ISOLATION OF DENSÖVIRUS AND PICORNAVIRUS FROM NATURAL FIELD POPULATIONS OF Spodoptera littoralis BOISD. (LEP. NOCTUIDAE) IN EGYPT.

Fédière, G.1; M. A. K. El-Sheikh2, Omima Khamiss1, Maha Masri1 and M. Salah1
1- Laboratoire d'Entomovirologie du Caire (LEC), IRD-Fac. of Agriculture, Cairo University, P. O. Box 26, Giza, Cairo, Egypt
2- Dept. of Econ. Entomol. and Pesticides, Fac. of Agric., Cairo Univ., Giza.

ABSTRACT

Two natural viral strains belonging to the Parvoviridae (Densovirus) and the Picornaviridae (Picornavirus) families were isolated from larvae of the Egyptian cotton leafworm Spodoptera littoralis. The strains were sampled from natural infestation of cotton field in Kafr El-Sheikh Governorate in May 1997. Electron microscopy observations revealed the presence of icosahedral particles with 22 nm in diameter for the viral capside related to the Densovirus (DNV), and 29 nm for the second one as a Picornavirus (PV). For the DNV four capsid proteins of 91, 63, 53 and 47 KDa have been separated, and were undifferentiated from that of the unique published Densovirus isolate from Egypt, the Mythimna loreyi DNV (MDNV). PV capsids contain three major structural proteins of 30, 32 and 33 KDa resembling the Drosophila C virus (DCV), a very well known Picornavirus of insect. The DNV genome consists of a single stranded DNA molecule of size 5.95 Kb. It was characterized using 12 restriction endonucleases. The DNA restriction profiles were identical with these of MDNV. The PV contain a single stranded RNA genome of size 9.4 Kb as the different reported isolates of DCV.

Using rabbit antisera, immunological comparison revealed a complete homology between the DNV and MDNV, as well as, the PV isolated from S. littoralis shows a complete serological identity with the DCV.

This results suggest that we can registrated the DNV isolated from S. littoralis as a strain of MDNV and the small RNA virus as an Egyptian strain of DCV.

Larvae of S. littoralis reared on artificial diet in the laboratory were susceptible to these two viruses.

Keywords: cell line S152, Cotton leafworm, Densovirus, Parvoviridae, Picornaviridae, Picornavirus, Spodoptera littoralis.

INTRODUCTION

Different invertebrate viruses were already isolated from the Egyptian cotton leafworm Spodoptera littoralis Boisduval in Egypt. Firstly two member of the family Baculoviridae were detected. A Nucleopolyedrovirus (SNPV) was isolated forty three years ago by Abul-Nasr, (1956), then a Granulovirus (SGV) was isolated from diseased larvae fifteen years ago at Bouskédin.
Fédière, G. et al.

Côte d'Ivoire and characterized it in Egypt through its biological, immunological and biochemical properties by Abol-Ela et al., (1994) and Abd-Alla et al., (1997). Recently two new strains of the SIGV, Egyptian isolates, were characterized from Gharbeia and Sharkeia governorates (Khamiss et al., 1999). Finally an unusual free virus, firstly recorded among lepidopterous as a Bunyavirus (SIBV) was isolated by Abol-Ela et al., (1995). Since now, no viral strains belonging to the Parvoviridae (Densovirus) and the Picornaviridae (Picornavirus) families were recorded from S. littoralis.

Densovirus share many properties with vertebrates Parvovirus, but their genome organization and their expression strategy are completely different (Bergoin and Tijssen, 1998). Therefore two subfamilies are recognized within the Parvoviridae family, the Densovirinae and the Parvovirinae (Tijssen and Bergoin, 1995). Despite their high virulence, their infectivity and their polyspecificity towards a broad host-range, the use of Densonucleosis Viruses (DNV) as biological pesticide has not yet been investigated in detail because of non achievement of inocuity tests. Nevertheless, the successful control of at least two insect pests of oil palm by DNVs was reported in Colombia (Genty and Marfa, 1975) and in Côte d'Ivoire (Fediere et al., 1986) against respectively Sibine fusca (Stoll) and Caspalia extranea (Walker). A Densovirus isolated in Egypt from the maize worm Mythimna loreyi Duponchel, designated as MIDNV (Fediere et al., 1995), is polyspecific and infects experimentally a large number of Egyptian Lepidopterous pests as well as S. littoralis, the European corn borer Ostrinia nubilalis Hubner, the maize pink borer Sesamia cretica Lederer, the nce purple line borer Chilo agamemnon Bleszynski, the cotton pink bollworm Pectinophora gossypiella Saunders and Galleria mellonella Linnaeus (Fediere, 1996). In view of the important property of host range for biological control, a complete homology between MIDNV and a natural strain from S. littoralis could approve the natural polyspecificity of MIDNV in the field.

In recent years the number of descriptions of small (less than 40 nm in diameter) nonoccluded RNA-viruses from insects has increased steadily although few have yet to be placed within a defined taxonomic framework. Exceptions to this are members of the Cricket Paralysis Virus (CrPV) and Drosophila C Virus (DCV) complex, placed within the Picornaviridae family, as Picornavirus (PV) of insects (Christian and Scotti, 1994).

This work is considered as a part of the program concerning the inter-relationship between the different viruses isolated from S. littoralis and their host, and the biodiversity of the viral strains regarding as well as the geographical distribution of this pest in Egypt and the host-crops.

In this paper, we describe two new viral strains from S. littoralis. The relevant research concerns the virus populations analysis among naturally infected S. littoralis from the fields and the laboratory rearing, and the identification of the viral isolates.
MATERIALS AND METHODS

The Virus isolates

The strains of DNV and PV of *S. littoralis* were sampled as well as, from larvae collected during a natural infestation of cotton field in Kafr El-Sheikh Governorate in May 1997, and from natural dead-larvae of the mass-rearing of this pest in the laboratory during the year 1998.

The strain of *M. loreyi* DNV designated as MIDNV was the original strain propagated for several years in our laboratory (Fediere et al., 1995). The strain DCV, Picornavirus, the rabbit immunserum anti-DCV and the cell line SI52 were obtained from Dr. F. X. Jousset, Saint-Christol-Les-Ales, France (personal communication).

Virus purification

The infected larvae were homogenized in Tris (0.05M)-SDS (0.06%) Buffer, pH 7.8. After filtration through gauze textile and clarification (10,000 g for 5 min), the virus was concentrated by high speed centrifugation (Ti 55 Beckman rotor, 35,000 rpm for 2 h). The pellets, resuspended in Tris (0.05M)-Buffer, pH 7.8, were dispersed by ultrasonication and then clarified (10,000 g for 5 min). The resulting supernatant, containing virus particles, was layered onto sucrose gradient (15-45%) prepared in Tris-Buffer and centrifuged at 120,000 g during 2 h 30, then the band of virions was collected and washed using Tris-Buffer. The virions were pelleted by centrifugation (Ti 55 Beckman rotor, 35,000 rpm for 2 h) and then, the virus was resuspended in 2 ml of Tris-Buffer, the concentration of the final suspension was measured on the spectrophotometer at 260 nm.

Electron microscopy

Purified viral suspension was negatively stained in 2% uranyl acetate, pH 7.4 and the grids were examined through electron microscope.

Serology

Double diffusion tests in 1% agar were done using antisera to DCV and to MIDNV and the different viral strains.

ELISA tests

For detecting the viral proteins, ELISA was conducted using the direct method. Specific conjugate was elaborated by extracting globuline from total rabbit immunosera anti-DCV and anti-MIDNV. IgG-enzyme conjugate was prepared and used at concentration of 1/1000. The same dilution was used for the globuline. For the samples several dilutions of 1/10, 1/100 and 1/1000 were used.

Electrophoresis of the viral proteins

Molecular weight and number of proteins were assessed by comparing their electrophoretic mobilities in 12% polyacrylamide gel...
according to Fediere et al. (1995) 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. (1993). The nucleic acid precipitate was resuspended in Tris (15mM)-EDTA (1mM)-Buffer, pH 7.5. The concentration of the final suspension was measured on the spectrophotometer at 260 nm.

To determine whether the nucleic acid nature is DNA or RNA, the two samples (1 μg in 10 ul) were treated with DNase and RNase (0.1 ug), then analyzed by electrophoresis on a 1% agarose gel.

**Estimation of RNA-genome size**

The RNA-genome size was estimated by electrophoresis in formaldehyde denaturing 1% agarose gel as recommended by Maniatis et al. (1982), using standard molecular weight RNA marker (Promega).

**Restriction enzyme digestion and electrophoresis of the viral DNA**

The DNV-DNA was digested with the 12 following endonucleases: Bam HI, Bgl II, Eco RI, Hae II, Hae III, Hha I, Hinc II, Hind III, Hpa I, Pst I, Sca I, Xba I, under conditions specified by the supplier (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).

**DNA probe**

A DNA probe prepared from the total extract DNA of the MDNV was used. The digoxygenin-labelled DNA probe was applied according to the protocol recommended by the supplier (Boehringer). The same protocol was applied for the "dot blot" hybridization technique to detecte homology between the MDNV-DNA and the DNV-DNA of S. littoralis.

**RESULTS AND DISCUSSION**

Examination of purified viral suspension by electron microscopy revealed the presence of large number of icosahedral non-enveloped particles of 22 nm and 29 nm in diameter (Fig. 1) resembling the Densovirus (DNV) of family Paroviridae and the Picornavirus (PV) of family Picornaviridae respectively.

Electrophoresis of viral proteins for the DNV revealed, four capsid polypeptides of 91 (VP1), 63 (VP2), 53 (VP3) and 47 (VP4) KDa, with the VP4 as a major band (Fig. 2). These values are undifferentiated from that of the unique published Densovirus isolate from Egypt, the MDNV. PV capsids contain three major structural proteins of 33 (VP1), 32 (VP2) and 30 (VP3) KDa (Fig. 2), resembling those of the DCV, a very well known Picornavirus of insect.
Extracted nucleic acid of the two samples digested by both DNAse and RNAse and then electrophoresed revealed the resistance to RNAse, prove the DNA nature for the DNV, and the resistance to DNAse, prove the RNA nature for the PV.

The DNV genome consists of a single stranded DNA molecule of size 5.95 Mb. It was characterized using 12 restriction endonucleases and the DNA restriction profiles were identical with these of MDNV (Fig. 3 and Table 1). The PV contain a single stranded RNA genome of size 9.4 Kb as the different reported isolates of DCV.

Fig. (1). Electron micrograph of a purified viral suspension of mixed S. littoralis Densovirus (d) and Picornavirus (p). Bar represents 100 nm.
Fig. (2). Electrophoretic analysis of purified viral DNV and PV particles from *S. littoralis*. The polypeptides were separated by 12 % SDS-PAGE. Lane 1: MIDNV; Lane 2: mixed *S. littoralis* DNV and PV; Lane 3: Proteins markers (94, 67, 43, 30, 20.1 and 14.4 KDa).

Fig. (3): Restriction endonuclease profiles of digested viral DNA from *S. littoralis* DNV and MIDNV in 1 % agarose gel. Lane 1: Standard molecular weight DNA marker VII (Boehringer); Lane 2: MIDNV-DNA digested by Hha I; Lane 3: *S. littoralis* DNV-DNA digested by Hha I; Lane 4: MIDNV-DNA digested by EcoR I; Lane 5: *S. littoralis* DNV-DNA digested by EcoR I; Lane 6: MIDNV-DNA digested by Hind III; Lane 7: *S. littoralis* DNV-DNA digested by Hind III; Lane 8: Standard molecular weight DNA marker II (Boehringer).
Table (1): Number and size (in Kb) of different restriction fragments generated by 12 endonucleases from the *S. littoralis* DNV-DNA

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bgl II</td>
<td>4.50</td>
<td>1.45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pst I</td>
<td>3.85</td>
<td>2.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bam HI</td>
<td>5.39</td>
<td>0.28</td>
<td>0.28</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hinc II</td>
<td>4.88</td>
<td>0.57</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hpa I</td>
<td>3.84</td>
<td>1.18</td>
<td>0.47</td>
<td>0.37</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eco RI</td>
<td>4.05</td>
<td>1.59</td>
<td>0.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hind III</td>
<td>4.74</td>
<td>0.92</td>
<td>0.16</td>
<td>0.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hae III</td>
<td>1.64</td>
<td>1.14</td>
<td>0.99</td>
<td>0.69</td>
<td>0.47</td>
<td>0.48</td>
<td>0.39</td>
</tr>
<tr>
<td>Hae II</td>
<td>3.35</td>
<td>2.03</td>
<td>0.57</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xba I</td>
<td>2.57</td>
<td>1.88</td>
<td>0.77</td>
<td>0.71</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sca I</td>
<td>3.28</td>
<td>1.24</td>
<td>0.71</td>
<td>0.49</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hha I</td>
<td>2.51</td>
<td>1.06</td>
<td>0.95</td>
<td>0.43</td>
<td>0.33</td>
<td>0.32</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Using rabbit antisera, immunological comparison revealed a complete homology between the DNV and MlDNV, as well as, the PV isolated from *S. littoralis* shows a complete serological identity with the DCV.

It will be interesting to clone the genomes of these two new strains of *S. littoralis* virus into bacteria using DNA and cDNA techniques and study the sequence homology with MlDNV and DCV.

These results suggest that, since the complete sequence homology was achieved, we can state that registrated the DNV isolated from *S. littoralis* as a new strain of MlDNV. This fact illustrates the wide host range of this virus, and the small RNA virus of *S. littoralis* as the first Egyptian strain of DCV.

It is important to note that during several assays performed positively to multiply in vitro the MlDNV by infection and transfection of a cell line from *S. littoralis* (Si52), two small viruses of 22 and 29 nm were also isolated (unpublished data). The quantity of these viruses was not quite enough to facilitate the detailed studies. It will be interesting to compare these two viruses chronically infecting the cells of this culture from the host, with the Densovirus and the Picornavirus isolated *in vivo*.

The confirmation of the natural polyspecificity of MlDNV in the field complete its biological characterization and would provide investigators a more completely known pathogen for use as microbial agent against *S. littoralis* larvae on cotton. It is possible that MlDNV may represent an important complementary agent alongside with SiNPV and SiGV in the IPM programmes of the pest, as the use of a Granulovirus (ScGV) for microbial control of the maize pink borer *S. cretica* in Egypt (Fediere et al., 1997). Whereas, concerning the Picornaviruses of insects, unfortunately, for several reasons, they were not accepted for direct use in biological control programs.
REFERENCES


مطبوعة من

مجلة جامعة المنصورة
للفنون الزراعية

مجلد 24 العدد (11)

نوفمبر 1999

جزء (ب)

تصدر منذ 1976
رقم الإيداع
بدار الكتاب المصرية 804

تصدرها
كلية الزراعة - جامعة المنصورة

فاكس: 2216288 (00)
تلفون: 2237569 (00)