

AIM: To study the hydrolysis of starch by microorganisms.

PRINCIPLE:

The following test detect the presence of exoenzymes. Exoenzymes are enzymes that are secreted into the surrounding medium and work on substrates found outside the cell. In general, these exoenzymes are hydrolytic and break down large biomolecules that are too large to be easily transported into the cell. These biomolecules must be broken down into their smaller building blocks before they can be made available as a nutrient source for the cell. Starch molecules are too large to enter the bacterial cell, so some bacteria secrete exoenzymes to degrade starch into subunits that can then be utilized by the organism. Starch is broken down into glucose, protein into amino acids, and triglycerides into fatty acids and glycerol.

STARCH HYDROLYSIS TEST

This is a test for the amylase exoenzyme released by some microorganisms that breaks down starch into glucose. Starch agar is a simple nutritive medium with starch added. Since no color change occurs in the medium when organisms hydrolyze starch, we add iodine to the plate after incubation. Iodine turns blue/black in the presence of starch. A clearing around the bacterial growth indicates that the organism has hydrolyzed starch.

Organisms: *E. coli* and *B. cereus*

Media: One starch agar plate (1% starch)

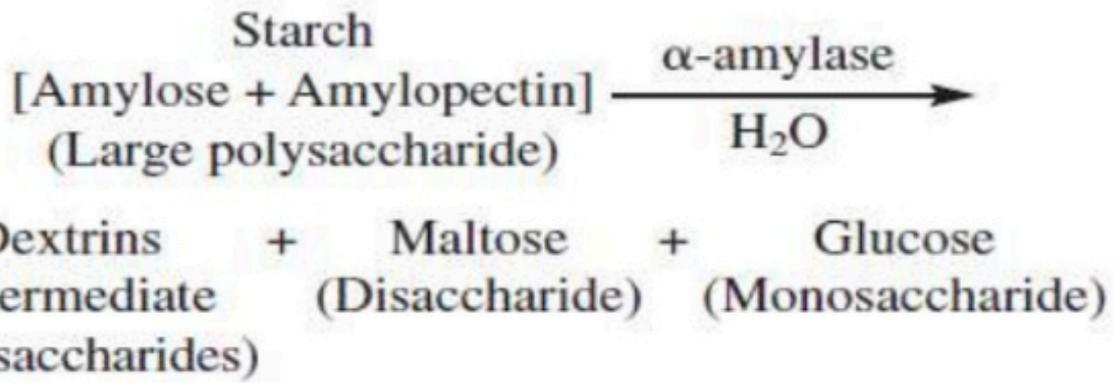
Chemical: Gram's Iodine solution

Procedure:

1. Divide plate in half and label each section with the appropriate organism. Do a straight line inoculation of each organism onto the plate. Incubate the plate, inverted, at 37° C for 48 hrs.
2. After incubation, flood the plate with Gram's iodine.

Observe for the presence of dark color surrounding the inoculation line. Dark color up to the edge of the inoculum indicates the absence of starch hydrolysis. A clear zone surrounding the inoculum indicates a positive reaction, meaning that the starch in the medium has been broken down (hydrolyzed).

RESULT: Activity of amylase is indicated by a clear zone around the streaking while the rest of the plate gives blue black colour after adding iodine. The *B. subtilis* produced a clear zone around the growth which is a **positive** reaction but *E. Coli* gives a negative reaction.



Interpretation:

- ✓ 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 colour change.
- ✓ If a clear area appears after adding Gram's iodine to a medium containing starch and bacterial growth:
 - Amylase has been produced by the bacteria.
- ✓ If there is no clearing, starch has not been hydrolyzed.

