Journal of American Science

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Electrophoretic analyses of proteins and enzymes in the midguts of palm weevil, *Rhynchophorus phoenicis* F. (Coleoptera: Curculionidae).

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Abstract: Proteins and enzymes are very important in living organisms. Proteins play crucial roles in the repairs and growth of organisms while enzymes which are proteinous in nature, are important in food digestion. Electrophoretic profile of the midgut homogenate of the larvae and adults of *Rhynchophorus phoenicis* F were carried out to determine their protein bands using sodium dodecyl-sulphate polyacrylamide gradient gel electrophoresis. The results of this work revealed eight distinct protein bands whose relative mobility Rm ranged from 0.06 – 0.75 in the larva and 0.08 – 0.89 in the adult. The molecular weights of the proteins in the larva are 17,780 Da, 25,120 Da, 31,620 Da, 50,120 Da, 63,100 Da, 79,430 Da, 100,000 Da and 199,500 Da while in the adult the molecular weights of the proteins are 11,220 Da, 22,390 Da, 25,120 Da, 31,620 Da, 50,120 Da, 95,500 Da, 177,800 Da and 190,500 Da. The midguts of *R. phoenicis* have so much proteins and enzymes that are responsible for the effective digestion of the fibrous tissues of the palm trees.

[Omotoso, O. T. **Electrophoretic analyses of proteins and enzymes in the midguts of palm weevil, *Rhynchophorus phoenicis* F. (Coleoptera: Curculionidae)**. *J Am Sci* 2024;20(5):28-32]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). http://www.jofamericanscience.org 05. doi:10.7537/marsjas200524.05.

Key words: Electrophoresis, buffer solution, enzymes, midgut, molecular weights, homogenate

Introduction

The palm weevil, *Rhynchophorus phoenicis* F. is a key pest of all palm trees in the tropics. The main hosts of the adult beetles are coconut, oil and date, raphia and ornamental palms. This beetle decomposes plant materials, including dead standing palms, stumps and logs that are lying on the ground, sawmill wastes and wastes from agricultural processing. Insects have played an important part in the history of human nutrition in Africa, Asia and Latin America (Bodenheimer, 1951).

Palm weevils are prominent as pests and edible insects but there is dearth of information on the electrophoretic patterns of the proteins and the enzymes in the midgut of the insect. Electrophoresis is a method used in biochemistry, molecular biology, genetics and clinical chemistry to separate a mixed population of DNA, RNA, proteins and enzymes. Electrophoresis has been used to analyze the various enzymes present in natural populations of the butterfly, *Coolias eurytheme* (Johnson, 1971). Electrophoretic patterns of some enzymes of three insect pest; *Distantiella, theobroma* (Distant),*Sahlbergella singularis* Haglund (both Heteroptera: Miridae) and *Bathycoelia thallassina* (Herrich-Schaeffer) (Heteroptera: Pentatomidae) have been analyzed by Coker (1974). Electrophoresis has also been used by some workers to analyze the haemolymphs of insects. Electrophoretic patterns of the haemolymph proteins of *Rhynchophorus ferrugineus* Olivier and *Galleria mellonella* L have been studied by Thomas and Nair (2011) and Hyrsl, *et al.*, (2011) respectively. In electrophoresis, biomolecules are separated by applying an electric field to move the charged molecules through an agarose matrix and the biomolecules are separated by size in the agarose gel matrix (Sambrook and Russel, 2001). Thus, the gel electrophoresis is a technique used in the laboratory to separate charged molecules such as proteins, RNA and DNA. Gel is a size selective sieve substance which separate proteins into bands during electrophoresis. As proteins move through a gel in response to an electric field, the smaller molecules travel more rapidly than larger proteins.

As a result of dearth of information on the electrophoretic profile of the proteins and enzymes of *R. phoenicis*, this research work was conducted to provide background information for future references.

Materials and methods

Collection and preparation of homogenate

The larvae and adult palm weevils, *R. phoenicis* used for this work were collected from Igbokoda in Ondo State and put in plastic bowls. The specimen

were immobilized in a deep freezer at -10° C for 30 minutes. The alimentary systems of the insects were dissected out. The midguts of the weevils were removed and homogenized in a chilled glass homogenizer. The homogenate was prepared from 1g of midgut/ml of sample buffer (Tris-HCl pH 6.8). The homogenate was centrifuged at 5,000 rev/min. for 20 minutes at 4° C in a Beckman Optima Model LE-80K refrigerated centrifuge. The supernatant was boiled for 5 minutes and then used for electrophoresis. A modified method of Laemmli (1970) was used in preparing 4% acrylamide concentration and stock solution.

Preparation of solutions

Sample buffer

Sample buffer was prepared by adding 6 ml of 1M tris-HCl ph 6.8, 2 g of SDS, 10 ml of glycin, 7.013 ml of 2 M Mercaptoethanol and 0.005 g of Bromophenol blue and making everything to 50 ml mark with distilled water inside a measuring cylinder.

1.5 M Tris-HCl pH 8.8

For the preparation of 1.5 M Tris-HCl pH 8.8, 138.054 g of Tris-base and 56.88 g of Tris-acid were measured and poured in 1000 ml measuring cylinder and made up to 1 litre with distilled water. This gave 1.5 M Tris-HCl pH 8.8.

1.0 M Tris-HCl pH 6.8

To prepare 1.0 M Tris-HCl pH 6.8, 3.7057 g of Tris-base and 153.102 g of Tris-acid were measured and poured in 1000 ml measuring cylinder and made up to 1litre with distilled water. This gave 1.0 M Tris-HCl pH 6.8

Reservoir buffer

For reservoir buffer preparation, 3.028 g of tris-base, 14.42 g of Glycin 192 mM, 1 g of SDS 0.1% were measured and dissolved in a measuring cylinder and made up to 1 litre with distilled water.

Stock solution for polyacrylamide gel electrophoresis

All the following compounds were measured and added together. They include 3.72 ml of water, 0.65 ml of 1.0 M Tris-HCl pH 6.8, Acrylamide 30% 0.70 ml, 10% SDS 0.052 ml, TEMED 0.0052 ml and 10% APS (1000 mg/ml) 0.052 ml.

Fixing solution

Ten percent (10%) acetic acid was added to 40% methanol (v/v) and the gel was put inside and shook at intervals of 20 minutes for 1 h.

Staining solution

The following compounds were measured; 10 g Ammonium sulphate, 1 ml Phosphoric acid and 0.1 g Coomassie G250 and added together. Everything was made up to 100ml mark with distilled water in a measuring cylinder.

Destaining solution

Distilled water was used to de-stain the gels. The distilled water was changed at intervals of 20 minutes for 8 h.

Electrophoretic assay

The gel components were mixed together and a glass slab was filled to mark with the gel solution using a pipette. Distilled water was carefully layered on the top of the gel solution to prevent the formation of air or gel meniscus. This ensured that a flat gel surface was obtained. The gel was left to polymerized after which it was ready for use. The sample mixture was applied on top of the individual gel with a micropipette after the layered distilled water had been discarded. Reservoir buffer was poured in the electrophoresis apparatus. Tracking dye was applied. The reference proteins used for the determination of molecular weights included Myosin (200,000 Da), β-Galactosidasee (119,000 Da), Serumalbumin (66,000 Da), Ovalbumin (43,000 Da), Carbonic anhydrase (29,000 Da), Trypsin (20,000 Da) and Lysozyme (14,500 Da). Electrophoresis of the samples and the protein standards were run together by applying a current of 10 mA per gel. Electrophoresis power pack, Vokam Model SAE 2761 was used to pass current through the gels. Electrophoresis was stopped when the tracking dye was 6/7th down the gel. The gel was removed from the slab by squirting water from a syringe between the gel and the glass wall. The gel was fixed for 1h and stained with Coomassie brilliant blue G250 for 8 h. The gel was de-stained in distilled water every 24 h until the bands became clearer. The de-stained gel was photographed. A graph of relative mobility was plotted against the log of molecular weight using the formula:

$$\text{Relative mobility} = \frac{\text{Distance of protein migration}}{\text{Distance of dye migration}}$$

The molecular weights of the proteins were determined from the standard curve obtained and the comparative relative mobility values were determined.

Results

The result of the electrophoretic profile of the larval and adult stages of palm weevil, *R. phoenicis* is shown in plate 1. The larva and adult have eight types of proteins each in their midguts (Table 1.). The

relative mobility value of the first protein band in the larva was 0.06 while it was 0.08 in the adult. The Rm value of the second protein band was 0.25 in the larva while it was 0.1 in the adult. The Rm value of the third protein band was 0.31 in the larva while it was 0.28 in the adult. The Rm values of the fourth, fifth, sixth and seventh protein bands in the larva were 0.39, 0.42, 0.58 and 0.64 respectively while in the adult the Rm values were 0.42, 0.58, 0.64 and 0.69 respectively. Protein bands with Rm values of 0.42, 0.58 and 0.64 were present in both the larva and adult stages. The Rm value of the eighth protein band was 0.75 in the larva while it was 0.89 in the adult

The molecular weights of the proteins in the larva ranged from 17,780 Da to 199,500 Da while in the adult it ranged from 11,220 Da to 190,500 Da. The proteins of molecular weights 25,120 Da, 31,620 Da and 50,120 Da were found in both the larval and adult stages. Proteins with molecular weights of 199,500 Da, 100,00 Da, 79,430 Da, 63,100 Da and 17,780 Da were specific to the larval stage while those with molecular weights of 190,500 Da, 177,800 Da, 95,500 Da, 22,390 Da and 11,220 Da were specific to the adult stage.

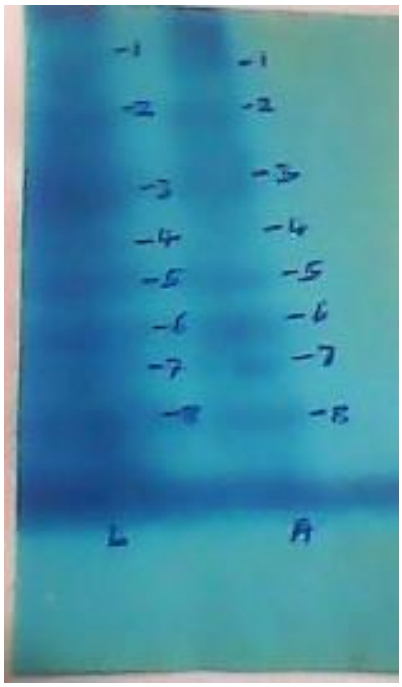

Plate 1. SDS-PAGE of the midgut of palm weevil, *R. phoenicis* The samples were loaded onto a 4% gel. **Lane L:** Larva of *R. phoenicis*, **lane A:** Adult of *R. phoenicis.*

Table 1. Molecular weights (Da) of the proteins of larva and adult palm weevil, *R. phoenicis*

Standard protein Name (Mol. Weight (Da)	Larva Rm (Mol. Weight (Da)	Adult Rm (Mol. Weight (Da)
1 Myosin 200,000	0.06 199,500	0.08 190,500
2 β-Galactosidase 119,000	0.25 100,000	0.10 177,800
3 Serumalbumin 66,000	0.31 79,430	0.28 95,500
4 Ovalbumin 43,000	0.39 63,100	0.42 50,120
5 Carbonic anhydrase 29,000	0.42 50,120	0.58 31,620
6 Trypsin 20,000	0.58 31,620	0.64 25,120
7 Lysozyme 14,500	0.64 25,120	0.69 22,390
8	0.75 17,780	0.89 11,220

Mol. Weight = Molecular weight
Rm = Relative mobility

Discussion

It is known that insects are good sources of proteins and as pests; they have enormous quantities of enzymes which help them in degrading their foods. The results of sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis showed that both the larva and adult palm weevils contained several protein bands with wide ranging molecular weights in their midguts (Plate 1.). Both the larval and adult stages of this insect have many protein bands which indicate different molecular weights. Similar proteins (carbohydrases) reported in the midgut of the larva of sweet potato weevil, *Cylas formicarius elegantulus* by Baker *et al.,* (1984) were also found in the larva and adult palm weevil (Table 1.) Baker *et al.,* (1984) reported that carbohydrases with relative mobility (Rm) values of 0.27 and 0.41 were found in *C. formicarius.* Baker *et al.*,(1984) reported a single proteinase band of relative mobility of 0.38 and two isozyme bands of relative mobilities of 0.84 and 0.95 respectively in *C. formicarius.* Similar protein band with Rm value of 0.39 was discovered in the larva while protein of Rm value of 0.89 was discovered in the adult stage of this insect. Detection of proteins with the same Rm in the larva and adult weevils connotes that the proteins are essential for their development. This finding agrees with what was reported by Nunamaker and Mckinnon (1989). These authors detected identical protein components in the egg, larva, pupa and adult stages of *Culicoides variipennis.* The authors reported further that the proteins detected were necessary for developmental changes, hence they were present in all the life stages and that under the right sets of metabolic conditions, the same proteins could be used to construct the necessary tissues and mediate the appropriate metabolic processes unique to the particular life stage.

Proteins with Rm of 0.58 were discovered in both the larva and adult palm weevil in this work. This finding agreed with the work of Thomas and Nair (2011) who had earlier reported protein bands with the same Rm value of 0.58 in the adult haemolymph of *Rhynchophorus ferrugineus* Olivier. A total of 15 protein bands were reported in the haemolymph of *R. ferrugineus* by Thomas and Nair (2011). Protein bands with Rm values of 0.24, 0.40, 0.66, 0.71 and 0.88 reported in the haemolymph of the developmental stages of *R. ferrugineus* by Thomas and Nair (2011) were observed in this work (i.e. larva and adult stages). The greater number of protein bands found in the midguts of both the larva and adult palm weevils could have been enzymes that are responsible for the faster digestion and enormous destruction of palm trees by the larval and adult stages of the insect.

Protein with Rm 0.06 reported in the larva stage has earlier been reported as malic dehydrogenase enzyme in the larva stage of *Distantiella theobroma* while protein with Rm 0.28 reported in the adult stage has also earlier been reported as adult enzyme in the same insect by (Coker, 1974). Baker (1978) and Aggrawal and Bahadur (1981) have reported an array of enzymes in the midguts of the larva of *Attagenus megatoma* and *Periplaneta americana* respectively. The presence of enzymes with the same molecular weights in both the larva and adult are expected since both stages feed on the same type of food. Coker (1974) reported the presence of esterase, leucine dehydrogenase and malic dehydrogenase enzymes in the larva and adult *Distantiella theobroma* (Distant) and *Sahlbergella singularis* Haglund. Amylase of molecular weight of 60,000 Da was reported in *P. americana* by Due *et al.* (2008). The enzyme of molecular weight 63,100 Da found in the larva of palm weevil was comparable to those reported for the α-amylases of *Thermus filiformis* and *Haloferax mediterranei* by Egas *et al.* (1998) and Perez-Pomares, *et al.,* (2003) respectively. The presence of esterase, leucine aminopeptidase, NAD linked alcohol dehydrogenase and lactate dehydrogenase have been confirmed in *Bathycoelia thallassina* (Herrich-Scaeffer) (Coker, 1974).

Acknowledgement

The author is very grateful to Dr. Adewale, I.O. of the Department of Biochemistry, Obafemi Awolowo University, Ile-Ife for his assistance with the electrophoretic pack and for the unrestricted access to his laboratory.

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5/12/2024