MODULE 34

Carbohydrate Fermentation

PREREQUISITE SKILL

Mastery of Module 2, "Preparing and Dispensing Media," and Module 7, "Aseptic Transfer of Microbes."

MATERIALS

sterile fermentation tubes of:*†
- glucose (dextrose) broth (5)
- lactose broth (5)
- sucrose broth (5)

slant cultures of:
- *Escherichia coli*
- *Proteus vulgaris*
- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*

For related experience:
- carbohydrate fermentation disks for rapid test‡

phenol red agar plate (4)*

tubes containing 1 ml sterile water and swab (4)*

* Prepared by the student if the instructor so indicates.

† Directions are at the end of the module.

‡ Available from Difco Laboratories, Detroit, Michigan.

OVERALL OBJECTIVE

Demonstrate that different bacteria produce enzymes that ferment specific carbohydrates.

Specific Objectives

1. Describe why different bacteria produce different enzymes.
2. Correlate the reason why different bacteria produce different enzymes with the individuality of bacteria.
3. Describe how the individuality of bacteria is used to identify closely related species.
4. List the ingredients of a Durham fermentation test.
5. State all the information that can be obtained by growing bacteria in fermentation broths.
6. List seven fermentable carbohydrates.

7. Define the terms substrate, end product, endoenzyme, Durham tube, pH indicator, aliquot, catabolic, and altered substrate.
8. Describe a rapid disk test for carbohydrate fermentation.

DISCUSSION

The most important criteria in the identification of bacteria are their physiological differences. Physiological differences in bacteria are manifested in the nutrients they can utilize and the end products that result from metabolism of these nutrients, both of which depend on the enzymes the bacteria can manufacture. The type and number of enzymes produced depend on the genetic code of the bacterial cell.

Thus not all bacteria produce the same enzymes; different substrates (nutrients) are degraded, resulting in different end products. For example, some microbes produce gelatinase, and others produce lipase but not gelatinase. Careful study of the substrates an organism attacks can establish a pattern of metabolic activities. This pattern can be used to identify bacteria of closely related species.

Most bacteria are very much like tissue cells in that they use various carbohydrates as their main source of energy. The simple sugars you will be studying in Module 34, "Carbohydrate Fermentation," are small enough in molecular size to diffuse from the surrounding environment to the interior of the cell and there be used for energy. Lactose, a disaccharide, must be transported into the cell by permeases located in the cell membrane. The catabolic endoenzymes within the cell remove energy from these carbohydrates, which results in an alteration of the substrate molecule. The substrate molecule usually becomes smaller, and the altered substrate is an end product of metabolism.

Substrate
(e.g., carbohydrate)

enzyme ⇓

altered substrate + energy
(end product)

In the fermentation of a nutrient, a series of enzymatic reactions (enzyme systems) are involved in the production of end products.

The activities in the module will demonstrate clearly that different bacteria can ferment the same or different carbohydrates, depending on the enzymes they can produce. The activities will also show that even though different bacteria ferment the same carbohydrate, the end products of metabolism can be different for each bacterial type. Thus it is the intent of the module to exemplify the individuality of bacteria by studying the nutrients they use and the end products they produce.

To study the physiological differences between bacteria, you will be using fermentation tubes. A fermentation tube is a culture tube that contains the following:

1. A Durham tube: a small tube that is placed upside down in the culture tube. The purpose of the inverted Durham tube is to show if gas has been produced as an end product of metabolism. This does not allow you to determine which gas is evolving. The most common gases produced by bacteria are hydrogen, carbon dioxide, and methane.
2. Phenol red broth base: a medium that contains the ingredients of nutrient broth plus a pH indicator. The nutrient-broth-like ingredients support the growth of most bacteria. The pH indicator is phenol red, which is red at a pH near neutral and turns yellow if organic acids are produced.
3. A specific carbohydrate: such as glucose, lactose, maltose, sucrose, or mannitol. If a carbohydrate such as glucose is fermented, an acid environment results, and the broth turns yellow. Gas is never produced unless the carbohydrate has been fermented. At the end of the module, you will learn how to prepare the different fermentation tubes for the activity.

ACTIVITY

Inoculation of the Fermentation Tubes

Figure 34-1 shows the most typical results for this activity. See also color plate 7.

1. Inoculate separate tubes of the three different sugar fermentation broths with each of the organisms listed in the materials section.
   - Four different organisms are inoculated into three different sugar fermentation tubes.
2. Keep one uninoculated tube of each fermentation broth as a comparative control.
3. Incubate all tubes at 37°C for 48 hours.

At your next lab session, fill in the table on the worksheet, using the symbols beneath the table to record results. Compare the inoculated sugar tubes with the control tubes. Only a decided yellow color change occurring within 48 hours is considered positive.

If necessary, refer to Figure 34-1 for a schematic representation of the different results. Consult Table 56-1 in Module 56 to determine whether your results are correct. Draw a short conclusion on how physiological differences can be used to identify bacteria.

Take the post test after you have performed or read thoroughly the related experience.

Related Experience: Rapid Disk Test for Carbohydrate Fermentation

Small filter paper disks are impregnated with different sugars or sugar alcohols. They may be used in a variety of carbohydrate-free nutrient broths, semisolid media, or solid media. Here is the procedure for solid medium:

1. Inoculate a phenol red agar plate for confluent growth. Organisms from solid media should be dispersed in a small amount of sterile water to obtain an even seeding of the agar surface. Use tubed sterile water and a sterile swab to inoculate for confluent growth.
2. Place the desired carbohydrate disks on the surface of the inoculated plate with a flamed forceps. Press the disks gently to ensure contact with the agar surface.
3. Incubate at the optimum temperature for 4 hours.
4. Observe after 4 to 8 hours and again after 18 hours of incubation to detect possible reversion of reactions.
5. Repeat the procedure for the remaining three organisms.

A color change surrounding the disk (yellow) indicates acid production. Often this is enough information to differentiate sugar-fermenting bacteria from nonfermenters.

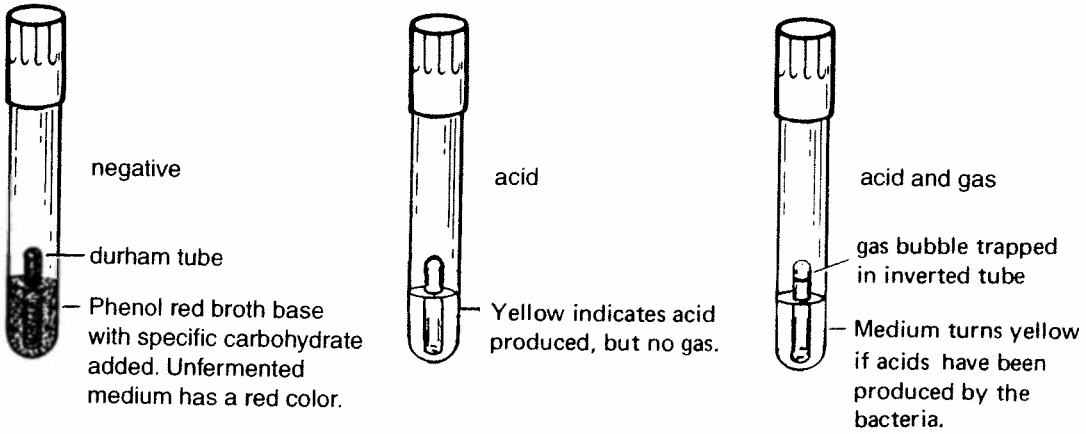

FIGURE 34-1
Three possible reactions in fermentation tubes. See color plate 7.

PREPARING CARBOHYDRATE FERMENTATION BROTH TUBES

1. According to the directions on the label of the medium bottle, rehydrate 300 ml of phenol red broth base.
2. Divide the phenol red broth into three 100 ml portions.
3. To each 100 ml aliquot of phenol red broth add 0.7 g of a different sugar (carbohydrate); i.e., glucose to one, lactose to another, and sucrose to the third. Mix each thoroughly to dissolve the sugar.
4. Place an inverted Durham tube in each of 15 culture tubes.
5. Dispense about 7 ml of glucose broth into five tubes. Label the glucose broth tubes immediately.
6. Dispense and label five tubes of lactose broth and five tubes of sucrose broth into the other culture tubes with inverted gas tubes. Label the tubes carefully because all tubes will look alike although the substrates differ.
7. Autoclave the carbohydrate fermentation tubes at 15 psi for 15 minutes. The pressure of the autoclave will force the air out of the Durham tube. Other carbohydrates such as fructose and xylose are decomposed by heat and must be sterilized by using a bacteriological filter.