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Qualitative estimation of digestive enzymes in midgut of *Gryllodes sigillatus* Walker

Experimental insects were kept in laboratory on a mixed diet consisting of bread, liver, and sugar. Specimens were dissected in ice cooled distilled water. Adhering tissues were removed. Midgut pieces were ground. The volume of each set was raised to 10 ml. Five specimens of *Gryllodes sigillatus* Walker: were taken for each set of experiment. The homogenate was then covered by a few drops of toulene and kept for an hour in refrigerator after which it was centrifused. The supnatant was taken for the experiment. For working controls side by side some of the homogenate in each case was first boiled and then treated alike side by side the experimental tubes. Tests were conducted for detection of following enzymes as detailed below.

Amylase: 1 ml of homogenate was added to 5 ml of substrate consisting of 0.5% freshly prepared boiled and cooled starch solution (B.D.H. soluble starch) and 3 ml of M/15 Sorenson phosphate buffer was added. The tube was incubated at 37 °C for 72 hours and then the reaction mixture was tested for presence of starch by potassium iodide iodine test. Two drops of potassium iodide iodine solution (0.5% iodine solution in 1.5% potassium iodide solution) was added to the tube. Absence of a blue colour indicated absence of starch and thus presence of amylase.

Invertase: 1 ml of homogenate was added to 5 ml of substrate consisting of 15% sucrose solution buffered with Sorenson phosphate buffer. The tube was incubated for 48 hours at 37 °C and the test for presence of fructose and glucose was tested by Barfoed test. A small quantity of acetic acid was added to a solution of copper acetate (40 parts of 6.7% copper acetate and 1 part of 3.5% acetic acid) and heated with tissue suspension. Reddish brown precipitate occurred showing presence of glucose and thus presence of invertase.

Maltase: 1 ml of homogenate was added to 5 ml of substrate consisting of 5% maltose solution buffered with Sorensons phosphate buffer and the presence of glucose was tested by Barfoed test, the positive reaction of which indicated presence of Maltase.

Lipase: 1 ml of homogenate was added to 5 ml of substrate which consisted of milk to which were added a few drops (4–6) of 1% solution of bromothymol blue and then 1% solution of potassium hydroxide till the solution turned blue. The reaction mixture was covered by toulene as usual and incubated for 24 hours. After 24 hours a change in colour of blue milk to yellow indicated conversion of fats into fatty acids and glycerol thereby confirming presence of lipase.

Lactase: 1 ml of homogenate was added to 5 ml of substrate consisting of 5% lactose solution buffered by Sorenson phosphate buffer and the presence of glucose was tested by Barfoed test, a positive reaction of which indicated presence of lactase.

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Protease: The white of egg was sucked in fine capillary tubing and then the ends were sealed by heating the capillary tubing which was then transferred to water bath for coagulating the white of egg. The tube was then cut in small pieces and transferred to reacting homogenate which was buffered at 6,7, and 8 pH. Albumen dissolves in the tubes in alkaline medium after sometime showing presence of protease.

Summary

Experiments showed the presence of amylase, invertase, maltase, lipase, lactase and protease in the midgut of *Gryllodes sigillatus* WALKER.

Zusammenfassung

Versuche ergaben, daß im Mitteldarm von *Gryllodes sigillatus* WALKER Amylase, Invertase, Maltase, Lipase, Laktase und Protease vorhanden sind.

Резюме

Исследования показали, что в средней кишке у *Gryllodes sigillatus* WALKER находятся амилаз, инвертаз, малтаз, липаз, лактаз и протеаз.

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