20. Demonstration of protease or amylase activity in different regions of an insect's gut

Grasshopper is a good material to be used for the study of digestive enzymes in insect's gut. It is relatively cheap and is available all seasons in Hong Kong. It is possible to keep them alive overnight by keeping them in a cool place and they can recover their activities on exposure to warmth. To ensure an adequate supply of active enzymes, grasshoppers should be fed with an ample supply of fresh grass one to two hours before killing.

Procedure

A. Extraction of enzymes from different regions of grasshopper's gut

1. Kill the grasshopper by decapitation. Remove its wings and limbs. Affix it with pins in a wax tray and cover it up with water or Insect Ringer. Dissection is done in water if it can be carried out quickly or in Insect Ringer if slowly.
2. Slit the grasshopper open dorsally and isolate the gut. Remove the thoracic muscles, fats and reproductive organs which cover the gut.
3. Transfer the gut onto a watch-glass. Slit open the gut to remove any food or faecal content. Rinse with chilled distilled water.
4. Separate the gut into fore-gut, mid-gut and hind-gut by cutting at the gastric caeca and Malpighian tubules as shown in Fig.1.
5. Place the 3 parts separately in three mortars. Add 2 cm^3 of chilled distilled water to each mortar and grind with a pestle. Allow the extract to settle.

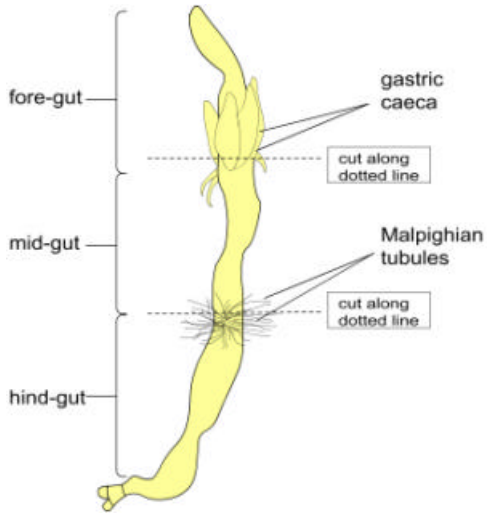

Fig.1 Different regions of an insect's gut

Note

1. When the grasshopper gut has been isolated, rinsing of its inside should only be done very briefly to avoid complete removal of enzymes from the digestive glands which are lining the epithelium.

B. Distribution of amylase in the fore-gut, mid-gut and hind-gut

1. Use a dropper to place one drop of iodine solution to each cavity on a spot plate.
2. Draw 2 cm^3 of 1% starch solution into each of 4 separate 5 cm^3 syringes. Label them as F, M, H and C respectively. Rinse the outside of the syringes with distilled water.
3. Draw 1 cm^3 of the fore-gut, mid-gut and hind-gut extracts into the syringes labelled as F, M and H respectively, while to syringe labelled as C add distilled water instead. Start the stopwatch immediately and note the room temperature. Suck in some air to quickly mix the contents.
4. At 2-minute intervals, carefully release one drop of reaction mixture from each syringe onto an iodine spot. Note for the disappearance of blue-black colour and record the time.
   (The test may be done at 1-minute intervals if the students can manage to do the test for all syringes within this time interval.)

Note

1. In carrying out the iodine test for starch in the assay of amylase activity, it is advisable to use a very dilute iodine solution.

2. As the brown colour will fade gradually, it is advisable not to leave the iodine solution in the cavities for too long.
3. A spot colour made up by mixing 0.01% of starch solution and iodine solution may be used as an arbitrary standard for the absence of starch.

C. Distribution of protease in the fore-gut, mid-gut and hind-gut

1. Cut 4 strips of film (0.5 x 3.5 cm) from an exposed and developed B/W photographic negative.
2. Mount the four strips onto a slide with adhesive tape. Label the strips as F, M, H and C respectively.
3. Put the slide in a moist chamber, e.g. a petri dish containing a piece of moist filter paper.
4. Add 2 drops of the fore-gut, mid-gut and hind-gut extracts onto the emulsion surface of the F, M and H strips respectively, while onto C add 2 drops of distilled water instead. Replace the lid.
5. Incubate the petri dish at 37°C for 2 hours. After 2 hours, wash the four strips and note for any clear spot formed.
6. Compare the enzyme activities by estimating the degree of clearing.

Note

1. The incubation time depends on the quality and source of photographic negatives. Some may take a longer time to obtain good results. It is advisable to try out the material first and determine the appropriate incubation time required.

2. B/W photographic negatives, which have been exposed, developed and fixed (without hardener), are good materials for this experiment.

Preparation of Reagent

1. Iodine solution

   A stock iodine solution is prepared by dissolving 1 g iodine and 2 g potassium iodide in 100 cm^3 distilled water. 0.5 cm^3 of the stock solution is added to 10 cm^3 distilled water prior to use.

2. Insect Ringer

   Dissolve 0.42 g potassium chloride, 0.24 g calcium chloride, 0.2 g sodium hydrogencarbonate and 6 g sodium chloride in 600 cm^3 distilled water, and bring the total volume to 1 litre. It is better to use freshly prepared solution.