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Identification of Three Nuclear Polyhedrosis Viruses Through Restriction Endonuclease Analysis

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Biophysical and biochemical properties of the baculoviruses (BV) have been extensively used to identify as well as to differentiate the subgroups of BV, the viruses within the subgroup and the variants of same virus species (Harrap and Payne, 1979). Nucleic acid analysis through electron microscopy, sedimentation techniques, reassociation kinetics and restriction endonuclease (REN) analysis are the tools generally employed. Of these, REN analysis is relatively a simple and well defined method for the accurate classification of more than 300 BV recorded from various insect hosts. This analysis relies on a selected enzyme cleaving the viral DNA at a specific nucleotide sequence to produce defined DNA fragments. Identification and subsequent grouping of genotypically similar BV will help in eliminating some of the confusion arising from classification based on host origin (Gettig and McCarthy, 1982). In the present study, the nuclear polyhedrosis viruses of *Amsacta albistriga* (AaNPV), *Spodoptera litura* (SlNPV) and *Spilosoma obliqua* (SoNPV) which have great potential in the microbial control of the respective host pest species (Jayaraj *et al.*, 1977; Ramakrishnan *et al.*, 1981; Battu and Ramakrishnan, 1989) were compared through REN analysis.

Virions were pelleted after alkali dissolution from the respective polyhedral inclusion bodies. The DNA of each virus was isolated (Sambrook *et al.*, 1989) and digested by Hind III for 2 h at 37°C. The restricted DNA was loaded on to a 0.7% agarose gel and electrophoresed at 30V for 20h using LKB Maxiphor unit and photographed on a thermal paper through UVP transilluminator. The genome size of the DNA fragments were estimated by comparison with lamda DNA Hind III + EcoR I Marker as per the method of Southern (1979).

The Hind III cleavage pattern of DNA was quite unique for each of the viruses (Fig.1). The number of fragments generated

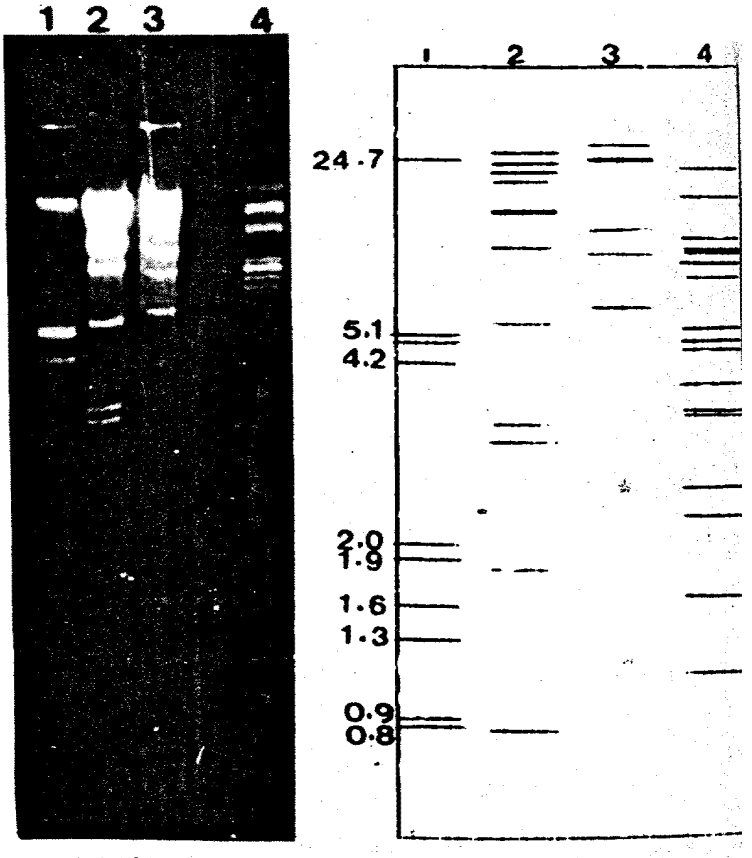

Fig.1. DNA restriction profile with Hind III
(A) 1. Lamda marker (EcoR I + Hind III digest) 2. SlNPV 3. AaNPV 4. SoNPV
(B) Schematic representation of the restriction profile

by Hind III was 12, 6 and 17 respectively for SlNPV, AaNPV and SoNPV. AaNPV had only a few low molecular weight fragments compared to SlNPV and SoNPV (Table 1). In SlNPV, the high molecular weight fragments were more whereas in SoNPV, the fragments were evenly distributed from high to low. The average genome size estimated from the electrophoretic mobility of the fragments from three gels was 134.6, 109.6 and 103.0 kb for SlNPV, AaNPV and SoNPV respectively. The enzymatic cleavage consistently resulted in the same pattern of fragments for the respective viruses. This clearly implies variations in the nucleotide sequences and thus the lesser homology between the genomes of these three NPVs. Vlak (1980) attributed these differences to the biological variations between the viruses like morphology, host range and biological activity: Such REN analysis were also used for comparing the different NPV isolates of *Mamestra* spp. (Erlandson, 1990) and *Spodoptera* spp. (Shapiro *et al.*, 1991). This method is quite useful in identifying SlNPV, AaNPV and SoNPV. The advantage of such method is that the genetic heterogeneity or changes, strain difference between isolates and virus mixtures can be identified.

Table 1. Size of Hind III restricted fragments (kb)

SlNPV	AaNPV	SoNPV
26.2	34.9	18.0
23.8	25.0	13.4
21.0	25.0	9.3
17.8	10.1	8.4
11.5	8.5	8.4
11.5	6.1	7.8
8.7		6.9
5.4		5.1
3.1		4.4
2.9		4.1
1.8		3.5
0.9		3.2
		3.1
		2.3
		2.1
		1.7
		1.3
Total 134.6	109.6	103.0
±4.7	±6.6	±5.2

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KEY WORDS : Baculoviruses, nuclear polyhedrosis viruses, *Amsacta albistriga, Spodoptera litura, Spilosoma obliqua,* endonuclease restriction analysis.

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