

Fermentation Tests (Phenol Red Broth with various carbohydrates)

Fermentations are energy-producing biochemical reactions in which organic molecules serve both as electron acceptors and donors. This typically means that an energy-rich organic substrate (such as a carbohydrate) is partially oxidized (loss of electrons) to yield a small amount of ATP, while the smaller metabolites of the incompletely oxidized substrate are reduced (gain of electrons) and released to the culture medium as waste products (Fig. 6-36 of the Atlas, p. 58). Sometimes these fermentation waste products are useful as beverages or flavoring, but they are not useful to the organism that made the fermentations products. Fermentation is a rather inefficient method of ATP production since the original substrate is not completely oxidized to carbon dioxide and metabolites are transformed into waste products. The ability of microorganisms to ferment carbohydrates and the types of products formed are very useful in identification. A given carbohydrate may be fermented to a number of different end products, depending on the microorganism involved. These end products (alcohols, organic acids, gases or other organic molecules are characteristic of the particular microorganisms. For example, if fermenting bacteria are grown in a liquid medium containing the carbohydrate glucose, they may produce organic acids as by-products of the fermentation. Consult Fig. A-5 and Table A-6, p. 207-208 of the Atlas for various types of fermentation reactions. These acids are released into the medium and lower its pH. If a pH indicator such as phenol red or bromcresol purple is included in the medium, the organic acid production will change the medium from its original color to yellow (Fig. 6-38 of the Atlas, p. 59).

Gases produced during the fermentation process can be detected by using a small, inverted tube called a Durham tube (named after Herbert Edward Durham, English bacteriologist, 1866-1945), within the liquid culture medium. After adding the proper amount of broth, Durham tubes are inserted into each culture tube. During autoclaving, the air is expelled from the Durham tubes, and they become filled with the medium. If gas is produced, the liquid medium inside the Durham tube will be displaced, entrapping the gas in the form of a bubble (p. 57 of the Atlas).

Results for each test organism are typically recorded as:

acid (yellow) / no gas

acid (yellow) / gas

no acid (red orange) / gas

no acid (red orange) / no gas.

It is useful to compare tubes against uninoculated control tubes of the same batch of medium. Note that after recording results, you should also check to see if the tubes are turbid, indicating growth. It may be necessary to tap the bottom of the culture tube several times to suspend cell that have settled to the bottom. If the tube is not turbid after tapping the tube several times, then the organism did not grow in the fermentation medium, and no conclusions about its fermentation ability can be made.

Also note that the formation of an intense pink color in phenol red tubes that have been incubated longer than 18 hours may be due to reversion (p. 57 of the Atlas) caused by the organism running out of the carbohydrate and switching over to metabolizing amino acids derived from the peptones included in the culture medium. Deamination of amino acids to ammonia results in the formation of alkaline conditions, causing the phenol red indicator to turn a bright pink color. It is possible for an organism to form acidic fermentation products

during carbohydrate metabolism followed by the formation of alkaline deamination products. Once again it is useful to compare the inoculated Phenol Red tubes to uninoculated control tubes of the same batch of medium.

Procedure:

To demonstrate the various fermentations reactions in the Phenol Red carbohydrate tubes we will use *Escherichia coli*, *Proteus vulgaris*, *Alcaligenes faecalis*, and *Staphylococcus epidermidis* as the test organisms.

1. Label the tubes as you take them from the rack. They are color-coded by cap. Do not mix up the tubes. Proper labeling is important.
2. Inoculate a loopful of one organism into Phenol Red Glucose (PR Glucose), PR Sucrose, and PR Lactose broth tubes. Inoculate each of the other organisms into their separate PR Glucose, PR Sucrose, and PR Lactose tubes. Be sure to label the tubes clearly and accurately. Also be sure not to cross contaminate the tubes with multiple organisms.
3. Incubate the tubes at 35 °C for 18 hours.
4. During the next laboratory period, observe tubes for the formation of a yellow color (acid) and/or gas production. Compare to uninoculated control tubes. Also check that the tubes are turbid. You may have to gently tap the bottom of the tube a few times to suspend any cells that have settled to the bottom. The tubes should be turbid otherwise you cannot make any conclusions about the fermentations reactions.
5. Record your results on the next page.

To demonstrate the carbohydrate utilization and fermentation ability of the Eukaryotic yeast, *Saccharomyces cerevisiae* (common baker's yeast), we will use fermentation broth media containing glucose or lactose, but lacking in the indicator Phenol Red.

1. Label the tubes as you take them from the rack. They are color-coded by cap. Do not mix up the tubes. Proper labeling is important.
2. Inoculate a loopful of *Saccharomyces cerevisiae* into both Fermentation Glucose and Fermentation Lactose broth tubes (remember these tubes lack the phenol red indicator). Be sure to label the tubes clearly and accurately. Also be sure not to cross contaminate the tubes with other organisms.
3. Incubate the tubes at 35 °C for 18 hours.
4. During the next laboratory period, observe tubes for the formation of turbidity and/or gas production. Compare to uninoculated control tubes. You may have to gently tap the bottom of the tube a few times to suspend any cells that have settled to the bottom. The tubes should be turbid otherwise you cannot make any conclusions about the fermentations reactions.
5. Record your results on the next page.

