HELSINGIN YLIOPISTO
MAATALOUS-METSÄTIETEELLINEN TIEDEKUNTA

OPETTAJIEN AKATEMIA

Amylasis and hydrolysis of starch

Pauliina Lankinen, Antti Savin and Sari Timonen
Division of Microbiology and Biotechnology, Department of Food and Environmental Sciences

Objectives

The objective of this exercise is to demonstrate the ability of microbes to produce starch-degrading enzymes.

Background

Starch is a chain-like polysaccharide that consists of thousands of glucose units. It occurs either as linear chain amylose or branched amylopectin. Plants often store the glucose produced as a result of photosynthesis as starch. The starch acts as an energy reserve, and especially the seeds of a plant contain a lot of starch. Starch is the main source of carbohydrates for the most of humanity. Cereal/grain crops (wheat, rice, maize) and potato are the main sources of starch (in human diet/nutrition),

In order to make use of the energy stored in starch, bacteria, fungi, plants and animals must break it down to smaller sugar/saccharide units. Organisms use enzymes called amylases to break down starch. All amylases break the 1,4-α-glycosidic bonds between the glucose molecules in starch. The most common starch-degrading amylase is α -amylase, which is produced by all organisms that utilize starch. Humans have alpha-amylase especially in the saliva and in the pancreatic juice. Alpha-amylase hydrolyses starch in random places creating glucose compounds of unequal lengths. The linear-chain amylose is broken into maltose (2 glucose molecules) and maltotriose (3 glucose molecules), the branched amylopectin is breaken down into maltose, glucose and dextrin (several glucose molecules). Alpha-amylase is active at a neutral pH. Many bacteria, fungi and plants produce beta-amylase. Beta-amylase breaks starch down into maltose.

Amylases have many different industrial applications. Amylases are used in laundry detergents to remove starchy stains. Amylases are used as a flour treatment agent to improve the growth of yeast. Alpha- and beta-amylases from malted barley are most often used as flour treatment agents.

Principles

In this exercise the ability of microbes to break down starch by producing starch-degrading enzymes will be studied. Microbes are grown on a medium that contains starch. If the microbe produces enzymes that break down starch, the starch disappears from around the growth. The degrading of starch is shown with an iodine solution. Iodine forms a dark blue complex when it binds to the starch. There will be a colourless area indicating the presence of degraded starch. A starch-degrading microbe (positive reaction) and a microbe that cannot break down starch (negative reaction) are cultivated on a same plate.

Questions about the subject of the exercise

- Why do plant cells store the sugar produced as starch instead of individual glucose molecules?
- What does malting mean?

Notes for the instructor

Step 4 does not require 30 mins! You might want to add microscoping and/or teach the students how to transfer microbes on empty plates or make their own mixed cultures to take back to their school: it is possible for the students to continue this at school (you can get iodine solution from a pharmacy). Then you need parafilm and more empty plates. Remember to ask for the plates from Mika Kalsi for the microbes that you will need to cultivate yourself.

Lab safety

The microbes used are not harmful to humans, but they should nonetheless be handled with care and contamination should be avoided. It is recommended that gloves are worn when working with microbes.

Time needed

30 minutes or 2-3 days, if the starch agar plates are not cultivated beforehand.

Equipment

- Iodine solution (such as Betadine from the pharmacy or Lugolin solution), use fresh solution
- Microbial pure cultures for a negative and a positive control
    - starch-degrading e.g. *Bacillus subtilis*
    - a strain incapable of degrading starch e.g. *Escherichia coli* or *Saccharomyces cerevisiae*.
- Solid starch growth media set in Petri dishes, such as TY or TGY. Add 2 g of starch and 15 g of agar to one liter of water. The warm, liquid medium is poured into sterile Petri dishes (15-20 ml / Petri dish).
- Sterile glass rods or transfer loops
- Disposable Pasteur pipets

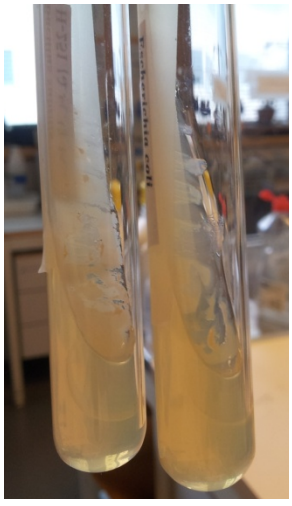

Figure 1 Microbial pure culures as a slant culture.

- Gloves

Instructions

If the exercise is done with plates cultivated beforehand, start from step 4.

1. Divide the starch agar plate into two by drawing a line to the bottom of the plate. Mark (on the bottom of the plate) on which side you are going to transfer which microbe.

2. Transfer some of the microbial growth from a pure culture or your own culture on a starch agar plate with a sterile glass rod or a transfer loop. Transfer the starch-degrading microbe on one side and the strain which is incapable of degrading starch on the other side of the plate. You can also test a mixed culture from a source of your choice. You can for example sprinkle some soil on the medium or touch the surface with your fingers.

3. Let the microbes grow for 2 to 3 days (figure 2). The ideal growth conditions depend on the microbes used, but room temperature is good for most microbes.

4. Cover the surface of the cultured plates with the iodine solution after the colonies have appeared. Watch/observe the reaction and write down the results. A dark colour around the microbial growth shows that the microbe does not produce starch-degrading enzymes, as the starch is still left (figure 3).

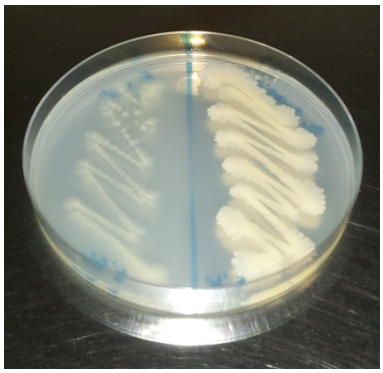

Figure 2 Microbial growth on a starch agar plate.

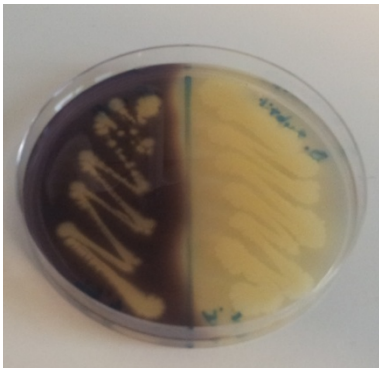

Figure 3 A plate covered with iodine solution (positive reaction on the right, negative on the left).

Observations

- How quick was the colour change after adding the iodine solution?

Sample	Reaction: + / -	Time needed for the reaction
E. coli		
B. subtilis		

Your own sample:		

Answers to the questions:

Why do plant cells store the sugar produced as starch instead of individual glucose molecules?

Individual glucose molecules are water-soluble. The free glucose molecules bind to the water molecules inside the cell taking up space. A high glucose concentration inside the cell results in osmotic stress for the cell. In starch amylose and amylopectin chains are linked with hydrogen bonds forming dense/compact starch granules. This structure makes starch water-insoluble. Therefore starch doesn't cause osmotic stress in the cells and glucose can be stored more compactly.

What does malting mean?

Malting means the germination of grain in order to convert the starch to a more soluble form through activation of amylase production. Malting is divided into three different stages: soaking, germinating and drying. In the soaking stage the humidity of the grain is raised to 45 %, when the grain starts to sprout. When the grains sprout, they produce amylases that convert the starch into a more soluble form. When the desired degree of sprouting is achieved, germination is stopped by drying the grain. The most common malted grain is barley, and it is used for example in brewing beer.

About the equipment needed

Microbial pure cultures can be purchased from HAMBI microbial culture collection, riitta.saastamoinen@helsinki.fi ca. 80 € / culture.

As the starch agar media, most (/any?) common nutrient media with 2 g of starch added to it will do. Common growth media are for example TY and TGY. Disposable Petri dishes and media can be purchased from VWR International. You can also enquire after ready-made plates from Mika Kalsi at the Department of Food and Environmental Sciences in the Faculty of Agriculture and Forestry (University of Helsinki), mika.kalsi@helsinki.fi