PRACTICAL BIOTECHNOLOGY

Plant protoplasts

PROTOPLASTS are cells (plant, fungal or bacterial) that have had their cell walls removed. This can be done mechanically, or by enzymic digestion. The 'naked' cells are surrounded only by a cell membrane and can be used in a variety of ways. For example, two or more protoplasts can be fused with the help of a detergent, polyethylene glycol, to produce hybrid cells with characteristics from each 'parent'. Infection of protoplasts with genetically-modified *Agrobacterium tumefaciens* is one way of introducing new genes into plant cells. Whole plants can be regenerated from protoplasts grown on solid or liquid media.

A mixture of carbohydrases can be used to degrade plant tissue, producing a protoplast suspension which can easily be seen under a microscope. For school work, this procedure does not require an aseptic environment if the protoplasts are not to be cultured.

Materials

Round green lettuce, 1 leaf
21% sucrose solution, 0.1 cm^3
13% sorbitol solution, 20 cm^3
(sorbitol is available from high street chemists as an alternative to sugar for diabetics e.g. *Sionon*)
Carbohydrase enzyme mixture, Novo *Viscozyme*®, 0.5 cm^3 (available from the NCBE)
Paper tissue
10 cm^3 plastic syringe } for dispensing liquids
1 cm^3 plastic syringes, 2 }
Test tube
Centrifuge tube
Fine glass stirring rod
Filter funnel
Strip of nylon gauze, to plug filter funnel
Microscope slide
Cover slip
Microscope with x40 objective
Water bath set at 37°C
Bench centrifuge

Practical details

Lettuce preparation

1. Cut the lettuce leaf into pieces roughly 5 mm x 5 mm.
2. Add 15–20 lettuce pieces to 9.5 cm^3 13% sorbitol solution in a test tube.
3. Incubate the tube in a water bath at 37°C for 5 minutes. *During this time the lettuce tissue will equilibriate to the osmotic potential of the sorbitol solution.*

Enzymic digestion of cell walls

1. Gently stir 0.5 cm^3 of the carbohydrase enzyme mixture into the sorbitol and lettuce preparation.
2. Return the tube to the water bath for another 20 minutes. Gently agitate the tube from time to time while it is incubating. *The carbohydrase mixture contains pectinases, cellulases and other cell wall-degrading enzymes.*

Recovery of protoplasts

1. Tightly pack the spout of the filter funnel with the nylon gauze. *This will produce a fine filter of roughly the correct pore size (able to trap cell debris greater than 50*µm *in diameter).*
2. Pour the digested lettuce suspension into the filter funnel.
3. Wash any trapped protoplasts through the filter using 10 cm^3 of 13% sorbitol solution. Collect all the filtrates in a centrifuge tube. *The protoplasts will pass through the filter while the majority of the undigested cell debris will remain on top of the gauze.*
4. Ensure that the centrifuge is correctly balanced then spin the filtrate for roughly 5 minutes at 2,000 rpm (you may need to adjust this according to the type of centrifuge available).
5. Carefully discard the supernatant, leaving the pellet of protoplasts at the bottom of the tube. Use a tissue to wipe away excess liquid from the inside wall of the tube.
6. Resuspend the pellet in approximately 0.1 cm^3 of 21% sucrose solution. *This is an osmotically-balanced (isotonic) medium in which the delicate protoplasts will not burst.*

Examination of protoplasts

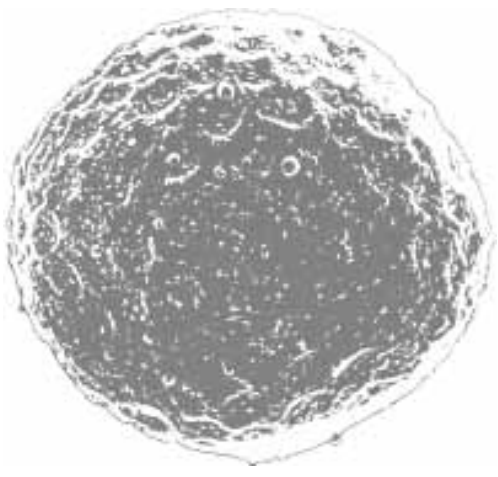
The protoplasts will remain stable in the sucrose solution for several hours and can easily be seen without staining using a microscope with a x40 objective.

Safety

Care should be exercised when loading and operating the centrifuge. Students using the centrifuge must be closely supervised. Spills of enzyme should be wiped up promptly using plenty of water.

Further activities

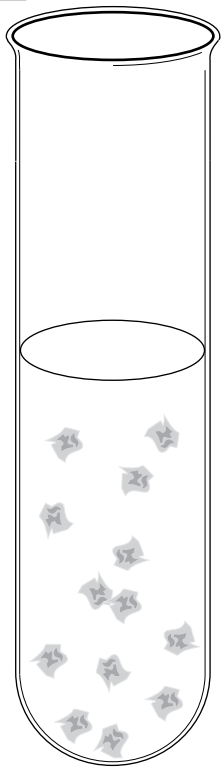
Plant protoplasts are a safe alternative to blood cells for observing crenation and osmotically-induced lysis. Students can investigate the effects of a range of different concentrations of sucrose solutions on the appearence of the protoplasts.



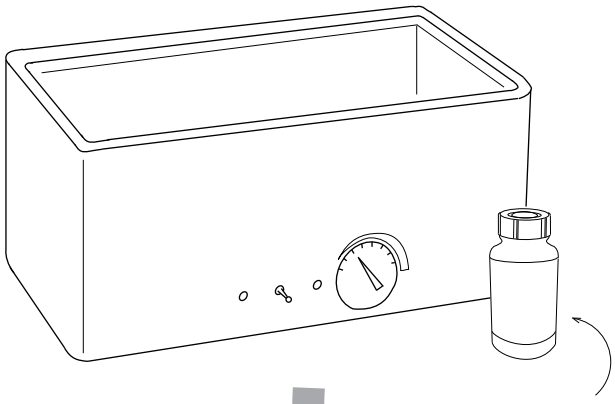
Additional Information

Plant protoplasts by Teresa Bengochea and John Dodds (1986) Chapman and Hall.
ISBN: 0 412 26640 7.

Plant protoplasts



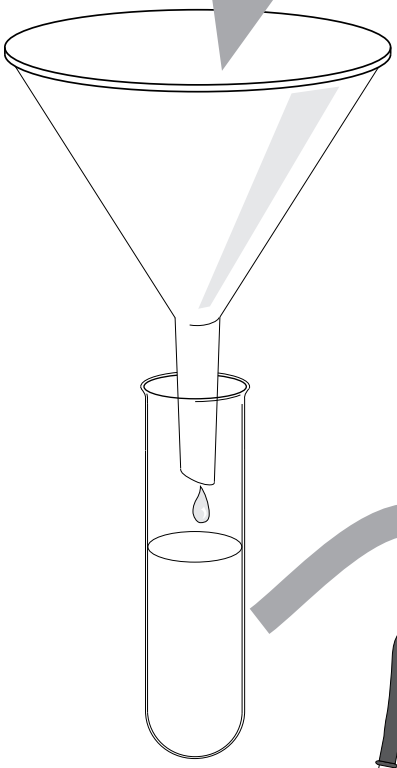
2. Incubate the tube at 37°C water bath for 5 minutes.



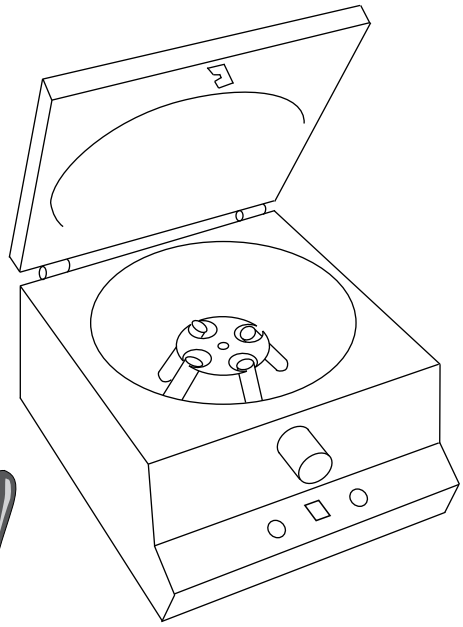
Enzyme mixture breaks down cell walls.

3. Add 0.5 cm^3 of enzyme – then incubate for another 20 minutes.

4. Filter the tissue suspension through a funnel plugged with gauze.

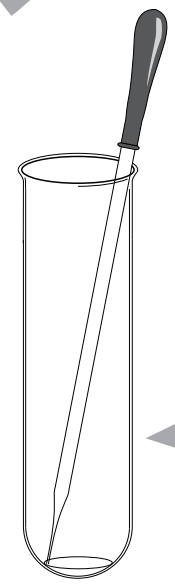


1. Cut the lettuce into pieces and put them into sorbitol solution.

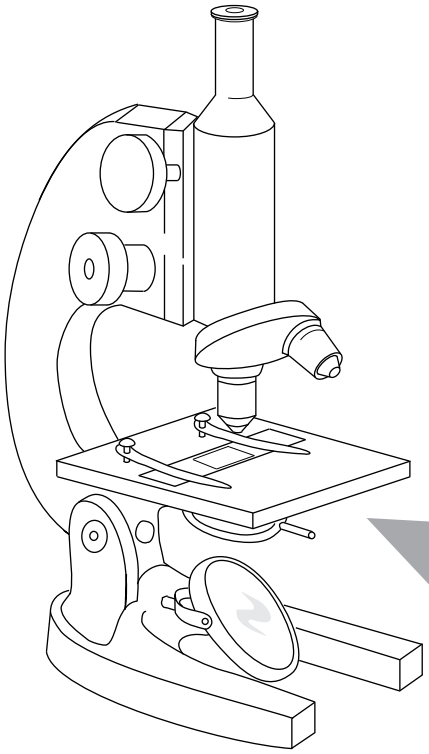


5. Spin down the protoplasts to the bottom of the tube.

Carefully pour away the liquid from the top.



6. Resuspend the protoplasts in 0.1 cm^3 of sucrose solution.



7. Look at the result.