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Carboxylesterase Activity of *Bemisia tabaci* (Hemiptera: Aleyrodidae): Subcellular Distribution, Temperature and pH Effects

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ABSTRACT

Carboxylesterases are major enzymes involved in the detoxification of insecticides in several insect species. The measurement and hydrolytic ability of these enzymes function best within specific temperature and hydrogen ion concentration (pH) conditions. The effects of temperature and pH on the hydrolysis of α-naphthyl acetate by Bemisia tabaci carboxylesterase and the subcellular fractionation of this enzyme were studied. The optimum assay conditions for measuring carboxylesterase activity of B. tabaci were a pH of 6.8 - 7.2 and 40 °C - 45 °C. Subcellular localization study of the enzyme in B. tabaci showed the presence of carboxylesterase activity in all the fractions.

***Keywords**: Bemisia tabaci, carboxylesterase, pH, temperature, subcellular distribution*

INTRODUCTION

The whitefly *Bemisia tabaci* is one of the world's most invasive species [1]. It is a devastating global pest that feeds on vegetables, cotton and several economically important crops [1,2, 3]. It is a phloem feeder and a transmitter of several plant viruses [3, 4]. It excretes honey dew that leads to the growth of sooty mold thereby affecting the photosynthetic ability of plants [3]. The damaging activity of *B. tabaci* could result in severe crop losses ranging from 20 % to total yield loss [5]. The widespread and frequent use of insecticides to bring its population under control is a dominant practice in Ghana [3], contributing to the development of insecticide resistance.

Carboxylesterases are a large group of hydrolyzing enzymes that cleave ester bonds [6]. These detoxification enzymes play an important role in insecticide resistance and have been associated with resistance to several insecticide classes in many insects [7,8,9]. Due to its role in insecticide metabolism, the measurement of carboxylesterase activity is used as a biochemical indicator of insecticide resistance in many insect species.

The study sought to monitor the effect of temperature and hydrogen ion concentration for the assay of carboxylesterase activity of *B. tabaci* so as to enhance assay sensitivity and observe the distribution of carboxylesterase activity in the subcellular fractions of this invasive insect pest.

MATERIALS AND METHODS

Insects

Unsexed *Bemisia tabaci* adults were aspirated from *Duranta sp* in an open-air enclosed environment at ARPPIS, University of Ghana campus and used for the carboxylesterase assay.

Temperature and pH effects The effects of temperature on carboxylesterase activity were monitored from 20 °C to 60 °C at 5 °C intervals. All the other assay parameters remained unchanged. For studies involving pH effects, carboxylesterase activity was monitored from a pH of 6.0 to 8.0 with all other parameters being unchanged.

Subcellular distribution of carboxylesterase activity

The subcellular fractions were obtained by slightly modifying the method used for *Aphis gossypii* [10]. About 50 whiteflies were homogenized in 2 ml of 0.07 M phosphate buffer (pH 7.0) in a glass

well using a glass rod. Half of the mixture was designated as crude homogenate whilst the other half was centrifuged at 500 g for 10 min. The supernatant was decanted and the pellet resuspended in the buffer and centrifuged at 10,000 g for 5 min. The pellet was again resuspended and centrifuged at 40,000g for an hour to yield pellet and supernatant. These successive centrifugation patterns yielded a series of subcellular fractions which were nuclear (500 g pellet), mitochondria (10,000 g pellet), 40,000 g pellet and 40,000 g supernatant. The carboxylesterase activity levels of the crude homogenate and the subcellular fractions were determined. Three replicates and three determinations were done for the subcellular fractions, temperature and pH assays.

Homogenate preparation

About 50 whiteflies were mass-pulverized for each parameter (temperature, pH and subcellular distribution) and this homogenate served as the enzyme source [10].

Carboxylesterase activity

Carbxylesterase activity was determined by using α-naphthyl acetate as substrate [11]. The assay consisted of 100 µl enzyme extract pipetted into test tubes containing 2.8 ml of phosphate buffer pH 7.0 [11]. This was incubated at 40 °C in a water bath for 10 min with 100 µl of 30 mM α-naphthyl acetate. After incubation, 0.5 ml of a solution mixture of sodium dodecyl sulphate-fast blue salt (SDS-FBS) was added to each test tube to stop the reaction and allow for colour development. The resultant optical density of each sample was measured at 600 nm against a control on a CAMSPEC M106 spectrophotometer.

Protein content assay

The method of protein content determination for *A. gossypii* was used for this assay [11]. Two millimeters of a mixture (50 ml of 2 % Na_2CO_3 in 0.1 M NaOH added to 0.5 ml each of $CuSO_4$ and $NaC_4O_62H_2O$ in a total ratio of 50: 0.5: 0.5) was added to 0.1 ml of enzyme extract in a test tube and allowed to stand for 20 min. A 0.25 ml phenol: water (1:1) mixture was added to the contents of the test tube and allowed to stand for 20 minutes to allow for a blue colour development. The optical density of the samples was read at 750 nm against a control on the spectrophotometer.

RESULTS AND DISCUSSION

Optimal carboxylesterase activity assay conditions

The carboxylesterase activity levels increased gradually from the initial temperature of 20 °C to 35 °C and thereafter increased sharply to 40 °C. Beyond 50 °C, there was a steep decline in the activity measured (Fig. 1). In monitoring the effect of various assay buffer pH conditions, the rate of α-naphthol formation increased gradually with a plateau from pH 6.8 to 7.2, though it peaked at 7.0. Carboxylesterase activity measured decreased at higher pH levels of 7.4 and above (Fig. 2).

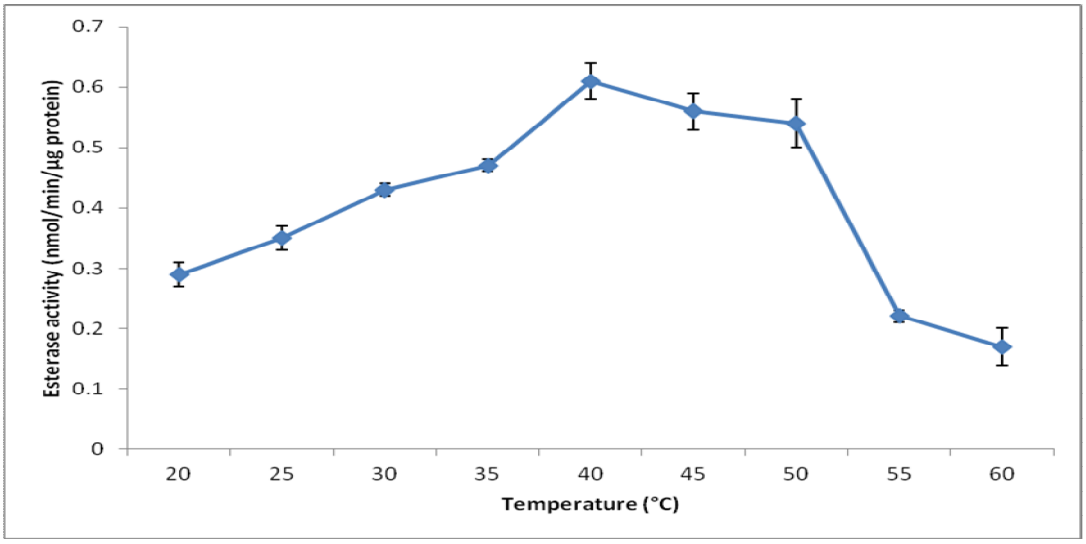

Fig. 1. Temperature (mean ± SE) effect on *B tabaci* carboxylesterase hydrolysis of α-naphthyl acetate

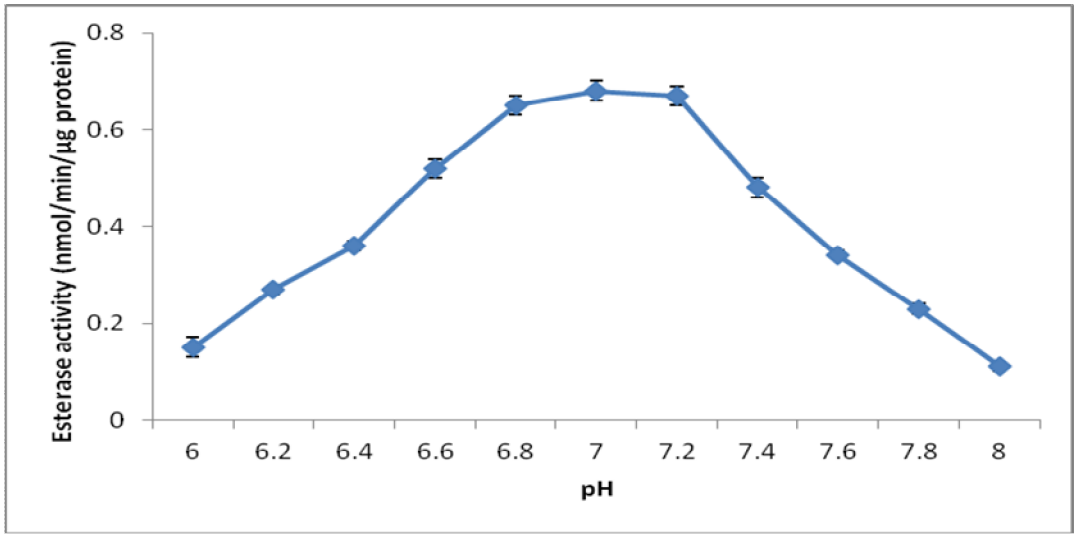

Fig. 2. Hydrolysis of α-naphthyl acetate by *B. tabaci* carboxylesterase at various pH conditions

Most enzymes have optimum activity within specific temperature and pH conditions and in this study, it was observed that the sensitivity of carboxylesterase assay conditions in *B. tabaci* is within an optimum range of 40 °C - 45 °C and a pH of 6.8 to 7.2. This falls within a similar range of assay conditions for *A. gossypii* [10]. In that study [10], the optimum temperature range for assaying carboxylesterase activity of *A. gossypii* was 40 °C to 45 °C with a pH range of 6.6 to 7.0, though a pH range of 6.8 to 7.2 was recommended. In characterizing esterases from populations of western corn rootworm *Diabrotica virgifera virgifera* resistant and susceptible to methyl parathion, an optimal assay temperature of 35 °C to 45 °C and a pH of 7.0 to 7.4 with the highest esterase activity recorded at a buffer pH of 7.4 was observed [12]. However, in this study of *B. tabaci* carboxylesterase, activity rapidly decreased beyond a pH of 7.2.

Distribution of carboxylesterase activity in subcellular fractions

Carboxylesterase hydrolyzing activity of α-naphthyl acetate was present in all the fractions and the distribution of total and specific carboxylesterase activities at the subcellular levels varied (Table 1).

Table 1: Subcellular fractionation of α-naphthyl acetate hydrolyzing activities in homogenate of *B. tabaci* aspirated from *Duranta sp*

Fractions	Total activity(T) µmoles/10min/ml	Yield[a] (%)	Specific activity nmoles/10min/µg protein	Yield[a] (%)
Crude homogenate	0.253 ± 0.036	___	21.20 ± 1.20	___
Nuclear	0.026 ± 0.006	13.0	3.40 ± 0.68	14.2
Mitochondrial	0.004 ± 0.002	2.0	0.30 ± 0.07	1.3
40,000 g pellet	0.013 ± 0.003	6.5	0.80 ± 0.26	3.3
40,000 g supernatant	0.157 ± 0.007	78.5	19.40 ± 0.25	81.2

[a] Denominator was the sum of activity of fractions except crude homogenate activity

The least specific activity was recovered in the mitochondrial fraction with the most activity distributed in the 40,000 g supernatant. An earlier study in *A. gossypii* [10] found that 82 % of α-naphthyl acetate hydrolyzing esterase activity in the insect was in the cytosolic portion and the least activity was distributed in the mitochondrial fraction. The presence of esterase activity in subcellular fractions of insects has also been observed in resistant populations. The recovery of both non-specific esterases and malathion carboxylesterases in both resistant and susceptible *Tribolium castaneum* has been reported [13] and the highest activity was recovered in the cytosolic fraction for both strains. Subcellular localization of esterolytic activity was also found in methyl parathion resistant and susceptible *D. v. virgifera* with the highest activity in the cytosolic fraction [12].

The distribution of esterase activity at the subcellular level is not only limited to whole organism studies as it has also been observed in tissue studies of insects. In studying the localization of esterase activities in the gut of the termite *Reticulitermes flavipes*, esterase isoforms were recovered from the mitochondrial, microsomal and cytosolic fractions [14]. Though in this experiment the carboxylesterase activity in the nuclear fraction was more than the mitochondrial fraction, it was lesser than the 40,000 g supernatant. The recovery of most activity from 40,000 g supernatant was not surprising since it contained both the microsomal and cytosolic cell fractions. However, more esterolytic activity is localized in the cytosolic fraction than the microsomal fraction [10,12,14].

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REFERENCES

1. GISD [Global Invasive Species Database] (2012). *Bemisia tabaci*. Available: http://www.issg.org/database/species/ecology.asp?si=106&fr=1&sts=&lang=EN. Accessed 10 Nov 2012.
2. Oliveira, M.R.V., Henneberry, T.J. & Anderson, P. (2001). History, current status, and collaborative research projects for *Bemisia tabaci*. Crop. Prot., 20(9): 709-723.
3. Obeng-Ofori, D. (2007). Major arthropod pests of root, tuber crops and plantains. (Ed. Obeng-Ofori, D.) Major pests of food and selected fruit and industrial crops in West Africa, City Publishers, Accra, Ghana, p. 59-75.
4. Navas-Castillo, J., Fiallo-Olivé, E. & Sánchez-Campos, S. (2011). Emerging virus diseases transmitted by whiteflies. Annu. Rev. Phytopathol., 49: 219-248.
5. Brown, J.K. & Bird, J. (1992). Whitefly-transmitted geminiviruses and associated disorders in the Americas and the Caribbean Basin. Plant Dis., 76(3): 220-225.
6. Oakeshott, J.G., Claudianos, C., Campbell, P.M., Newcomb, R.D. & Russell, R.J. (2005). Biochemical genetics and genomics of insect esterases. (Eds. Gilbert, L.I., Latrou, K. & Gill, S.S.) Comprehensive Molecular Insect Science, Vol. 5, Elsevier BV, Oxford, UK, p. 309-381.
7. Ranson, H., Claudianos, C., Ortelli, F., Abgrall, C., Hemingway, J., Sharakhova, M.V., Unger, M.F., Collins, F.H. & Feyereisen, R. (2002). Evolution of multigene families associated with insecticide resistance. Science, 298(5591): 179-181.
8. de Carvalho, R.A., Torres, T.T. & de Azeredo-Espin, A.M. (2006). A survey of mutations in the *Cochliomyia hominivorax* (Diptera: Calliphoridae) esterase E3 gene associated with organophosphate resistance and the molecular identification of mutant alleles. Vet. Parasitol., 140(3-4): 344-351.
9. Li, X., Schuler, M.A. & Berenbaum, M.R. (2007). Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annu. Rev. Entomol., 52: 231-253.
10. Owusu, E.O., Komi, K., Horiike, M. & Hirano, C. (1994). Some properties of carboxylesterase from *Aphis gossypii* Glover (Homoptera: Aphididae). Appl. Entomol. Zool., 29(1): 47-53.
11. Owusu, E.O., Kim, C-S. & Horiike, M. (1995). Susceptibility of cotton aphid *Aphis gossypii* Glover (Homoptera: Aphididae) to dichlorvos and its relationship to activity levels of some esterases. Research Reports, Kochi University, Kochi Japan, 44: 60-67.
12. Zhou, X., Scharf, M.E., Meinke, L.J., Chandler, L.D. & Siegfried, B.D. (2003) Characterization of general esterases from methyl parathion-resistant and -susceptible populations of western corn rootworm (Coleoptera: Chrysomelidae). J. Econ. Entomol., 96(6): 1855-1863.
13. Haubruge, E., Amichot, M., Cuany, A., Berge, J.B. & Arnaud, L. (2002). Purification and characterization of a carboxylesterase involved in malathion-specific resistance from *Tribolium castaneum* (Coleoptera: Tenebrionidae). Insect Biochem. Mol. Biol., 32(9): 1181-1190.
14. Wheeler, M.M., Tarver, M.R., Coy, M.R. & Scharf, M.E. (2010). Characterization of four esterase genes and esterase activity from the gut of the termite *Reticulitermes flavipes*. Arch. Insect Biochem. Physiol., 73(1): 30-48.