* Gram Pos. rod

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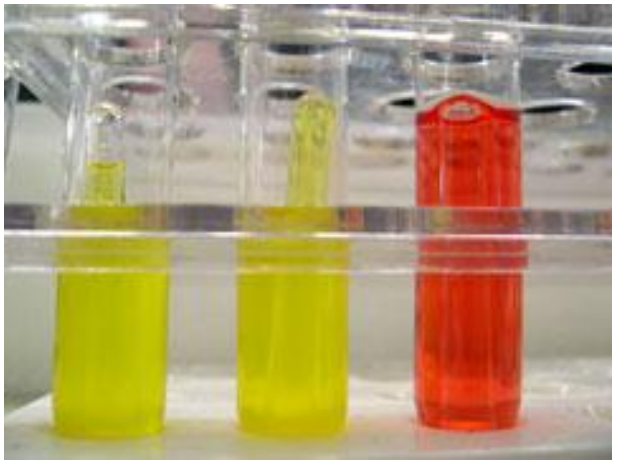
- **Phenol Red Dextrose Broth (PRDB):** does the microbe ferment glucose or dextrose?
  - Yellow with gas: +
  - Yellow without gas: –
  - Red with or without gas: –
  - Yellow/red with or without gas: +/–



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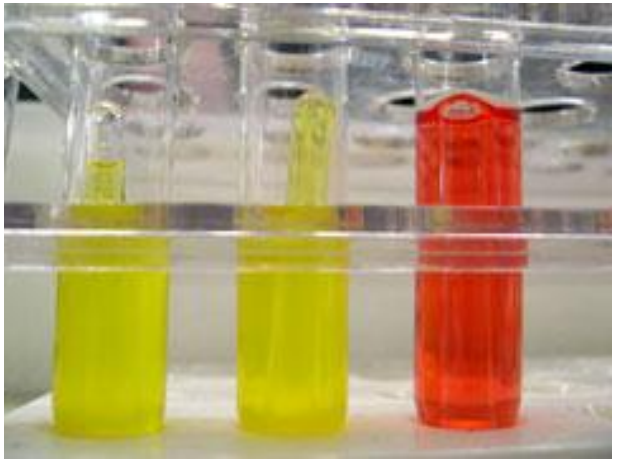
- **Phenol Red Lactose Broth (PRLB):** does the microbe ferment lactose?
  - Yellow with gas: +
  - Yellow without gas: –
  - Red with or without gas: –
  - Yellow/red with or without gas: +/–



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

Nitrate  Nitrite

Nitratase

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

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- **Nitrate Broth**

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

\- +

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- **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

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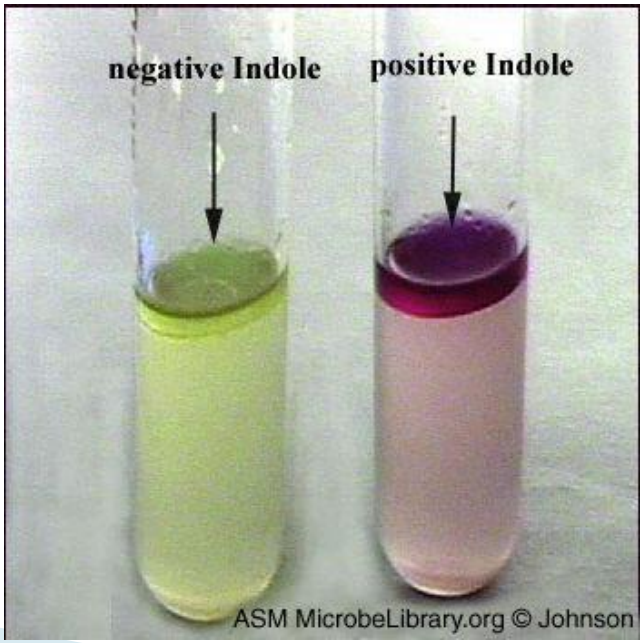
- Tryptone Broth

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



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- **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

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- **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

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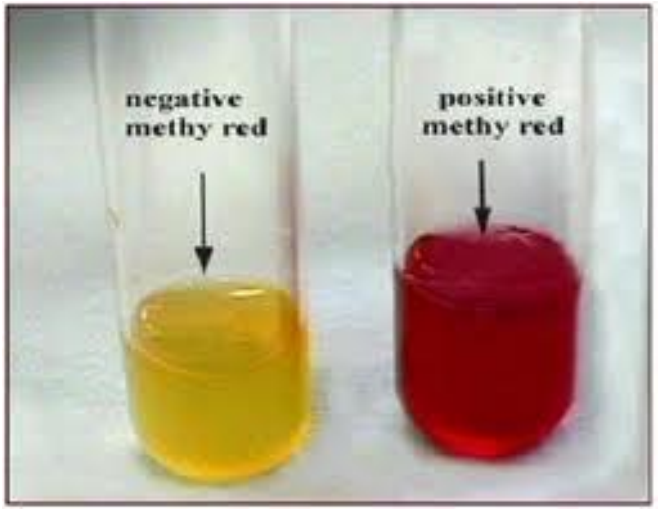
- **Methyl Red Test**

RESULTS: Pink: +
Not Pink: –



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

Glucose  Acetoin

ADD: 18 drops of Barritts A Reagent
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.**

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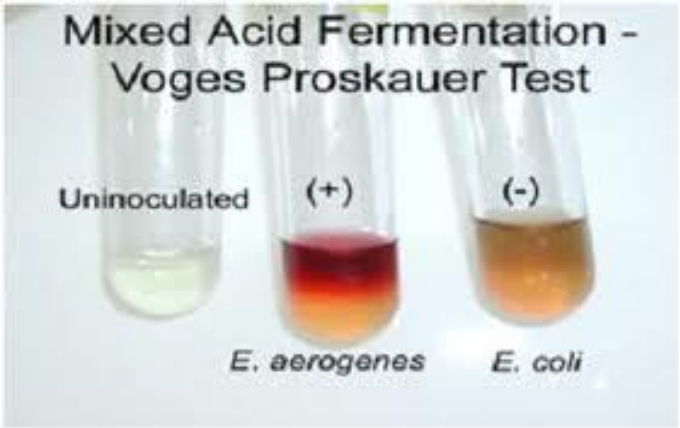
- **Voges Proskauer Test**

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



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

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

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- **Simmon's Citrate Test**

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

Blue slant: +

Green slant: -

\- +

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- **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 broken down to CO_2 & ammonia, pH increases to 8.4.

Urea → Ammonia (pH 8.4)

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- **Hydrolysis of Urea**

RESULTS: Observe for a color change

Magenta/purple: +

Not magenta/purple: –

– +

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- **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.

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- **Hydrogen Sulfide (H_2S) Test**

RESULTS: Black color in butt of tube: +
No back color in butt: -

\- - + -

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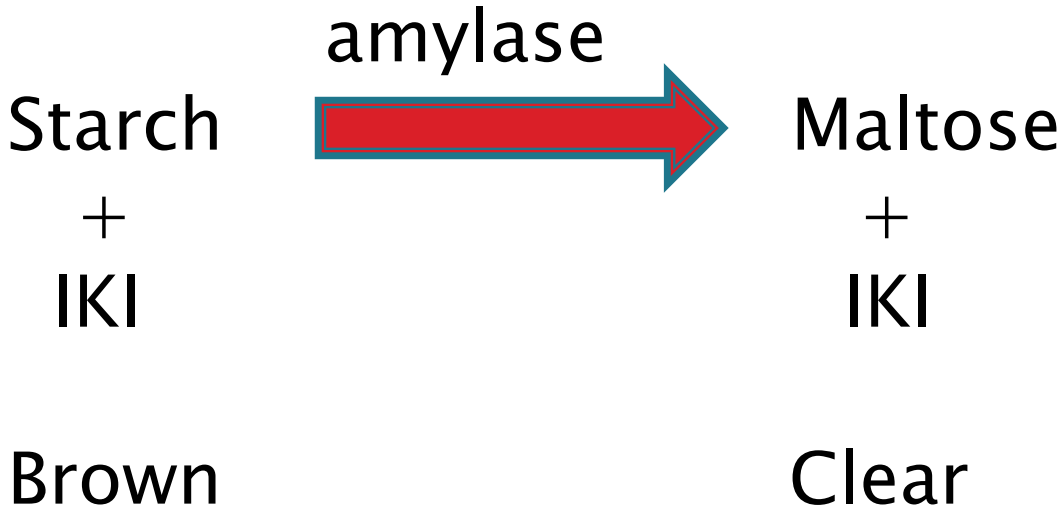
- **Hydrolysis of Starch:** Does the microbe produce an exoenzyme called amylase that breaks starch into maltose?



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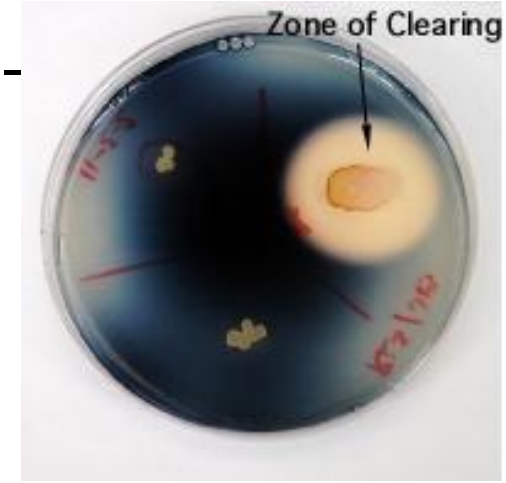
- **Hydrolysis of Starch**

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



BIO 201 Lab 12
Exp. 16: Week 1 Unknowns

Professor Diane Hilker

Overview

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

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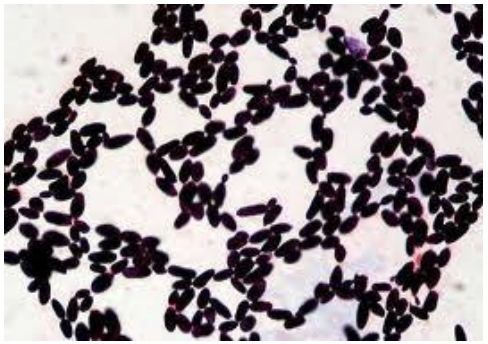
- **Purpose:** To determine the identity of unknown microorganisms by using various test methodologies.

- 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

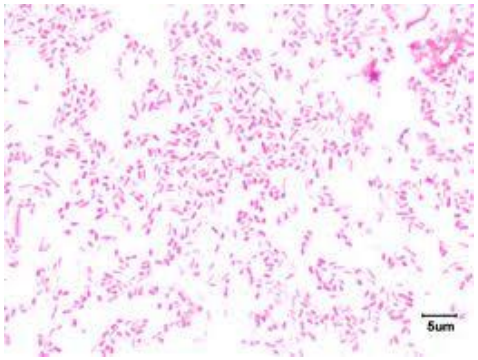
I. Exp. 16: Identification of Unknown Microorganisms

- Gram Stain

Yeast



Gram Neg.Rod



Gram Pos. Cocci