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Isolation and Characterization of the Enzymes Present in the Digestive Tract of Slugs and Snails

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ABSTRACT

Aim of this study: *Identify enzymes present in an herbivore and carnivore gastropod. Identify the digestive enzymes present in slugs, representative of the herbivore gastropod.***Methodology:** *Bicinchoninic acid protein assay done to identify protein enzymes in two groups of samples. Purified pectinase identified using the pectinase assay. Protein assays include BCA plate assay and pectinase assay.***Results:** *Findings of this study would reveal significantly high levels of purified pectinase in slug samples***Conclusion:** *The types of enzymes present in the gastropod samples would be consistent to their feeding habit and activity.*

KEYWORDS : Enzymes, Digestive Tract, Slugs and Snails, Protein assays

Introduction

Belonging to the phylum Mollusca, gastropods are biologically important and are classified as filter feeders, carnivores and herbivores. They do not only constitute an important link in the ecosystem's food chain but are also used as indicator organisms on the health and status of the environment. Many studies have been devoted to the feeding habits of these organisms and earlier studies have pointed out locations of enzymes in the bodies of these organisms. Studying their feeding habit and the enzymes present to digest their food would be important since it would provide information on how these organisms feed and digest their food. Despite the presence of earlier literature on feeding habits, there appears to be a paucity of literature on the enzymes present in the bodies of gastropods.

MATERIAL AND METHODS

Bicinchoninic acid (BCA) protein assay

Biocinchoninic Acid or is also known as Smith Assay or simply BCA, This is a biochemical assay for determining the total level of protein in a given solution of 0.5µg/mL to 1.5mg/ml). BCA assay first was explained by Smith, et al. (1985).

Pectinase Assay

Purified pectinase is very efficient multi-component depolymerizing plant pectins with differentiated degrees of esterification. Pectin lyase, activities on polygalacturonic acid (pectin) and nonmethylated polygalacturonic acid (pectate) are significant enzymatic activities. The product contains hemicellulase, cellulase, pectinesterase and xylanase activities. Those work synergistically with pectin lyase and polygalacturonase to digest wall tissues of plant cell.

Method: One unit liberates 1 umole of D-galacturonic acid from polygalacturonic acid per minute at 37°C, pH 5.0.

Reagents: M Citric Acid/Phosphate Buffer (Assay Buffer), pH 5.0 adjust the pH of buffer by adding Disodium hydrogen phosphate (Na2HPO4).

Color Reagent A: Dissolve 40g sodium carbonate anhydrous Na2CO3 in 600 ml reagent grade H2O. Add 16gm glycine and stir until dissolved. Add 0.450 gm CuSO4 5H2O, stir until dissolved. Bring to 1 liter with reagent grade H2O.

Color Reagent B: Dissolve 1.2 g neocuprine - HCl in 1 liter reagent grade H2O. Store at 4°C in a brown bottle. D-galacturonic acid standard, 0.01mg/ml 0.5% Polygalacturonic Acid Substrate:

Heat 100 ml Assay Buffer on a hot plate. While heating and stirring, slowly add 0.5 gmpolygalacturonic acid. Heat and stir until dissolved. It will be slightly viscous and opaque. Do not allow solution to boil. Dialysed the substrate in distil water for 2 hours in at 4°C with changing the water in each hour. Store at 4°C.

Enzyme: Make up fresh daily. Keep solutions on ice until used. Make serial dilutions 0.1, 0.01, 0.001 mg/ml in assay buffer.

Procedure: Prepare three tubes, each containing 0.1 ml of buffer and 0.6 ml of substrate (Reagent Blanks). Prepare three tubes, each containing 0.6 ml of substrate and 0.1 ml of enzyme sample at 0.1 mg/ml (Test), other three tubes at 0.01 mg/ml (Test) and three tubes at 0.001 mg/ml (Test). Prepare three tubes, each containing 6 ml of buffer and 1 ml of enzyme sample at 0.1 mg/ml (Sample Blanks) other three tubes at 0.01 mg/ml (Sample Blanks) and three tubes at 0.001 mg/ml (Sample Blanks).

Table 1. Sample Assay using Pectinase.

	Reagent samples	Test samples	Blank samples
Assay buffer	100		600
Enzyme (pectinase) with three concentrations (0.1,0.01,0.001)		100	100
Polygalacturonic Acid substrate	600	600	

Mix the tubes well by inversion, then Incubate tubes in 37°C water bath for 60 minutes ± 1 minute. After the assay tubes have incubated for 60 minutes, place rack immediately into ice water to stop reaction about 10 mints. Aliquot from each tube 25 µl into another set of tubes (also on ice). To each reaction tube and standard tube add 0.5 ml of Color Reagent A and 0.5 ml of Color Reagent B. Mix well by inversion. Place rack into hot plate for 15 minutes ±1 minute. Cool tubes. Read absorbance at A450 against a water blank using disposable cuvettes. Calculate mean absorbances for each set of triplicates.

Plot a standard curve:

$x = \mu g$ D-galacturonic acid

$y = A_{450}$

Calculate rate of change. The curve should be linear up to 100 ug D-galacturonic acid, if not, repeat curve with fresh reagents.

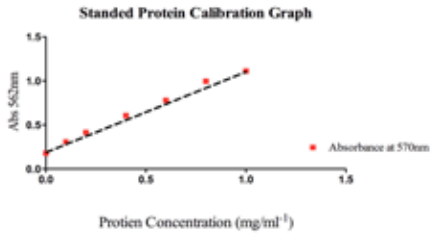
RESULTS AND DISCUSSION

BCA Plate Assay

Protein concentration in the assay was measured with BCA assay plate, which uses 96 wells plate. The snail samples in this result were measured. The preparation of the standard reagent involves mixing 25ml BCA with 0.5ml copper. The 96 wells plate was separated into different segments. Standard protein was emptied into the first section. In the other columns, the aliquoted two different concentrations of standard protein samples were loaded in quadruples.

Figure 1. Standard Curve for Bovine Serum Albumin (BSA)

Figure1 shows the reaction progress of BCA assay as measured at 562 nm. A BSA concentration of 1.0 mg/mL was used. Incubation of the plate was at 37°C for 30 minutes.



Pectinase assay

At 37^{0}C and pH of 5.0, a unit gives out 1 µmole of Dgalacturonic acid from ploygalacturonic acid every minute.

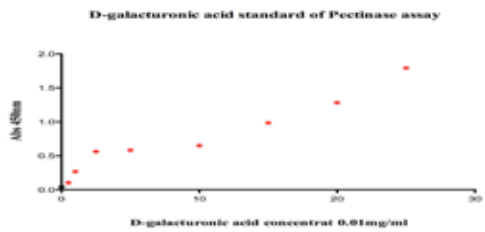


Figure 2. D-galacturonic acid standard of Pectinase Assay

Figure 2 shows the reaction progress of D-galacturonic acid standard as measured at 450 nm. Dgalacturonic acid standard of 0.01 mg/mL was used. Incubation of the plate took placed at 37°C for 60 minutes.

Similarities between the digestive tract of slugs and snails to humans are many (Baker, 2001). Earlier studies (Henry et al., 1991; Herrera-Fernandez et al., 2000) have pointed out that the digestive diverticulary gland of gastropods contains digestive enzymes, which are mainly responsible for the extracellular digestion of both snails and slugs. Apart from absorbing important nutrients, the cells present in the digestive tract of these organisms are also responsible for intracellular digestion and in the transport of proteins. Antioxidant enzymes are also found to be present in digestive glands of molluscs (Orbea et al., 2000). Despite the relative abundance of snails and slugs and their biological importance to the ecosystem, there appears to be a paucity of literature on the actual identification and classification of digestive enzymes present in these marine organisms. Understanding the digestive process is important since it would allow researchers to understand the mechanisms involved in digesting the different organisms where snails and slugs fed on.

A study by Zaral et al. (2010) would confirm the presence of digestive phospholipase A2, also termed mSDPLA2 in the hepatopancreas of H. trunculus, which would confirm that this organism produce bile salts. Similar to humans, the mollusc H. trunculus would also produce bile salts. The hepatopancreas has been repeatedly identified by earlier studies (Nelson and Morton, 1979; Morton, 1983; Beeby and Richmond, 1988; Henry et al., 1991) as the main site for digestion in molluscs and also the main site for nutrient absorption. Other studies (Pal, 1971 and 1972; Owen, 1973; Henry et al., 1991; Franchini and Ottaviani, 1993) also suggested that the hepatopancreas is the site for lipid storage. While all these studies have strongly suggested and identified the main site of digestion, there appears to be a paucity of literature on the list of enzymes present in snails and slugs. In the present study, protein assay was done to measure the presence of protein digestive enzymes. Results would show that specifically pectin was present from the samples tested in the study as evidenced by a significant increase in the absorbance.

Finally, this study would reveal high levels of purified pectinase in slug samples, This would indicate that this group is highly suited to digesting cellulose, which is present in plant cells but absent in animal cells.

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