Student's Guide

Life forms and processes

Breakdown of protein by microbes

Proteins are large molecules made up of amino acids. They have to be broken down by organisms before they can be used. Milk protein (casein) is white and when mixed with nutrient agar, makes it cloudy. You are going to use the disappearance of this cloudiness as an indicator of protein breakdown by microbes.

Learning objectives

To show:

- ▷ that microbes produce proteases
- ▷ the chemical nature of proteins
- ▷ the use of proteases in industry

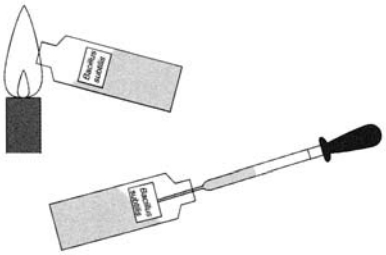
Techniques required

See *Basic Practical Microbiology*

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

Procedure

1. Label the base of each agar plate with either *Bacillus subtilis* or *Saccharomyces cerevisiae* plus your name and the date.

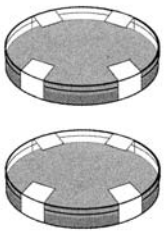


2. Open the culture of *Bacillus subtilis*. Flame the neck of the bottle in the Bunsen burner and use one of the dropping pipettes to remove a small amount of culture. Reflame the neck of the bottle and replace the top.

3. Lift the lid of the appropriately labelled plate and release one drop of culture on to the middle of the agar. Replace the lid. Place the dropping pipette into the beaker of disinfectant.

4. Repeat using the other culture and other plate plus a fresh dropping pipette.

5. Tape up both of the dishes. Keep the plates upright until the drops have dried, then invert. The plates will be incubated until the next lesson.



Safety! *Do not open the plates.*

Next lesson...

5. Examine your agar plates. Answer the questions.

Teacher's Guide

Life forms and processes

Breakdown of protein by microbes

Protease enzymes are produced by several bacteria and fungi. They catalyse the hydrolysis of proteins to amino acids. Industrial applications of proteases include detergent manufacture, brewing and baking, meat tenderisation and leather preparation. Microbes, including fungi of the genus *Aspergillus*, are used to produce proteases on a commercial scale.

Learning objectives

To show:

- ▷ that microbes produce proteases
- ▷ the chemical nature of proteins
- ▷ the use of proteases in industry

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 *Saccharomyces cerevisiae* should be inoculated at least 48 hours before the lesson.
2. Plates must be treated carefully after inoculation to prevent the spreading of the drops of culture. They should not be inverted until the drops have dried.
3. When the milk agar is made, it is assumed that the microbial population of the milk will not affect the outcome of the investigation. A control plate could be used to cover this possibility, although the uninoculated area of the plate really suffices.
   This aspect could form the basis for a question exploring students' understanding of the difference between pasteurised and sterilised milk.

Notes

1. The milk agar is opaque due to the milk protein casein. After inoculation and incubation, clear areas around microbial colonies indicate protease activity.
2. An alternative procedure using paper discs can be used in this experiment (see page 8).

Materials (each group)

- ▷ bottles containing ca 2 cm^3 each of:
  Bacillus subtilis nutrient broth culture
  Saccharomyces cerevisiae malt extract broth culture
- ▷ 2 milk agar* plates
- ▷ 2 sterile dropping pipettes
- ▷ Bunsen burner
- ▷ beaker of disinfectant
- ▷ marker pen
- ▷ adhesive tape

*Milk agar. Make up and sterilise nutrient agar. Allow to cool to 45–50 °C and add pasteurised milk (10 % by volume) aseptically and mix carefully. The milk should be freshly bought and pasteurised. Skimmed, semi-skimmed or full cream milk can be used.

Questions

Session 1

1. What do proteins consist of?
2. Why are proteins important in our bodies?
3. How are proteins broken down in our bodies?
4. What is causing the cloudiness of the agar in your Petri dishes?
5. If the microbes you are using break down proteins, what do you expect your agar plates to look like next lesson?

Session 2

6. Describe the appearance of your agar plates.
7. Is there any evidence of protease production by the microbes?
8. If so, how is this beneficial to the microbes?
9. Suggest some uses of protease enzymes in industry.
10. How are these enzymes produced on a large scale?
11. What factors might affect the activity of the enzymes?
12. Suggest a procedure for investigating the effects of one of these factors.

