LAB LECTURE NOTES FOR WEEKS 13 AND 14

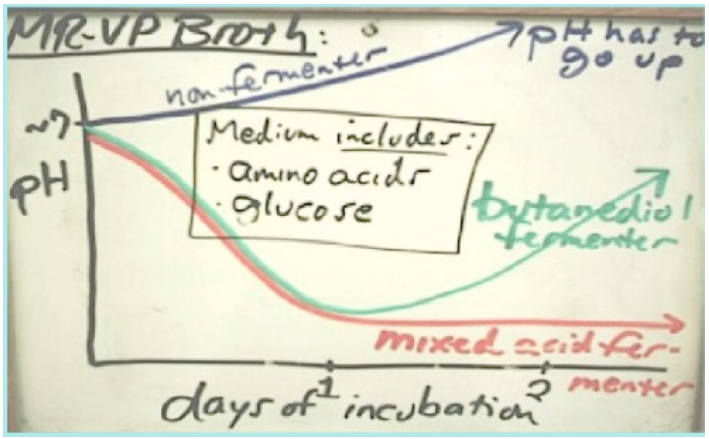
EXPERIMENT 14A – PERIOD 4

(ISOLATION AND IDENTIFICATION OF "ENTERICS")

1. In Period 4, we are reading the tubes of the test media according to the procedure in the lab manual. Among other things, we can tell if (1) lysine and/or ornithine are **decarboxylated** by the unknown (an **anaerobic alkaline reaction**) – and also if (2) the unknown is a "**mixed-acid fermenter**" or a "**butanediol-fermenter**" with the **methyl red test**:

   a. **Amino acid decarboxylation** is another reason why an organism can produce an **alkaline** reaction in a medium. Unlike amino acid deamination, this occurs under **anaerobic** conditions. Decarboxylation of certain amino acids is useful in enteric identification tests, and our usual whiteboard summary of our tests for ornithine and lysine decarboxylation is shown here: http://www.jlindquist.com/microbiology102/M102images/NewDecarbox.jpg

   b. Our usual diagram on the **methyl red test** is shown on the right, and it goes along with the explanation given in the manual. The methyl red reagent is added to the MR-VP medium culture after at least two days of incubation, as differentiation between the two types of fermentation is not achieved at one day.



EXPERIMENT 15A – PERIOD 5

(ENRICHMENT AND ISOLATION OF COLIFORMS)

1. We do some of the same tests that are used to identify enterics. This time our identifications are only tentative. One usually finds that coliform isolates turn out to be identified as various species of the enteric group.
2. Coliforms should never be thought of as a subcategory of the enteric group (or ever as a taxonomic group) as explained in the introduction to Exp. 15 in the manual.

EXPERIMENT 17A – PERIODS 2+3

(THE LAST ISOLATION EXPERIMENT)

1. This "experiment" is basically a review of some things we have done before. There are no precise identifications to be made, but we can determine whether or not our isolates from HIA and/or MacConkey Agar can perform any (or all) of the **three catabolic processes associated with chemotrophy**: aerobic respiration, anaerobic respiration and fermentation. For any isolate, we could have **any number** of these three processes checked off. (There are many organisms that can perform all three, such as enterics according to the Experiment 14 handout.)

   a. The **catalase** test will be positive for those that can perform respiration – **at least aerobic respiration**, as we will have to test for anaerobic respiration separately. (And we do.)

   b. **Nitrate reduction** will be positive for those that can perform **anaerobic respiration** which is having the ability to utilize an "oxygen substitute" as the electron acceptor in respiration. Recall Experiment 7A where either nitrite (NO_2^-) formation or N_2 gas production constitute a positive reaction for nitrate reduction.

   c. We use the **glucose fermentation** test to see if any of our isolates can perform **fermentation**.

2. We will also determine gram reaction and shape for each isolate, as well as any outstanding features that may appear (such as unusual colony pigmentation).

3. For the checklist that will be handed in on Period 4, we don't grade on accuracy of the identification which is certainly not done with any precision. Note that the identification handout only lists a limited number of genera which can at least get us in the ballpark of the actual genus for each isolate pending the performance of tests we would not have time or money for.

EXPERIMENT 9A – PERIODS 1
(ENUMERATION OF BACTERIOPHAGES)

1. We are setting up a modification of the usual dilution plating experiment. We are working with **bacteriophages** (viruses which infect bacteria) which require a living bacterial "host" in order to replicate. So in this experiment, we use *Escherichia coli* strain B as the "medium" which the phage (strain JL-1) can utilize in order to make more copies of itself. Instead of inoculating directly from the dilution of bacteriophage onto the plate, we inoculate it into a tube of top agar which includes the host culture, and then the **whole mixture gets dumped** onto the bottom agar plate. The colonies of bacteriophage will show up as "gaps" in the otherwise solid growth of the host culture on the plate. This will be more easily visualized in Period 2.

2. Note that it could not matter how much top agar is used or how much host culture is inoculated into the top agar. (Such would never be considered as we have learned from dilution theory before.) All that matters mathematically is **how much of the phage dilution** eventually winds up on the plate, and it is 0.1 ml in each case.

3. We are using the **same dilution theory principles** as we do when we count bacterial colonies to determine CFU's per ml of a sample. So, in period 2 we will be simply counting plaques on the countable plate and then determining the no. of plaque-forming units (PFUs) per ml of the original phage suspension. (Just like with bacterial cells, we consider this a **suspension**, not a solution!) Plaques are basically colonies of phages.

Analogies:
cell ~ phage
CFU ~ PFU
colony ~ plaque