LAB LECTURE NOTES FOR WEEK 8

covering the laboratory work for this week including some general information regarding **Enrichment and Isolation of Bacteria** *and* **pH-based Differential Media**. What we can't get through today in lab, we will finish up discussing after Spring Break.

EXPERIMENT 8A – PERIOD 1

(DEMONSTRATION OF CONJUGATION AND RECOMBINATION)

1. Set up in groups of four. Just simple streaking today according to directions in manual.
2. Discussion after break.

EXPERIMENT 11B – PERIOD 2

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

1. **The General Plan**: Following are some of the things we need to consider when isolating a particular type of organism from a natural source, and for each of these items we tailor our procedures to go along with the type of organism we are after such that growth is enhanced and growth of competing organisms is suppressed. These are discussed in some detail in the introduction to Experiment 11 and more specifically in the opening paragraphs of Exps. 11A, 11B and 11C. **Today we note these things relevant to Experiment 11B which concerns the Purple Non-Sulfur Photosynthetic Bacteria.**

   a. **Appropriate sample containing our organism**? This can be the habitat or a sample from where we expect significant contamination of the organism. Examples:

      (1) The habitat for **purple non-sulfur photosynthetic bacteria** is mostly shallow ponds and lakes where anaerobic conditions prevail at the bottom and there is still enough light for photosynthesis. As purple non-sulfur photosynthetic bacteria can also respire with oxygen, they are not inhibited by aerobic conditions. We can find them as significant contaminants in various stages of the water cycle and we are usually successful in getting them from snow, rain, icicles and hailstones.

      (2) Also, *Streptomyces* and *Bacillus* (two other organisms we isolate in Exp. 11) have their habitat in the soil where they grow to large concentrations as they participate in biodegradation.

   b. **Do we need to use an enrichment**? Keep in mind the strict definition of enrichment which is discussed in the introduction to Experiment 11.

      (1) Our bottles in Exp. 11B contain a broth (that is, liquid) medium that enhances the growth of the organisms we are after in that it includes sodium **succinate** as **carbon source** and **electron donor**. **Anaerobic conditions** and **light** are necessary for these organisms to photosynthesize, and they show themselves by their **reddish pigment** which are photosynthetic pigments. This is specifically a **selective enrichment** in that many other organisms are inhibited such as those that can't grow anaerobically and those that can't use succinate as a carbon source or electron donor. **We continue these medium and incubation conditions** when we streak for isolated colonies on plates containing the same medium (plus agar) and incubate them anaerobically with light.

      (2) The other two experiments don't utilize an enrichment as those organisms are in such high numbers, we have to make dilutions in order to get plates that have isolated colonies of them. So, instead of enrichment and then plating, we do a direct plating.

   c. **Selection**. This can be accomplished by the use of **selective media and/or special procedures** that will inhibit organisms that we don't want to interfere with what we are after. Each part of Exp. 11 has its own selective features to get the organisms we want and inhibit as many other types as possible so they don't "get in the way."

d. **Detection**: Even though we may use selective methods, we still examine our isolation plates for appropriate kinds of colonies that are consistent with what we are after. So, **for the photosynthetic bacteria, we will look for reddish colonies**.

e. **Isolation and Testing**: We eventually will run tests and observations to see that we did isolate the intended type of organism in each experiment. We will also run a special test for a characteristic that may be present, such as the expected **confirmation of anoxygenic phototrophy** for the photosynthetic bacteria. (We also test for antibiotic production for *Streptomyces* and starch breakdown for *Bacillus* in the other two parts of Exp. 11.)

2. We will follow this up with a **Lab Report** which will be due near the end of the Semester. **Appendix I** (the last appendix in the manual) gives guidelines.

3. The following table *presented in these lab lecture notes* is meant to reiterate the **general plan** in the isolation of microorganisms – emphasizing what we have to consider **specifically** for the types of organisms in Experiment 11 (and also Exp. 15A which follows the same general plan). **Today *in lab* we emphasize the photosynthetic bacteria** (Exp. 11B), and **the rest of the table will be finally discussed by the end of next period**.

ENRICHMENT AND ISOLATION OF BACTERIA IN EXPERIMENT 11.
Also adding Experiment 15A which follows the same general concept.

	11A: *Streptomyces*	**11B:** The purple non-sulfur photosynthetic bacteria	**11C:** *Bacillus*	**15A:** Coliforms*
source	soil	water	soil	water
need for enrichment?	no	yes	no	yes
cell type(s)	**vegetative cells** (filamentous) **reproductive spores**	**vegetative cells** (rods, spirals, and other types)	**vegetative cells** (rods) **endospores**	**vegetative cells** (rods)
selection, enhancement and detection	selective medium with **starch and casein** (p. **107**) distinct, **hard** colony	set up media & conditions for **photoorganotrophs**, using **anaerobic incubation and light** and also potassium or sodium **succinate as carbon source and electron donor**. **red**-pigmented growth	select for **endospores** with use of 80°C water bath **aerobic** incubation	**inhibit gram-positive** organisms include **lactose** in the media along with **Durham tube or pH indicator** as needed

* By definition, coliforms ferment lactose with production of acid and gas. Therefore they are detected in lactose-containing broth media with Durham tube and on solid media with pH indicator.

4. **One more thing about Experiment 11B today:** Wet mounts of the enrichments are optional. They usually don't tell us much, although we sometimes recognize certain cell shapes as typical of a couple specific genera of these organisms as described in the lab manual. *Rhodospirillum* is spiral-shaped, and *Rhodomicrobium* is a very short rod with the rods connected by extremely thin filaments.

EXPERIMENTS 11A AND 11C – PERIOD 1 FOR EACH
(ISOLATION OF *STREPTOMYCES* AND *BACILLUS* FROM SOIL)

1. **Both of these experiments can be set up to share the same soil sample and also some of the dilutions:**

   a. Dilutions of a soil sample and platings are made for Experiment 11A, and then (with one more dilution blank) we can use the same dilutions to do the plating for Exp. 11C.

   b. Then, there is a heating step where we "heat-shock" the soil sample and make new dilutions and platings for Exp. 11C.

2. We do the dilution plating method primarily to get well-isolated colonies, and quantitation will only be important for Exp. 11C. As will be explained, **selective methods** are used in each experiment to obtain the desired organism (a selective medium in 11A, and a "selective procedure" in 11C).

3. These plates must be specially incubated, as we don't want overgrowth or drying out of the plates. Trays will be provided up front.

4. More about Experiments 11A and 11C after Spring Break when we have more time.

pH-BASED DIFFERENTIAL MEDIA – THE INFLUENCE OF ALKALINE AND ACIDIC REACTIONS IN CERTAIN GLUCOSE-CONTAINING TEST MEDIA (especially related to experiment 7)

There will be a brief demonstration based on the information on the whiteboard in the back of the lab that is effectively summarized on this web page: http://www.jlindquist.com/microbiology102/GFBphotoessay.pdf