

## Polyacrylamide Gel Electrophoretic Band Pattern of Esterase in the Pupae of *Bactrocera papayae* and *Bactrocera carambolae* (Diptera: Tephritidae)

<sup>1,2</sup>M. Hasanuzzaman and <sup>1</sup>A.B. Idris

<sup>1</sup>Department of Environmental and Natural Resource Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor Darul Ehsan, Malaysia

<sup>2</sup>Department of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment, Bangladesh Atomic Energy Commission, Ganakbari, Savar, Dhaka-1349, Bangladesh

**Abstract:** The study was undertaken to compare the electrophoretic banding patterns of esterase isozyme between the pupae of *Bactrocera papayae* and *Bactrocera carambolae* (Diptera: Tephritidae) by using polyacrylamide gel. These two *Bactrocera* species are the major agricultural pests, especially fruits and vegetables. One esterase band, EST-1 was detected and the relative mobility value was 0.15 which was close to the cathode. The EST-1 band was present in the pupae of both *Bactrocera* species. There was no difference in the esterase patterns of both species. The thickness and activation of the band varied slightly. So, the results prove that the pupae of the two *Bactrocera* species have almost similar esterase band pattern in the same polyacrylamide gel.

**Keywords:** Electrophoresis, fruit fly, isozyme, polyacrylamide gel, tephritidae

### INTRODUCTION

Asian papaya fruit fly, *Bactrocera papayae* Drew & Hancock and carambola fruit fly, *Bactrocera carambolae* Drew & Hancock (Diptera: Tephritidae) are major agricultural pests in Malaysia, like other Asian countries. These two *Bactrocera* species are belonging to the *Bactrocera dorsalis* complex and considered as sibling species (Chua *et al.*, 2010). According to Clarke *et al.* (2005), the *Bactrocera dorsalis* complex of tropical fruit flies contains 75 described species, largely endemic to Southeast Asia; and within the complex are a small number of polyphagous pests of international significance, including *Bactrocera dorsalis* sensu stricto, *Bactrocera papayae*, *Bactrocera carambolae*, and *Bactrocera philippinensis*. Asian papaya fruit fly, *B. papayae* has been recorded from 193 host plant species in 114 genera and 50 families in Asia; *B. carambolae* attacks more than 151 kinds of fruits and vegetables; and they are distributed in Southeast Asia (Malaysia, Singapore, Indonesia, Thailand, Brunei) and South-America (Suriname, Brazil, French Guiana) (Drew and Hancock, 1994; Allwood *et al.*, 1999). In Malaysia, economic losses were estimated at 12.8 million ringgit due to fruit flies in the year of 1987 (Vijaysegaran, 1988).

The ester hydrolyzing enzyme, esterase, comprises a multi-functional and heterogeneous group of enzymes. They have as a shared characteristic participation in ester hydrolysis. Esterase patterns are

important tool for analysis of genetic differentiation and evolutionary relationship of insects (Nascimento and De Campos Bicudo, 2002). They are also stage-specific and tissue-specific in insects (Lima-Catelani *et al.*, 2004) and are closely associated with morphological, physiological, or biochemical ontogenetic alterations (Cohen *et al.*, 1977). In insects, they are related to several metabolic processes, such as food digestion, insecticide resistance, pheromones and juvenile hormone hydrolysis (Campbell *et al.*, 2003). The present study was undertaken to compare the esterase isozyme band patterns in the pupae (9 days old) of *B. papayae* and *B. carambolae* in the same polyacrylamide gel.

### MATERIALS AND METHODS

**Biological samples:** Pupae of *Bactrocera papayae* and *B. carambolae* were collected from the Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor and reared in Universiti Kebangsaan Malaysia (UKM). Rearing was maintained in a controlled room at 25±2°C with 70-80% relative humidity and 14 h light: 10 h dark cycle. Adult diet were yeast and sugar (1:3) and cotton in water. For egg collection and as larval media, fresh sweet gourds were used for *B. papaya* and fresh star fruits for *B. carambolae*.

The study was carried out between 2010 and 2011 at the Entomology laboratory, School of Environmental

and Natural Resource Sciences, Faculty of Science and Technology, UKM, Bangi, Selangor Darul Ehsan, Malaysia.

value of esterase isozymes was calculated in the gel using the formula described by Raja *et al.* (2009).

## RESULTS AND DISCUSSION

**Electrophoresis:** Pupae (9 days old) of *B. papayae* and *B. carambolae* were used for the experiments which were collected from the laboratory reared stock. According to Bernardo and de Campos Bicudo (2009), samples were homogenized in 200  $\mu$ L of buffer solution (0.1 M Tris-HCl plus 10% glycerol at pH 8.8). Ten micro liter of bromophenol blue was added into each sample as a tracking dye. Homogenates were centrifuged at 10,000 rpm for 15 min at 5°C. Esterase isozyme patterns were analyzed in 10% polyacrylamide gels (Laemmli, 1970). After the sample application (30  $\mu$ L), the gels were subjected to electrophoresis (vertical type) for 4 h at room temperature (25°C) using a constant voltage of 200 V and 0.1 M Tris-glycine (pH 8.3) as the running buffer (Bernardo and de Campos Bicudo, 2009). Esterase isozymes were identified in the gels following the technique described by Johnson *et al.* (1966) and Steiner and Johnson (1973), using  $\alpha$ - and  $\beta$ -Naphthyl acetates as substrates. The esterase isozyme bands were numbered according to the Recommendations of the Standard Committee of Enzyme (Webb, 1964). The Relative Mobility (RM)

Polyacrylamide gel electrophoresis band pattern of nonspecific esterase isozyme was found in the pupae (9 days old) of *B. papayae* and *B. carambolae* (Diptera: Tephritidae) which was shown in Fig. 1. One esterase band, EST-1 was detected and the relative mobility value was 0.15 which was close to the cathode. The EST-1<sup>0.15</sup> band was present in the pupae of both *Bactrocera* species. There was no difference in the esterase patterns of both species. The thickness and activation of the band varied slightly. The pupae of the two *Bactrocera* species have almost similar esterase band pattern in the same polyacrylamide gel.

Hasanuzzaman and Idris (2012a) observed three esterase isozymes (EST-1<sup>0.61</sup>, EST-2<sup>0.46</sup> and EST-3<sup>0.15</sup>) on Polyacrylamide Gel Electrophoresis (PAGE) during the different life stages of *Bactrocera carambolae*. EST-1<sup>0.61</sup> and EST-2<sup>0.46</sup> were present in larvae (3 and 6 days old); EST-2<sup>0.46</sup> and EST-3<sup>0.15</sup> were observed in adults; whereas EST-3<sup>0.15</sup> was found in pupae (3 and 6 days old); and esterase activity was not detected in eggs. Hasanuzzaman and Idris (2012b) reported that

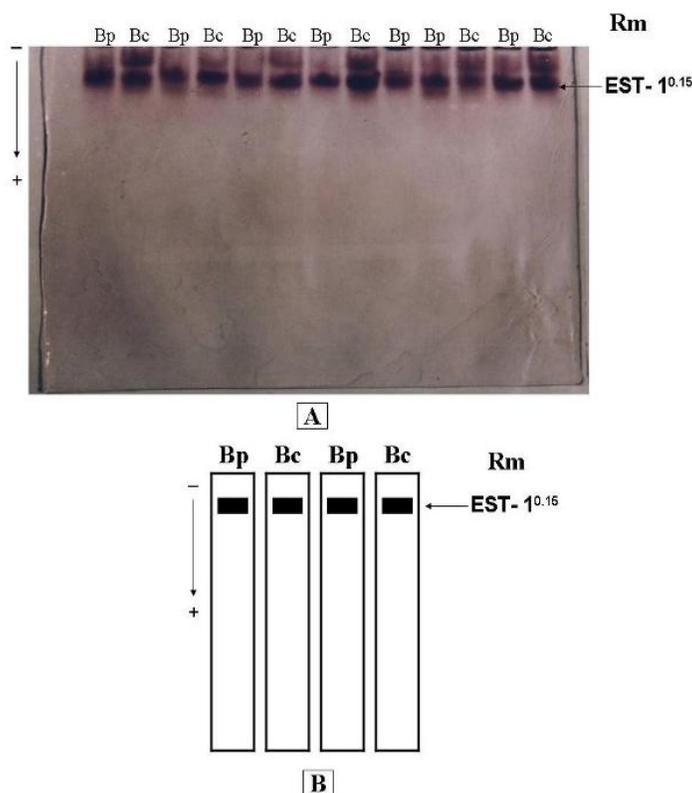


Fig. 1: Esterase isozyme patterns in the pupae (9 days old) of *Bactrocera papayae* and *Bactrocera carambolae* in polyacrylamide gel, (A) photographic representation, (B) schematic diagram. Bp: *Bactrocera papayae*; Bc: *B. carambolae*

EST-1<sup>0.61</sup> and EST-2<sup>0.46</sup> bands were present in 9 days old larvae of *B. papayae* and *B. carambolae*. The thickness and the staining degree of bands varied and EST-2<sup>0.46</sup> band was comparatively thicker than the EST-1<sup>0.61</sup> band. There was no difference in the esterase isozyme patterns of these two *Bactrocera* larvae. Hasanuzzaman and Idris (2012c) also found in the adult flies that two bands, EST-1<sup>0.46</sup> and EST-2<sup>0.15</sup> were present in *B. carambolae*, whereas only one band, EST-2<sup>0.15</sup> was observed in *B. papayae*. EST-2<sup>0.15</sup> band was thick and highly stained than the band of EST-1<sup>0.46</sup> in *B. carambolae*, but there was no difference in male and female adults, when comparing among the same species. Hasanuzzaman (2003) reported seven esterase bands, controlled by two esterase loci (EST-1 and EST-2) during the different life stages of *Bactrocera cucurbitae* on PAGE gel and their relative mobility values were 0.17, 0.27, 0.37, 0.46, 0.58, 0.87, 1.00. Borja *et al.* (2010) found four esterase isozyme bands, controlled by two loci (EST-1, EST-2) in *Bactrocera occipitalis* and *Bactrocera philippinensis*.

Two esterase *as* loci (EST-1 and EST-2) were detected in the adult brown planthopper, *Nilaparvata lugens* by Bashar *et al.* (2002) on PAGE gel. Cohen *et al.* (1977) studied the expression of esterases during ontogenesis of the flour beetle *Tribolium castaneum* in PAGE gel. Two nonspecific esterases were detected and designated F (Fast) and S (Slow) according to their relative migration distances. In the adults of *Drosophila virilis* the maximum activity/intensity of esterase patterns was detected by Sasaki (1974). Ruvolo-Takasusuki *et al.* (2006) investigated the pattern of esterase expression in the stingless bees *Tetragonisca angustula* and *Tetragona clavipes* using polyacrylamide gels. Seven bands of esterase activity were detected in *T. clavipes* (EST-1 to EST-7) and two bands in *T. angustula* (EST-1 and EST-2). Khuda-Bukhsh (1990) observed non-specific esterase profiles in *Aphis gossypii*, *A. craccivora* and *Lipaphis erysimi* by using polyacrylamide gel electrophoresis. Samples of *Lipaphis erysimi* produced 2 esterase bands, while those of the other species produced a single band. Raja *et al.* (2009) assessed on the variations in esterase pattern of six populations of *Callosobruchus maculatus* on polyacrylamide gel electrophoresis and found that Coimbatore population recorded three isozyme fractions (rm 0.17, 0.28 and 0.41). Santos *et al.* (1985) reported that six esterase isozymes were investigated during the development of *Anopheles darlingi* using polyacrylamide gel electrophoresis. Esterases 5 and 6 were detected in all developmental stages; esterases 1 and 2 were more intensively stained if larvae; while esterases 3 and 4 were better visualized in pupae and adults. Strong differences in intensity of some of the isozymes were observed during the pupal stage. Gruppe (1988) differentiated three subspecies of *Myzus cerasi* in Central Europe. *M. cerasi* strain is characterized by two esterases with R (f)-values of 0.9 and 0.4; whereas

*Prunus cerasus*, *Prunus avium* by three esterases with R (f)-values of 0.9, 0.4 and 0.2. Wool *et al.* (1989) studied electrophoretically adults of some species of whiteflies in Israel and Colombia. The patterns of esterase isozymes were species-specific and reported that it may be used to identify whitefly adults.

## CONCLUSION

In conclusion, electrophoresis can be used to study the biochemical characterization of the esterase isozyme patterns using vertical polyacrylamide gel in the pupae of the two *Bactrocera* species and also the developmental stages of the other *Bactrocera* pest species. The information presented here will also be helpful for further study on the determination of insecticide resistant and non-resistant pest species in the population; the phylogenetic relationships among these *Bactrocera* species; and also the esterase isozyme polymorphism in natural population.

## ACKNOWLEDGMENT

This work was supported by Universiti Kebangsaan Malaysia (Project grant, OUP-2012-043). Sincere thanks to Prof. Dr. Mahani Mansor Clyde for providing the laboratory facilities to carry out this research program.

## REFERENCES

- Allwood, A.J., A. Chinajariyawong, R.A.I. Drew, E.L. Hamacek, D.L. Hancock, C. Hengsawad, J.C. Jinapin, M. Jirasurat, C. Krong, S. Kritsaneepaiboon, C.T.S. Leong and S. Vijaysegaran, 1999. Host plant records for fruit flies (Diptera: Tephritidae) in South-East Asia. *Raffles Bull. Zool.*, Supplement No., 7: 1-92.
- Bashar, K., R.M. Shahjahan, M.A. Baqui and M. Hasanuzzaman, 2002. Electrophoretic banding pattern of esterase isozymes in adult brown planthopper, *Nilaparvata lugens* (Stal) (Hom.: Delphacidae). *Univ. J. Zool. Rajshahi University*, 21: 27-29.
- Bernardo, A.A. and H.E.M. de Campos Bicudo, 2009. Variability of esterase patterns in adult flies of the *saltans* species group of *Drosophila* (subgenus *Sophophora*). *Genetica*, 137: 111-124.
- Borja, F.N.N., M.S. Mendiolo, C. dR. Medina and L.R.I. Velasco, 2010. Isozyme Polymorphism in the Oriental Fruit Fly [*Bactrocera occipitalis* (Bezzi) and *B. philippinensis* Drew and Hancock] from Puerto Princesa, Palawan, Philippines. *Philipp. Agric. Sci.*, 93: 232-237.
- Campbell, P.M., G.C. Robi, L.N. Court, S.J. Dorrian, R.J. Russell and J.G. Oakeshott, 2003. Developmental expression and gene/enzyme identifications in the alpha esterase gene cluster of *Drosophila melanogaster*. *Insect Mol. Biol.*, 12: 459-471.

- Chua, T.H., B.K. Song and Y.V. Chong, 2010. Development of allele-specific single-nucleotide polymorphism-based polymerase chain reaction markers in cytochrome oxidase I for the differentiation of *Bactrocera papayae* and *Bactrocera carambolae* (Diptera: Tephritidae). J. Econ. Entomol., 103(6): 1994-1999.
- Clarke, A.R., K.F. Armstrong, A.E. Carmichael, J.R. Milne, S. Raghu, G.K. Roderick and D.K. Yeates, 2005. Invasive phytophagous pests arising through a recent tropical evolutionary radiation: The *Bactrocera dorsalis* complex of fruit flies. Annu. Rev. Entomol., 50: 293-319.
- Cohen, E., E. Sverdlov and D. Wool, 1977. Expression of esterases during ontogenesis of the flour beetle *Tribolium castaneum* (Tenebrionidae; Coleoptera). Biochem. Genet., 15: 253-264.
- Drew, R.A.I. and D.L. Hancock, 1994. The *Bactrocera dorsalis* complex of fruit flies (Diptera: Tephritidae: Dacinae) in Asia. Bull. Entomol. Res., Suppl., 2: 1-68.
- Gruppe, A., 1988. Electrophoretic studies on different subspecies of *Myzus cerasi* F. (Homoptera: Aphididae). J. Appl. Entomol., 105(5): 460-465.
- Hasanuzzaman, M., 2003. Esterase isozyme variation and polytene chromosome analysis in the melon fly, *Bactrocera cucurbitae* (Diptera: Tephritidae), M. Phil. Thesis, University of Dhaka, Dhaka, Bangladesh.
- Hasanuzzaman, M. and A.B. Idris, 2012a. Isozymes variation during development, revealed by esterase band patterns of *Bactrocera carambolae*. GSTF J. BioSci., 1(2): 31-35.
- Hasanuzzaman, M. and A.B. Idris, 2012b. Comparative study on esterase isozyme patterns between the larvae of *Bactrocera papayae* and *Bactrocera carambolae* (Diptera: Tephritidae). Acad. J. Entomol., 5(2): 122-125.
- Hasanuzzaman, M. and A.B. Idris, 2012c. Electrophoretic banding patterns of esterase isozymes in the adult fly of *Bactrocera papayae* and *B. carambolae* (Diptera: Tephritidae) by using polyacrylamide gel. Serangga, 17(1), (In Press).
- Johnson, F.M., C.G. Kanapi, R.H. Richardson, M.R. Wheeler and W.S. Stone, 1966. An operational classification of *Drosophila* esterases for species comparison. University Texas Publ., 6615: 517-532.
- Khuda-Bukhsh, S.S., 1990. Non specific esterase profiles in three species of aphids (Homoptera: Aphididae). National Acad. Sci. Lett., 13(10): 395-397.
- Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.
- Lima-Catelani, A.R.A., C.R. Ceron and H.E.M. de Campos Bicudo, 2004. Genetic variation during development, revealed by esterase patterns of *Aedes aegypti* (Diptera: Culicidae). Biochem. Genet, 42: 69-84.
- Nascimento, A.P. and H.E.M. De Campos Bicudo, 2002. Esterase patterns and phylogenetic relationships of *Drosophila* species in the *saltans* subgroup (*saltans* group). Genetica, 114: 41-51.
- Raja, M., S.J. William and K.J. Hussain, 2009. Genetic diversity of *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae) populations. Int. J. Integr. Biol., 8: 15-18.
- Ruvolo-Takasusuki, M.C.C., L.H.O. Viana, T.V. Baitala, K.C. Nicolin and V.A.A. Toledo, 2006. Characterization of esterases in *Tetragonisca angustula* and *Tetragona clavipes* (Hymenoptera; Meliponinae). Braz. J. Morphol. Sci., 23(3-4): 431-434.
- Santos, J.M.M., E.P.B. Contel and W.E. Kerr, 1985. Biology of Amazonian mosquitoes III: Esterase isozymes in *Anopheles darlingi*. Acta Amazonica, 15: 167-177.
- Sasaki, F., 1974. Esterase isozymes of *Drosophila virilis*: Developmental aspects of the  $\alpha$ - and  $\beta$ -esterases and their distribution in their body parts. Japanese J. Genet., 49: 223-232.
- Steiner, W.W.M. and W.E. Johnson, 1973. Techniques for electrophoresis of Hawaiian *Drosophila* US-IBP. Island Ecosystems Technical Report, 30: 1-21.
- Vijaysegaran, S., 1988. The Current Situation on Fruit Flies in Peninsular Malaysia. In: Vijaysegaran S. and A. Ibrahim (Eds.), Proceedings of the 1st International Symposium on Fruit Flies in the Tropics, Kuala Lumpur: Malaysian Agricultural Research and Development Institute, pp: 125-139.
- Webb, E.C., 1964. Nomenclature of multiple enzyme forms. Nature, 203: 821.
- Wool, D., D. Gerling, B.L. Nolt, L.M. Constantino, A.C. Bellotti and F.J. Morales, 1989. The use of electrophoresis for identification of adult white flies (Homoptera: Aleyrodidae) in Israel and Colombia. J. Appl. Entomol., 107(4): 344-350.