

Starch Hydrolysis

Many bacteria produce enzymes called hydrolases. Hydrolases catalyze the splitting of organic molecules into smaller molecules in the presence of water. This exercise will present the hydrolysis of the large carbohydrate polysaccharide starch. The starch molecule consists of two constituents: amylose, an unbranched glucose polymer (200 to 300 units) and amylopectin, a large branched polymer. Both amylopectin and amylose are rapidly hydrolyzed by certain bacteria, using their α -amylases, to yield glucose (a monosaccharide), as shown in Fig. 6-83 (p.75-76). Some intermediary breakdown products, dextrans (shorter chain oligosaccharides), and maltose (disaccharide) are also formed in the reaction.

Gram's iodine can be used to indicate the presence of starch. When it contacts starch, it forms a blue to brown complex. Hydrolyzed starch does not produce a color change. If a clear area appears after adding Gram's iodine to a medium containing starch and bacterial growth, α -amylase has been produced by the bacteria (Fig. 6-84, p. 76). If there is no clearing, starch has not been hydrolyzed.

Procedure

First Period: Starch Hydrolysis Test

1. With a marker, draw a line on the bottom of a starch agar plate dividing it in half. Label each half with the bacterium to be inoculated. (Do not draw lines showing where the organisms will be inoculated as this may interfere with correctly reading the results). Add your name and date to the plate.
2. Using aseptic technique, streak the respective bacteria onto the plate in a **straight line** within the section.
3. Incubate the plate for 24 to 48 hours at 35°C.

Second Period

1. Place several drops of Gram's iodine on each of the line streaks on the starch agar plate. Let the iodine react with the starch for a few minutes. If the area around the line of growth is clear, starch has been hydrolyzed, and the test is positive; if it is not clear or the entire medium turns blue, starch has not been hydrolyzed, and the test is negative. (Note: with some types of starch, it is possible to determine the results of starch hydrolysis without using the iodine. In such a case record your results in the absence of iodine, and then use the iodine and record your results again.)
2. Record your results in the space provided.

Review Questions

1. Describe the function of hydrolases
2. Describe the chemistry of starch hydrolysis.
3. The chemical used to detect microbial starch hydrolysis on starch plates is _____
4. What does starch hydrolysis by a bacterium indicate?
5. Amylase is an enzyme that attacks starch. The smallest product of this hydrolysis is called _____.
6. How is it possible that bacteria may grow heavily on starch agar but not necessarily produce a-amylase?
7. What are the ingredients of starch agar?