Bacterial Identification Tests

Some tests may be absent from this ppt presentation. These pictures are from students. If you see an error, please email me at fester@unlv.nevada.edu. Most of these pictures were given to me by Austin McDonald, from 351 Fall 2007. Thanks Austin!!
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 breakdown of carbohydrates
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 😊.
Phenol Red (PR) Fermentation

glucose, sucrose, lactose for
Saccharomyces cerevisiae

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

Why did S. cerevisiae NOT change color?
Starch hydrolysis

- Zone of clearing +
- No zone –
- *Bacillus subtilis* +,
  *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
Lipid Hydrolysis

- Dark blue precipitant zone / clearer blue zone +
- No color change –
- *Bacillus subtilis* & *Staphylococcus epidermidis* + w / clearer blue zone around bacterial growth
- Spirit blue agar w/3%Bacto lipase reagent is used to see if triglycerides are hydrolyzed into glycerol and free fatty acids/lipase
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* –
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* +
Gelatin hydrolysis

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

• Bubbles +
• No bubbles –
• Reagents 3% H₂O₂

Tests for the ability to break down toxic O₂ products/superoxide dismutase (catalyzes the destruction of superoxide) & catalase or peroxidase (catalyzes the destruction of hydrogen peroxide)

\[2 \text{O}_2^- + 2 \text{H}^+ \rightarrow \text{superstable dismutate} \rightarrow \text{O}_2 + \text{H}_2\text{O}_2\]

\[2 \text{H}_2\text{O}_2 \rightarrow \text{catalase} \rightarrow 2 \text{H}_2\text{O} + \text{O}_2\]
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 $\rightarrow$ Wurster’s blue + red cyt C

clear $\rightarrow$ dark purple $\rightarrow$ oxidized
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$_2$S reacts w/ ferrous sulfate forming the black precipitate. Sodium thiosulfate is reduced to sulfite/thiosulfate
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
Indole (IMViC tests)

- *Enterobacter aerogenes* – 
- *Escherichia coli* (pink/red) + 
- Kovac’s reagent detects if tryptophan has been hydrolyzed to indol/tryptophanase
Methyl Red (MR) (IMViC tests)

- *Enterobacter aerogenes* (left) –

- *E. coli* (bright red) +

- Reagent: Methyl red indicator identifies pH change due to mixed acid fermentation
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
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
Urease

\[ \text{E. coli} \quad \rightarrow \quad \text{Proteus vulgaris} \quad + \]

Phenol Red a pH indicator turns tube bright pink because NH\(_3\) decreases the pH

\[ \text{CO(NH}_3\text{)}_2 + 2 \text{H}_2\text{O} \rightarrow \text{urease} \rightarrow \text{CO}_2 + \text{H}_2\text{O} + 2 \text{NH}_3 \]
B-galactosidase

- *E. coli* (yellow) +
- no color change clear – (not shown)

- Reagent ONPG
- Hydrolysis of lactose to glucose and use of lactose as sole carbon source / B-galactosidase
- We use ONPG disks for this test
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
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* –
Mannitol salt

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

• Check which bacteria are capable of lysing red blood cells (RBCs) by using blood agar (sheep blood).
• $\alpha = \text{partial lysis of red blood cells blood looks greenish}$
• $\beta = \text{complete lysis of blood clearing}$
• $\gamma = \text{no lysing}$
• Clockwise starting from the left: $\textit{Staphylococcus aureus} \beta$, $\textit{Staphylococcus epidermidis} \gamma$, teeth $\alpha$
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)
Normal Microbiota on the human body

• Both pictures show examples of normal microbiota that grow on the ear, arm, palm, and feet.

• What bacteria do you think these colonies represent?
Temperature

• *Escherichia coli, Serratia marcesens, Bacillus stearothermophilus* are on all three plates
• At 4°C (top) no bacteria grew
• At 30°C (middle) both *Serratia marcesens* and *Escherichia coli* grew
• At 60°C (bottom) only *Bacillus stearothermophilus* grows
• What range of temperatures categorizes bacteria into psychrophiles, mesophiles, and thermophiles?
• Why isn’t *Serratia marcesens* red?
pH

- Bacterial tolerance to different pH varies much more than human tolerance.
- Can you remember the pH ranges for acidophiles, neutrophiles, and alkalophiles?
- No Demos – will not be a station
Osmotic Pressure
NaCl%= 0 on TSA plate

• Detects ability of an organism’s salt tolerance

• *Staphylococcus epidermidis* +

• *Escherichia coli* +

• *Halobacterium salinarium* --
Osmotic Pressure
NaCl% = 0.5 on TSA plate

- Detects ability of an organism’s salt tolerance
- *Staphylococcus epidermidis* +
- *Escherichia coli* ++
- *Halobacterium salinarium* --
Osmotic Pressure
NaCl%= 5 on TSA plate

- Detects ability of an organism’s salt tolerance
- *Staphylococcus epidermidis* ++
- *Escherichia coli* ++
- *Halobacterium salinarium* --
Osmotic Pressure
NaCl% = 10 on TSA plate

- Detects ability of an organism’s salt tolerance

- *Staphylococcus epidermidis* +
- *Escherichia coli* --
- *Halobacterium salinarium* --
Osmotic Pressure
NaCl\% = 20 on TSA plate

- Detects ability of an organism’s salt tolerance
- *Staphylococcus epidermidis* --
- *Escherichia coli* --
- *Halobacterium salinarium* ++
Compare how well the 3 bacteria grow on each plate of different NaCl conc. Is the streak thickness the same on all of them?