

Lecture 20

I. Biochemical tests for Gram Positive rods

A. NSM (Nutrient Sporulation Media)

1. Used to induce spore formation
2. Limited nutrients are required “stress” the cells
3. Recall that some genera produce dormant spores as a means of protection.
4. Must stain culture to determine if the organism is capable of forming spores

Review: What stain would you use to determine if organisms grown on an NSM agar produce spores?

II. Biochemical test for gram negative rods

A. Methyl Red Voges-Proskauer (MR-VP)

1. Used to determine _____ is used _____.

Review: Which tests would you likely perform before an MR-VP test?

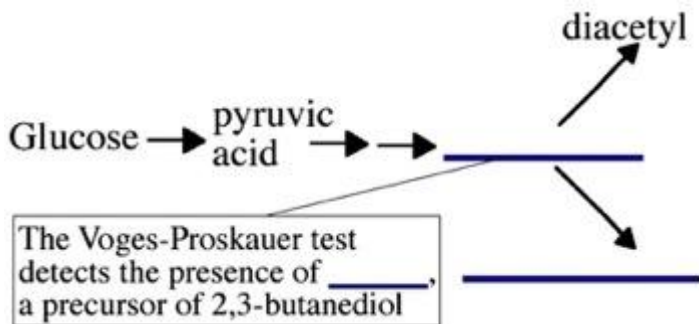
2. _____ Fermentation Pathway

i. In this pathway, glucose is fermented to produce several _____ (lactic, acetic, succinic, and formic acids). The stable production of enough acid to overcome the phosphate buffer will result in a _____.

ii. _____ is a pH indicator. If this indicator is added to the culture broth and the pH is below 4.4, a _____. If the MR turns _____, pH is above 6.0 and the mixed acid fermentation pathway has _____ been utilized.

3. _____ Fermentation Pathway

i. In this pathway, glucose is fermented to produce a _____ instead of organic acids.



Note: A culture will usually only be _____: either MR-positive or VP-positive. But remember, some bacteria are non-fermenters and will thus _____.

ii. In order to detect acetoin, alpha-naphthol and KOH are added. Acetoin reacts with the alpha-naphthol in the presence of KOH to produce a red color. Thus, if the culture is _____, it will turn _____.

B. The MR-VP test is used to differentiate between _____ organisms based on the way they _____ (See How Does It Work?, below).

Interpreting MR-VP results:

Color	Addition of Methyl Red		Addition of α -naphthol and KOH	
	Yellow	Red	Brown-green/yellow	Brown-pink
Result	_____, not able to perform _____ fermentation	_____, able to perform mixed acid fermentation	_____, from the degradation of glucose	_____, forms acetoin from the degradation of glucose

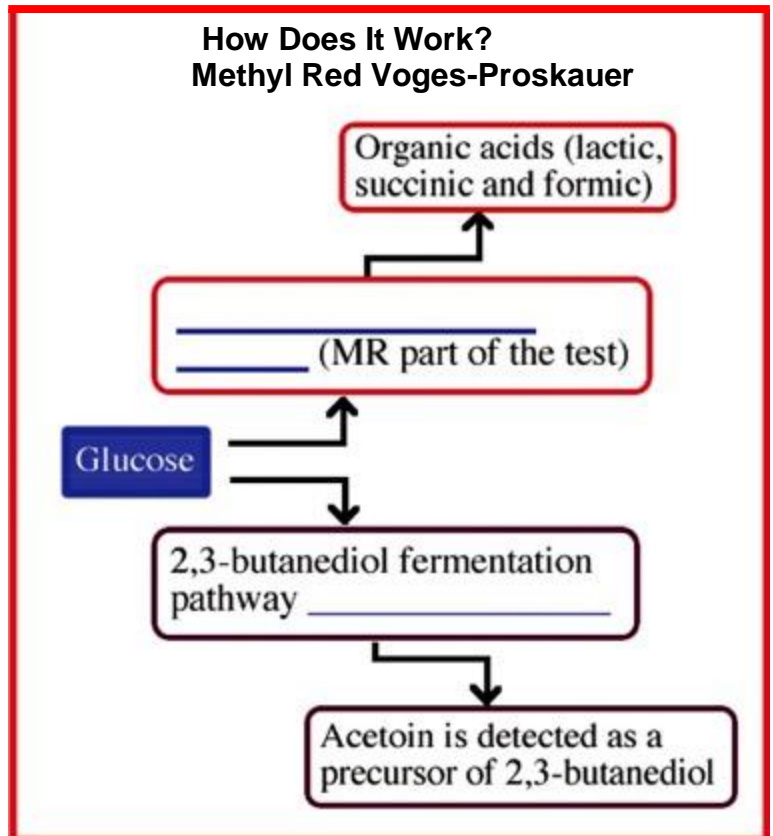
Note: Organisms can usually only be _____. However, organisms can be MR- and VP-.

For example:

Escherichia coli is _____

Enterobacter aerogenes is _____ and VP+.

Pseudomonas aeruginosa is MR- and _____



Things to remember today:

1. Perform _____ needed to help determine your unknown.
2. Determine your unknown using the dichotomous keys in the Appendix and _____.
3. Consult the lab notebook for a description of the report format. If you have any questions about the report, be sure to ask today.
4. Remember the late policy: _____ that a report is late. Late reports are not accepted after one full week.
5. Reports are due on _____.
6. Once you have correctly identified your unknown, do the following: remove all tape — including that on the original plates and stocks — and dispose of it in the trash; discard all plates and slants in the biohazard containers; and wash all microscope slides. Two points will be taken off the unknown report for failure to properly clean up.