Microbiology Laboratory 8

Observations from Last Week's Lab

TSI slants:

Result (slant/butt)	Symbol	Interpretation
Red / Yellow	K/A	Only the small amount of glucose was fermented. The acid in the slant was oxidized by the presence of O_2. Also, with the presence of O_2 the bacteria metabolized the peptones which also produced basic (alkali) products. The butt remained acidic because of the low O_2 amount and the slow growth.
Yellow / Yellow	A/A	The lactose and/or sucrose was fermented with and without O_2 to produce acidic waste products.
Red / Red	K/K	No carbohydrate was fermented.

Black slant - H_2S production - If the organism can reduce sulfur it produces hydrogen sulfide which is a gas. The gas will react with the iron present in the medium to produce iron sulfide which appears as a black precipitate. If you have the black precipitate report the butt as acid (A).

Cracks or bubbles - CO_2 production - Look for gas bubbles that have formed between the medium and the glass tube. You may also see fissures (breaks / separations) in the medium or all the medium may be pushed upwards from the bottom of the tube.

SIM medium

1. S - If the media turned black - the sulfur in the amino acid cystine was reduced.
2. I - Place a small amount (5 -6 drops) of Kovack's reagent on the top of the agar. Be careful Kovack's reagent is toxic. Look for the reagent to turn red. If it turns red it is positive for indole production
3. M - Look for bacterial growth away from the stab line. If observed, this is a positive result for motility which means the bacteria has flabella. If your culture reduced the sulfur and turned black you cannot probably see the stab line. If you can't detect movement record the result at N/A.

Deeps

1. Look for bacterial growth along your stab line. If bacteria grew below the surface, this is a positive result for anaerobic growth.

Sucrose Broth

1. Growth (turbidity)
2. Acid production (turn the media yellow)
3. Gas production (bubble in the Durham tube)

Record your results on the black board.

Endospore Staining

The organisms in the genera *Bacillus* and *Clostridia* can produce **endospores** when environmental conditions become unfavorable. The dormant form of the bacteria allow the organisms to survive the harsh environmental conditions. If conditions improve, the endospore can undergo a process called **germination** which forms a new metabolizing and growing cell. Endospores are dehydrated structures that are not actively metabolizing. Endospores are resistant to heat, radiation, acids, and many other chemicals such as disinfectants. Much of their resistance is due to the low amount of water in the endospore (as low as 10% the normal water content of a cell). Another factor contributing to their resistance is the tough protein coat (**exosporium**). As a result of their ability to survive many adverse conditions, endospores define the conditions required to achieve sterility. Usually autoclaving is done which raises the temperature to 121°C for 15 - 20 minutes. Water based liquids can only achieve this temperature when under pressure.

The properties of endospores that allow them to survive harsh conditions also make them difficult to stain. If using a typical dye like methylene blue, the spores appear as unstained areas in the growing cells. To overcome the poor staining of endospores, heat is used to slightly spread open the exosporium and allow the stain malachite green to enter the endospore. When removed from the heat the exosporium closes trapping the stain (malachite green) inside. Notice that here the heat is acting as a mordant to the malachite green. A secondary stain, like safranin is used to stain the vegetative cells.

Lab Assignment - Quick Spore Stain

1. Prepare a smear from a known culture. Allow it to air dry.
2. Clamp your slide with a clothespin and pass it through a Bunsen burner flame slowly 10 times.
3. Put a drop of water on the end of your slide. If the water boils your slide is too hot. When the water does not boil . . .
4. Immediately (while the slide is still hot) flood your smear with malachite green and allow it to sit for 5 min.
5. Wash the smear with water.
6. Stain with safranin for one minute.
7. Wash the smear with water.
8. Blot dry and observe under the microscope. If spores are present, they will appear green and the vegetative cells will be red.
9. Report your findings (spore + or -) on the chalk board.

Ultraviolet Radiation

Ultraviolet (UV) light can damage DNA and thus upon exposure kill cells. UV is nonionizing short wavelength light. It falls between 4 nm and 400 nm. Visible light begins at 400 nm. Thus we cannot see UV light.
The most mutagenic affect of UV light occurs at 260 nm which is absorbed by DNA. When two pyrimidines (Ts or Cs) are beside one another in the DNA sequence, UV light causes the formation of a covalent bond between the bases themselves. Normally the bases are attached to each other through their deoxyribose sugar, phosphate backbone and not directly. UV light formes a covalent bond directly between the bases. These bonded bases are called **dimers**. The dimers deform the DNA such that the transcription and replication enzymes can not determine what bases are supposed to be there. As a result the bacteria cannot divide, nor can it express those affected genes.
Cells have in their DNA, several genes that code for enzymes that can repair these mutations in the nucleotide sequence. These repair enzymes are quite effective under normal exposure. However, when bombarded with enough UV radiation, so many dimers (mutations) are formed, that the repair enzymes cannot keep up. Under these conditions the cells die.
UV light is often used to sterilize surfaces (like laminar flow hoods) or liquids (like in a sewage treatment plant). The killing ability of UV light depends on several factors: Time is an important factor. Exposure has to induce the formation of more dimers than the repair enzymes can fix. Blocking agents can also abrogate the effect of UV light. One item not easily apparent is clear plastic. While visible light can pass through clear plastic, UV light is blacked. Thus, it is used to protect our eyes from UV light damage. Finally, endospores themselves are resistant to UV light damage.

Lab Assignment - UV exposure

1. Obtain four NA plates.
2. Transfer 100 µl of liquid culture to each plate and spread around with a EtOH sterilized hockey stick.
3. Allow the plate to dry.
4. Remove the lid from a plate and place half the plate upside down on the UV portion of the transilluminator for 15 seconds. Then replace the lid.
   - Be sure to remove the lid. Remember plastic can block UV light.
6. Repeat step 4 with another plate for 30 seconds of exposure.
7. Repeat step 4 with another plate for 1 min. of exposure.
8. With your fourth plate, expose half of the plate for 2 minutes.
9. Incubate your plates inverted at 37°C.