International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 3 Number 8 (August-2015) pp. 81-87
www.ijcrar.com

Restriction endonuclease fragment analysis of *Hyposidra talaca* nucleopolyhedrovirus genome

Bappaditya Ghosh[1], Ananda Mukhopadhyay[2], Ananya Das[1] and Min Bahadur[1]*

[1]Genetics and Molecular Biology Laboratory, Department of Zoology, University of North Bengal, Dist. Darjeeling, 734013, India
[2]Entomology Research Unit, Department of Zoology, University of North Bengal, Dist. Darjeeling, 734013, India

Corresponding author

KEYWORDS

Hyposidra talaca, Biopesticide, Nucleo polyhedrovirus, Restriction endonuclease, *Camellia sinensis*

A B S T R A C T

A nucleopolyhedrovirus isolated from *Hyposidra talaca*, a major defoliating pest of Tea in Northern region of West Bengal, India, is pathogenic to this pest. Restriction digestion of *Hyposidra talaca* nucleopolyhedrovirus (HytaNPV) genome with *Eco*RI, *Xho*I, *Kpn*I, *Bam*HI and *Pst*I produced 29, 21, 13, 10 and 9 fragments, respectively ranging from 0.62 to 62.00 Kb. *Pst*I produced the largest fragment of 62 kb. Based on the restriction analysis the mean size of the HytaNPV genome was estimated to be 174.76 kb. The present study will help to develop this virus as a potential biopesticide in the Integrated Pest Management strategy of Tea.

Introduction

Hyposidra talaca Walker (Lepidoptera: Geometridae), commonly known as 'Black Inch Looper' is a major leaf-eating lepidopteran pest of tea (*Camellia sinensis*) in Terai-Dooars region of Eastern Himalaya (Das *et al.,* 2010). A number of synthetic chemical pesticides, especially organophosphates and pyrethroids, which are regularly applied in the tea gardens for controlling the looper pests including *H. talaca*, proved to be hazardous to the environment and human health (Mobed et al., 1992). Studies have shown that gradually the pests have developed tolerance to these synthetic chemical pesticides and become less susceptible, often resulting in control failures (Gurusubramanian *et al.,* 2008; Roy *et al.,* 2010). To minimize the use of synthetic pesticides, alternative approaches of pest management in agriculture have been contemplated, among these microbial pesticides based management appears to be more eco-friendly and effective.

An effective biopesticide that has been successfully applied in the management of many crops, orchard and forest are prepared based on baculoviruses (nucleopolyhedrovirus and granulovirus). Baculoviridae is a large family of pathogens that infect insects, particularly the order: Lepidoptera (Blissard *et al.,* 2000). Baculoviruses have a large circular, super-coiled and double-stranded DNA genome ranging between 80-180 kb packaged into rod-shaped virions (King *et al.,* 2011). More than 700 baculoviruses have been identified from the insects of the orders Lepidoptera, Hymenoptera, and Diptera (Moscardi, 1999).

Baculoviruses as microbial insecticides are ideal tools in integrated pest management (IPM) programs as they are usually highly specific to their insect hosts, thus, they are safe to the environment, humans, other plants, and natural enemies (Yasuhisa, 2007). Based on 30 core baculovirus genes they are phylogenetically divided into four genera: Alphabaculovirus (lepidopteran-specific nucleopolyhedrovirus), Betabaculovirus (lepidopteran-specific granulovirus), Gammabaculovirus (hymenopteran-specific nucleopolyhedro-virus), and Deltabaculovirus (dipteran-specific nucleopolyhedrovirus) (King *et al.,* 2011).

Alphabaculoviruses can be further subdivided into group I and II nucleopolyhedroviruses (NPVs) (Herniou *et al.,* 2003). In nucleopolyhedroviruses (NPVs) nucleocapsids are occluded in large protein crystals forming Occlusion body (OB). NPV produces two types of viruses during their infection cycle: occlusion-derived viruses (ODVs), which transmit infections among insects (oral infection), whereas budded viruses (BVs) spread infection to neighbouring cells (Keddie *et al.,* 1989). At the late stage of infection, the infected larvae show enhanced dispersal behaviour (Goulson, 1997), followed by dramatic degradation of the host cadavers by liquefaction (Federici, 1997) and this pathogenicity is highly species-specific.

A strain of *Hyposidra talaca* NPV (HytaNPV) has been found pathogenic to the concerned pest in laboratory condition (Mukhopadhyay *et al.,* 2011). Therefore, HytaNPV can be developed as an alternative to the synthetic chemical pesticides to control the *H. talaca* in the tea gardens. The present study has been initiated to characterize the HytaNPV genome by restriction endonuclease analysis.

Materials and Methods

Stocks of HytaNPV OBs were built-up from cadavers of NPV infected *H. talaca* larvae following the method of Kawarabata and Matsumoto (1973) with some modifications. Cadavers of *H. talaca* showing typical symptoms of NPV infection were collected and stored in 1 ml of distilled water at room temperature for putrefaction to enable the release of OBs from the infected tissues. The putrefied suspension was homogenized, the homogenate was then filtered through double layers of cheese cloth and the filtrate was centrifuged at 1000x g for 20 min at 20°C. The supernatant was removed and the sedimented polyhedra were suspended in 25% (w/v) sucrose dissolved in distilled water and centrifuged at 1000x g for 20 min at 20°C. The pelleted polyhedra were re-suspended in 25% sucrose solution in a volume of 10ml and were layered on 30 ml of 50% sucrose solution and centrifuged at 1800xg for 40 min at 20°C. The last step was repeated twice and the polyhedra were washed several times with de- ionized distilled water and stored at -20°C for future use.

OBs of HytaNPV were suspended in Tris-EDTA (10mM Tris, 1mM EDTA, pH-8.0) and then were dissolved by adding dissolution buffer (0.1M Na_2CO_3, 0.01M EDTA, 0.17M NaCl, pH 10.8). Viral DNA was extracted by proteinase K (1 mg/ml) digestion in presence of 1% SDS followed by phenol-chloroform purification as described by O'Reilly *et al.* (1994). The DNA was then ethanol precipitated, dried, dissolved in Tris-EDTA (pH 8.0) and stored at -20°C. Restriction digestion of HytaNPV DNA by *Eco*RI, *Xho*I, *Kpn*I, *Bam*HI and *Pst*I were carried out following the method of Sambrook and Russell (2001). About 1.5–2 µg of HytaNPV DNA was set up for digestion with 10 units Restriction enzyme in presence of 1x restriction buffer and 1x acetylated-BSA at 37°C for overnight for complete digestion. The digested fragments were separated in 0.4-0.7% agarose gel at 70 V for 6 hours and gels were viewed and photographed in Gel Documentation System (SPECTROLINE BI-O-VISION, UV/WHITE Light Transilluminator) and analyzed using the software: ImageAide: Version 3.06.

Results and Discussion

Restriction digestion of DNA of HytaNPV with *Eco*RI, *Xho*I, *Kpn*I, *Bam*HI and *Pst*I produced 29, 21, 13, 10 and 9 fragments, respectively ranging between 0.62 kb to 62 kb. Restriction profiles of these digestions are shown in figure 1 and size of the restriction endonuclease fragments is summarized in table 1. The size of the each fragment was determined comparing restriction endonuclease fragment mobilities with those of *Hin*dIII digested λDNA as molecular weight standard. The fragments were designated alphabetically starting with 'A' according to the size from higher to lower as proposed by Vlak and Smith (1982). This number represents the minimum number of cleavage sites for each of these five enzymes, since fragments smaller than 0.62 kb were not detectable.

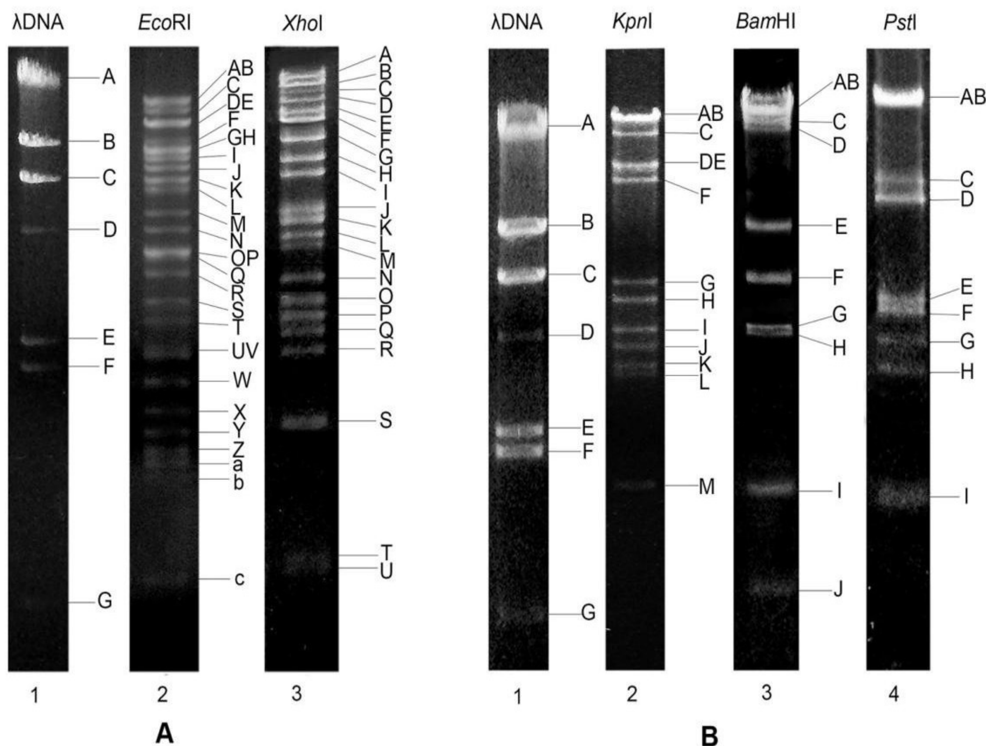
To resolve the high molecular weight fragments (particularly more than 23 kb), the digested DNA were separated in 0.4% agarose gel and to detect the fragments smaller than 23 kb, the digested fragments were separated in 0.7% agarose gel. *Eco*RI digestion produced 29 fragments ranging from 0.62-19.53 kb while 21 fragments could be resolved ranging from 0.67 kb to 25.56 kb with *Xho*I digestion (Figure 1A). Digestion with *Kpn*I produced 13 fragments ranging from 1.46 to 37.50 kb. Other restriction digestions with *Bam*HI and *Pst*I produced 10 and 9 bands, respectively ranging from 0.71 to 48 kb for *Bam*HI and from 1.68 kb to 62 kb kb for *Pst*I (Figure 1B). The results showed that HytaNPV DNA digested with *Kpn*I, *Bam*HI and *Pst*I have high molecular weight DNA above 23 kb which were resolved as doublet. The fragments AB in *Kpn*I, *Pst*I and *Bam*HI are more than 23 kb in size and appear to co-migrate (Figure 1B). The fragments AB in *Eco*RI and DE in *Kpn*I digestion were resolved by running the digests in 0.4% agarose gel for 6 hours (data not shown). Another doublet DE in *Eco*RI was confirmed by double digestion with *Eco*RI and *Pst*I (data not shown). The mean size of the HytaNPV genome was estimated to be 174.76 kb (Table 1).

Restriction endonuclease fragment analysis of different NPVs has been carried out to estimate the genome size (Hu *et al.,* 1998; Chen *et al.,* 2000; Lin *et al.,* 2012) and has been shown to be relatively stable and is often used as an effective tool to differentiate closely related NPVs (Woo *et al.,* 2006).

Table.1 Size of HytaNPV DNA fragments (in kb) after restriction digestion with respective enzyme (*Hin*dIII digested λ DNA as molecular weight marker has been shown also)

Fragments	λ DNA	*Eco*RI	*Xho*I	*Kpn*I	*Bam*HI	*Pst*I
A	23.130	19.53	25.56	37.50	48.00	62.00
B	9.416	18.13	22.39	37.00	47.50	61.00
C	6.557	15.62	20.31	21.57	27.50	14.74
D	4.361	12.87	18.01	17.68	22.85	13.57
E	2.322	12.72	15.38	16.87	10.48	6.08
F	2.027	9.28	13.89	15.17	6.92	5.66
G	0.564	8.53	10.42	6.75	4.97	4.17
H		8.30	8.36	5.89	4.68	3.49
I		7.89	7.18	4.66	1.40	1.68
J		7.11	5.21	4.17	0.71	
K		6.34	4.83	3.49		
L		5.89	4.36	3.24		
M		4.82	4.06	1.46		
N		4.25	3.33			
O		3.76	2.85			
P		3.60	2.64			
Q		3.57	2.42			
R		3.35	2.20			
S		2.89	1.44			
T		2.60	0.68			
U		2.18	0.67			
V		2.10				
W		1.90				
X		1.63				
Y		1.47				
Z		1.36				
a		1.27				
b		1.17				
c		0.62				
Total		174.75	176.19	175.45	175.01	172.39
Mean				174.76		

Figure.1 Restriction fragment profiles of HytaNPV DNA. (A) *Eco*RI (lane 2) and *Xho*I (lane 3) in 0.7% agarose gel (B) *Kpn*I (lane 2), *Bam*HI (lane 3) and *Pst*I (lane 4) in 0.4% agarose gel. *Hin*dIII digests of λDNA was used as molecular weight marker (lane 1). Restriction fragments of individual restriction profiles are designated alphabetically starting with 'A' for the largest fragment



Though the isolation and bioassay study related to the lethal concentrations and lethal time of HytaNPV from Terai-Dooars tea plantations were carried out by Mukhopadhyay *et al.* (2011), no information on the total size of the genome or restriction digestion analysis is available till date. So the present study is the first report on the restriction profiling of HytaNPV. Genome size of baculoviruses ranges from 80 to 180 kb (King *et al.,* 2011). The size of HytaNPV genome estimated in this study falls within the above range. The Restriction endonuclease fragment profile along with restriction mapping and estimation of genome size of BusuNPV isolated from another looper pest of tea *Biston(=Buzura) suppressaria*, has been reported in China with a mean genome size of 129 kb (Liu *et al.,* 1993), however Hu *et al.* (1998) documented a genome size of 120.9 kb. Recently the whole genome organization and sequence of BusuNPV was reported by Zhu *et al.* (2014) with a genome size of 120.42 kb. BusuNPV is reported as a close relative of HytaNPV (Antony *et al.,* 2011) but the estimated genome size of HytaNPV in the present study is higher by about 55 kb than BusuNPV. It appears that such genome size variation is not unusual between closest relatives of NPV. Chen *et al.* (2000) reported the genome size of *Helicoverpa armigera* NPV (HearNPV) to be 130.1 kb but the NPV isolated from *Helicoverpa assulta*, a closest relative of HearNPV was shown to have a genome size of 138 kb (Woo *et al.,* 2006) which is 8 kb higher than that of the former. The present

study will provide some idea about the genome of HytaNPV broadly revealing the size and the cleavage sites of the five restriction endonucleases. Further studies are contemplated to determine the exact size, restriction map and constitution of the HytaNPV genome by restriction digestions using different endonucleases and subsequent sequencing.

Acknowledgement

Authors are thankful to the Head, Department of Zoology for providing the Departmental Central laboratory facility which is supported by the Fund for Improvement of Science and Technology Infrastructure programme, Department of Science and Technology, New Delhi, India and the Special Assistance Programme of the University Grants Commission, New Delhi. The fellowship to Mr. Bappaditya Ghosh under the Basic Scientific Research support of University Grants Commission, Bahadur Shah Zafar Marg, New Delhi, India (Ref. No. 4-1/2006(BSR)/7-134/2007 (BSR) dated 25.02.2013) is sincerely acknowledged.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Antony, B., Sinu, P.A., Das, S., 2011. New record of nucleopolyhedroviruses in tea looper caterpillars in India. *J. Invertebr. Pathol.,* 108: 63–67.

Blissard, G., Black, B., Crook, N., Keddie, B.A., Possee, R., Rohrmann, G., Theilmann, D., Volkman, L. 2000. Family Baculoviridae. In: van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E.B., Estes, M.K., Lemon, S.M., McGeoch, D.J., Maniloff, J., Mayo, M.A., Pringle, C.R., Weckner, R.B. (Eds.), Virus taxonomy: classification and nomenclature of viruses. Seventh Report of the International Committee for the Taxonomy of Viruses. Academic Press, San Diego. Pp. 195–202.

Chen, X., Li, M., Sun, X., Arif, B.M., Hu, Z., Vlak, J.M., 2000. Genomic organization of *Helicoverpa armigera* single-nucleocapsid nucleopolyhedrovirus. *Arch. Virol.,* 145: 2539–2555.

Das, S., Roy, S., Mukhopadhyay, A. 2010. Diversity of arthropod natural enemies in the tea plantations of North Bengal with emphasis on their association with tea pests. *Curr. Sci.,* 99: 1457–63.

Federici, B.A. 1997. Baculovirus pathogenesis. In: Miller, L.K. (Eds.), The Baculoviruses. Plenum Publishing Corporation, New York. Pp. 33–59.

Goulson, D. 1997. Wipfelkrankheit: modification of host behaviour during baculoviral infection. *Oecologia,* 109: 219–228.

Gurusubramanian, G., Rahman, A., Sarmah, M., Roy, S., Bora, S. 2008. Pesticide usage pattern in tea ecosystem, their retrospects and alternative measures. *J. Environ. Biol.,* 29: 813–826.

Herniou, E.A., Olszewski, J.A., Cory, J.S., O'Reilly, D.R. 2003. The genome sequence and evolution of baculoviruses. *Annu. Rev. Entomol.,* 48: 211–234.

Hu, Z.H., Arif, B.M., Jin, F., Martens, J.W.M., Chen, X.W., Sun, J.S., Zuidema, D., Goldbach, R.W., Vlak, J.M. 1998. Distinct gene arrangement in the *Buzura suppressaria* single-nucleocapsid

nucleopolyhedrovirus genome. *J. Gen. Virol.,* 79: 2841–2851.

Kawarabata, T., Matsumoto, K. 1973. Isolation and structure of nuclear polyhedrosis virus from polyhedra of silkworm, *Bombyx mori*. *Appl. Entomol. Zool.,* 8: 227–233.

Keddie, B.A., Aponte, G.W., Volkman, L.E. 1989. The pathway of infection of *Autographa californica* nuclear polyhedrosis virus in an insect host. *Science,* 243: 1728–1730.

King, A.M.Q., Adams, M.J., Carstens, E.B., Lefkowitz, E.J. 2011. Virus taxonomy ninth report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, London, San Diego.

Lin, T., Liu, L., Chang, R., Lang, G., Xu, W. 2012. Molecular Characterization of a Nucleopolyhedrovirus Newly Isolated from *Ophiusa disjungens* in China. *Indian. J. Virol.,* 23: 379–381.

Liu, M., Hu, Z., Liang, B., Jin, F., Li, M., Xie, T. 1993. Physical mapping of buzura suppressara nuclear polyhedrosis virus genome. *Arch. Virol.,* 128: 357–362.

Mobed, K., Gold, E.B., Schenker, M.B. 1992. Occupational health problems among migrant and seasonal farm workers. *West. J. Med.,* 157: 367–373.

Moscardi, F. 1999. Assessment of the application of baculoviruses for control of Lepidoptera. *Annu. Rev. Entomol.,* 44: 257–289.

Mukhopadhyay, A., Khewa, S., De, D. 2011. Characteristics and virulence of nucleopolyhedrovirus isolated from *Hyposidra talaca* (Lepidoptera: Geometridae), a pest of tea in Darjeeling Terai. *Indian Int. J. Trop. Insect. Sci.,* 31: 13–19.

O'Reilly, D., Miller, L.K., Luckow, A.V. 1994. Virus methods. In: Baculovirus expression vectors: a laboratory manual. Oxford University Press, New York. Pp. 124–138.

Roy, S., Mukhopadhyay, A., Gurusubramanian, G. 2010. Baseline susceptibility of Olignychus coffeae to Acaricides in North Bengal tea plantations, India. *Int. J. Acarol.,* 36: 357–362.

Sambrook, J., Russell, D.W. 2001. Restriction endonuclease digestion of DNA in agarose plugs. In: Molecular clonning: A laboratory manual, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Harbor, New York. Pp. 5.68–5.70.

Vlak, J.M., Smith, G.E. 1982. Orientation of the genome of *Autographa californica* nuclear polyhedrosis virus: a proposal. *J. Virol.,* 41: 1118–1121.

Woo, S., Choi, J.Y., Je, Y.H., Jin, B.R. 2006. Characterization of the *Helicoverpa assulta* nucleopolyhedrovirus genome and sequence analysis of the polyhedrin gene region. *J. Biosci.,* 31: 329–338.

Yasuhisa, K. 2007. Current status and prospects on microbial control in Japan. *J. Invertebr. Pathol.,* 95: 181–186.

Zhu, Z., Yin, F., Liu, X., Hou, D., Wang, J., Zhang, L., Arif, B., Wang, H., Deng, F., Hu, Z. 2014. Genome sequence and analysis of *Buzura suppressaria* nucleopolyhedrovirus: A Group II Alphabaculovirus. *PLos One,* 9: 1–8.