Amylase Content of Saliva

Introduction

Ever leave a soda cracker in your mouth long enough to have it turn to sugar? Amylase, an enzyme in saliva, breaks down starch into simpler sugars. How much amylase is in our saliva?

Concepts

- Enzymes
- Digestion

Materials (for each student group)

Amylase solution, 0.1%, 3–5 mL
Potassium iodide–iodine staining solution, KI/I_2, 10% 5–10 mL
Beral-type pipets, graduated, 10
Distilled water, 100 mL
Graph paper
Saliva, 3–5 mL
Ruler
Starch-agar plates, 2%, 2
Test tube rack
Test tubes with screw caps, 12

Safety Precautions

Iodine solution is irritating to the eyes and skin. Iodine will stain clothing, books, hands, and anything containing starch. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Please review current Safety Data Sheets for additional safety, handling, and disposal information.

Preparation

Prepare 2, 2% starch–agar plates pre group prior to class time. Aseptic techniques are not required for this activity except during the disposal phase as described in the *Disposal* section. The following recipe is given for 1 L of agar. One liter of agar will make 50 Petri dishes if poured in a thin layer at the bottom of each dish (just enough to cover the bottom). Adjust the recipe proportionately to prepare more or less than 50 plates.

Ingredients

Agar, 15 g
Corn starch, 20 g (grocery store brands are fine)
Distilled water, 1 L

Directions

Add the dry ingredients slowly to the water while heating and stirring to avoid clumps. Heat solution to near boiling while continuing to stir. When all ingredients are dissolved, remove from heat and let cool until the container can be handled comfortably and the media is still liquid. Pour plates and let cool. When completely cool, invert the agar plates and store upside down. Since the plates are not sterile, they should be kept cool and used within one day. Prepare amylase solution by adding 0.1 g of amylase to 100 mL of water. Stir until the amylae dissolves.

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Procedure

Day 1

1. Obtain two starch–agar plates.
2. Using a plastic drinking straw, disposable spatula or other, similar-sized device, cut wells completely down through the agar to the Petri dish in the "numbered clock positions," as shown in Figure 1.
3. Using a marker, write very small on the back of the Petri dish and label the wells on one dish as follows: S0 (12), S1 (2), S2 (4), S3 (6), S4 (8) and S5 (10). Label the wells on the other dish as follows: A0 (12), A1 (2), A2 (4), A3 (6), A4 (8) and A5 (10).
4. Have one volunteer from each team collect 3–5 mL of saliva in a clean, small beaker or plastic drinking cup.
5. Label 10 test tubes: S1, S2, S3, S4, S5, A1, A2, A3, A4, and A5.
6. Place the collected saliva in test tube S1. Place 5 mL of 0.1% amylase enzyme in test tube A1. These are the stock solutions.

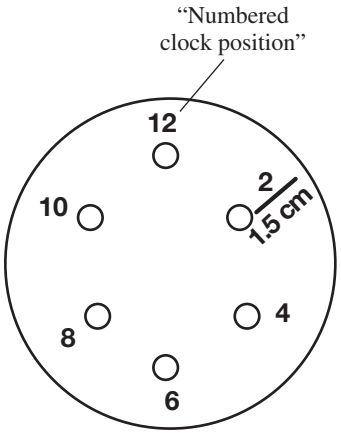

Figure 1. Placement of wells in starch agar plates.

7. Using a graduated cylinder and graduated pipets, make the following serial dilutions to both the saliva and amylase stock solutions, as shown in Figures 2 and 3.

For each dilution, cap the test tube and gently invert to mix each new solution well before doing the next dilution. Failure to do so will affect the results of the experiment. Use a different pipet for each transfer. It may help to label pipets.

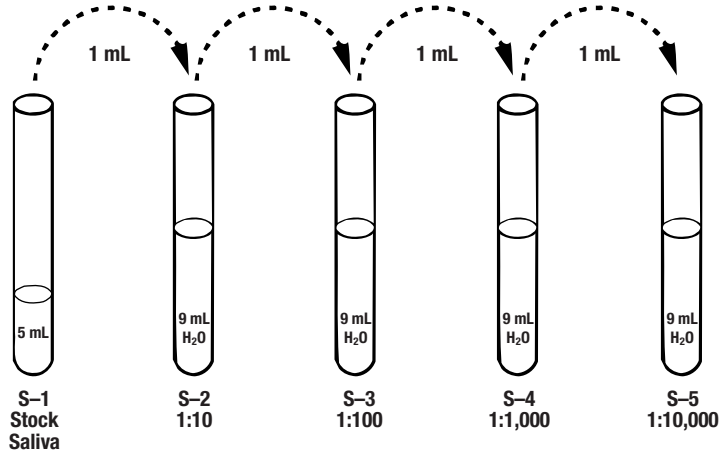

Figure 2. Serial dilution of stock saliva solution.

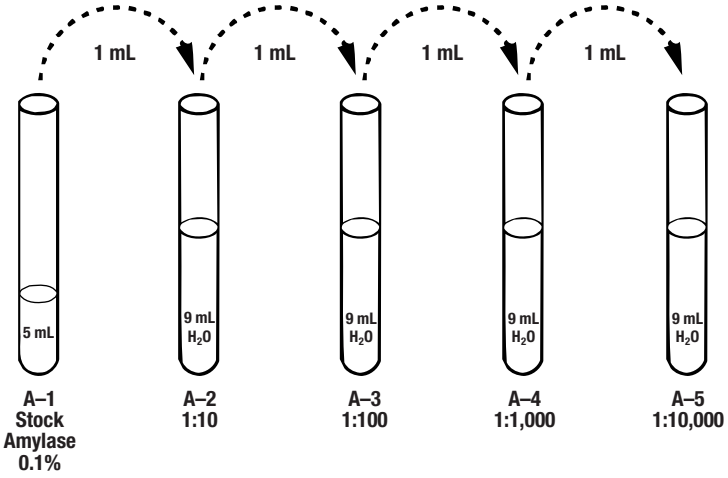

Figure 3. Serial dilution of stock amylase solution.

8. Using a clean pipet, place one drop of distilled water in wells S0 and A0.
9. Using the same, labeled pipets used for the dilution, place one drop of each of the diluted solutions into its appropriately labeled well, i.e., S1 in S1, S2 in S2, A1 in A1, etc. Working from the least concentrated solutions up to the most concentrated will minimize the effect of cross-contamination in the pipets. Recall that the numbers are on the bottom, so add drops in a counter-clockwise direction
10. When the solutions have been added to all of the wells, replace the lids on the Petri dishes and incubate the plates at or near 37 °C (body temperature) for 24 hours.
11. Wash all test tubes, pipets, beakers, and other used materials thoroughly with soap and water.

Day 2

Preparation: Make 10% iodine solution and dispense to dropper bottles or test tubes with screw tops.

12. Using a dropper or pipet, place several drops of the iodine staining solution in the center of each plate. Replace the cover and gently swirl the Petri dish to allow the iodine to cover the entire suface of the plate. Pour off any excess iodine that remains once the surface of the agar has been covered.

Caution: Be careful with the iodine solution—it will stain anything with starch in it, including clothing, books, hands, etc.

13. Observe the clear "halos" that develop around the wells in each Petri dish. Colorless areas indicate where starch digestion has occurred, since iodine tests for the presence of starch.
14. Measure the diameter of the halo that has formed around each well. Record the measurements in an appropriate data table.
15. Construct a graph by plotting the diameter of the clear halo versus the dilution factor for the solution in each well. Plot both the saliva data and amylase data on the same graph.
16. Analyze the graph and answer the following questions.

a. Describe the relationship between the concentration of the enzyme and the size of the halos.

b. By comparing the two series of points on the graph, estimate the concentration of amylase in the saliva used in your experiment. How does your estimate compare to saliva samples tested by other groups in the class?

Saliva Plate	
Well	Size of Halo (mm)
S0	
S1	
S2	
S3	
S4	
S5	

Amylase Plate	
Well	Size of Halo (mm)
A0	
A1	
A2	
A3	
A4	
A5	

Disposal

Non-nutrient agar was used in this experiment. Since there was no nutrient source, microorganisms should not have grown during the short duration of this experiment. The iodine used to flood the surface of the agar should also kill many microbes that may be present. Despite the high probability that there are no pathogenic organisms in the Petri dishes, good laboratory practice is to treat them as if they are contaminated. Petri dishes should be sterilized using one of two methods. After they have been sterilized, the used Petri dishes can be packaged in plastic bags and placed in the regular trash.

1. Autoclave the plates before disposal. Use a standard autoclave or pressure cooker and follow directions carefully. Plastic petri dishes will melt in the autoclave so place them in an autoclave bad or use chemical sterilization.
2. Soak the Petri dishes in a 10% household bleach solution before disposal.
3. Countertops should also be thoroughly cleaned after performing this activity.

Tips

- There are several variables in conducting this experiment that can greatly influence the results. Using contaminated pipets or graduated cylinders in the dilutions and transfers is one potential problem area. Pipets must be continually washed and dried during the entire experiment. Disposable pipets are convenient for this lab.
- The size of the drop in each well sample is another potential variable that will affect results. A free-hanging drop that falls into the well will likely have a different volume than a drop that is touched down the side of the well. The key in the experiment is trying to be consistent in delivering the drops. Have students hold the pipets vertically rather than at an angle and practice delivering drops. Fine-tuning their technique and "controlling" variables in an experiment are valuable laboratory skills for students to develop.

Discussion

Starches are very large carbohydrate molecules consisting of long chains of glucose "units" joined together. Saliva, which is secreted by the salivary glands (about one liter per day), contains an enzyme that catalyzes the hydrolysis (digestion) of starch. The enzyme catalyzes the digestion through a series of intermediate steps and ultimately produces maltose, which consists of two glucose molecules bonded together. Hydrolyzing maltose to glucose requires another enzyme, maltase, that is not present in saliva. (It is secreted by both the pancreas and the small intestine.)

The salivary enzyme that hydrolyzes starch to maltose is called salivary amylase. Amylase has been isolated, crystalized, and purified for years and is used in industry to produce maltose, which is used in many food products.

The action of amylase on starch can be easily followed using the iodine starch test. Iodine yields a deep blue color with starch. As starch is hydrolyzed, repeated tests with iodine will go from blue to red to reddish-brown, eventually turning colorless when the starch has been completely digested to maltose. The clear halos in the starch agar occur as a result of the starch being digested into maltose.

By comparing the graph of saliva digestion versus that obtained using different concentrations of amylase, the amount of amylase present in saliva can be estimated. The amount of amylase in the saliva will vary greatly from one individual to another.

NGSS Alignment

This laboratory activity relates to the following Next Generation Science Standards (2013):

Disciplinary Core Ideas: Middle School
MS-LS1 From Molecules to Organisms: Structures and Processes
LS1.C:: Organization for Matter and Energy Flow in Organisms
MS-PS3 Energy
PS3.D: Energy in Chemical Processes and Everyday Life

Disciplinary Core Ideas: High School
HS-LS1 From Molecules to Organisms: Structures and Processes
LS1.C: Organization for Matter and Energy Flow in Organisms

Science and Engineering Practices
Analyzing and interpreting data
Using Mathematics and omputational Thinking

Crosscutting Concepts
Patterns

Materials for *Amylase Content of Saliva* are available from Flinn Scientific, Inc.

Catalog No.	Description
A0012	Agar
S0125	Starch
I0027	Iodine–Potassium Iodide Solution
A0302	Amylase
AP8170	Petri Dishes
AP1721	Pipets, Beral-type, Graduated
AP7116	Test Tubes with screw caps, plastic
GP9158	Test tubes with screw caps
AP7296	Spatulas, disposable

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.