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

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ABSTRACT

A new Densonucleosis virus was isolated from the maize worm, Mythimna (= Leucania) loreyi Dup., in Egypt. This virus may play an important role, as a regulating factor of pest populations. The virus has icosahedral non-enveloped particles of 25 nm in diameter. The virions of Mythimna loreyi Densovirus (M/IDNV) contained 4 structural proteins with molecular weights of 41000, 53000, 63000 and 91000 daltons, respectively. The size of the DNA molecule which composes the viral genome was estimated to be 5950 bases. Southern hybridization and ELISA tests revealed a relationship between M/IDNV and both Galleria mellonella DNV and Junonia coenia DNV, but failed to reveal any homology with Casphalia extranea DNV. Restriction profiles of the M/IDNV DNA showed essential differences between M/IDNV and GmDNV as well as with JcDNV.

It is suggested that M/IDNV is a member of the Densovirus genus (Parvoviridae family).

Key words: Densovirus, Mythimna loreyi, Parvoviridae.
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 molecule, 5-6 Kilobases (Kb) in length (Barwise and Walker, 1970; Jousset et al., 1990). Owing to their properties, DNVs have been classified within the genus Densovirus of the Parvoviridae family (Francki et al., 1990).

The present work records, for the first time in Egypt, a virus isolated from the maize worm, Mythimna loreyi Dup. (Noctuida). The work is a part of a program concerning the interrelationship between corn pests and their complex of viral diseases. The objective is to select pathogenic viruses which could be proposed as biological control candidates. Therefore, two new viruses infecting the most important corn borer in Egypt, Sesamia cretica (Lepidoptera, Noctuidae) were recently isolated. The first is a small RNA virus belonging to family Picornaviridae (Fediere et al., 1991a), and the second is a Granulosis virus from family Baculoviridae (Fediere et al., 1993). This baculovirus is already included in the research strategy.

The present investigation aims to characterize a new Densovirus recorded in M. loreyi designated M. loreyi Densonucleosis Virus (MDNV).

MATERIALS AND METHODS

The Virus isolate:

Virus was purified from naturally infected larvae of M. loreyi, collected from maize fields at the Western Farm of Cairo University, Agricultural Experimental Station, Giza, in September, 1993. This virus isolate was propagated in laboratory reared larvae of M. loreyi, infected per os. The viral suspension is available at the Entomovirology Laboratory, Faculty of Agriculture, Cairo University.

Three other Densoviruses were used for the comparison: G.mellonella DNV and J.coenia DNV (from Prof. Max Bergoin, Molecular Virology Laboratory, INRA-CNRS Research Station of St. Christol-Les Ales, France), and C.extranea DNV (from ORSTOM collection).
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 and Osborn (1969) with those of standard marker proteins (Pharmacia).

Extraction and analysis of viral nucleic acid:

The extraction of nucleic acid from the purified virus was carried out using the procedure of Fediere et al., (1991b). The nucleic acid precipitate was resuspended in Tris buffer and the purity and concentration of the solution checked by UV spectrophotometry. The purified suspension was digested by both DNase and RNase then electrophoresed to reveal the nature of the nucleic acid.
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 title 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).

Antisera and ELISA test:

Antisera were prepared in rabbits according to Fediere et al., (1990). The indirect method of ELISA test "Enzyme Linked Immuno Sorbent Assay" using the alkaline phosphatase was conducted according to Kelly et al., (1978).

RESULTS AND DISCUSSION

Examination of purified viral suspension by electron microscopy revealed large number of icosahedral non-enveloped particles of 25 nm in diameter (Fig. 1). All the virions were identical in shape and size. Few particles were interiorly stained by uranyl acetate showing the viral capsid.

U.V. extinction spectra of the viral suspension showed a maximum absorption at 260 nm and a minimum at 240 nm, which characterize the viral nucleoproteins. The average ratio of extinction at 260 nm to that at 280 nm was 155.

Electrophoresis of viral proteins revealed one major band (VP4) with molecular weight of 41 Kd as well as three minor bands with molecular weight of 53 Kd (VP3), 63 Kd (VP2) and 91 Kd (VP1) (Fig.2). These values are closely related to those of some other Densovirus, especially with JcDNV and GmDNV (Table 1).
Table (1): Comparison of size (in kd) of the capsid proteins of MlDNV, Jc DNV, Gm DNV, Ce DNV and Bm DNV.

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Ml DNV</th>
<th>Jc DNV (Fediere, 1983)</th>
<th>Gm DNV (Kelly et al, 1980)</th>
<th>Ce DNV (Fediere, 1983)</th>
<th>Bm DNV (Nakagaud &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>71.000</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. MlDNV DNA had no restriction site for Kpn I. The enzymes Bgl II and Pst I cleaved the MlDNV DNA once; the others, Bam HI, Hind II, Hae II and Eco RI, twice. The sizes of the restriction fragments of the MlDNV genome are shown in Table (2). This restriction profile showed essential differences with the JcDNV, GmDNV and CeDNV genomes (Table 2) and suggests that MlDNV 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 Ml DNV, Jc DNV, Gm DNV and Ce DNV.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>MlDNV DNA</th>
<th>JcDNV DNA (Jousset et al, 1990)</th>
<th>GmDNV DNA (Jousset et al, 1990)</th>
<th>Ce 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>3.50; 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>
<tr>
<td>Pst I</td>
<td>3.75; 2.20</td>
<td>NCS</td>
<td>NCS</td>
<td>NCS</td>
</tr>
</tbody>
</table>

NCS = No cleavage site recognized by the enzyme.
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 DNVs, 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**


الذي أجري على الحمض النووي الخاص بفيروس الميغمنا لوريا (MIDNV DNA)
وجوجد اختلافات أساسية بين هذا الفيروس GmDNV وفيروس MIDNV و كل منهما مع فيروس
DNV
واحد الفيروسات MIDNV والخاصة بنوع Densovirus genus (عائلة باروفيزيدي).

المجلة العلمية لكلية الزراعة - جامعة القاهرة - المجلد (46) العدد الرابع
Fig. (1): Electron micrograph of purified suspension of *Ml* DNV stained by uranyl acetate (X 180,000)

Fig. (2): 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).