ISOLATION OF A NEW DENSONUCLEOSIS VIRUS FROM
MYTHIMNA LOREYI DUP. (LEP. NOCTUIDAE) IN EGYPT

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By


* Entomovirology Laboratory, ORSTOM, Dept. of Econ. Entomol. & Pesticides, Faculty of Agriculture, Cairo University, Giza, Egypt.
** Molecular Virology Laboratory, INRA-CNRS, Comparative Pathology Research Station, St-Christol-Les-Ales, France.

ABSTRACT

A new Densonucleosis virus was isolated from the maize worm, Mythimna loreyi Dup. (Lep. Noctuidae) in Egypt. This virus may play an...
INTRODUCTION

Densonucleosis Viruses (DNVs) are small icosahedral DNA viruses, 20 to 25 nm in diameter isolated from several species of insects, mainly Lepidoptera, where they produce highly contagious lethal diseases (Meynadier et al., 1964; Tijssen and Arella, 1991). Their capsid contains 4 polypeptides (Tijssen et al., 1976) and their genome is a single-stranded linear DNA.
Virus Purification:

The infected larvae were homogenized in Tris (0.05M)-SDS (0.06%) buffer, pH 7.8. After filtration through cheese cloth and clarification (9,000g, 5min), the virus was concentrated by high speed centrifugation (Ti.55 Beckman rotor, 40,000 rpm, 2h). The viral pellets, resuspended in Tris (0.05M, pH 7.8) buffer were dispersed by ultrasonication and then clarified (9,000g, 5min). The resulting supernatant, containing virus particles, was layered onto a 15-45% sucrose gradient prepared in Tris buffer and centrifuged (SW28 Beckman rotor, 27,000 rpm, 2h30). The virus band was collected and the purified virus particles were then concentrated as above and stored at -20°C in Tris buffer.

Electron microscopy:

Purified viral suspension was negatively stained with 2% uranyl acetate, pH 7.4.

Spectrophotometric measurements:

U.V. absorption of purified virus was examined through wavelengths between 320 and 220nm. The average ratio of optical densities at 260 and 280nm was measured.

Electrophoresis of the viral proteins:

Molecular weight and number of proteins were assessed by comparing their electrophoretic mobilities in 9% polyacrylamide gels according to Weber.
Restriction enzyme digestion and electrophoresis of the viral DNA:

The DNA was digested with the following endonucleases: Bam HI, Bgl II, Eco RI, Hae II, Hind II, Kpn I and Pst I, under conditions specified by the suppliers (Boehringer). The digested fragments were analysed by electrophoresis on horizontal 1% agarose gel. The gel was visualised and photographed on a UV transilluminator. The size of the DNA fragments was estimated by comparison with standard marker DNA (Boehringer).

Nucleic probe and blot hybridization:

The digoxigenin-labelled MIDNV DNA probe was applied according to the protocol recommended by the suppliers (Boehringer). The dot blot method was applied to determine the probe titre and for detecting homology between the MIDNV DNA, the JcDNV DNA (Jousset et al., 1990), the GmDNV DNA (Jousset et al., 1990) and the CeDNV DNA (Fediere et al., 1991a).
Table (1): Comparison of size (in kd) of the capsid proteins of *M* DNV, *Jc* DNV, *Gm* DNV, *Ce* DNV and *Bm* DNV.

<table>
<thead>
<tr>
<th>Proteins</th>
<th><em>M</em> DNV</th>
<th><em>Jc</em> DNV (Fediere, 1983)</th>
<th><em>Gm</em> DNV (Kelly et al., 1980)</th>
<th><em>Ce</em> DNV (Fediere, 1983)</th>
<th><em>Bm</em> DNV (Nakagawa &amp; Kawase, 1980)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP1</td>
<td>91.000</td>
<td>101.000</td>
<td>107.000</td>
<td>82.000</td>
<td>77.000</td>
</tr>
<tr>
<td>VP2</td>
<td>63.000</td>
<td>68.000</td>
<td>70.000</td>
<td>74.000</td>
<td>70.000</td>
</tr>
<tr>
<td>VP3</td>
<td>53.000</td>
<td>58.000</td>
<td>61.000</td>
<td>54.000</td>
<td>57.000</td>
</tr>
<tr>
<td>VP4</td>
<td>41.000*</td>
<td>49.000*</td>
<td>43.000*</td>
<td>49.000*</td>
<td>50.000*</td>
</tr>
</tbody>
</table>

* = Major protein

Extracted nucleic acid, digested by both DNase and RNase and then electrophoresed revealed the resistance to RNase which prove the DNA nature of the viral genome. DNA extraction from DNV virions in high salt buffer produced the formation of one double-stranded molecule. The average size of this DNA molecule was estimated to be 5.95 kb. The viral genome was cleaved with 7 endonucleases. *M* DNV DNA had no restriction site for Kpn I. The enzymes Bgl II and Pst I cleaved the *M* DNV DNA once; the others, Bam HI, Hind II, Hae II and Eco RI, twice. The sizes of the restriction fragments of the *M* DNV genome are shown in Table (2). This restriction profile showed essential differences with the *Jc* DNV, *Gm* DNV and *Ce* DNV genomes (Table 2) and suggests that *M* DNV is a new member of the genus Densovirus.

Table (2): Comparison of number and size (in Kb) of the restriction fragments from the genomic DNA of *M* DNV, *Jc* DNV, *Gm* DNV and *Ce* DNV.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th><em>M</em> DNV DNA (Jousset et al., 1990)</th>
<th><em>Jc</em> DNV DNA (Jousset et al., 1990)</th>
<th><em>Gm</em> DNV DNA (Jousset et al., 1990)</th>
<th><em>Ce</em> DNV DNA (Fediere et al., 1991a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kpn I</td>
<td>NCS</td>
<td>NCS</td>
<td>NCS</td>
<td>NCS</td>
</tr>
<tr>
<td>Bgl II</td>
<td>4.60; 1.35</td>
<td>NCS</td>
<td>3.58; 1.37; 0.90</td>
<td>NCS</td>
</tr>
<tr>
<td>Hae II</td>
<td>3.38; 2.04; 0.53</td>
<td>5.35; 0.50</td>
<td>NCS</td>
<td>NCS</td>
</tr>
<tr>
<td>Bam HI</td>
<td>5.35; 0.30; 0.30</td>
<td>5.25; 0.31; 0.29</td>
<td>5.25; 0.31; 0.29</td>
<td>NCS</td>
</tr>
<tr>
<td>Hind II</td>
<td>4.78; 0.62; 0.55</td>
<td>3.45; 1.35; 1.05</td>
<td>4.20; 1.20; 0.45</td>
<td>1.68; 1.43; 1.13; 0.66</td>
</tr>
<tr>
<td>Eco RI</td>
<td>4.10; 1.55; 0.30</td>
<td>5.0; 1.50; 0.55; 0.30</td>
<td>4.05; 1.50; 0.30</td>
<td>3.49; 0.58; 0.51; 0.32</td>
</tr>
</tbody>
</table>
Dot blot hybridization revealed relationships between MDNV DNA, GmDNV DNA and JcDNV DNA, but failed to reveal any homology with CeDNV DNA.

ELISA test was conducted with MDNV rabbit antiserum against GmDNV, JcDNV and CeDNV. Strong serological relationships were observed between the first three DNV's, but a weak relationship was revealed with CeDNV.

Different characteristics indicate that there are at least two groups of DNVs. The first group, with a genome length averaging 6Kb and the profile of structural proteins in 4 well separated polypeptides, is represented by GmDNV and JcDNV. The second group, with a genomic size of about 5 Kb and the profile of structural proteins separated in two groups of two proteins, square with CeDNV and Bombyx mori DNV (Bando et al., 1987). The different relationships between MDNV and both JcDNV and GmDNV, and the lack of homology with the member of the second group, may reflect its closeness to the first group. MDNV was assumed the causal agent of the frequent disease outbreaks occurring among M. loreyi stock culture in the laboratory. However, further investigations are required to study the virulence as well as the host range of MDNV, especially on the corn pests. Fundamental studies are necessary for a better understanding of the molecular biology of this virus.

REFERENCES


(MiDNV DNA) profiles و الذي أجري على الحمض النووي الخاص بفيروس البيومينا لورباي (Jc) و فيروس GmDNV و فيروس MiDNV DNV وكان ذلك مع فيروس Jc.

و يوجد إختلافات أساسية بين هذا الفيروس MiDNV و فيروس يمكن أن يُعتبر فيروس دنوسيروس نسبتاً ( قناة بارنوفيدى، Densovirus genus).

المجلة العلمية لكلية الزراعة - جامعة القاهرة - المجلد (43) العدد الرابع (أكتوبر 1995) : 192-207.
Fig. (1): Electrophoretic analysis of MlDNV polypeptides in 9% polyacrylamide-SDS gel.

Lane A: MlDNV
Lane B: Protein standards: phosphorylase (MW:94.000), bovine serum albumin (67.000), ovalbumin (43.000), carbonic anhydrase (30.000), Trypsin inhibitor (20.100).