LAB LECTURE NOTES FOR WEEK 10

EXPERIMENT 11A – PERIOD 3

(ISOLATION OF STREPTOMYCES)

1. Note that the plates we streaked in the previous period were onto an all-purpose medium. If we had unknowingly picked some non-*Streptomyces* along with our desired colonies (which is very easy to do), they should all really grow well on this medium, and **we can then avoid those non-*Streptomyces* colonies easier** on the all-purpose medium than if we used the initial selective medium again for re-streaking.

2. Today we need to pick **isolated** *Streptomyces*-like colonies to continue, and then we make just a **single streak** across the middle of the new plates to grow up cultures to test for antibiotic activity. The **known culture** (*Streptomyces griseus*, which we made available last period is a "control") will simply **demonstrate what positive and negative antibiotic activity look like** and should also have a general colony type consistent with what we have been trying to maintain all along.

EXPERIMENT 11B – PERIOD 4

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

1. The following Q and A should help in interpreting the results of the "light/dark test" on our isolates of purple non-sulfur photosynthetic bacteria:
   a. Is anaerobic growth seen in one or both tubes? Is it red-pigmented? (Note: Anaerobic, red-pigmented growth is what we have seen on the plates and in the original enrichment, and it has been our main indicator for the presence of these organisms.) What catabolic process is responsible? The three catabolic processes associated with anaerobic growth are **anaerobic respiration**, **fermentation** and **anoxygenic phototrophy**.

      Answer: As there is nothing in the medium to promote anaerobic respiration or fermentation (that is, there are no such items as nitrate or sugars), the only appropriate choice would be **anoxygenic phototrophy**. So, in which tube – light or dark – do you see the anaerobic, red-pigmented growth? It only occurs in one condition and not the other.

   b. Is aerobic growth seen in one or both tubes of the Succinate Agar? If so, what catabolic process is responsible? The two catabolic processes associated with aerobic growth are **aerobic respiration** and **oxygenic phototrophy**.

      Answer: **Aerobic respiration** is what is responsible for the aerobic growth. (We aren't dealing with oxygenic phototrophs in this course; these are organisms such as algae and cyanobacteria that are plant-like in that they use water as the electron donor, and when that happens the water is oxidized with the formation of O_2.)

2. As the typical purple non-sulfur photosynthetic bacterium can behave as a phototroph (performing anoxygenic phototrophy) and as a chemotroph (performing aerobic respiration), it is then termed a **facultative phototroph**.

3. With the tubes that were incubated in the light, we may see considerable **gas production**. This is **molecular hydrogen (H_2)!**

EXPERIMENT 11C – PERIOD 2, POSTPONED FROM LAST WEEK

(ISOLATION OF BACILLUS; COPIED FROM WEEK 9 NOTES)

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. Next week we will check the isolates 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!

We will just touch on the following two experiments today,
and it will only take a few minutes to set them up for next week.

EXPERIMENT 15A – PERIOD 2

(WATER ANALYSIS – SPECIFICALLY COLIFORM ENRICHMENT AND ISOLATION)

1. Recall that we are skipping the dilution plating (which according to the manual is "optional") and are just doing the enrichment and isolation procedure for coliforms in this experiment.

2. Today we check our LLTB tubes to see if we have presumptive evidence of coliforms from the water sample we inoculated into it. This medium is designed for the selective **enrichment** and **isolation** of coliforms, as it is **inhibitory to gram-positive bacteria** and contain **lactose to detect lactose fermentation**, as coliforms are **gram-negative** bacteria that **ferment lactose to acid and gas**.

3. Today we do a **confirmatory** test in media which are similar to LLTB but more highly selective for gram-negative bacteria:

   a. We inoculate **Brilliant Green Lactose Bile Broth** (BGLB) to **confirm** whether or not we have **coliforms** in the LLTB tube.

   b. We also inoculate **EC Broth** and incubate it at 44.5°C to **confirm** whether or not we have **fecal coliforms** in the LLTB tube. Fecal coliforms are a subset of coliforms which are generally associated with fecal matter.

   c. Next period, **growth and gas** will indicate a positive result for coliforms in general (in the BGLB Broth) and fecal coliforms (in the EC Broth).

4. We start isolation from these enrichments next week.

EXPERIMENT 14A – PERIOD 1

(ISOLATION AND IDENTIFICATION OF THE "ENTERICS")

1. Each person has an unknown containing a mixture of organisms which we need to streak out for **isolated colonies.** (The three-phase method is always preferred as we learned back in Exp. 3A.) Note the handout, and there will be more about what we are doing here next time.