LAB LECTURE NOTES FOR WEEK 9

THE ENRICHMENT/ISOLATION EXPERIMENTS IN GENERAL

1. For Experiments 11A, 11C and 15A (which we haven't mentioned much in lab yet – but will do so this week), refer to the table in Week 8's lab lecture notes on page 20.

2. Note the last appendix in the manual (**pages 125-128**) about the **Lab Report** associated with Exp. 11. More about these guidelines will be explained in lab as necessary.

EXPERIMENT 11A – PERIOD 2

(ISOLATION OF STREPTOMYCES)

1. **Selective isolation:** Accomplished by direct plating (no need for enrichment) on Actinomycete Isolation Agar which contains starch and sodium caseinate as major nutrients (sole sources of carbon and energy). For an organism to grow on this medium, it needs to have **extracellular enzymes to break down the starch** to simple sugars **and the casein** to amino acids. (Very few genera of bacteria can do this.) Then, the sugars and amino acids can be easily transported into the cell. (Recall amylase from Exp. 7 as the extracellular enzyme that breaks down starch – and how we did the test for starch breakdown.) A good explanation of Actinomycete Isolation Agar is on **page 107**.

2. We do the **dilution plating method primarily to get good isolated colonies** rather than to perform any quantitation. As for the *Bacillus* experiment (11C), we do not know how much soil we are putting in the screw-capped saline tube, nor do we know the amount of saline in the tube.

3. **Detection of *Streptomyces*** on the selective medium**:** Note that ***Streptomyces* will not be the only thing that will grow on the medium**. We must pick the typical and distinctive *Streptomyces*-like colonies that are isolated and totally avoid (1) the bacteria that produce "soft" colonies and also (2) molds from the soil. Even though a mold inhibitor is part of the isolation medium, we still get some mold growth on these plates. We will also **streak the culture** of *Streptomyces griseus* as a control culture to go along with our isolates.

4. Note that the plates we streak in Period 2 are onto an all-purpose medium. If we unknowingly pick some non-*Streptomyces* along with our *Streptomyces* colonies in Period 2 (very easy to do), they should all really grow well on this medium, and **we will then be able to avoid those non-*Streptomyces* colonies easier** on the all-purpose medium than if we used the initial selective medium again for re-streaking.

EXPERIMENT 11B – PERIOD 3

(ENRICHMENT AND ISOLATION OF PURPLE NON-SULFUR PHOTOSYNTHETIC BACTERIA)

1. As mentioned previously, this an example of a type of organism that can be recovered from nature easily – not only from its **natural habitat** but also where it can be likely found as a **significant contaminant**, as these organisms can be swept by water currents throughout the depths of ponds, lakes and streams, and then swept by air currents all over the general environment to eventually be found in snow, ice, hailstones and probably soil.

2. Purple non-sulfur photosynthetic bacteria are **extremely versatile**:

   a. They can switch between being **photoautotrophs**, **photoheterotrophs** and **chemoheterotrophs** depending on conditions. Recall these definitions from Appendix D as they apply to **energy source** and **carbon source**.

   b. **More specifically about catabolism**: Depending on the conditions they find themselves, this organisms are capable of switching between aerobic respiration and anoxygenic phototrophy. Anoxygenic phototrophy is another catabolic method that we are considering this semester which is associated with anaerobic growth. (The other two are fermentation and anaerobic respiration which we have considered already.)

3. In today's period, we simply examine our streak plates and pick a representative of the different colony types (no more than three) to make smears which we can heat-fix, stain and observe later. One of the colonies we choose is also used in our special "light vs. dark" test in which we observe growth reactions based on whether or not they can aerobically respire, and whether or not they can show anoxygenic phototrophy. This is, of course, not the oxygen relationship test that we have described earlier.

4. For identifying any isolate to genus (not all the way to species), we will have a handout. Also, the spiral-shaped genus (*Rhodospirillum*) will be shown on our website in a demonstration of **phototaxis**.

EXPERIMENT 11C – PERIOD 2
(ISOLATION OF BACILLUS)

1. Today we will look for any difference in the relative **variety** of colonies: simply greater vs. lesser between the plates inoculated from the unheated soil suspension and those inoculated from the "heat-shocked" soil suspension.

2. Noting any difference in the **CFUs/ml** of the soil suspension between the two sets of plates is probably going to be difficult due to how some of the colonies spread over the plate and also over other colonies.

   a. Do the best you can in **estimating** the number of colonies.

   b. What **types of CFUs** would be expected in the non-heat-shocked soil suspension? What **type of CFU** would be expected in the heat-shocked soil suspension?

3. Note that we are doing the **gram stain procedure rather than the endospore stain**. In the gram stain, the vegetative cells will show up stained, and the endospores will be recognized as unstained oval-shaped bodies within or outside of the vegetative cells. We are also setting up tests to determine the oxygen relationships of our isolates (each would be either a strict aerobe or a facultative anaerobe as we have defined those terms already) and also whether or not they can break down starch.

4. Proceed as indicated in the manual for the catalase, starch and glucose tests. Note why we do the "slide catalase test" (Method 2 in Appendix G) and how it can be done without the slide!

EXPERIMENT 15A – PERIOD 1
(WATER ANALYSIS)

1. In this experiment, we are using the principles of enrichment and isolation from Experiment 11 to detect and isolate **coliforms**. Included in the definition of coliform is the fact that **they are gram-negative, and they ferment lactose to acid and gas**. Therefore we start off by inoculating a broth medium which is selective for gram-negative bacteria, and it includes lactose whose gas from fermentation is detected by the formation of a bubble in the Durham tube.

2. More about this next week. Note that we are **skipping the dilution plating** of the water sample.

EXPERIMENT 8A – PERIOD 2
(CONJUGATION AND RECOMBINATION)

1. **Conjugation** is just one of several ways in which organisms can **transfer** DNA from one to another. Details about these DNA transfer processes (which also include transformation and transduction) are best left to the lecture course. **Recombination** is a completely separate process and involves what goes on in the recipient cell (a **genetic exchange**) when DNA from the donor cell is inserted into the recipient's chromosome.

2. It should be pointed out that our Hfr (donor) strain also happens to be a mutant in that it is unable to synthesize the amino acid methionine, and our F-minus (recipient) strain is also a mutant, being unable to synthesize the amino acid threonine. Thus, neither strain will grow on a minimal medium which is formulated for "normal" strains of *E. coli* which can make all of their amino acids from the medium components. (See Appendix D.)

3. In our experiment, note that there is growth on the cross-streaked plate of Minimal Medium where the two strains are mixed, not where they are still "pure." This represents growth (into colonies) of F-minus cells which have <u>received the gene for threonine synthesis from the Hfr cells</u> and thus can grow like "normal" strains of *E. coli* – able to produce all of their amino acids from the medium components.