351 Practical II
Differential Tests Review

Ex. 5-2

Phenol Red (PR)- Fermentation glucose, sucrose, lactose for *Escherichia coli*

- Lac (left) gas+
- Glu(middle) gas +
- Suc (right) no gas –

- Phenol red indicator used to see if fermentation has occurred. Durham tubes are red before any fermentation has occurred. Fermentation produces gas and/or acid from the breadkdown of carbohydrates

Ex. 5-2

Phenol Red (PR) Fermentation glucose, sucrose, lactose for *Alcaligenes faecalis*

- Suc (left) –
- Lac (middle) –
- Glu (right) –

- Think about why *A. faecalis* could not breakdown glu,suc, or lac?

This is a negative result, must have full yellow to be positive. Don't worry the exam ones will be more obvious ☺!

Ex. 5-2

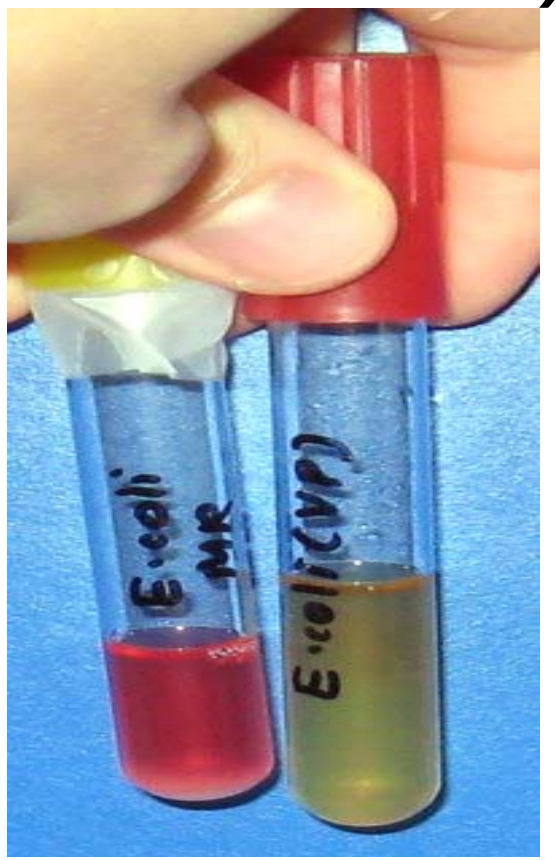
Phenol Red (PR) Fermentation glucose, sucrose, lactose for *Saccharomyces cerevisiae*

- Lac (left) –
- Glu (middle) gas
- Suc (right) –

Why did *S. cerevisiae* NOT change color?

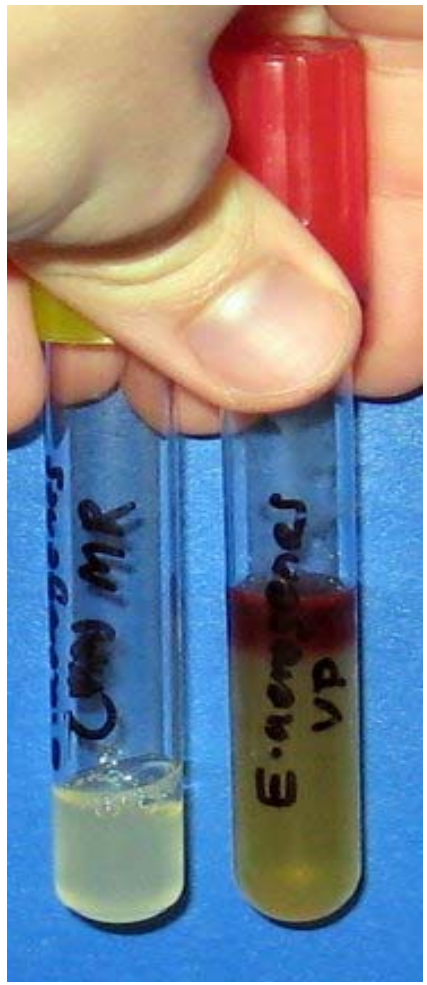
Ex. 5-4
Methyl Red (MR) (IMViC tests)

- *Enterobacter aerogenes* (left) –
- *E. coli* (bright red) +
- Reagent: Methyl red indicator identifies pH change due to mixed acid fermentation



Ex. 5-4
Voges – Proskauer (VP)
(IMViC tests)

- *Enterobacter aerogenes* +
- *E. coli* – (left)
- Barritt's reagent Tests for acetoin, precursor to 2,3 butanediol fermentation
- Addition of alpha-napthol and KOH



This is the beginning of the reaction, you should see a cherry red color throughout inoculation!

Ex. 5-5
Catalase

- Bubbles +
- No bubbles –
- Reagents 3% H_2O_2

Tests for the ability to break down toxic O_2 products/superoxide dismutase (catalyzes the destruction of superoxide) & catalase o peroxidase (catalyzes the destruction of hydrogen peroxide)

$2\ O_2^- + 2\ H^+$ **---**superstable dismutate→ $O_2 + H_2O_2$

$2\ H_2O_2$ ---catalase→ $2\ H_2O + O_2$

Ex. 5-6
Oxidase

- Blue (30 sec) +
- No color change –
- Tests done on Oxidase strips
- Tests for the oxidation of reduced cytochrome c to form water and reduced cytochrome c / Cytochrome oxidase

Oxidized cyt C + reagent → Wurster's blue + red cyt C

clear

dark purple

oxidized

Ex. 5-7
Nitrate

- Red color after reagents/no color after zinc +

  Escherichia coli (right)
- No color change after zinc is a + for denitrification to nitrogen gas or ammonia

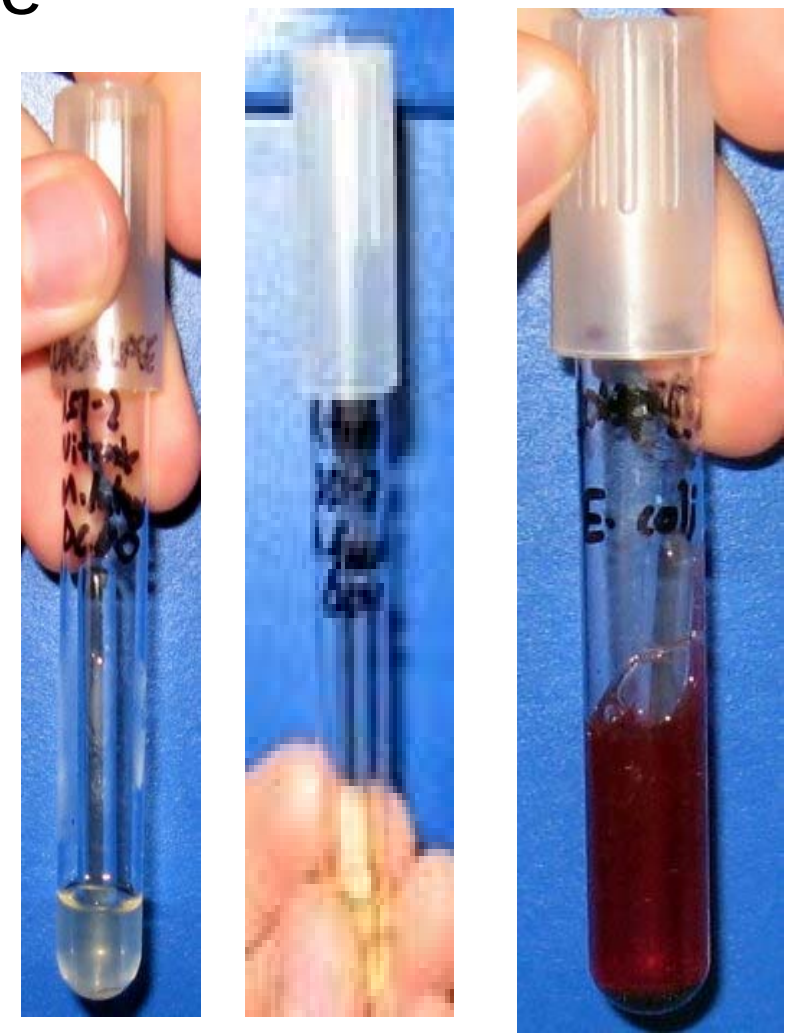
  Soil- (not pictured, would have a gas bubble in durham tube)

- Color change after Zn added will be – for nitrate reductase

  Micrococcus luteus (left)

  Alcaligenes faecalis (middle)

- Reduction of nitrate to nitrite to be used as a final electron acceptor/Nitrate reductase



Ex. 5-8
Citrate (IMViC tests)

- *E. coli* (left green) –
- *Enterobacter aerogenes* (right royal blue) +
- Reagent: Bromothymol blue indicator tests for ability to use citrate as sole carbon source/citrate permease

Ex. 5-13
Starch hydrolysis

- Zone of clearing +
- No zone –
- *Bacillus subtillis* +,

  Alcaligenes faecalis –

  Escherichia coli – (Clockwise)
- Iodine must be on the plate to visualize the zone of clearing surrounding the bacteria. This zone indicates starch was broken down to dextrins, maltose, and glucose/alpha-amylase

Ex. 5-15

Urease

E. coli – (left)

Proteus vulgaris +

Phenol Red a pH indicator turns tube bright pink because NH_3 decreases the pH

$CO(NH_3)_2 + 2\ H_2O$ –urease→ $CO_2 + H_2O + 2\ NH_3$

Ex. 5-16

Casein hydrolysis

- Zone of clearing +
- No zone –
- Test used to see If casein is degraded into amino acids for use as a carbon source/proteolytic enzymes
- *Escherichia coli* – , *Alcaligenes faecalis* – *Bacillus subtilis* +

Ex. 5-17
Gelatin hydrolysis

- Liquid on gelatin +
- No liquid –
- Hydrolysis of gelatin into amino acids to be used as nutrients/gelatinase
- *Escherichia coli* (top) –
- *Bacillus spp.* +

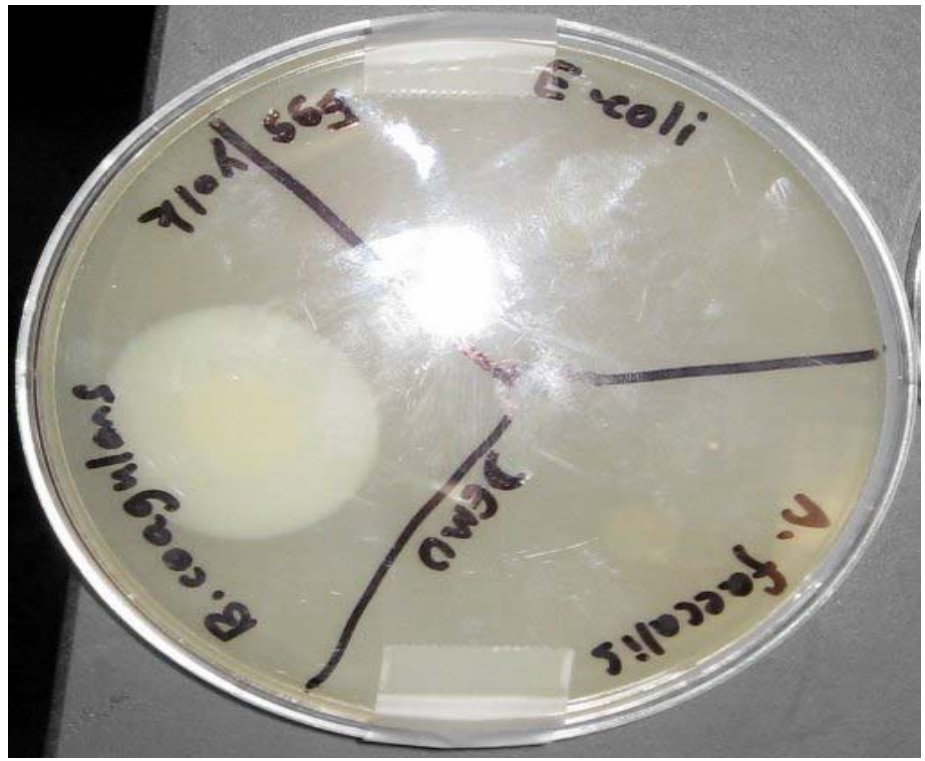
Ex. 5-19
Lipid Hydrolysis

For the Egg Yolk agar, the growth must have a white halo around the colony growth if it utilizes the lipids therefore having the enzyme lipase (hard to see in pics!).

Bacillus spp. +

Escherichia coli –

Alcaligenes faecalis –



Ex. 5-20

Sulfur reduction test, Indole production, Motility (SIM) deeps

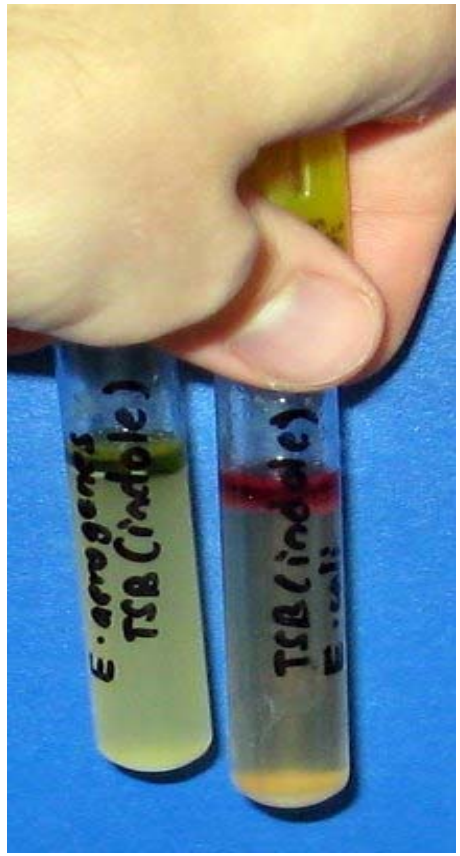
all 3 tests done w/SIM deeps just add Kovac's reagent for Indole test

- Alcaligenes faecalis (left) -
- *Escherichia coli* (middle) –
- *Proteus vulgaris* (black precipitate) +

- Reagent: Ferrous ammonium sulfate-indicator. H_2S reacts w/ ferrous sulfate forming the black precipitate Sodium thiosulfate is reduced to sulfite/thiosulfate

Indole (IMViC tests)

- *Enterobacter aerogenes* –
- *Escherichia coli* (pink/red) +
- Kovac's reagent detects if tryptophan has been hydrolyzed to indol/tryptophanase



Ex. 5-23
Litmus Milk

From Left to Right:

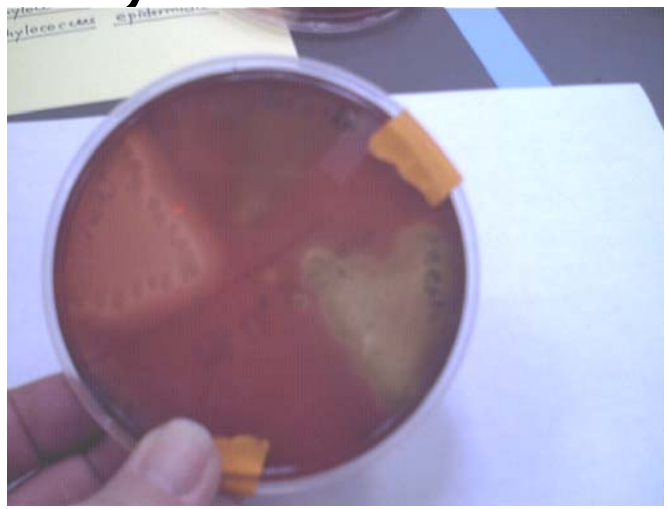
1. *Control* **(NC)**
2. *Enterococcus faecalis* **(R)**
3. *Bacillus megaterium* **(D)**
4. *Proteus vulgaris* **(C)**
5. *Alcaligenes faecalis* **(K)**
6. *Latococococcus lactis* **(AC)**
7. *Escherichia coli* **(A)**

****See Page 187 in book for classifications**

Ex. 5-24
Bacitracin Susceptibility

Ex. 5-26
Blood Agar: Hemolysis

- Check which bacteria are capable of lysing red blood cells (RBCs) by using blood agar (sheep blood).
- α = partial lysis of red blood cells blood looks greenish
- β = complete lysis of blood clearing
- γ = no lysing
- Clockwise starting from the left: *Staphylococcus aureus* β, *Staphylococcus epidermidis* γ , teeth α



Ex. 5-27
Coagulase

- Results:

+ clotting in the bottom of the broth

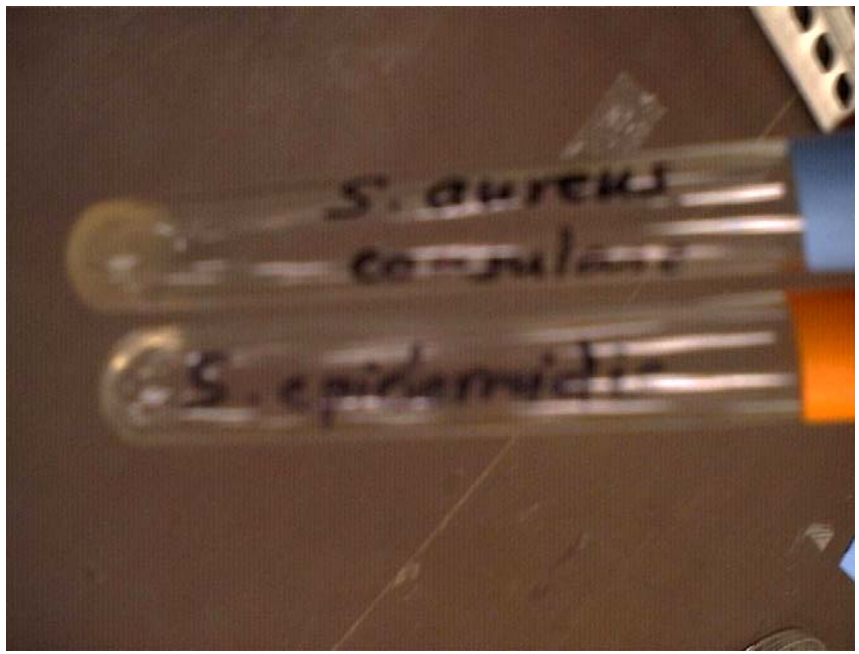
- Reagents:

Plasma

- Reason/Enzymes

Clots plasma to avoid attack by host's defenses/Coagulase

Staphylococcus aureus +;
Staphylococcus epidermidis –



Ex. 5-28
Motility

- Spreading growth +

(Spreading growth looks like a mascara brush in the deep)

*Escherichia coli (*right)

Proteus vulgaris (left)

- Linear growth –

Staphylococcus epidermidis (middle)

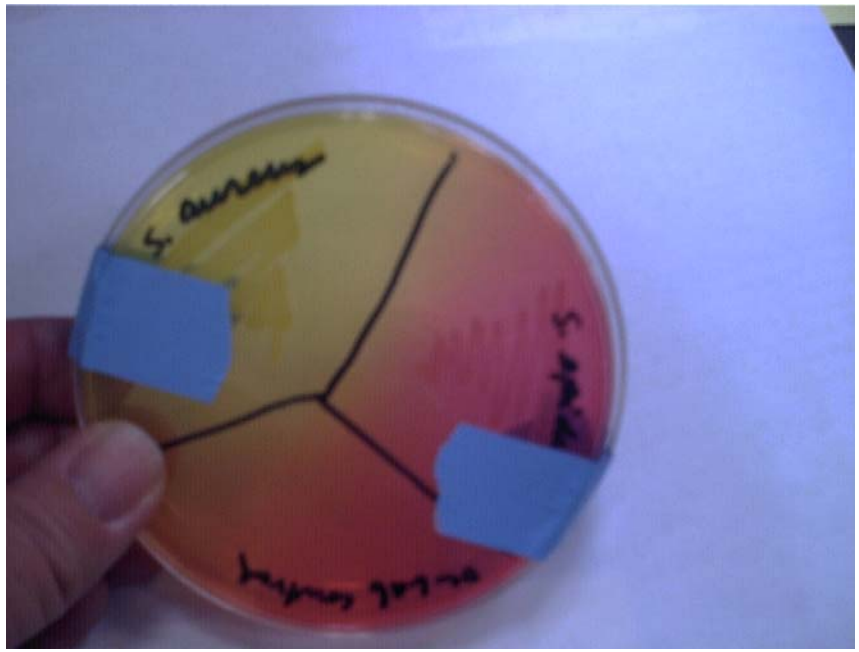
- To test for the ability of bacterium to migrate in solid agar deep

Ex. 7-3
Antibiotic

- Ability of antibiotics to inhibit growth on Mueller-Hinton agar plates (Whether bacteria are susceptible, intermediate, or resistant depends on the amount of antibiotic and the diameter of zone of inhibition, check table 43.1 of your lab manual)

Mannitol salt

- Mannitol salt agar is a selective and differential medium used for differentiating between different stapylococci
- *Staphylococcus aureus* changes medium to yellow
- *Staphylococcus epidermidis* will not change the medium
- Why does *S. aureus* change the color of this medium?



About…
351 Practical II Review Slides

- These slides are manufactured by students, if you see some error, please contact me at fester@unlv.nevada.edu
- Most of these slides were contributed by Austin McDonald from the 351 Fall 2007 Class. Thanks Austin!!