Microbiology Laboratory 7

Observations from Last Week's Lab

Selective & Differential Media
Record your data from the cultures from last lab and write the results on the chalk board using "**+**" for positive and "**-**" for negative.

Eosin Methylene Blue Agar (EMB):

Growth (G)

Lactate metabolism (L) indicated by dark centered colonies.
Indicate if a metallic green sheen is present.

MacConkey Agar (MCA):

Growth (G)

Lactate metabolism (L) (pink or red colonies)

Mannitol Salt Agar (MSA):

Growth (G)

Mannitol metabolism (M) (turns the media yellow)

Simmons' Citrate Agar (SCA):

Growth (turns the media blue)

Lactose broth:

Growth (G) (turbidity)

Acid production (A) (turn the media yellow)

Gas production (Gas) (bubble in the Durham tube)

Starch Plate - Iodine forms a bluish - black precipitate in the presence of starch.

- Transfer 700-750 µl of iodine to your starch plate with a transfer pipet.
- Tilt the plate so that all the iodine covers as much of the plate as possible.
- Wait a few minutes to allow the iodine to react with the starch.
- Pick up the plate and hold it up to the light while observing through the bottom of the plate.
- If the colony degraded the starch in the media there will be a light staining region (yellow halo) around the colony.

TSI - Triple Sugar Iron

TSI is a selective and differential media used to identify several gram negative enteric bacteria. For nutrients the media contains three sugar sources (**triple sugar**): 1% sucrose, 1% lactose, a much smaller amount (0.1%) of glucose and peptone (which is not fermentable). The pH indicator phenol red is present to detect the production of acids. (acid - yellow, basic - red) All organisms we will examine can use glucose so they will all begin to grow. If only glucose is fermented, only the butt will turn yellow. There is not enough glucose to turn the top of the slant yellow. If the bacteria can not use either lactose or sucrose they will use the peptone (aerobically) which produces an alkaline waste-product. In this case the slant may turn a deeper red. On the other hand if the bacteria can degrade either sucrose or lactose they will produce acid waste products in both the butt (yellow) and the slant (yellow). If no fermentation occurs, no acid waste products are produced so both the butt and the slant will be red.

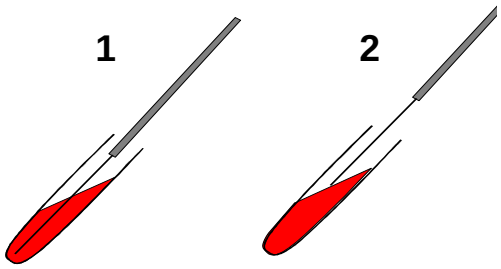
If gas (CO_2) is produced bubbles between the glass tube and the agar will appear. Another indicator of gas production, occurs when the agar slant is lifted off the bottom of the tube or broken apart.

Ferrous (**iron**) sulfate is also added to TSI medium. If the bacteria can use the iron as a terminal electron acceptor it will produce hydrogen sulfide (H_2S) which forms a black precipitate.

A TSI slant should be observed within 48 hours to ensure that all the carbohydrates have not been depleted by bacterial metabolism.

Lab Assignment Inoculate a TSI slant

- Use a straight inoculating needle to pick up an isolated colony (or from a slant).
- Pick up a TSI slant & remove the cap with your dominant hand.
- Flame the to of the tube.
- Inoculate the TSI slant by first stabbing your needle into the slant all the way down to the bottom of the butt.
- Withdraw the needle but before removing the needle from the tube streak the surface of the slant in a serpentine motion.
- Now completely remove your needle and flame the top of the TSI slant tube.
- Replace the cap and flame the entire needle.



Sucrose Broth

A very useful way bacteria are identified is by what organic compounds they can break down. The range of compounds used depends on the collection of genes and thus enzymes a species of bacteria can make. Sucrose (saccharose) is a sugar that some bacteria can use because of an enzyme that separates the disaccharide into its monosaccharides (glucose and fructose). A pH indicator (phenol red) is added to the medium to detect the production of acid by-products. Small inverted tubes called Durham tube is also immersed in the medium to test for the production of the gas (hydrogen or carbondioxide).

Lab Assignment Inoculate a Sucrose broth

- Inoculate a tube of sucrose broth with your bacteria of the day.
- Label your tube and place it in the 37° incubator.

SIM - Sulfide Indole Motility Medium

Sulfide production. This medium tests for the presence of hydrogen sulfide which arrises from the breakdown of the amino acid cysteine. The hydrogen sulfide will combine with the ferrous salts in the medium to produce a black precipitate in the tube. This is useful in differentiating enteric bacteria.

Indole production. Next this medium tests for the production of indol. If the bacterium makes the enzyme tryptophanase it can hydrolyze the amino acid tryptophan to indol and pyruvic acid and ammonia. The addition of a few drops of Kovac's reagent after culturing will test for the presence of indole. If indole is present, the Kovac's solution will turn red.

Motility. Lastly this medium can be used to test for motility of the bacteria. To inoculate the medium, you use the needle and stab the agar. The medium contains a low amount of agar (0.7%) which makes the medium semisolid. This allows the bacteria that are motile to swim in the medium. After culturing, you look for a diffuse growth out from the line of inoculation or by turbidity throughout the tube. If the bacteria (like *Staphylococcus aureus*) are nonmotile they will only grow along the line of the inoculation.

Lab Assignment - SIM culturing

- Using your inoculating needle, pick up some of a known culture.
- Stab your needle into the center of the SIM media in the test-tube.
- Label your tube and incubate the tube at 37°C.

Anaerobic culturing of bacteria

Bacteria can be divided into groups based on their need for oxygen, which is about 20% of our atmosphere.

Obligate aerobes require oxygen to grow because they utilize cellular respiration. In this process, oxygen is the terminal electron acceptor and forms a water molecule in the cell. These organisms, like us, can not survive without the presence of oxygen. Examples include *Pseudomonas* and *Micrococcus*.

Microaerophiles like lower concentrations of oxygen, usually 5-10% oxygen. Usually their sensitivity to higher oxygen concentrations is the result of having oxygen-sensitive proteins or are limited in their ability to carry out cellular respiration. One example of a microaerophile is *Helicobacter pylori* which causes stomach ulcers.

Facultative aerobes can grow with and without oxygen. Usually better growth is observed in the presence of oxygen since they use cellular respiration to get energy from glucose. Without oxygen other metabolic processes are utilized (like fermentation) which yield significantly less energy so growth is much slower. A classic example of this bacteria is *E. coli.*

Anaerobes are bacteria that do not utilize oxygen in their metabolism. **Obligate anaerobes** can not even tolerate oxygen and must be grown in conditions without oxygen present. Examples of this group include *Clostridium* and *Bacteroides*. **Aerotolerant anaerobes** can grow in the presence of oxygen but still do not utilize oxygen in their metabolism. They usually contain some enzymes that protect them from oxygen. An aerotolerant example is *Streptococcus pyogenes*.

To culture obligate anaerobes special conditions that eliminate oxygen are required. This is usually accomplished in anaerobic incubators or in anaerobic jars that use chemical catalysts to eliminate oxygen. A candle jar does not eliminate enough oxygen for obligate anaerobes.

Lab Assignment - culturing deeps

- Using your inoculating needle, pick up some of a known culture.
- Stab your needle into the center of the "deep" agar in a culture test-tube.
- Label your tube and incubate the tube at 37°C.