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

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By

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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 (*Ml*DNV) contained 4 structural proteins with molecular weights of 41 000, 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 *Ml*DNV and both *Galleria mellonella* DNV and *Junonia coenia* DNV, but failed to reveal any homology with *Casphalia extranea* DNV. Restriction profiles of the *Ml*DNV DNA showed essential differences between *Ml*DNV and *Gm*DNV as well as with *Jc*DNV.

It is suggested that *Ml*DNV is a member of the Densovirus genus (Parvoviridae family).

Key words: Densovirus, *Mythimna loreyi*, Parvoviridae.

INTRODUCTION

Densonucleosis Viruses (DNV's) 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, DNV's 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 (*Ml*DNV).

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,pH7.8) 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, pH7.4.

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 *Ml*DNV 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 *Ml*DNV DNA, the *Jc*DNV DNA (Jousset *et al.*, 1990), the *Gm*DNV DNA (Jousset *et al.*,1990)and the *Ce*DNV 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 Densoviruses, especially with *Jc*DNV and *Gm*DNV (Table1).

Table (1): Comparison of size (in kd) of the capsid proteints of *Ml*DNV, *Jc* DNV, *Gm* DNV, *Ce* DNV and *Bm* DNV.

Proteins	*Ml* DNV	*Jc* DNV (Fediere, 1983)	*Gm* DNV (Kelly *et al.*1980)	*Ce* DNV (Fediere, 1983)	*Bm* DNV (Nakagaki&Kawase,1980)
VP1	91.000	101.000	107.000	82.000	77.000
VP2	63.000	68.000	71.000	74.000	70.000
VP3	53.000	58.000	61.000	54.000	57.000
VP4	41.000*	49.000*	43.000*	49.000*	50.000*

* = Major protein

Extracted nucleic acid, digested by both DNase and RNase and then elecrophoresed 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. *Ml*DNV DNA had no restriction site for Kpn I. The enzymes Bgl II and Pst I cleaved the *Ml*DNV DNA once; the others, Bam HI, Hind II, Hae II and Eco RI, twice. The sizes of the restriction fragments of the *Ml*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 *Ml*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 *Ml* DNV, *Jc* DNV, *Gm* DNV and *Ce* DNV.

Enzymes	*Ml*DNV DNA	*Jc*DNV DNA (Jousset *et al.*1990)	*Gm*DNV DNA (Jousset *et al.*1990)	*Ce* DNV DNA (Fediere *et al.*1991a)
Kpn I	NCS	NCS	NCS	NCS
Bgl II	4.60;.1.35	NCS	3.58;1.37;0.90	NCS
Hae II	3.38;2.04;0.53	5.35 ;0.50	NCS	NCS
Bam HI	5.35;0.30; 0.30	5.25;0.31; 0.29	5.25;0.31;0.29	NCS
Hind II	4.78; 0.62; 0.55	3.45; 1.35;1.05	4.20;1.20 ;0.45	1.68;1.43 ;1.13;0.66
Eco RI	4.10;1.55; 0.30	3.50; 1.50; 0.55; 0.30	4.05;1.50; 0.30	3.49; 0.58; 0.51; 0.32
Pst I	3.75 ;2.20	NCS	NCS	NCS

NCS = No cleavage site recognized by the enzyme.

Dot blot hybridization revealed relationships between *Ml*DNV DNA, *Gm*DNV DNA and *Jc*DNV DNA, but failed to reveal any homology with *Ce*DNV DNA.

ELISA test was conducted with *Ml*DNV rabbit antiserum against *Gm*DNV, *Jc*DNV and *Ce*DNV. Strong serological relationships were observed between the first three DNV's, but a weak relationship was revealed with *Ce*DNV.

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 *Gm*DNV and *Jc*DNV. 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 *Ce*DNV and *Bombyx mori* DNV (Bando *et al.*,1987). The different relationships between *Ml*DNV and both *Jc*DNV and *Gm*DNV, and the lack of homology with the member of the second group, may reflect its closeness to the first group. *Ml* DNV 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 *Ml*DNV, especially on the corn pests. Fundamental studies are necessary for a better understanding of the molecular biology of this virus.

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عزل فيروس دينسونيكلوزيس جديد من حشرة الميثيمنا لورياى فى مصر

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ملخص

تم عزل فيروس جديد من نوع *الدينسونيكلوزيس* Densonucleosis من حشرة دودة الذرة ميثيمنا لورياى فى مصر. و قد وجد أن هذا الفيروس يمكن أن يلعب دورا هاما كعامل ضبط لتعدادات هذه الآفة. للفيروس جزيئات غير مغلفة كروية تقريبا ذات قطر ٢٥ نانومتر. تحتوى فريونات فيروس الدينسو(*Ml*DNV) الذى يصيب الميثيمنا على ٤ حزم للبروتينات التركيبية ذات أوزان جزيئية ٤١٠٠٠ ، ٥٣٠٠٠، ٦٣٠٠٠ ، ٩١٠٠٠ دالتون على التوالى. و قد قدر حجم جزىء الحامض النووى (DNA) بـ ٥٩٥٠ قاعدة. كشفت إختبارات الـ Southern hybridization و الـ ELISA عن وجود علاقة بين هذا الفيروس و فيروسات الدينسو الخاصة بحشرتى *Junonia coenia*، *Galleria mellonella* ولكنه لم يثبت وجود تشابه مع فيروس الدينسو الخاص بحشرة *Casphalia extranea* و قد أظهر إختبار البصمة بإستخدام إنزيمات القطع Restriction

profiles و الذى أجرى على الحمض النووى الخاص بفيروس الميثيمنا لوريای (*Ml*DNV DNA) وجود إختلافات أساسية بين هذا الفيروس *Ml*DNV و فيروس *Gm*DNV و كذلك مع فيروس *Jc* DNV .

و من خلال تلك الخصائص ، تقترح الدراسة أن يكون فيروس *Ml*DNV أحد الفيروسات التابعة لجنس Densovirus genus(عائلة بارفوفيريدى) .

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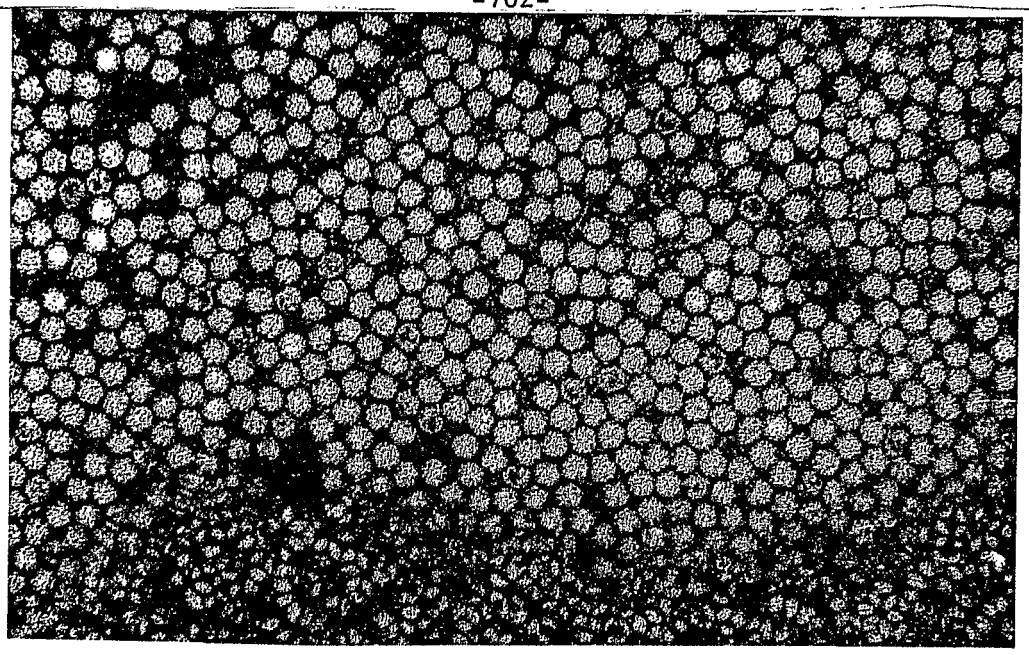

Fig. (1) : Electron micrograph of purified suspension of *MI* DNV stained by uranyl acetate (X 180.000)

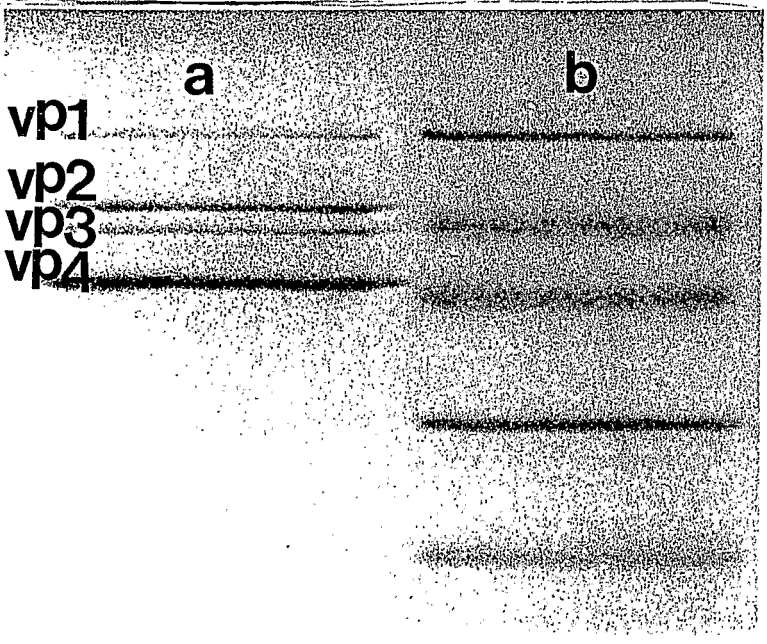

Fig. (2) : Electrophoretic analysis of *MI*DNV polypeptides in 9% polyacrylamide-SDS gel.
Lane A : *MI*DNV
Lane B : Protein standards : phosphorylase (MW:94.000), bovine serum albumin (67.000),ovalbumin (43.000), carbonic anhydrase(30.000), Trypsin inhibitor(20.100).

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