Student's Guide

Life forms and processes

Breakdown of starch by microbes

Amylase is an enzyme which breaks down starch molecules into sugars and is produced by our bodies to break down the starch we eat. Certain microbes also produce amylase. Some of these are used as a source of amylase for the starch processing industry. You are going to compare the activity of amylase from different sources.

Learning objectives

To show:

- ▷ that microbes produce amylase
- ▷ a simple assay technique for amylase activity
- ▷ an aspect of the industrial importance of microbes

Techniques required

See *Basic Practical Microbiology*

- ▷ flaming the neck of a bottle (pp. 10–11)

Procedure

1. Turn a starch agar plate upside down and divide the base into four sections by drawing on it with a marker pen. Label the sections A, B, C and D. Write your name and date on the plate. Turn the plate the correct way up. You may like to keep a key to the sections:

A	*Bacillus subtilis*
B	*Escherichia coli*
C	0·1 % amylase solution
D	Distilled water

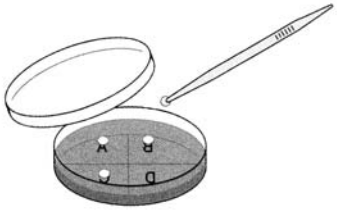
2. Pass the forceps through the Bunsen burner flame, allow them to cool and use them to pick up one of the paper discs. Open the culture of *Bacillus subtilis*. Flame the neck of the bottle and dip the disc into the broth. Allow any excess to drain off, reflame the neck and replace the top on the bottle. Transfer the disc to the middle of section A on the agar plate. Flame the forceps.

   Repeat using another disc, the culture of *Escherichia coli* and section B of the plate.

   Repeat again using another disc, the amylase solution and section C of the plate.

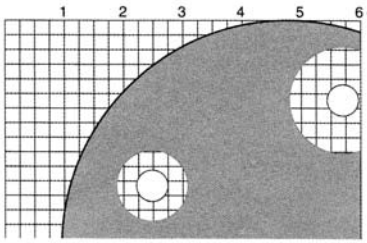
   Finally, use the same procedure to place a disc soaked in sterile distilled water on section D of the plate. Place the forceps in the beaker of disinfectant.

   Tape up the dish. The plates will be incubated until the next lesson.



Next lesson...

3. Lift the lid of your Petri dish. This is permitted because the microbes have previously been killed. Using a dropper, place just enough iodine solution to cover the surface of the agar. Replace the lid.



4. Measure the diameter of any clear zones around the discs, by placing the agar plate on the graph paper. Record your results.

Teacher's Guide

Life forms and processes

Breakdown of starch by microbes

Amylase enzymes are widespread in the animal, plant and microbial worlds. The amylases produced by microbes are much used in industry, particularly in starch processing, where the products of starch degradation (sugars, syrups) are used in the manufacture of foods and beverages. Microbial enzymes are secreted by cells and degrade starch molecules that are too large to pass through the cell wall.

Learning objectives

To show:

- ▷ that microbes produce amylase
- ▷ a simple assay technique for amylase activity
- ▷ an aspect of the industrial importance of microbes

Age range

Year 9 and above

Duration

Session 1	40 minutes
Session 2	30 minutes
Incubation period:	min. 48 h between sessions

Recommendations

1. The cultures of *Bacillus subtilis* and *Escherichia coli* should be inoculated into nutrient broth at least 48 hours before the lesson. *B. subtilis* (catalogue no. B53) from Sciento produces amylase.
2. The paper discs can either be purchased (Whatman Antibiotic Assay discs) or punched from filter or chromatography paper with a cork borer (ca 6 mm diameter) or a hole punch.
3. After the first lesson and incubation at room temperature for a few days, the agar plates should be inverted and filter papers soaked in 40% methanal (formaldehyde)* solution placed in the lids. These should be left overnight to kill the microbes and then removed, after which the students can irrigate the plates with iodine solution.

* *Safety! Methanal is toxic and corrosive. Avoid breathing in the vapour. Use eye protection, gloves and a fume cupboard.*

Notes

Some strains of *B. subtilis* produce amylases whereas *E. coli* does not. Other microbes suitable for school use, such as the fungus *Aspergillus oryzea*, also show marked amylase activity. This requires starch malt agar, which is made by adding 100 cm^3 4% starch suspension to each 100 cm^3 malt agar (made with light malt). If using *A. oryzea*, a culture grown in malt extract broth for 7 days is required.

Materials (each group)

Session 1

- ▷ test tubes/bottles containing 2 cm^3 each of:
  Bacillus subtilis nutrient broth culture
  Escherichia coli nutrient broth culture
- ▷ starch nutrient agar plate
  (Heat 4 g soluble starch in 100 cm^3 distilled water to form a suspension. Allow to cool and mix with 100 cm^3 molten nutrient agar before sterilisation.)
- ▷ 0·1 % amylase solution
- ▷ 4 paper discs (see recommendations)
- ▷ forceps
- ▷ sterile distilled water
- ▷ Bunsen burner
- ▷ beaker of disinfectant
- ▷ marker pen
- ▷ adhesive tape

Session 2

- ▷ iodine solution and dropper
- ▷ graph paper and ruler

Questions

Session 1

1. What reaction is catalysed by amylase enzymes?
2. Why do microbes produce amylase enzymes?
3. What is the purpose of the control disc soaked in distilled water?

Session 2

4. Describe the appearance of your agar plate after it has been flooded with iodine.
5. Do all microbes produce amylases?
6. What physical factors might affect the amylase activity?
7. Suggest some reasons why microbes are used as a source of industrial amylases.
8. Suggest some uses of amylase enzymes in industry and the home.