Lab Notes

10/14 Lab Metabolic Tests (Ex 34-39)

Phenol Red (PR) Broth -Durham Tubes **pg. 267**

- A simple broth that has a sugar in it (can vary)
    - PR **glucose, lactose & sucrose** (commonly used in lab)
- PR is a pH indicator
    - pH < 6.8 → yellow
    - 6.9 < pH < 7.3 → red
    - pH > 7.4 → pink/magenta
- Carbohydrate fermentation results in lower pH (acidic end products) or acid end products + gas
- Gas will only be an end product if there is also acid

Results/Observations: turbidity → growth, color → pH, gas → fermentation
→ EXAM: understand what each result means & explain

Methyl Red (MR), Voges-Proskauer (VP) pg. 263

- Methyl red test
    - 15 drops methyl red to MRVP broth tube
    - Mix
    - Instant color change
    - Methyl red is a pH indicator

Glucose fermentation → stable acids

Results/Observations: Acid = red; (+) result
Basic = yellow; (-) result
no acid = no color
S. epidermidis (+)

- VP test (Do this test before MR Test!!!)
    - Transfer 1mL to another tube
    - Add 9 drops VPA (use blue nutcracker to break glass/release contents)
    - Mix (shake contents of tube)
    - Add 3 drops of VPB (use blue nutcracker to break glass/release contents)
    - Mix
    - Wait 15-20 min
    - Read every 10 min for 45 min
    - Do not shake tubes

Glucose fermentation → acetoin + butanediol
After VPA and VPB

Results/Observations: red = (+) result; don't confuse with copper color

S. epidermidis (-)

Nitrate test (NO3) -pg 271

- Anaerobic respiration involving reduction of an inorganic molecule other than oxygen
  - N2O (nitrous oxide)
  - NO (nitric oxide) -gas, hormone, chemical signal: increases blood flow
  - NOS
- Nitrate reduction broth
  - Denitrification (converting nitrate to N2)
  - Nitrate → nitrite → nitric oxide → nitrous oxide → molecular nitrogen
    - This occurs when O2 is not the final electron acceptor

Nitrate test

- Add 6 drops of nitrate reagent A and B
- Within 2 min, change in color occurs

Results/Observations: if red = (+) for nitrite (if nitrite is detected) NO3 was reduced to NO2 (or nitrate was reduced to nitrite meaning denitrification took place) TEST done if red!
If no red color, add a pinch of zinc powder

- Within 2 min, color change occurs
- Zinc + NO3 = Red (nitrogen reduction did not take place) = (-) test result for NO3 reduction
- If no red after zinc, (+) test because nitrate went through nitrogen reduction to a product further than nitrite

Citrate Test pg. 269 -slant (stab and smear)

- Used to differentiate organisms based on ability to grow when essential nutrient (like carbon) is limited
- Sodium citrate = sole C source
- Ammonium phosphate = sole N source (nitrogen used for proteins/amino acids, nucleic acid -DNA; nucleotides; nitrogenous base)
- Citrate → alkaline (NH3) + ammonia products + Bromthymol blue (blue when pH inc/green when pH is neutral)
  - Ammonium is basic (causes pH to go up)

Results/Observations: blue when pH increases (BASIC)
Green when pH is neutral
S. epidermidis (-) -appeared green with little to no growth on slant

Tryptic Soy Agar (TSA) -slant
Toxic buildup

- The ETC can lead to a buildup of Hydrogen Peroxide (H2O2)
  - 2H2O2 —--- (via catalase) —--> 2H2O + O2

- In order to engage in cellular respiration, bacteria need to make/have catalase

- Catalase test
    - Use glass slide
    - Use applicator stick to smear your cells
    - Add **1**-2 drops of H2O2
    - Look for bubbling (determines if catalase is present of not)
    - S. epidermidis bubbled immediately (+) test
- Oxidase test
    - Tests for cytochrome oxidase -enzyme in ETC
        - Take sterile cotton swab to pick up cells
        - Apply 2 drops of oxidase reagent
        - Within 2 min, color change occurs

Purple color = (+) result (cytochrome oxidase is present)
No color change (-) result (cytochrome oxidase is not present)
S. epidermidis = (-) test no color change occurred; cytochrome oxidase is not present

- **Spirit Blue Test(Fat Hydrolysis) pg. 276**
    - Lipase (exoenzyme)
        - Break down lipids
        - Lipids (too big to enter cell) —(lipase) —> C,H (smaller molecules that can enter cell)
        - Look for halos around colonies (transparent blue)
        - Use stock (slant) to inoculate sprint blue plate with just a straight line
        - S. epidermidis shows a halo around the colony; conclusion s. Epidermidis appeared to be capable of making the enzyme lipase; typically it shouldn't but somehow it does show a (+) test
        - No you got a negative test which was supposed to happen

- **Starch Hydrolysis test pg. 275**
    - Starch is a polysaccharide made of glucose monomers
    - Some cells can breakdown starch to glucose (requires an enzyme)
        - Amylase (some bacterial cells make it and some don't)
    - Starch is too big to enter cell, needs to be broken down via amylase into glucose
    - Starch —(amylase)-→ glucose
    - Inoculate starch hydrolysis plate with a straight line
    - Add iodine -dark blue/black in presence of starch.
    - After addition of iodine; No zone of hydrolysis around streak; (-) test; does not create amylase

- **Casein Hydrolysis Test p. 275**
  - Casein -protein found in milk
  - Can be broken down by casease
  - Casein —- (casease) —-> peptides/nitrogen behaving compounds (small enough to get into cell)
  - Bacteria get smaller peptides; source of amino acids
  - Inoculate starch hydrolysis plate with a straight line
  - No white appearance of white halo around bacteria; S. epidermidis (-) test result

- **Mannitol Salt Agar pg. 387**
  - Ingredients include: beef extract, peptone, NaCL (75g in 1L H2O = 7.5%)
    - Typical [salt] in bacterial cell = 0.85%
  - Mannitol (fermentable; get acids)
  - Phenol red (pH indicator)
    - Acidic = yellow
    - Neutral = red
    - Basic = hot pink
  - Which ingredient is selective? -salt (not a lot of bacterial cells will be able to tolerate the salt concentration)
  - Which ingredient is differential? -mannitol (not an essential ingredient, but allows us to differentiate the cells via color; doesn't restrict in growth or anything)
  - A differential and selective medium
  - Favors growth of staphylococcus
    - Differentiates: pathogenic staphylococcus (ferments mannitol) from nonpathogenic staphylococcus (does not ferment mannitol)
  - Streak the slant
  - Appears bright pink and orange reddish towards the bottom; small amount of growth = able to tolerate salt concentration → basic; more alkaline; tolerant of salt = (-) MSA test

- **EMB Agar (pg. 414)**
  - Used to detect coliforms (fecal contamination)
    - gram negative, bacillus (rod shaped), lactose fermentation
  - Selective medium -encourages growth of gram (-) organisms
    - Inhibits growth of gram (+) organisms
  - Ingredients include: lactose, color indicators: sodium sulfate and basic fuchsin
    - Detects changes in pH

        - Also double as gram (+) inhibitors
    - Inoculate plate with wavy streak
    - Little to no growth on plate; colorless/color of medium; lactose nonfermenters
    - Red with gold metallic sheen -indicates vigorous lactose fermentation; indicative of coliform bacteria (fecal contamination)
    - S. epidermidis is Gram (+) → (-) test

- **<u>Urease test pg. 276</u>**
    - Urea is a nitrogen containing compound; broken down by urease
        - Products: carbon dioxide and NH3 ammonia (contributes to an alkaline pH)
    - Urease is a hydrolytic exoenzyme
    - If rxn takes place, pH should increase = basic)
    - Basic = bright pink
    - Acidic = yellow
    - Inoculate culture broth tube; just drop cap on tube; do not push down
    - Organism appeared bright pink = basic = (+) test

- **<u>Indole Test (Tryptophan Degradation) pg. 276</u>**
    - Inoculate culture broth tube w/loop full of cells
    - Tryptophanase is enzyme needed to
    - Tryptophan (amino acid)—(tryptophanase) —> indole + pyruvic acid
    - Add 15 drops of Kovac's Reagent
    - Yellow/no color change = (-) test result
    - Pink/red = indole (+) test result; makes enzyme tryptophanase
    - Indole is byproduct therefore if rxn occurred, then indole (pink/red color) should appear
    - S. epidermidis = (-) result; no change in color occurred

- **<u>Phenylalanine Deaminase pg. 277</u>**
    - Phenylalanine(amino acid) — (phenylalanine deaminase) —> phenylpyruvic acid + ammonia (bi-product)
    - Streak the slant w/loop
    - Add 6-8 drops of Ferric chloride added to slant
    - Detects phenylpyruvic acid → Green color appears in 5-10 min
    - Yellow (remained the same color) = negative test = no phenylpyruvic acid is detected
    - S. epidermidis remained same color after addition of acid (-) test

- **<u>Motility pg. 132 ex. 17</u>**
    - Semisolid motility agar -allows cells to move if capable
    - Use needle to stab center of the media (do not go all the way down) using your organism
    - Motility test has TTC -Turns red when reduced

- Organisms shows some signs of movement; diffuses away from line (+) motility

- **Pg. 137 (use organisms provided to view under microscope)**
  - Loop full of cells on slide; use toothpick and vaseline; place vaseline on corners of cover slip; place cover slip using vaseline to stick

- **KIA (Kligler Iron Agar) pg. 279**
  - Glucose -1g in 1L
  - Lactose & Sucrose -10g each in 1L
  - Phenol red
  - Sodium thiosulfate
  - Ferrous sulfate
  - Inoculate slant by stabbing with needle & streaking slant
  - Glucose is preferred sugar; monosaccharide; easy to breakdown; but its only 0.1% -so it runs out within 12 hours
  - 0.1% glucose → acid (small amount); then goes to alternate source
  - Beef extract/peptone → NH3 -ammonia (raises pH)
    - Reversion -slant reverts, but not the butt
    - Basic slant, acidic butt (red slant yellow butt)
    - Glucose only fermenter results: basic slant/acidic butt, H2S (+/-) -may or may not have, gas (+/-) -may or may not have
  - Glucose and lactose and/or sucrose fermenter
  - 1% lactose, 1% sucrose (10x) more than glucose
  - lactose/sucrose → pH drops -acid (large amounts)
    - No reversion -Tube is all yellow
    - acidic/acidic, H2S (+/-), gas (+/-)
  - Cysteine (amino acid) + H2O + H+ —- (cysteine desulfurase) —-> pyruvate
    - Byproducts include H2S and NH2
    - Sugar fermentation produces acids (H+)
    - Cysteine desulfurase works only in an acidic environment
      - Breakdown of Cys to pyruvate
      - Produces hydrogen sulfide (H2S) gas -stinks
    - H2S + Fe → FeS (ferric sulfide), a black precipitate
    - Results: any black is (+) for H2S

Red = basic;
yellow; acidic;
presence of gas = bubbles; slant moved to the top;
presence of H2S = black precipitate; stinky → ONLY works in acidic environment

Plaque Assays Ex. 21

- Bacteriophages -virus that specifically targets bacterial cells
- The Lytic Cycle
    - Virus attaches to cell wall
    - Injects viral genetic info into cell
    - Directs cell to make more virus
    - Cell lyses
    - Virus released
- Plaques -clearings in lawn of bacteria
    - Where cells have been infected and died
    - One plaque was the result of one virus infecting one cell
    - You can count the number of plaques to determine the original phage concentration
- Conc D.F. = DF(dilution factor) x Volume Plated
    - DF(dilution factor) = depends on plate; A = 10^-1 x 10^-1 = 10^-2
        - A: 10^-2
        - B: 10^-3
        - C: 10^-4
    - Volume plated = 10^-1
        - Countable plaques range from 25-250
- PFU(plaque forming unit)/mL = (# plaques/FDF)

Soil Antibiotics
Obtain dirt in bag
Tare paper; weigh out 1g of dirt
Add 1g dirt into 99ml bottle of water = 100 fold dilution of dirt
Mix & let settle
Use disposable pipette to draw up 1mL of liquid
Transfer to 1st plate & spread
Transfer 0.1mL to plate & spread (about 2 and a half drops)
Let plates sit right side up
Leave at room temperature

Results: should expect bacterial growth; more so on 1.0ml plate

Gram Stain Unknown -Gram Negative Bacilli
Metabolic tests -flow chart

1. Citrate Test

11/6/25

Ex. 31: Soil Antibiotics (cont'd)

- Observations:
    - 1ml: lots of colonies; many different shapes/sizes; crescent shapes colonies
    - 0.1ml: significantly less than 1mL, as expected
- Next Step: Inoculate loop with a single colony and add one streak onto a new plate (1 per pair)

Ex: 56 Epidemic

- Candy #23 → inoculate bacteria and shake hands w 4 people; inoculate plate after each person
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11/18 LAB
Ex 131: soil Abx
Ex 43: Bacterial Food Counts
Ex 60: ELISA
Enzyme-Linked Immunosorbent Assay (ELISA)

- Direct (Sandwich) ELISA
    - Detects Ag in patient sample
    - Start with bound Ab
    - Add sample
    - Add enzyme linked Ab
    - Both Abs bind different epitopes
    - Add substrate
- Indirect ELISA
    - Detects Abs in patient sample
    - Start with bound Ag
    - Add sample
    - Add enzyme linked Ab
    - Add substrate

→ sample c & e appeared clear → negative result

Ex 61: Blood Typing
Blood Types

- Detects presence of A and/or B antigens
- Surface of RBC is covered with these proteins
- Helps with identification and signaling
- You develop antibodies against the antigens that your RBC lacks

Agglutination Test (test subject: 20)

- Blood typing
- Add anti-A, anti-b, anti Rh antiserum (separately) to blood
- Look for agglutination
- Pg 452

→ AB negative blood type

Ex 54: Blood Agar

Blood Agar Plates (BAP)

- Tryptic Soy Agar (TSA)
- 5% sheep's blood
- Swab your throat

Streptococci

Lancefield Classification system for streptococci

- Group A strep
    - Streptococcus pyogenes
    - Cause pharyngitis, scarlet fever
- Group B Strep
    - Streptococcus agalactiae
    - Part of normal flora of gut and female urogenital tract
- During delivery baby can acquire and develop neonatal sepsis, meningitis

NonLancefield Groups

- Viridans: Streptococcus mutans
    - Found in human oral cavity
    - Contributes to tooth decay
    - Adheres to tooth surface
    - Metabolizes sucrose to glucan dextran (sticky)
    - Biofilm forms (other cells collect)
    - Sugars fermented
    - Produce acid
    - Tooth decay

Differentiation of streptococci

- Group A and Group B
    - <u>B-hemolytic</u>
    - Complete destruction of RBC
    - Produces a clear zone on Blood Agar Plate
- Viridans and Strep. Pneumoniae
    - <u>A-hemolytic</u>
    - Incomplete destruction of RBC
    - Greenish discoloration

- Y-hemolytic
    - No apparent RBC damage
    - Enterococcus faecalis

Ex 15: Endospore Staining

- Endospore -cell formed within cells of certain bacteria
- Extremely resistant to adverse conditions
    - Temperature
    - Desiccation
    - UV light
    - Lack of nutrients
    - pH
- Can be formant for a long time
- Bacterial growth curve
    - Towards end of stationary phase, sporulation occurs

Endospore stain → differential stain

- Use Bacillus subtilis
- Make smear, air dry, and heat fix slide
- Flood with malachite Green (basic)
    - Use paper towel
    - Keep moist
- Steam 10 minutes
    - Allows dye to penetrate spores
- Rinse thoroughly with water
- Counterstain: Safranin (basic) for 5 min
- Water rinse
- Oil immersion

Acid Fast Stain Ex 16

- Tests for mycolic acids in the cell wall
- Mycolic acid -the waxy mycolic acid repels typical aqueous stains
- Carbolfuchsin is a lipid soluble stain, and thus penetrates the waxy cell wall
- Staining enhanced by steaming

Procedure

- Prep bacterial heat fixed smear with Mycobacterium smegmatis AND E. coli
- Add carbolfuchsin 12 min
- Rinse
- Add acid-alcohol 15 seconds
- Rinse
- Counterstain: Methylene blue 60 seconds
- Rinse
- Oil Immersion

Cells that have mycolic acid will be magenta/reddish (acid-fast)
Cells with no mycolic acid will be blue (nonacid-fast)
E. Coli (nonacid-fast)
M. smegmatis (acid-fast)

Capsules

- Protective polysaccharide layer
- Makes cell antiphagocytic
- Prevents desiccation

Capsule Stain Ex 13

- Bacillus megaterium
- Prepare smear
- Air dry (NO HEAT FIX -you'll shrivel the capsule)
- 1% crystal Violet for 2 min
- Rinse with Copper Sulfate
- Oil Immersion

Capsule Stain Ex 13 (mod)

- Bacillus megaterium
- Make negative stain using Congo Red (add congo red to edge, add cells, push/pull method)
- Air dry (NO HEAT FIX -you'll shrivel the capsule!)
- Counterstain: Maneval's Stain for 2 min
    - Background becomes dark blue and cells remain pink
    - Maneval's Stain is acidic
    - Turns Congo Ref blue
    - Stains cells pink
- Rinse gently to rid excess Maneval's stain
- Oil immersion