

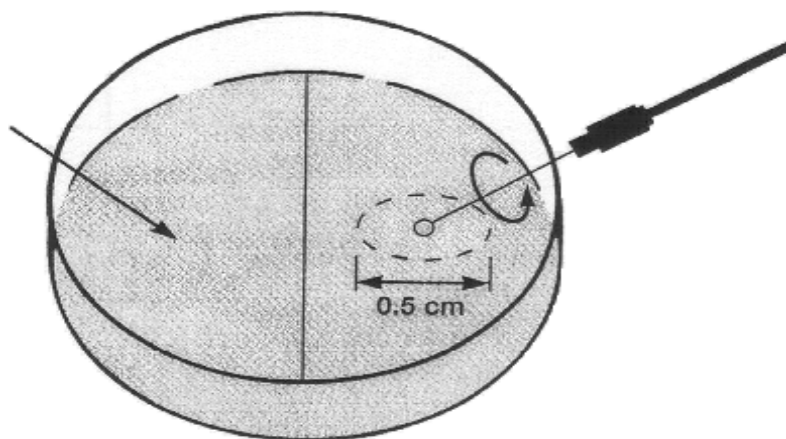
Lipid Hydrolysis, Lipase Test

Lipids are high molecular weight compounds possessing large amounts of stored energy. The two common lipids catabolized by bacteria are the triglycerides (triacylglycerols) and phospholipids. Triglycerides are hydrolyzed by the enzymes called lipases into glycerol and free fatty acid molecules as indicated in the following diagram. Glycerol and free fatty acid molecules can then be taken up by the bacterial cell and further metabolized through reactions of glycolysis, β -oxidation pathway, and the citric acid cycle (Fig. 6-47, p. 62-63). These lipids can also enter other metabolic pathways where they are used for the synthesis of cell membrane phospholipids. Since phospholipids are functional components of all cells, the ability of bacteria to hydrolyze host-cell phospholipids is an important factor in the spread of pathogenic bacteria. In addition, when lipase-producing bacteria contaminate food products, the lipolytic bacteria hydrolyze the lipids, causing spoilage termed **rancidity**.

When these same lipids are added to an agar- solidified culture medium and are cultured with lipolytic bacteria, the surrounding medium becomes acidic due to the release of fatty acids. By adding a pH indicator to the culture medium, it is possible to detect the hydrolysis of lipids by a color change. For example, spirit blue agar with Bacto lipase reagent has a lavender color. It turns royal blue around lipolytic bacterial colonies due to the acid pH.

First Period

1. With a marker, divide the bottom of a spirit blue agar plate in half and label half the plate *Bacillus subtilis* and the other half *Staphylococcus epidermidis*. Place your name and date on the plate. Do not make a mark on the plate indicating where the bacteria will be inoculated because this may interfere with interpretation of the results.
2. Spot-inoculate the spirit blue agar plates with the respective bacteria. Make a dime-sized circular spot of inoculum. (This circular pattern is preferred over the linear inoculation shown in Fig. 6-49)
3. Incubate the plate in an inverted position for 24 to 48 hours at 35°C.



Second Period

1. Examine the plate for evidence of lipid hydrolysis (Fig. 6-49). Hydrolysis is evidenced by an intense dark blue zone around the bacterial growth. If no lipid hydrolysis has taken place, the zone around the colony will remain lavender.
2. Record your results in the space below.

Review Questions

1. What is the function of lipases?
2. How can one determine whether a bacterium is lipolytic?
3. What are two functions of lipids in bacterial cells?
4. Give some examples of foods that might be spoiled by lipolytic bacteria.
5. How is the ability of certain bacteria to attack phospholipids related to pathogenicity?
6. What is the difference between a triglyceride (triacylglycerol) and a phospholipid?
7. What are several pathways that bacteria use to metabolize lipids?