ISOZYME BANDING PATTERNS OF ALCOHOL DEHYDROGENASE (ADH) LOCI IN PUPAE OF MELON FLY, BACTROCERA CUCURBITAE (COQ)

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Abstract: Isozyme banding patterns of alcohol dehydrogenase (ADH) in two days old pupae of the melon fly, Bactrocera cucurbitae were observed on 5% Polyacrylamide Gel Electrophoresis (PAGE). Two loci of ADH isoenzymes, designated as ADH-1 and ADH-2, were identified. Altogether four allelomorphs or electromorphs were found. The ADH-1 locus showed two electromorphs (Adh 0.18 and Adh 0.30). ADH-2 was also found with two electromorphs (Adh 0.06 and Adh 0.10). The bands of ADH-1 had high mobility, stained faintly and was close to the anode. ADH-2 allelomorphs showed a low mobility but stained deeply and was close to the cathode. The results are encouraging in the future development of a Sterile Insect Technique (SIT) programme for melon fly control and eradication.

Key words: ADH, isozymes, electrophoresis, Bactrocera cucurbitae, melon fly

INTRODUCTION

The melon fly, Bactrocera cucurbitae is a serious pest of fruits and vegetables in Bangladesh (Shahjahan et al. 2001) and its presence in any country cause severe limitations to the export of agricultural produce. It is also found in India, South-East Asia, Thailand, the Ryukyu Islands of Japan, Pacific Islands including Hawaii and Africa (Shahjahan et al. 1998). Isozymes are multiple forms of a single enzyme and therefore can be separated by electrophoresis. The different monomers of the isozymes are specified by different gene loci and the term allozyme is used to refer to variant proteins produced by allelic forms of the same locus (King 1974). ADH isozyme system is a common example which is under genetic control and for which the genetic locus has been identified (Grell et al. 1965). ADH is one of the potential genes thought to be useful as marker (Riva and Robinson 1986). In Ceratitis capitata, the primary interest to study alcohol dehydrogenase (ADH) was as a candidate enzyme for the development of a genetic sexing system (Gasperi et al. 1992). This enzyme is abundant in Drosophila, and it is generally accepted that it is responsible for alcohol detoxification and alcohol utilization (Van Delden 1982 and David 1988).
The study of the electrophoretic banding patterns of ADH isoenzymatic systems in *Anastrepha fraterculus* is very important, not only because they respond quickly to environmental changes, but also because if they show dynamic expression through development. This knowledge may provide for its control and management (Nascimento and Oliveria 1997).

In the melon fly, *B. cucurbitae*, four major larval serum protein (LSP) bands (Shahjahan et al. 1998) and two distinct sets of ADH bands (ADH-1 and ADH-2), showing eleven allelomorphs (Shahjahan et al. 2001) were reported in third instar larvae using 5% PAGE as biochemical markers. In this study the electrophoretic banding patterns of ADH isozymes of two days old pupae were observed on 5% PAGE at 5°C and the banding patterns were recorded.

**MATERIAL AND METHODS**

*Fly stocks:* The population of *B. cucurbitae* was obtained from a laboratory reared stock (Generation-65), which maintained using sweet gourd as larval feed at 25 ± 2°C temperatures and about 70-80% Relative Humidity. The adults were supplied with an artificial diet made of sugar, yeast extract and casein (2:1:1) in 1% agar gel as water media.

*Electrophoresis:* Electrophoretic banding patterns of ADH isozymes were observed on 5% PAGE using a discontinuous buffer system. Individual samples of two days old pupae of *B. cucurbitae* were macerated into 200 µl gel loading buffer (5% sucrose in Tris-Borate-EDTA (Ethylendiamine Tetraacetic acid) buffer, pH 8.5) with 20 µl bromophenol blue. After centrifugation at 13000 rpm for 20 min at 5°C, the supernatants were subjected to electrophoresis. Polyacrylamide gels (5%) were prepared using the standard protocol (Shahjahan et al. 2001) with slight modifications. A prepag was used containing 5 ml acrylamide and bis-acrylamide in the ratio of 29:1:11 ml TBE (IX) buffer containing 10.8 g Tris, 5.5 g Boric acid, 0.5 M EDTA 4 ml, pH 8.0: TEMED (N,N,N',N'-Tetramethyl-ethylene diamine) 250 µl; 10% AMPS (Ammonium persulfate) 200 µl. The gels were pre-run at 150 volts for at least 30 min at 5°C. The samples were loaded in 10 µl aliquots. The electrophoresis was done at 5°C with 150 volts for at least 3 hours. The staining solution for ADH contained 50 ml of TBE buffer (0.05 M, pH 8.5), 0.08 g NAD (Nicotinamide adenine dinucleotide), 0.04 g PMS (Phenazine methosulfate), 0.10 g NBT (Nitro Blue Tetrazolium) and 0.75 ml ethanol. The gels were destained in 30% ethanol and 12% acetic acid.

*Numbering of ADH isozymes:* The electrophoretically separated bands of ADH isozymes were numbered from the anodal end of the gel according to the recommendations of the Standard Committee of Enzyme (Webb 1964).
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Measurement of Relative Mobility (RM): The value of most mobile band was considered as one when the RM values of different bands of ADH isozymes were recorded (Shahjahan et al. 2001).

RESULTS AND DISCUSSION

The scanned photographic electrophoretic banding patterns of ADH isozymes in two days old pupae of B. cucurbitae are shown in Fig. 1A. No marked differences were observed in ADH isozyme patterns of the pupae. Two loci of ADH isozymes such as ADH-1 and ADH-2 showing four electromorphs were found in the pupae, while eleven allelomorphs or electromorphs were reported in the third instar larvae (5 days old) of the same species (Shahjahan et al. 2001). However, all electromorphs were not observed in any one individual pupae (Table 1 and Fig. 1B). The locus ADH-1 showed low activity whereas the ADH-2 showed comparatively higher activity. The ADH-1, anodic locus, had two electromorphs and ADH-2, cathodic locus, also possessed two allelomorphs (Table 1 and Figs. 1A and 1B). The RM values of ADH-1 were adh-0.18 and Adh-0.30 and for ADH-2 were Adh-0.06 and Adh-0.10 (Table 1).

Table 1. Electrophoretic bands of two ADH isozymes at 5°C based on their relative mobility (RM)* in two days old pupae of B. cucurbitae.

<table>
<thead>
<tr>
<th>Relative mobility</th>
<th>Sample slot Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 3 4 5 6 7 8 9 10 11 12 13</td>
</tr>
<tr>
<td>ADH-2</td>
<td>0.06 + + + + + + + + + + + +</td>
</tr>
<tr>
<td></td>
<td>0.10 + + + + + + + + - + + + +</td>
</tr>
<tr>
<td>ADH-1</td>
<td>0.30 - + + + + + + - + - + +</td>
</tr>
</tbody>
</table>

*Scored from the stained gels. + means present and – means absent.

Two intensely staining bands were detected in most of the specimens. In some cases, however, they were less strongly stained. These bands showed RM values of 0.06 and 0.18; whereas four intensely staining bands (RM values of 0.06, 0.30, 0.50 and 0.90) were found in third instar larvae of B. cucurbitae. Among these two bands, the band having RM 0.06 always stained deeply in pupae which was similar in larvae (Shahjahan et al. 2001). But the band having RM 0.18 was less strongly stained compared to the band at RM 0.06.

Matioli et al. (1992) found two ADH loci in Anastrepha grandis, A. striata and A. fraterculus. Three ADH loci were also reported in A. serpentina, A. obliqua and A. bistrigata (Matioli et al. 1992). Gasperi et al. (1992) observed two
genetically independent dimeric proteins, ADH-1 and ADH-2, in Ceratitis capitata. At least two ADH loci were also shown in Dacus tryoni and D. neohumeralis (McKechnie 1974). Steck (1991) detected only two ADH loci in adult individuals of A. fraterculus and related species.

Fig. 1. Isozyme banding patterns of ADH loci at 5°C in two days old pupae of B. cucurbitae. (a) Scanned photograph and (b) Diagrammatic sketch.

However, the objective of the current experimental work of the insect is to develop a methodology for the ultimate replacement of the chemical control of the fly by an ecologically less damaging integrated system which will rely heavily on non-insecticidal methods as suggested by Economopoulos (1979). In order to achieve the target we require an efficient method for culturing the melon fly in the laboratory and to identify the biochemical markers which will facilitate in comparing between genetically or biochemically altered laboratory-reared and wild-reared fly species.
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Acknowledgements: This research was supported by a grant from the Bangladesh University Grants Committion (UGC). The financial assistance in the form of a Research Fellowship from UGC to M. Hasanuzzaman is thankfully acknowledged.

LITERATURE CITED


