

LAB LECTURE NOTES FOR WEEK 11

EXPERIMENT 11A – PERIOD 4

(ISOLATION OF STREPTOMYCES)

1. When doing the cross-streaks of each of the four test organisms in Period 4, we streak from the **edge of the plate to the very edge of the center streak** of the *Streptomyces* isolate – and only in one direction (out to in). These test organisms will be used to see if they are inhibited in the presence of the *Streptomyces* culture. The known culture (the only one we can label with a species name – i.e., *Streptomyces griseus*) will demonstrate what inhibition and non-inhibition of the test organisms by an antibiotic will look like, and we should not expect our *Streptomyces* isolates to affect the test organisms in the same way. Different antibiotics can affect different test organisms, and many strains of *Streptomyces* do not show any antibiotic production at all!
2. If we are not putting the plates in a special tray up front for 2-day incubation, the regular incubators will be used, and they will be cooled down to refrigerator temperature after a couple days.

EXPERIMENT 11C – PERIOD 3

(ISOLATION OF BACILLUS)

1. 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!
2. We know all of our isolates have been grown in an aerobic environment (tolerating oxygen). As all species of *Bacillus* can perform **aerobic respiration**, we will expect to see positive catalase reactions for our isolates. So, how can we tell if any isolate is a **strict aerobe** or a **facultative anaerobe**? Remember (from Exps. 5A and 7) that one type can ferment and the other cannot; thus we use the Glucose Fermentation Broth to distinguish between the two types.

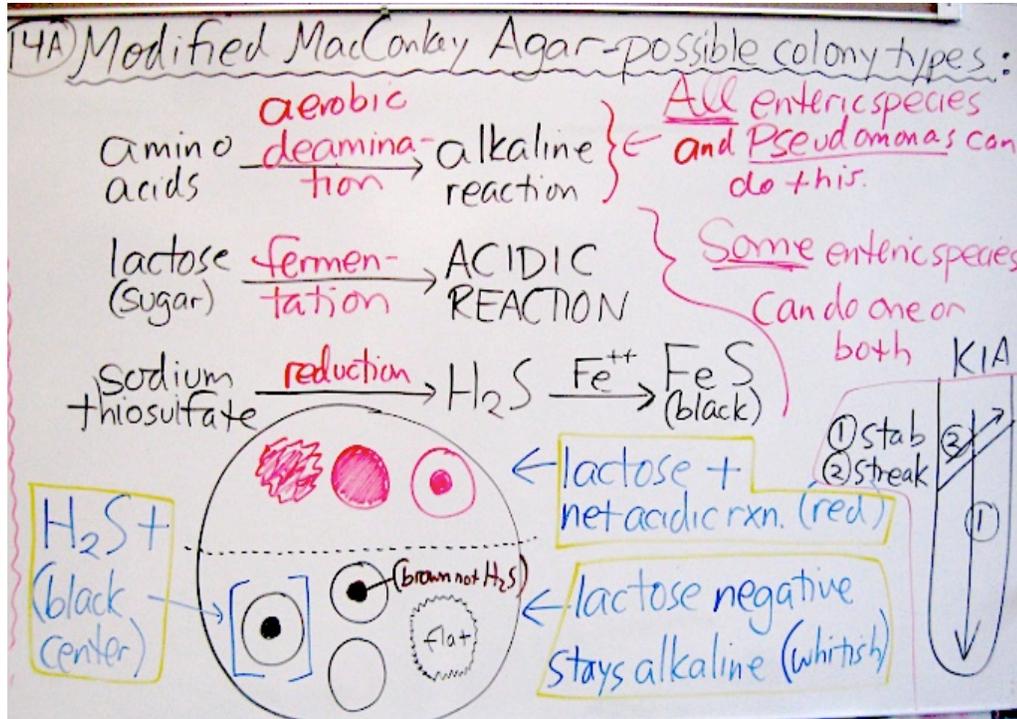
EXPERIMENT 15A – PERIOD 3

(ENRICHMENT AND ISOLATION OF COLIFORMS)

1. As for the LLTB Broth (which we inoculated these tubes from), a “**positive**” result in BGLB and EC Broth will be indicated by **growth and gas**.
 - a. A positive result in BGLB indicates the presence of **coliforms** among the various kinds of gram-negative organisms able to grow in the medium, as coliforms would be the ones fermenting lactose to form acid and the detectable gas.
 - b. The same goes for the EC Broth; however, this medium (along with the higher incubation temperature) is more selective, and a positive result will indicate the presence of those coliforms which fit the description of **fecal coliforms**.
2. Of course, selective enrichments are not pure cultures. Streaking plates of EMB Agar from BGLB Broth and/or EC Broth should result in **isolated colonies** of the various kinds of gram-negative organisms that are present. EMB Agar is a lot like MacConkey Agar in that it allows only growth of gram-negative bacteria, and it also distinguishes between lactose-positive and lactose-negative colonies. The lactose-positive colonies will be the coliforms that we will continue on with. If you have no gas (with or without growth) in the EC Broth, that’s OK; that just means no fecal coliforms are confirmed to be present, and you can streak both EMB plates with your BGLB tube.
3. Be sure to bring the plates up front for 1-2 day incubation.

EXPERIMENT 14A – PERIOD 2
(ISOLATION AND IDENTIFICATION OF “ENTERICS”)

- Today we try to find three different types of colonies on the Modified MacConkey Agar. As in regular MacConkey Agar, we can tell whether or not **lactose** is fermented. Also, the medium has been modified to detect **hydrogen sulfide**, so if the organisms in a colony can reduce thiosulfate, the hydrogen sulfide which is produced will react with the iron in the medium, producing a black color in the colony.



- From each of the three selected colonies, inoculate a tube of KIA as directed in the manual. This medium cannot be incubated for more than a day, so the tubes must be brought up front for one-day incubation.

Try to bring in a sample for Experiment 17A which starts next week. As mentioned on page 82, a solid sample from outdoors is recommended. A food sample can be substituted if it is not made from a controlled fermentation such as yogurt.