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Biochemical Characterisation of Starch Hydrolysing Bacteria

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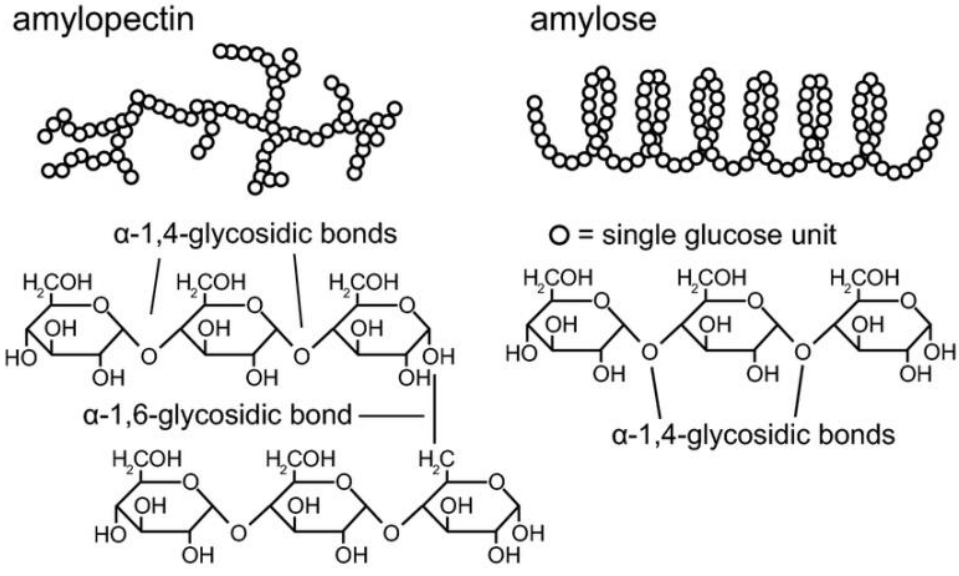
SUMMARY

We started witnessing the bacteria after the discovery of the microscope. We can distinguish bacteria up to genus level with the aid of a microscope but not up to species level. Biochemical studies given hands to overcome this backlog. Biochemical studies utilize the ability of bacteria to produce enzymes, utilization of different energy sources, oxidation and reduction of substrates and so on. Single biochemical test will not provide accurate information. Hence, we rely on different biochemical tests to identify a microorganism. In this popular article emphasis is given for the Starch hydrolysis test. Some bacteria produce alpha amylase enzymes to hydrolyse starch. It is visualized by adding iodine and interpreting the result. In this article we explained the principle and procedure behind it.

INTRODUCTION

Starch hydrolysis test is used to differentiate the members of *Streptococcus, Fusobacterium, Clostridium, Bacillus, Corynebacterium, Enterococcus and Pseudomonas.* In above mentioned genera some species are amylase-positive and some are amylase-negative. This test is employed to identify the potential of an organism to hydrolyse starch and to identify the ability of an organism to produce alpha amylase enzymes. Starch is a complex polysaccharide found in abundance in the plant as granular material stored in the cytoplasm as a reserve food material. Starch is composed of amylose and amylopectin. In amylose, D-glucose monomers are linked by α-1,4-glycosidic bonds. In amylopectin short side chain of D-glucose monomers linked by α-1,6-glycosidic bond is connected with a straight chain of D-glucose monomers linked by α-1,4-glycosidic linkage and gives a branched molecule. Hydrolysis is a chemical reaction, in which the water molecule breaks one or more chemical bonds. The starch can be hydrolysed by the enzyme alpha amylase. Some bacteria produce alpha amylase enzymes. So starch hydrolysing ability is used as a criterion for identifying alpha amylase producing bacteria.



(Source: https://microbeonline.com/starch-hydrolysis-test/)

Fig. 1) Chemical structure of starch amylopectin and amylose

Principle:

In particular, what is the need for the bacteria to produce starch hydrolyzing enzyme, amylase? Bacteria is a living organism, isn't it? It needs food to survive. As it is a heterotroph it can't produce food on its own. So it has to outsource the food material from its surroundings. Bacteria prefer glucose as the prime food material because it is very simple to assimilate. In case, if glucose is not available, will it starve until the glucose arrives? No, it has the ability to utilize other sources as well. In particular, we will discuss here about the ability of bacteria to utilize the starch. Starch is a complex polysaccharide; bacteria can't take starch as such. So, it excretes extracellular enzymes to hydrolyse starch into simpler compounds. By doing so bacteria can utilize starch in its energy producing metabolic pathway. To identify starch

hydrolysing bacteria, a general nutrient media with starch is made, in which the bacterial suspension is streaked in the centre of the plate as a straight line. After incubation the starch around the bacterial growth is hydrolysed. Since it is colourless we cannot distinguish the hydrolysed and un-hydrolysed starch. By adding an indicator, iodine we can visualize the starch hydrolysis reaction. The non-hydrolysed starch turns dark blue. The hydrolysed portion remains colourless.

Media and its composition:

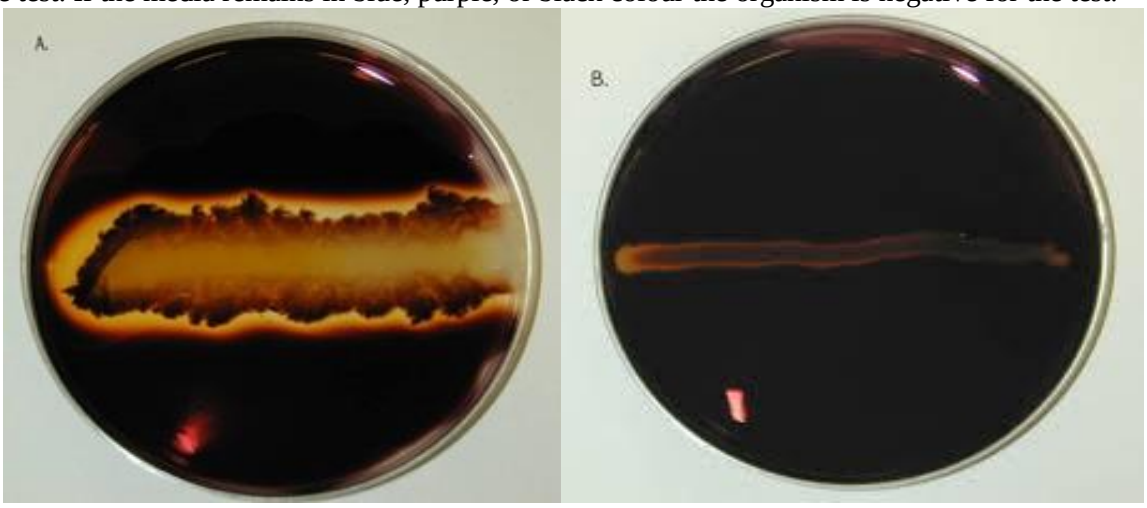
Starch agar is a simple nutritive medium in which starch is the carbon source. Agar, solidifying agent with starch added. Nitrogen, vitamins, carbon and amino acids are supplied through beef extract and pancreatic digestion of gelatine.

Following are the media constituents:

- Beef extract: 1.50g/l
- Sodium chloride: 5.00 g/l
- Yeast extract :1.500 g/l
- Animal Peptide: 5.00g/l
- Starch (soluble): 2.00 g/l
- Agar: 15.00 g/l
- p^H: 7.2 - 7.6 at 25°C

Procedure:

Streak the suspension of the organism in question at the centre of the petri plate aseptically. Incubate it for 48 hours at 37°C. After incubation, add iodine solution to the petri plate with the aid of a dropper for 30 sec, drain excess iodine. Examine the plate for the clear zone. If a clear zone is observed along the bacterial growth, the organism in question is positive for the test. If the media remains in blue, purple, or black colour the organism is negative for the test.



Positive test **Negative test**

(Source:https://homepages.wmich.edu/~rossbach/bios312/LabProcedures/Starch%20Hydrolysis%20Medium.html)

Fig. 2) Test positive (Left) with starch degraded around bacterial growth and negative result (Right) in which starch around bacterial growth is not degraded

Limitations of Starch hydrolysis test

- For proper identification of an organism mass spectrophotometry, immunological and biochemical testing should be done using a pure culture of the organism.
- Sub-culturing of the organism after this test cannot be done because the oxidative nature of the Gram's iodine will kill the cell.

CONCLUSION

Identification of microbes is one of the important aspects of microbiology which makes study of infectious diseases easier. Methods of microbial identification should be reliable and accurate, which are significant to deal the life-threatening health situations in scientific fields. The analytical techniques used to carry out microbial identification have, therefore, become essential to a number of applications so here, we cover the Starch hydrolysis biochemical tests that are used for this task.

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