Abstract

Introduction

The family Baculoviridae (nucleopolyhedroviruses and granuloviruses) includes a large group of DNA viruses that are pathogenic to several arthropod species, primarily to the insects of the order Lepidoptera but a few also infect members of the orders Hymenoptera and Diptera. The four genera of this virus family, Alphabaculovirus (lepidopteran-specific nucleopolyhedrovirus), Betabaculovirus (lepidopteran-specific granulovirus), Gammabaculovirus (hymenopteran-specific nucleopolyhedrovirus), and Deltabaculovirus (dipteran-specific nucleopolyhedrovirus), are host specific, highly pathogenic, and safe for industrial production and field applications. Baculoviruses, particularly nucleopolyhedroviruses (NPVs), have been considered an effective alternative to synthetic chemical pesticides to control different agricultural insect pests. An NPV (HytaNPV) isolated from a major leaf-eating lepidopteran tea pest, *Hyposidra talaca,* from the Terai-Dooars region of West Bengal and Assam in India, is found to be pathogenic against this defoliating pest and can be developed as an effective bioinsecticide against *H. talaca* in this region. Therefore, the information regarding the genome of HytaNPV and the phylogenetic relationship among different geographic isolates of HytaNPVs and the NPVs infecting other insects will be helpful to develop this virus as a biopesticide.

The present study contemplates the restriction endonuclease fragment analyses using *Bam*HI, *Bgl*I, *Eco*RI, *Hin*dIII, *Kpn*I, *Pst*I and *Xho*I, to construct the restriction profiles and to estimate the approximate genome size of nucleopolyhedrovirus isolated from *Hyposidra talaca* from Terai region of Darjeeling foothills. For comparison, a Dooars isolate has also been included in the study. The isolates collected from the two, east and west terrains of the mighty river Teesta, were designated as HytaNPV-ITK1 (Terai isolate) and HytaNPV-ID1 (Dooars isolate). Six genes (*polyhedrin, lef-8, lef-9, pif-1, pif-2* and *pif-3*) from both the isolates, those of Terai and Dooars, were sequenced. Partial restriction maps based on the obtained sequences of both the isolates were constructed and compared with the Dooars isolate of HytaNPV earlier reported by Nguyen et al. (2018). This reference isolate was designated as HytaNPV-R (Nguyen et al., 2018). Phylogenetic studies based on six gene sequences were carried out to know the taxonomic position and relationship of HytaNPVs (HytaNPV-ITK1 and HytaNPV-ID1) with the other reported baculoviruses.

The survey, sampling and maintenance of virus stock

A field survey, before the application of pesticides in tea plantations, suggested that *H. talaca* caterpillars (loopers) were found in abundance from March – November except during winter season. Adult moths become active in dimmed light during the early morning while their looper stage either perforated or defoliated the young tea leaves. NPV-infected dead looper caterpillars were noticed hanging from the tea leaves and twigs with their prolegs in a head-down position exhibiting liquefaction of the body.

NPV-infected cadavers of *Hyposidra talaca* caterpillars were collected separately from different tea plantations in the Terai region of Darjeeling foothills and also from the Dooars region of West Bengal, India for comparison. Occlusion bodies (OBs) of NPV of *H. talaca* were collected from the cadavers and purified by differential centrifugation. The stock of OBs was maintained by infecting the laboratory-reared 3^{rd}-5^{th} instar healthy larvae. OBs in large quantities were obtained from the larvae.

Viral DNA isolated from the OBs was used for restriction endonuclease fragment analyses. PCR amplification and sequencing of the target genes were carried out using gene-specific primers.

Restriction fragment analyses

Restriction fragment analysis using *Bam*HI, *Bgl*I, *Eco*RI, *Hin*dIII, *Kpn*I, *Pst*I and *Xho*I of both the Terai and the Dooars isolates, HytaNPV-ITK1 and HytaNPV-ID1 of the present study revealed 18, 8, 26, 9, 11, 7, and 20 fragments, respectively, ranging from 0.72 kb to 57.10 kb. A comparison with *in silico* restriction profiles of HytaNPV-R (Nguyen et al., 2018) revealed almost similar restriction profiles except for two restriction fragments, 3530 bp *Hin*dIII-fragment and 4857 bp *Xho*I-fragment. Such differences in restriction profiles among different geographic isolates are not uncommon, that represent a polymorphic state. Based on the restriction endonuclease fragment analyses the mean genome sizes of the HytaNPV isolates in the present study were estimated to be 138.20 kb for the Terai isolate (HytaNPV-ITK1) and 138.46 kb for the Dooars isolate (HytaNPV-ID1). The minor difference in genome sizes were either due to some undetected low molecular weight restriction fragment(s) or due to unresolved change in size(s) of restricton fragment(s) produced by observed site polymorhism, in agarose gel electrophoresis. The estimated genome size of both the isolates of the present study was found to be very similar to HytaNPV-R (Nguyen et al., 2018), which was 139.089 kb.

PCR and Sequencing of genes

Being most conserved, *polyhedrin* was sequenced along with the other five core baculoviral genes, *lef-8, lef-9, pif-1, pif-2* and *pif-3*. A total of 6.96 kb for the Terai (HytaNPV-ITK1) and 5.60 kb for the Dooars (HytaNPV-ID1) isolates of the HytaNPV genome (139.089 kb) were sequenced with some gaps. Nucleotide and protein BLAST analyses of all the gene sequences of both the isolates showed the highest sequence identity of >98% for nucleotides and >99% for amino acids with the reference HytaNPV-R (Nguyen et al., 2018).

Pairwise alignment of the obtained sequences of both the isolates (HytaNPV-ITK1 and HytaNPV-ID1) using the Dooars isolate of HytaNPV-R as a reference (Nguyen et al., 2018) revealed more than 98.99% and 99.98% sequence identity for nucleotide and amino acid, respectively. Moreover, a total of 26 non-synonymous substitutions leading to the substitution of 25 amino acids were detected among the isolates of HytaNPVs. Among the NPVs pathogenic to the specimens of other genera than *Hyposidra*, the NPV (BusuNPV) pathogenic to *Buzura suppressaria*, the old looper pest of tea in the Terai-Dooars region in India, exhibited a maximum overall amino acid sequence identity of 90.9% and nucleotide sequence identity of 85.8% with both the HytaNPV isolates of the present study for all the six genes.

In the *polyhedrin* gene, all seven (7) variable sites were synonymous suggesting conservative properties of the gene. Moreover, the overall sequence comparison among the HytaNPV isolates of the Terai-Dooars region revealed a ratio of non-synonymous to synonymous substitution <1 for *lef-8* and *pif-1* (2/6=0.33 for *lef-8* and 6/10=0.6 for *pif-1*) whereas, the ratio for *lef-9* was 16/6=2.66 and for *pif-2* was 20/12=1.66, indicating that *pif-2* and *lef-9* genes are under positive selection.

A higher proportion of variable sites for nucleotides (16 out of 1001) were detected in the *pif-2* suggesting that despite being one of the most conserved genes, the genetic variations in *pif-2* among the geographic isolates of NPV infecting the host of the same species may exist in different populations.

Partial restriction maps of the HytaNPV isolates

The partial restriction maps based on the obtained sequences of the two isolates of HytaNPV (HytaNPV-ITK1 and HytaNPV-ID1) in the present study were constructed. Two restriction sites for each of *Eco*RI and *Kpn*I in *polyhedrin* sequence, and a single site for each of *Xho*I, *Eco*RI, *Bgl*I and *Bam*HI in *lef-9, lef-8, pif-2* and *pif-1* sequences, respectively, were found to be common in both the isolates. However, no site for any of the six restriction endonucleases

analysed in the present study was detected in the *pif-3* sequences in both the isolates. Another *Bam*HI restriction site in *pif-1* which was present in HytaNPV-ID1 and HytaNPV-R was found to be absent in the Terai isolate (HytaNPV-ITK1).

Phylogenetic analysis

Both the nucleotide and amino acid substitution-based phylogenetic trees using the concatenated sequence alignment of *polyhedrin*, *lef-8*, *lef-9*, *pif-1*, *pif-2* and *pif-3* genes showed that both the group I and group II alphabaculoviruses have diverged from a common ancestor. The delta, gamma and beta- baculoviruses have diverged before the divergence of alphabaculoviruses into group I and group II. HytaNPV isolates of the present study (HytaNPV-ITK1 and HytaNPV-ID1) and the reference, HytaNPV-R reported by Nguyen et al. (2018) from the Dooars region, were phylogenetically placed under the group II alphabaculovirus. Both, HytaNPV-ITK1, and HytaNPV-ID1, appeared to be closer to the HytaNPV-R. Among the NPVs infecting the host of other genera, BusuNPV exhibited the closest relationship with the isolates of HytaNPVs. The phylogenetic analyses also revealed that the NPVs infecting the members of the family Geometridae such as BusuNPV, EcobNPV, ApciNPV and SujuNPV including HytaNPVs showed close relationships among themselves.

Conclusion

The present study revealed that though there were few genetic differences present in polymorphic conditions among the Terai and Dooars isolates of HytaNPV in West Bengal, by and large, a single variant of HytaNPV is infecting *Hyposidra talaca* in these regions. An extensive investigation using more restriction endonucleases and sequencing on a wider area may reveal more distinguishable genetic features.