DTSH Starch Hydrolysis Test

Learning Objectives

The student will
- Use aseptic techniques in the safe inoculation of various forms of media.
- Follow oral and written instructions and manage time in the lab efficiently.
- Apply correct terminology regarding microbiological techniques, instruments, microbial growth, biochemical testing, media types and forms when making observations.
- Correctly perform various inoculation techniques and describe each technique’s purpose.
- Make accurate observations and appropriate interpretations of biochemical test results and use them in the identification of potentially disease causing microbes.

Background/Theory

Starch is a polysaccharide composed of glucose subunits. The glucose rings can be joined by a 1, 4-glucosidic bond resulting in long, straight chains of glucose. (“1, 4” refers to a bond between the number 1 carbon of one glucose molecule and the number 4 carbon of the next glucose molecule.) Branches off the straight chain can be formed by joining carbon number 1 to carbon number 6 of another glucose molecule. If the starch has all 1, 4 bonds, it is composed of straight chains of glucose called amylose. If there are also branches formed by 1, 6 bonds, it is called amylopectin. See Figure 1.

The amylose or amylopectin molecules are much too large to enter the bacterial cell. If a bacterial cell is to utilize this carbon source from its environment, it must hydrolyze the long chains into glucose subunits for transport across the plasma membrane. The enzyme α-amylase will hydrolyze 1,4 bonds and α-1,6 glucosidase will hydrolyze the 1,6 bonds. Because these enzymes are released outside the cell, they are called extracellular enzymes.

Starch agar contains soluble starch, beef extract, agar and water. If the specific enzymes are produced, they will diffuse through the medium and breakdown the starch in the area immediately surrounding the growth. After incubation, iodine will be added to the agar surface, turning a dark purple/black in the areas where starch is present and leaving an amber zone where the starch has been hydrolyzed to glucose.

![Figure 1 The molecular structures of amylose and amylopectin. (OpenStax, 2018)](image)
Materials per student pair
1 starch agar plate
Lab 2 scratch paper
Lab 2 iodine

Cultures
Fresh overnight plate cultures
*Bacillus subtilis*
*Staphylococcus epidermidis*
One other from the available organisms this week.

Procedure Lab 1
1. On the bottom, divide the plate into four sections. Label each with the name of an organism. For the forth section, label it “sham.” Complete the label with the other components. See figure 2.
2. Aseptically inoculate each of the sections with the corresponding organism using a short **spot inoculation**. For the section labeled “sham,” sterilize your loop and make a short spot on the surface without obtaining cells.
3. Be sure to use short lines. If your inoculations are too large the possible areas of hydrolysis will overlap making it difficult to determine results.
4. Place the plate for incubation for 24 hours at 25°C.

Procedure Lab 2
1. After incubation, you will test the medium to determine where the starch remains.
2. On a piece of white scratch paper or a paper towel, turn the plate right side up.
3. Remove the lid and cover the ENTIRE surface with iodine (Gram’s iodine is convenient) drop by drop. Do not flood the slide.
4. The parts of the media where starch is present will turn dark purple, dark brown or black. The areas where starch has been hydrolyzed to glucose, will be the color of the iodine, a light amber color.
5. Record your observations in the data table. Observations refer to the color of the media near the growth after adding the iodine. Results will be positive or negative (for starch hydrolysis) and interpretation will be either “produces enzymes that hydrolyze starch” or “does not produce enzymes that hydrolyze starch.”
6. To dispose of the plate, replace the lid and wait until all iodine is absorbed into the agar. Carefully fold the paper around the plate. Carry the bundle back to the plate disposal container and place paper and plate inside.
Lab Report: Starch Hydrolysis

Name ______________________________
Lab Section __________

Data and Observations

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<tr>
<th>Organism</th>
<th>Observations</th>
<th>Result +/-</th>
<th>Interpretation</th>
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Post Lab Questions
1. Is this medium considered selective, differential or both?

2. Is this medium defined or undefined?

3. What is the purpose of the sham inoculation?

4. Suppose you performed this test on one organism. You divided a starch agar plate in half and performed a sham inoculation on one side. You inoculated the second side with your test organism. After incubation and the addition of iodine, you noticed an amber colored area around your test organism. Most of the remaining agar surface appeared dark purple except for some small circles of amber on the sham side.
   a. Is your test organism most likely positive or negative for starch hydrolysis?
   
   b. What observation leaves you less confident of your test results? Explain.

   b. Give at least 2 possible explanations for this problem with your test system. (Be more specific than “contamination.”)
References