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Mannose Phosphate Isomerase Isoenzymes in *Plutella xylostella* Support Common Genetic Bases of Resistance to *Bacillus thuringiensis* Toxins in Lepidopteran Species

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Received 2 June 2000/Accepted 14 November 2000

A strong correlation between two mannose phosphate isomerase (MPI) isoenzymes and resistance to Cry1A toxins from *Bacillus thuringiensis* has been found in a *Plutella xylostella* population. MPI linkage to Cry1A resistance had previously been reported for a *Heliothis virescens* population. The fact that the two populations share similar biochemical, genetic, and cross-resistance profiles of resistance suggests the occurrence of homologous resistance loci in both species.

The efficacy of pest control with products derived from the bacterium *Bacillus thuringiensis* is limited by the capacity of the insects to develop resistance. To date only one insect pest, *Plutella xylostella*, has evolved resistance to *B. thuringiensis* in open field populations (3). Laboratory selection experiments have shown that other lepidopteran species, such as *Plodia interpunctella* (9), *Spodoptera exigua* (10), or *Heliothis virescens* (4), can also evolve resistance to *B. thuringiensis* formulations or toxins. Some of these resistant populations share a similar resistance profile: extremely high resistance to at least one Cry1A toxin, no or minimal cross-resistance to Cry1C, recessive or partially recessive inheritance of resistance, and reduced binding of at least one Cry1A toxin to proteins of the insect midgut. This different insect species suggest a similar genetic basis of resistance.

A genetic analysis with isoenzyme loci was performed on the YHD2 strain of *H. virescens* (5), which showed the resistance profile described above. The authors found that the mannose-6-phosphate isomerase (MPI; EC 5.3.1.8) locus mapped at 10 centimorgans from a locus (BrR-4) involved in resistance to the Cry1Ac *B. thuringiensis* toxin. Considering that there is a certain degree of conserved synteny among insect species, MPI linkage to *B. thuringiensis* resistance can be a common feature in lepidopterans. Chromosomal map studies have shown the existence of a conserved synteny of genes among different animal or plant species (1, 18). Particularly in insects, chromosomes of mosquito species have been shown to have high levels of synteny when mapped with restriction fragment length polymorphism (RFLP) markers (13, 14) and isoenzyme loci (8). Obviously, some levels of chromosome rearrangement (i.e., chromosome translocations or inversions) would change the linear order of the markers between species (14).

In the present work we tested the correlation of the MPI isoenzymes to Cry1A resistance in a *P. xylostella* population (PHI) (17) which showed a resistance profile similar to that of the *H. virescens* YHD2 population. We measured differences in the frequencies of MPI isoenzymes before and after selection with Cry1A toxins. A significant change in frequency for a given MPI isoenzyme would be indicative of linkage of the locus for this isoenzyme to the Cry1A resistance locus.

At the time of the experiments, the resistance ratio for Cry1Ac and Cry1Ab of the PHI population had decreased drastically and was around fourfold with respect to a susceptible control population (LAB-V) of the same species (data not shown). Third-instar larvae from the PHI population were selected using cabbage leaves dipped into aqueous solutions of Cry1A toxins. Cry1Ab and Cry1Ac were obtained from *B. thuringiensis* strains EG7077 and EG11070, respectively (Ecogen Inc.), and were prepared as active toxins (12). A sample of 250 larvae was selected with 4 μg of Cry1Ab/ml (this produced a larva-to-adult mortality of 75%), and another sample of 700 larvae was selected with 10 μg of Cry1Ac/ml (this produced a larva-to-adult mortality of 93%). Both doses produced 100% mortality in larvae from the LAB-V population. After 2 days of exposure to the Cry1A toxins, surviving larvae were transferred to untreated cabbage leaves and reared until adult emergence. Adult insects were frozen and kept at −80°C. MPI isoenzyme analysis was performed as described by Pasteur et al. (11).

Single adult insects were homogenized in 30 μl of 10 mM Tris-HCl–1 mM EDTA–0.4% NADP (pH 6.8). Separation of MPI isoenzymes was carried out using polyacrylamide gel electrophoresis (6) with 6% acrylamide in the resolving gel and 4% in the stacking gel. The protein-denaturing agent (i.e., sodium dodecyl sulfate) was omitted from the electrophoresis buffers. The enzymatic activity was developed with an overlay system as described by Pasteur et al. (11). No band was observed when control experiments were performed by omitting either the substrate or any of the coupling enzymes.

MPI analysis was performed for the LAB-V population, the PHI population, the Cry1Ab-selected sample (Sel.Ab), and the Cry1Ac-selected sample (Sel.Ac) (n = 50).

The susceptible LAB-V population and the PHI population (Table 1) showed four MPI isoenzymes with different electrophoretic mobilities. They were designated A to D relative to their migration patterns (A was the slowest band) (Fig. 1). The same MPI isoenzymes plus an additional one (band E) were

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found in insects from the Sel.Ab and Sel.Ac samples. Isoenzyme E was detected in approximately 15% of the insects in both selected samples but was not observed in any insect from the unselected PHI population or in the LAB-V population. Evidently, a low frequency of this isoenzyme form must be present in the PHI population. We conducted single-pair mating to unravel the genetic bases of MPI isoenzymes in *P. xylostella*. However, no conclusions could be drawn from these experiments, suggesting a complex genetic basis, perhaps due to the involvement of more than one locus encoding the protein or other loci involved in the posttranslational modification of the protein (data not shown).

No significant differences in isoenzyme frequencies (Table 2) were detected between the LAB-V and the PHI populations or between the two selected samples (Sel.Ab and Sel.Ac). However, MPI isoenzyme frequencies in the selected samples were different from the frequencies found in the unselected populations (LAB-V and PHI) (Table 2). Data from the unselected populations were pooled and compared with pooled data from the selected samples. Analysis of the pooled data for every single isoenzyme (using Fisher’s exact test on a contingency table for presence-absence) showed significant differences ($P < 0.05$) in the frequencies for the D and E forms.

Change in the frequencies of isoenzyme forms can sometimes be due to induction by exposure to a toxic agent, but then this change is not inherited. Band E was detected only in the selected samples; thus, if this MPI isoenzyme was due to a physiological induction, we would not expect to observe it in the offspring from the selected insects. A third sample of insects from the PHI population was selected with 50 $\mu$g of Cry1Ab/ml (which produced 90% mortality); survivors were mated, and the offspring was reared on cabbage leaves without Cry1Ab. Analysis of a sample of the offspring ($n = 35$) showed the occurrence of isoenzyme E in 8 insects (16%). This result supports the appearance of isoenzyme E by genetic selection and not by physiological induction.

Our results show a strong correlation between the occurrence of the D and E forms of MPI and resistance to Cry1A toxins. Previous studies showed that a multitoxin *B. thuringiensis* resistance gene in *P. xylostella* confers resistance to at least four Cry toxins: Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F (16, 17). Selection for resistance with either Cry1Ab or Cry1Ac affected similar changes in the MPI isoenzyme frequencies, in agreement with a common genetic basis of resistance to both toxins.

Conserved mechanisms of resistance have been found to insecticides such as pyrethroids or DDT. A homologous locus for resistance to these pesticides has been found in several insect species (2, 7). The fact that the *P. xylostella* PHI population and the *H. virescens* YHD2 population belong to the same resistance type, along with the results with MPI isoenzymes, also suggests the occurrence of homologous resistance loci in lepidopteran species for type I resistance to *B. thuringiensis* toxins.

We thank L. Calzada for help in the rearing of insect colonies and Ecogen Inc. for providing the bacterial strains used to prepare the Cry1A toxins.

### Table 1. Frequencies of insects showing a given isoenzyme in samples of nonselected and toxin-selected insects

<table>
<thead>
<tr>
<th>Sample</th>
<th>Frequency of insects showing isoenzyme type:</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAB-V</td>
<td></td>
<td>0.14</td>
<td>0.38</td>
<td>0.58</td>
<td>0.18</td>
<td>—</td>
</tr>
<tr>
<td>PHI</td>
<td></td>
<td>0.22</td>
<td>0.54</td>
<td>0.48</td>
<td>0.12</td>
<td>—</td>
</tr>
<tr>
<td>Sel.Ab</td>
<td></td>
<td>0.28</td>
<td>0.38</td>
<td>0.44</td>
<td>0.28</td>
<td>0.14</td>
</tr>
<tr>
<td>Sel.Ac</td>
<td></td>
<td>0.32</td>
<td>0.36</td>
<td>0.60</td>
<td>0.32</td>
<td>0.16</td>
</tr>
</tbody>
</table>

*LAB-V is a control susceptible population, PHI is a revertant population, and Sel.Ab and Sel.Ac are toxin-selected samples from PHI.

### Table 2. $P$ values from a $\chi^2$ test on a contingency table for MPI isoenzyme distribution between pairs of samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>LAB-V</th>
<th>PHI</th>
<th>Sel.Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHI</td>
<td>0.360</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sel.Ab</td>
<td>0.019</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Sel.Ac</td>
<td>0.041</td>
<td>0.009</td>
<td>0.918</td>
</tr>
</tbody>
</table>

* Differences were considered significant when the $P$ value was $<0.05$.

### References