Starch Agar Protocol

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Author Archana Lal
Naowarat Cheeptham

Information

History

In 1812, the Russian chemist Gottlieb Kirchhoff hydrolyzed starch into glucose by boiling its suspension with sulphuric acid. In 1814, J. J. Colin and H. F. Gaultier de Claubry showed that iodine develops blue color with starch (6). In 1815, S. S. Stromeyer confirmed this test. In 1833, Payen and Persoz (12) isolated a white, water soluble substance from germinating barley by ethanol precipitation. The substance was capable of hydrolyzing starch and was named diastase. In 1883, Duclaux introduced the custom of designating an enzyme by the substrate on which its action was first observed and adding the suffix, "-ase." In 1835, the Swedish chemist Jons Berzelius called the hydrolysis process "catalysis" and demonstrated that the germinating barley extract

days, after which there was no apparent increase in the density of the growth. He also found the starch agar suitable for routine use as most of his stock cultures grew as well or better than on plain agar. In addition, the cultures grown on starch agar were suitable for antigen preparation as starch did not have any adverseeffect on the cultures and the growth was dense. Furthermore, it is knownla1s166.32 684-6(n)-18(l)21et.8( )6722.39t -20(Tf

predominant (8). Alpha-amylose is a linear polymer of several thousand -D-glucose linked by 1,4- -glycosidic bonds. The amylopectin is larger than amylose. -D-glucose linked by 1,4- - glycosidic bonds but is a branched molecule with 1,6- -glycosidic branch points every 24 to 30 glucose residues (14).

FIG 1 Structure of starch molecule showing the 1,4- -glycosidic and 1,6- -glycosidic linkages.

Polymers such as starch molecules are too large to be transported into the bacterial cells through the plasma membrane. Some bacteria -amylase and oligo-1,6-glucosidase and hydrolyze starch molecules outside the cell by breaking the glycosidic linkages between glucose subunits. The resulting dextrin, maltose, or glucose molecules are more readily transported into the bacterial cell to be used inmetabolism.

-1,4- -1,6-glycosidic linkages that hold the starch polymer together. Alpha-amylase -1,4-glycosidic linkages of starch. It attacks the interior of polysaccharide chains resulting in the formation of a mixture of fragments of 5 to 9 units of the alpha configuration (10). Amylase completely splits amylose into glucose subunits. The enzyme oligo-1,6-glucosidase acts on 1,6- -1,4-glycosidic linkages in starch. It cleaves glucose units from the nonreducing ends of the polysaccharide starch and results in the formation of linear or branched dextrins and maltose. Dextrins and maltose are transported into the bacteria and are hydrolyzed by specific intracellular enzymes (10).

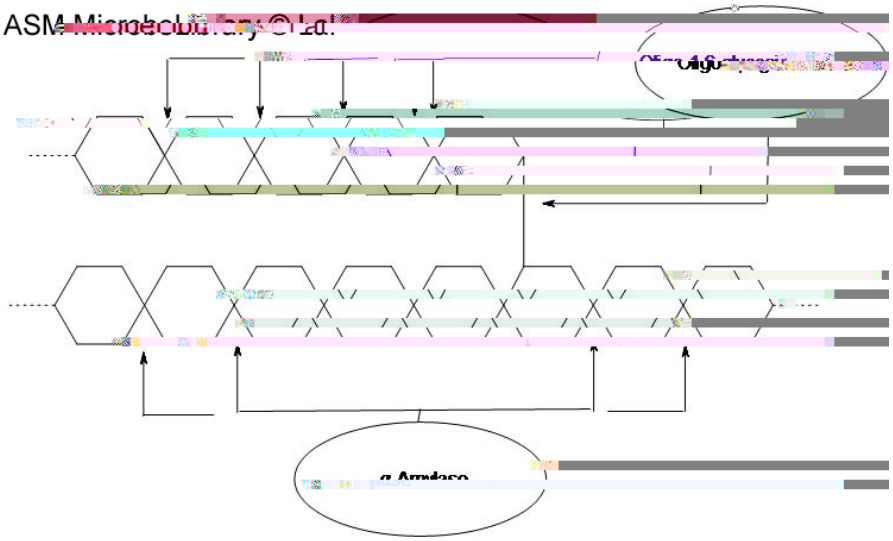

FIG 2 -amylase and oligo-1,6-glucosidase. Alpha- -1,4-glycosidic linkages of starch whereas oligo-1,6-glucosidase acts on 1,6- -1,4-glycosidic linkages in amylopectin.

-amylase and oligo-1,6-glucosidase are grown on starch agar, they secrete these enzymes into the surrounding areas and hydrolyze the starch (8). To detect the hydrolysis of starch, Gram's iodine (I_2KI solution or Lugol's iodine) is used. Gram's iodine reacts with starch to form a dark blue, purple, or black complex depending upon the concentration of iodine. -linkages give the amylose chain a spiral conformation which is responsible for a soluble dark blue starch-iodine complex with iodine reagent (10) and when triiodide ions in the iodine reagent slip into this spiral structure, the complex becomes blue. If this spiral conformation disintegrates, the blue color is lost. Highly branched chains of amylopectin form a red insoluble complex with iodine because they do not coil effectively (10). Upon hydrolysis of amylopectin, the first dextrin formed is erythrodextrin, which gives a color progressing from blue to violet to red-brown after the addition of iodine. With further hydrolysis, the iodine color is not produced because of the formation of colorless achroodextrins.

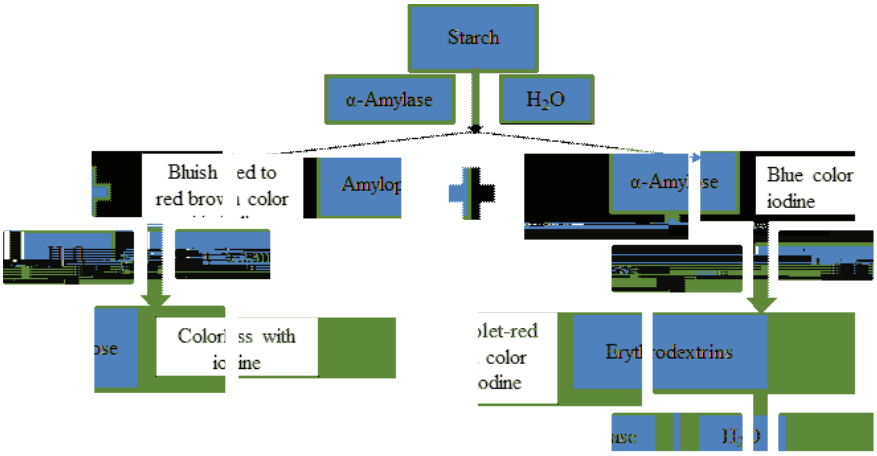

FIG 3 Diagrammatic representation of complete hydrolysis of starch showing all the intermediates and their reaction to iodine.

RECIPE (18)

Starch agar composition (g/liter)

Beef extract	3 g
Soluble starch	10 g
Agar	12 g
Distilled water	1 liter

Suspend the first three ingredients in 1 liter of distilled water. Mix thoroughly. Heat with frequent agitation and carefully bring to just boiling. Do not allow to boil as excessive boiling may hydrolyze the starch (5). Autoclave at 121°C for 15 min at 15 psi. Final pH of the medium should be 7.5 $\pm$ 0.2 at 25°C. After sterilization, pour the melted medium into sterilized petri plates (approximately 20 to 30 ml per plate) and let it solidify before use. Prepared medium is light amber to slightly opalescent. The prepared starch agar plates become opaque if refrigerated (10). The prepared medium can be dispensed into screw-

cap tubes and stored for up to 2 weeks. After 2 weeks the starch changes and reddish purple spots may develop upon addition of iodine (1). The stored medium in the tubes should be melted in a boiling water bath, poured into individual plates, and brought to room temperature before use.

Starch agar medium is also commercially available as premixed dehydrated powder from biological supply companies. The manufacturer's instructions should be followed to prepare the plates. This medium can also be purchased as premade agar plates from biological supply companies.

PROTOCOL

Inoculation. Use a fresh (16- to 18-hour) pure culture of test bacteria as an inoculation source. Pick a single isolated colony and either single streak or spot inoculate the surface of the agar medium.

FIG 4 Growth of *Bacillus subtilis* on starch agar plate before the addition of iodine solution (A) and after the addition of iodine solution (B). After the addition of iodine the clearing surrounding the bacterial growth indicates starch hydrolysis (+).

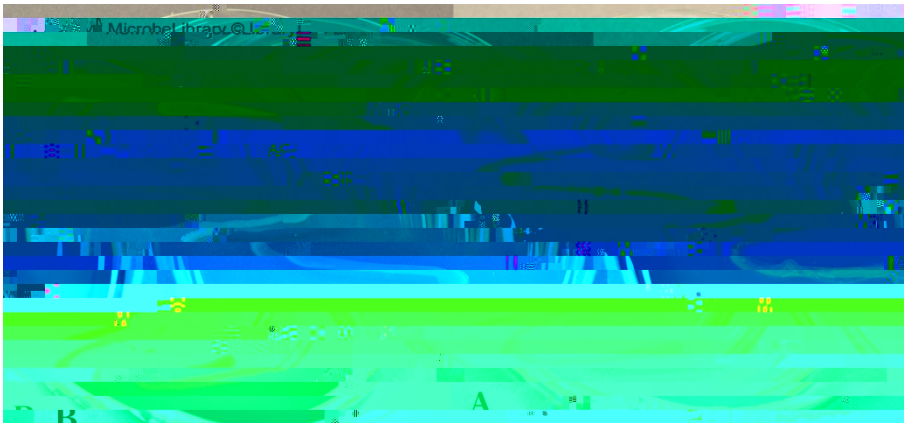

FIG 5 Growth of *Escherichia coli* on a starch agar plate before the addition of iodine solution (A) and after the addition of iodine solution (B). After the addition of iodine the dark blue or black color surrounding the bacterial growth (lack of a clear zone) indicates absence of starch hydrolysis (-).

SAFETY

The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the ASM Curriculum Recommendations: Introductory Course in Microbiology and the Guidelines for Biosafety in Teaching Laboratories.

COMMENTS AND TIPS

By placing a white sheet under the plate, the yellow zone going clear will be more easily observed.

If reincubation is anticipated, do not flood the plate with the iodine reagent. Apply a few drops of iodine reagent to a small area of the agar around the growth. Then if needed, the plate can be reincubated and retested. There are two reasons for this variation: (i) iodine, as an antiseptic, may kill or inhibit further growth of the bacteria and (ii) wa1(1)3(ya)(i)e96( )1y((4(o)1(au)cn 166.32 657.72 374.4 12560.52Ef BT 0 scn /

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Michael Hanophy
St. Joseph's College, Brooklyn, NY

Zoe Hawk
Arizona Western College, Yuma, AZ

Geoffrey Holm
Colgate University, Hamilton, NY

Norrenna Hubbard
Hondros College, West Chester, OH

Tamara McNealy
Clemson University, Clemson, SC

Elizabeth Mitchell
Central Piedmont Community College, Charlotte, NC

Jeffrey Pommerville
Glendale Community College, Glendale, AZ

S. N. Rajagopal
University of Wisconsin, Lacrosse, WI

Jackie Reynold
Richland College, Dallas, TX

Harlan Scott
Howard Payne University, Brownwood, TX

Lisa Spring
Central Piedmont Community College, Charlotte, NC

Jacqueline Washington
Nyack College, Nyack, NY