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Characterization of alphabaculovirus: HearNPV-IIPR05 isolate infecting *Helicoverpa armigera* (Hubner) larvae

Sanjay M. Bandi^{1*}, P. S. Shanmugavadivel¹, Lalit Kumar² and A. Revanasidda¹

Abstract

Background: The alphabaculoviruses are lethal pathogens of lepidopteran caterpillars including a polyphagous and globally recognized pest, *Helicoverpa armigera* (Hubner) infesting economically important agriculture crops worldwide. The biological and molecular characterizations of indigenous *nucleopolyhedrovirus* of the genus *Alphabaculovirus* isolated from *H. armigera* in chickpea fields are described.

Results: The virulence of virus isolate was tested in 3rd instar *H. armigera* larvae, and LC₅₀ (median lethal concentration) was estimated to be 2.69×10^4 OBs ml⁻¹. The ST₅₀ (median survival time) was 4 days post-inoculation, when the 3rd instar *H. armigera* larvae were inoculated by OB (occlusion body) concentration equivalent to LC₉₀. An average incubation period of the virus isolate in 3rd instar ranged between 4 and 6 days post-inoculation. The OBs of a virus isolate appeared irregular in shape and variable in size with diameter ranging from 0.57 to 1.46 μm on the longest edge and average of 1.071 ± 0.068 μm (mean ± SE). On the basis of phylogenetic analysis of *polh*, *pif-1*, and *lef-8* genes, the isolate was found to be a member of the genus *Alphabaculovirus*. The isolate showed a genetic affinity with species of group II *Alphabaculoviruses* and appeared to be a group II NPV.

Conclusions: On the basis of molecular phylogeny and associated host insect, this indigenous isolate was designated as HearNPV-IIPR05 isolate, which could be a potential candidate for the biological control of *H. armigera* infesting legumes and other commercial crops.

Keywords: Alphabaculovirus, *Nucleopolyhedrovirus*, *Helicoverpa armigera*, Phylogeny, Virulence, Biological control

Background

The baculoviruses are regarded as potential biological control agents because of their safety to vertebrates, high pathogenicity, host-specificity, and ability to cause epizootics in the insect population. They have been reported worldwide from over 600 host species (Martignoni and Iwai 1986), mostly from the insect species of orders Lepidoptera, Diptera, and Hymenoptera (Herniou et al. 2012). On the basis of genome sequence analysis and associated arthropod host, 4 genera, viz., *Alphabaculovirus*, *Betabaculovirus*, *Gammabaculovirus*, and *Deltabaculovirus*, were recognized within the family

Baculoviridae (Herniou et al. 2012). The genus *Alphabaculovirus* that included lepidopteran-specific *nucleopolyhedroviruses* (NPVs) is further classified into group I and group II NPVs, based on the presence of envelope fusion proteins in budded viruses, viz., GP64 and F protein, respectively (Lauzon et al. 2006).

Nearly 38 genes are reported to be conserved among all sequenced genomes of baculovirus till date, and these genes are assigned as core genes (Williams et al. 2016; Blissard and Theilmann 2018). Degenerate primers amplifying conserved genomic regions of a broad phylogenetic range of baculoviruses were used to study the relationship between viruses based on nucleotide variations. Oligonucleotides that amplify partial sequences of baculovirus core genes, *polyhedrin* encoding a major

* Correspondence: sanjaysmb@gmail.com

¹ICAR-Indian Institute of Pulses Research, Kanpur 208024, India
Full list of author information is available at the end of the article

matrix protein of OBs and *late expression factors* (*lef-8* and *lef-9*) encoding subunits of baculovirus RNA polymerase from various baculovirus genomes, were commonly used for characterization of baculovirus species (Herniou et al. 2003; Lange et al. 2004; Jehle et al. 2006b; Jose et al. 2013). Few of the genes coding for ODV envelope protein, *per os* infectivity factors (*pif-1* and *pif-2*) mediating oral infectivity were also reported to be conserved in baculoviruses (Herniou et al. 2003; Ohkawa et al. 2005; Okano et al. 2006), and used for species discrimination.

Baculovirus isolates from diverse ecological regions differed in their biological traits (Erlandson et al. 2007; Ali et al. 2018) due to their evolution over a period of time and space. The variations in their biological traits in terms of pathogenesis, virulence, and speed of kill were reported among the distinct geographical isolates of baculovirus (Rabindra 1992). The genotypic variations within or between baculovirus populations have been reported. Alphabaculoviruses comprised of lepidopteran-specific NPVs isolated from the same host species in different geographical regions indicated heterogeneity in DNA profiles (Gettig and McCarthy 1982). The genetic variants isolated from single host insect differed in their phenotypic traits for pathogenicity and speed of kill (Murillo et al. 2006). The intraspecific genetic diversity among the 8 strains of *H. armigera* NPV (HearNPV) was identified (Figueiredo et al. 2009), and coexistence of genotypic variants is frequently reported within the natural baculovirus populations (Cory and Myers 2003). The NPVs isolated from *Helicoverpa armigera* (Hubner) in different locations exhibited significant variations in virulence measured in terms of median lethal concentration (Rabindra 1992). Hence, development of viral bio-control agents involves selection of naturally occurring region-specific isolates and their virulence against local host-insect population for the effective management of associated host-insect pest.

In the present study, an indigenous baculovirus was isolated from naturally infected *H. armigera* larvae considering its adaptability to given set of climatic conditions of agroecosystem and virus-host interaction influencing characteristics of concentration-response and survival time of infected host. Biological activity, OB morphology, and phylogeny of 3 core genes (*polh*, *pif-1*, and *lef-8*) of a virus isolate were described.

Methods

Test insect

The culture of *H. armigera* used in this study was established in the laboratory from the field collected larval population. Larval colonies were provided by chickpea-based semi-synthetic diet as described by Nagarkatti and Satyaprakash (1974). The moths obtained from the field

collected larvae were examined by mother moth examination method for every generation to obtain disease-free colony (FAO 2011). The abdominal content from the spent moths was excised and homogenized in sterilized water. A droplet was smeared on a slide and examined under light microscope ($\times 100$) for the presence of disease-causing organisms including baculovirus. All the progenies from infected moths, if any, were discarded. The eggs and larvae obtained from the healthy moths were raised carefully. The disease-free colonies were reared for 3 successive generations under controlled conditions ($26 \pm 1^\circ\text{C}$ and $70 \pm 5\%$ RH) and a photoperiod (14:10 h light:dark) in BOD incubator, before being used for virus multiplication.

Virus isolate

The virus was isolated from naturally infected larvae of *H. armigera* collected from chickpea fields in New Research Farm, IIPR, Kanpur (India) ($26^\circ 31' 13.41''$ N; $080^\circ 14' 49.69''$ E) on the basis of typical baculovirus-induced symptoms described by Whitlock (1974). The presence of OBs of virus in the cadavers was confirmed by light microscopy. The virus was propagated in the healthy early instar (~ 3 rd instar) larvae of *H. armigera* by establishing infection with OB contaminated artificial diet, and cadavers exhibiting baculovirus symptoms were harvested for extraction of OBs.

Purification of viral occlusion bodies

The OBs from the cadavers were recovered by 2 differential cycles of centrifugation following the procedures of Rabindra et al. (2003) with slight modifications as described below. Infected insect cadavers were homogenized in a small volume of sodium dodecyl sulfate (SDS) (0.1%) and maintained overnight at room temperature. The homogenate was filtered through 4 layers of muslin cloth to remove the insect tissue debris. The filtrate was initially centrifuged at 500 rpm for 1 min to remove larger contaminants, and supernatant was again spun at 5000 rpm twice for 15 min each to pellet the occlusion bodies. The pellet was washed 3 times by distilled water and re-suspended in double-distilled water, and aqueous suspension of virus was preserved at $\leq 4^\circ\text{C}$. The OBs were enumerated, using improved Neubauer's Hemocytometer (0.1 mm depth) (Fein-Optik Jena, Germany) at $\times 400$ magnification for bioassay studies.

Electron microscopy

Morphology of the purified OBs extracted from individual larvae was studied under scanning electron microscope (SEM) (Model: FEI Quanta 250, The Netherlands) and transmission electron microscope (TEM) (Model: Jeol JEM 1400, Japan) at EM Unit-SAIF, CSIR-CDRI, Lucknow (India), and photographed at different

magnifications. The shape and average size on the longest edge of OBs were recorded.

Virus bioassay

Biological activity of the virus isolate was tested in 3rd instar larvae of *H. armigera* infected *per os* by “diet surface contamination” method (Eberle et al. 2012). To study the concentration-mortality response, 6 concentrations ranging between 3×10^6 and 3×10^2 OBs ml⁻¹ were prepared. About 50 µl of viral suspension was dispensed over the surface of chickpea-based semi-synthetic diet and spread uniformly over the surface using blunt glass rod, which was allowed to dry for about 10 min. Each treatment consisted of 15 pre-starved larvae (for 4 h) including the untreated control. The larvae were maintained individually, and each test was repeated thrice. The bioassay was carried out under controlled conditions at $26 \pm 1^\circ\text{C}$ and $70 \pm 5\%$ RH. Observations on mortality were recorded at 24 h interval starting from 4th day post-inoculation, and viral infection was confirmed by smear test. The mortality data were corrected depending upon the mortality in the control following Abbott’s formula (Abbott 1925). Any assays with more than 10% larval mortality in controls were rejected. The corrected mean larval mortality for each concentration was subjected to probit analysis (Finney 1971) for determination of median lethal concentration (LC₅₀) using SPSS statistical program 25.

The time-mortality response was studied in the 3rd instar larvae of *H. armigera* that was inoculated at 2 concentrations representing LC₅₀ and LC₉₀ previously determined in concentration-mortality assay. A group of 15 larvae, which were pre-starved for 4 h, were inoculated by 50 µl of viral suspension and incubated as described under the concentration-mortality assay. Larvae in controls that had not consumed viral inoculum were incubated at similar conditions, and larval mortality was recorded at every 24 h interval for 10 days. Time-mortality results of individual larvae that died due to viral infection were subjected to Kaplan-Meier estimate for survival analysis using SPSS statistical program 25. A test for significant variation between survival curves was performed by a log rank test (level of significance, $P \leq 0.05$).

Phylogenetic analysis of *polh*, *pif-1*, and *lef-8* genes

Viral DNA was extracted from purified OB suspension according to the procedure described by Woo (2001) with some requisite modifications described hereafter. The purified OBs were re-suspended in 0.1 M sodium carbonate solution. About 0.5 mg ml⁻¹ of proteinase K and 1% SDS was added to the final suspension and incubated at 37 °C for overnight. This was followed by extraction with phenol:chloroform:isoamylalcohol solution (25:24:1). The DNA was precipitated in ice cold absolute

ethanol, and the pellet was re-suspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The quality of extracted DNA was confirmed by electrophoresing in 0.8% agarose gel. The coding region of core genes, *polyhedrin (polh)*, *per os infectivity factor (pif-1)*, and *late expression factor (lef-8)*, was targeted to amplify using specific primers. The primer pairs targeting *polh* and *pif-1* genes were synthesized as described previously (Ashika et al. 2017). A set of 3 primer pairs amplifying 2.2 kb of *lef-8* gene from HearNPV were designed with published sequences available in the GenBank database of National Center for Biotechnology Information (NCBI) using Primer3 tool (Untergasser et al. 2012) (Table 1). Each polymerase chain reaction (PCR) mixture consisted of 50 ng template DNA sample, 1 U of *Taq* DNA polymerase, 10× *Taq* buffer, 2.5 mM MgCl₂, 2.5 mM of dNTP mix (Genei™), and 5 µl of each forward and reverse primer in a final volume of 50 µl reaction mixture. Amplification of partial sequences of *polh*, *pif-1*, and *lef-8* genes was done using BioRad Thermal Cycler with an amplification program as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 48 to 60 °C (annealing temperature varied for primer sets based on *Tm* value and given in Table 1) for 30 s and extension at 72 °C for 45 s, final extension at 72 °C for 10 min, and storage at 4 °C till further usage. The amplicons were checked in 2% agarose gel for their target size amplification.

The PCR amplicons were purified prior to sequencing and sequenced using both forward and reverse primers by Genei Laboratories (Bengaluru) and Bioserve Biotechnologies (Hyderabad). For each gene, 3 independent amplification and sequencing reactions were performed. The sequence data of *polh*, *pif-1*, and *lef-8* were assembled into contigs by using contig assembly module present in the BioEdit Software (Hall 1999). The sequences obtained in this study were subjected to similarity search using NCBI BLAST programs. The sequences generated were used to determine the phylogenetic relationships between the isolate under study and other baculovirus species recognized by the International Committee on Taxonomy of Viruses (ICTV). The deduced amino acid sequences of core genes from the isolate and GenBank retrieved sequences were aligned by MUSCLE using MEGA6 software suite. Phylogenetic analysis of aligned amino acid sequences was performed to study the taxonomic relationship of the isolate under study using MEGA6 tool, following UPGMA method (Tamura et al. 2013) with bootstrap of 1000 replications.

Results

Virus isolate

The virus isolated from the naturally infected *H. armigera* larvae collected from chickpea plant manifested typical signs of virus infection (Fig. 1a). The presence of

Table 1 Details of the baculovirus target core genes, primer sequences, and annealing temperature for amplification of DNA fragments

Target genes	Primer sequence	Amplicon size (bp)	Annealing temperature (°C)
Polyhedrin (<i>polh</i>)	F: 5'-ACTCGTTACAGTTACAGCCCTACT-3' R: 5'-CAGTGTATAGCGGAGCGTCA-3'	659	54
<i>Per os</i> infectivity factor-1 (<i>pif-1</i>)	F: 5'-CCGCTACCAATTCACGAAG-3' R: 5'-TCATGTGTACGTGGAATAATTGG-3'	1392	48
Late expression factor-8 (<i>lef-8</i>)	F: 5'-GACGTGATTGCCGATTTTC-3' R: 5'-TTTCAATGTTGTTGTTGCTG-3'	940	58
	F: 5'-GATCGTAGACACTATAGTCAATGG-3' R: 5'-GCGTTTTTCAAATTGGTC-3'	855	60
	F: 5'-CAATAATCGAAAAGGACACAG-3' R: 5'-TTGAAAATATTATCGGGAGTG-3'	949	58

polyhedral occlusion bodies was detected in the discharged body fluid of cadavers under phase-contrast light microscope at $\times 400$ magnification (Fig. 1b). These POBs exhibited Brownian movement in wet mounts. This isolate of virus established lethal infection when healthy and early instar larvae of *H. armigera* were fed with purified OB contaminated semi-synthetic diet in the laboratory (Fig. 1c).

Virulence of the virus isolate

Biological activity of the virus isolate was determined by measuring the concentration-mortality response (LC_{50}) of 3rd instar larvae of *H. armigera*. The isolate of virus induced lethal infection in the inoculated 3rd instar larvae within 5 or rarely 6 days post-inoculation. Untreated larvae developed normally until pupation. The LC_{50} value of virus isolate was estimated to be 2.69×10^4 OBs ml^{-1} (95% fiducial limits, 2.83×10^3 to 2.34×10^5). The slope of the concentration-response line was 0.38 ± 0.12 (χ^2 0.45) (Fig. 2).

To test the time response of *H. armigera*, a survival time analysis was conducted on 3rd instar larvae inoculated with

OBs at concentrations representing LC_{50} and LC_{90} values, in contrast to full-range bioassay. The survival rate of larvae differed significantly at different concentrations by log rank test (log rank test; $\chi^2 = 4.32$; $P \leq 0.05$), and 2 patterns in Kaplan-Meier survival curves were visible (Fig. 3). The larval mortality did not occur before 3 dpi, and probability of survival started to decline at 3 dpi for both concentrations tested. The survival rate for larvae declined gradually, irrespective of OB concentrations received by larvae. The median survival time (ST_{50}) was 4 and 6 dpi for larvae receiving OB concentration equivalent to LC_{90} and LC_{50} values, respectively, which differed significantly according to log rank test ($P = 0.038$). The survival rate of *H. armigera* larvae was 15 ± 0.08 and $40 \pm 0.11\%$ at LC_{90} and LC_{50} , respectively.

OB morphology

A distinguishing feature of baculoviridae is the presence of OBs which are critical for transmission of virus in host insects and survival of the virus outside the host (Blissard and Rohrmann 1990). In the present study, the OBs of virus isolate exhibited irregular shape, a typical character of OBs of NPV belonging to *Alphabaculovirus*.

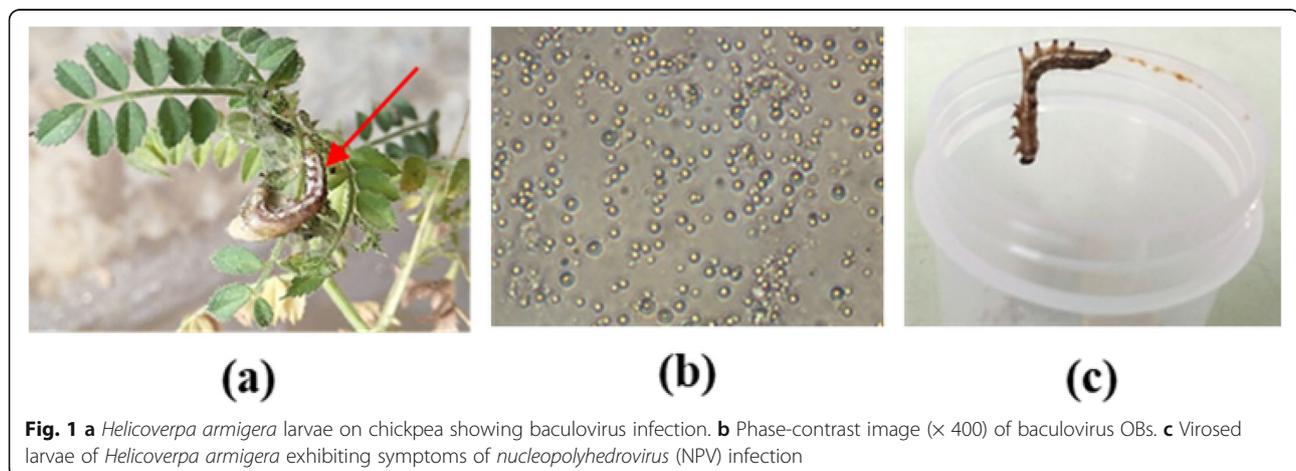


Fig. 1 a *Helicoverpa armigera* larvae on chickpea showing baculovirus infection. b Phase-contrast image ($\times 400$) of baculovirus OBs. c Virosed larvae of *Helicoverpa armigera* exhibiting symptoms of nucleopolyhedrovirus (NPV) infection

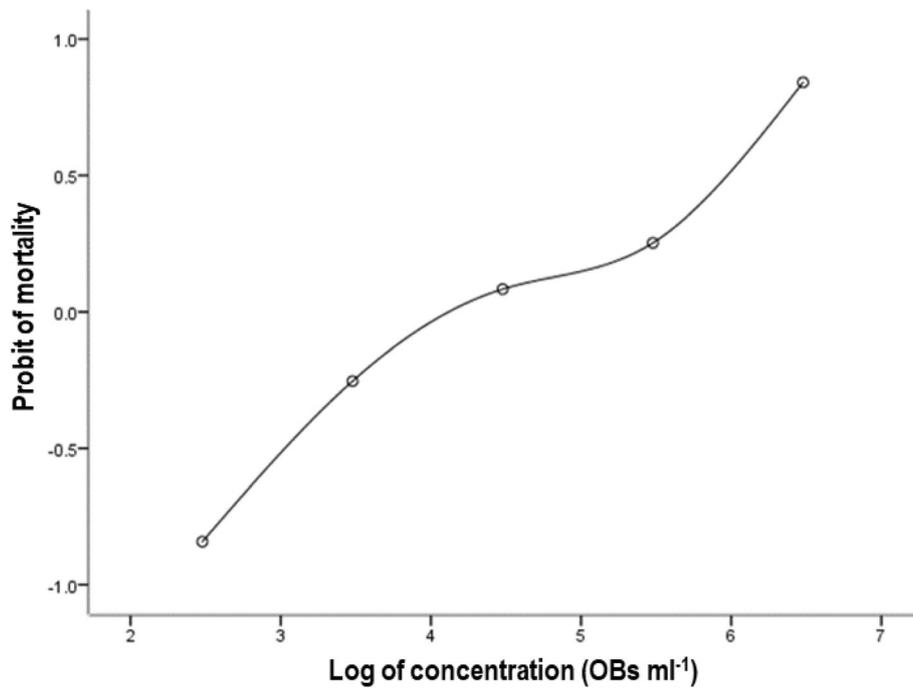


Fig. 2 Log (conc.)-probit mortality curve for *Helicoverpa armigera* larvae at different occlusion body (OB) concentrations

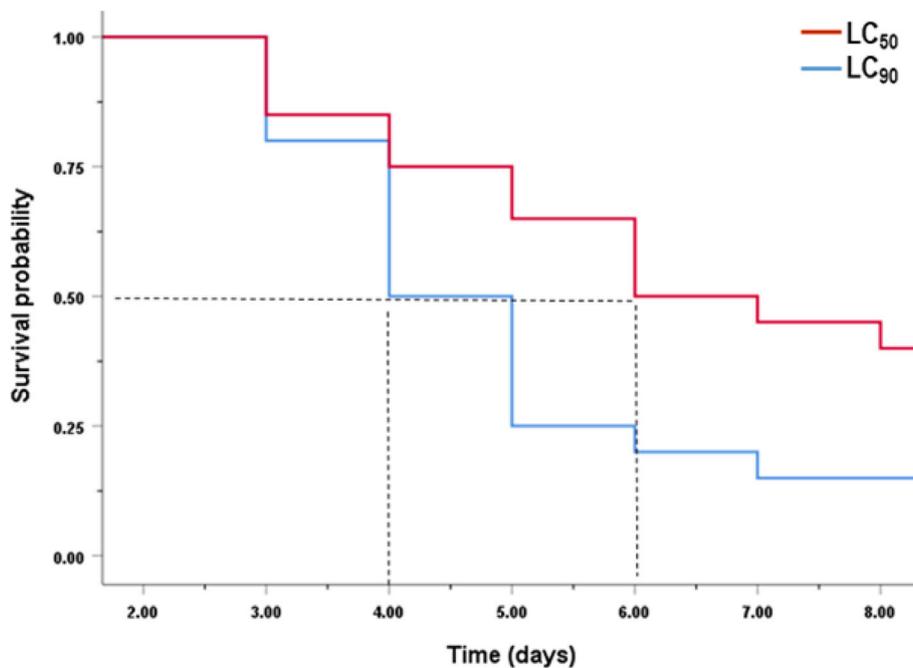


Fig. 3 Survival function of *Helicoverpa armigera* larvae at different occlusion body (OB) concentrations

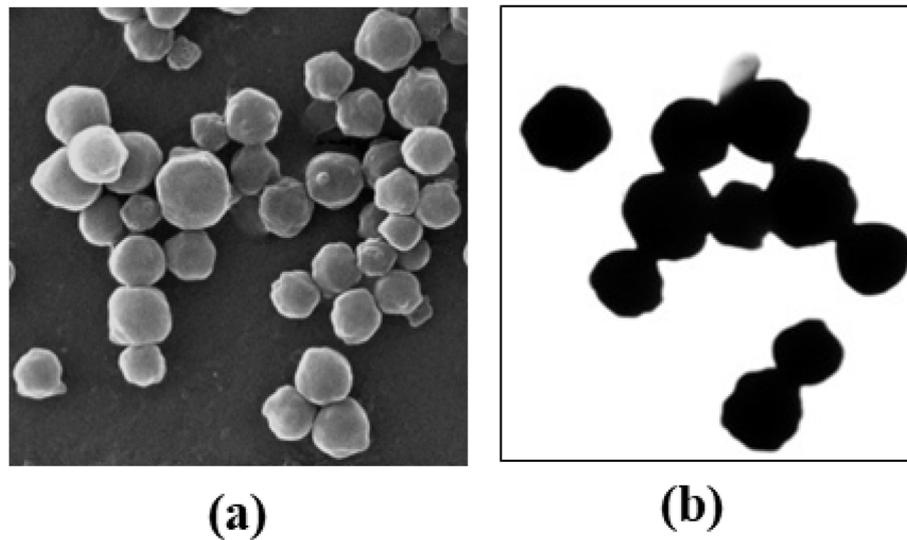


Fig. 4 **a** SEM and **b** TEM micrographs of viral occlusion bodies (OBs)

The OBs in SEM micrograph measured between 0.57 and 1.46 μm in diameter on the longest edge, with an average of $1.071 \pm 0.068 \mu\text{m}$ (mean \pm SE, $n = 20$) (Fig. 4).

Phylogeny of *polh*, *pif-1*, and *lef-8* genes

Nucleotide sequences of baculovirus core genes, *polh*, *pif-1*, and *lef-8*, were generated to determine the taxonomic position of virus isolate. PCR amplification of *polh*, *pif-1*, and *lef-8* genes from the present isolate using gene-specific primers resulted in amplicons of 596, 1375, and 2199 bp size,

respectively (Fig. 5). The partial nucleotide sequences of 3 genes from the present isolate were submitted in GenBank (Accession Numbers MN603765, MN603764, and MN603763, respectively). The amplicon size obtained in the present study for each gene corroborated with published reports (Jose et al. 2013; Ashika et al. 2017). The full-length ORF of completely sequenced *lef-8* genes varied between 2484 and 2757 bp (Pang et al. 2001).

The top matches of BLASTx queries for the present isolate were species of *alphabaculovirus* infecting host

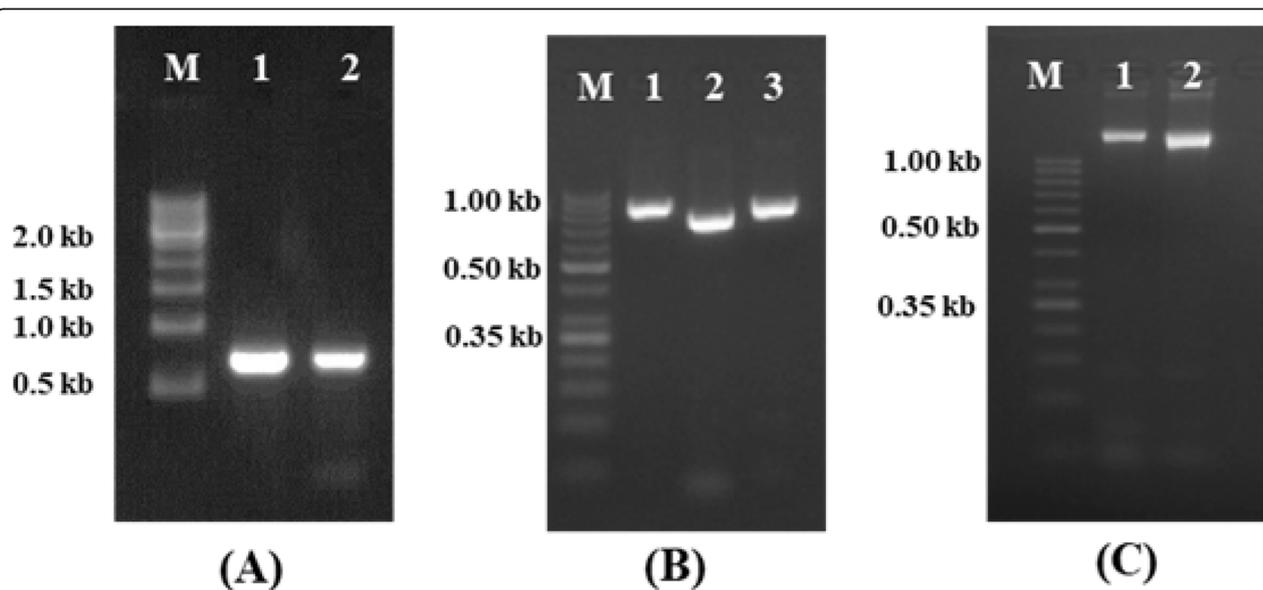


Fig. 5 PCR amplification of *polh*, *lef-8*, and *pif-1* gene coding regions. **a** Amplification of *polh* gene. M—1 kb ladder; 1 and 2—659 bp (2 replicas). **b** Amplifications of *lef-8* gene. M—50 bp step up ladder; 1—940 bp; 2—855 bp; and 3—949 bp. **c** Amplification of *pif-1* gene. M—50 bp step up ladder; 1 and 2—1392 bp (2 replicas)

species of the genus *Helicoverpa*, indicating genetic closeness with these viruses. The *polh* gene sequence exhibited more than 99% nucleotide identity with 17 isolates of HearNPV and 6 isolates of HzNPV available in GenBank. The sequences of *lef-8* revealed more than 99% homology with 16 isolates of HearNPV and 4 isolates of HzNPV. The *pif-1* gene analysis revealed greater than 99% sequence identity with 8 isolates of HearNPV and 3 isolates of HzNPV. All the GenBank isolates exhibiting maximum identity with our isolate belonged to the genus *Alphabaculovirus*.

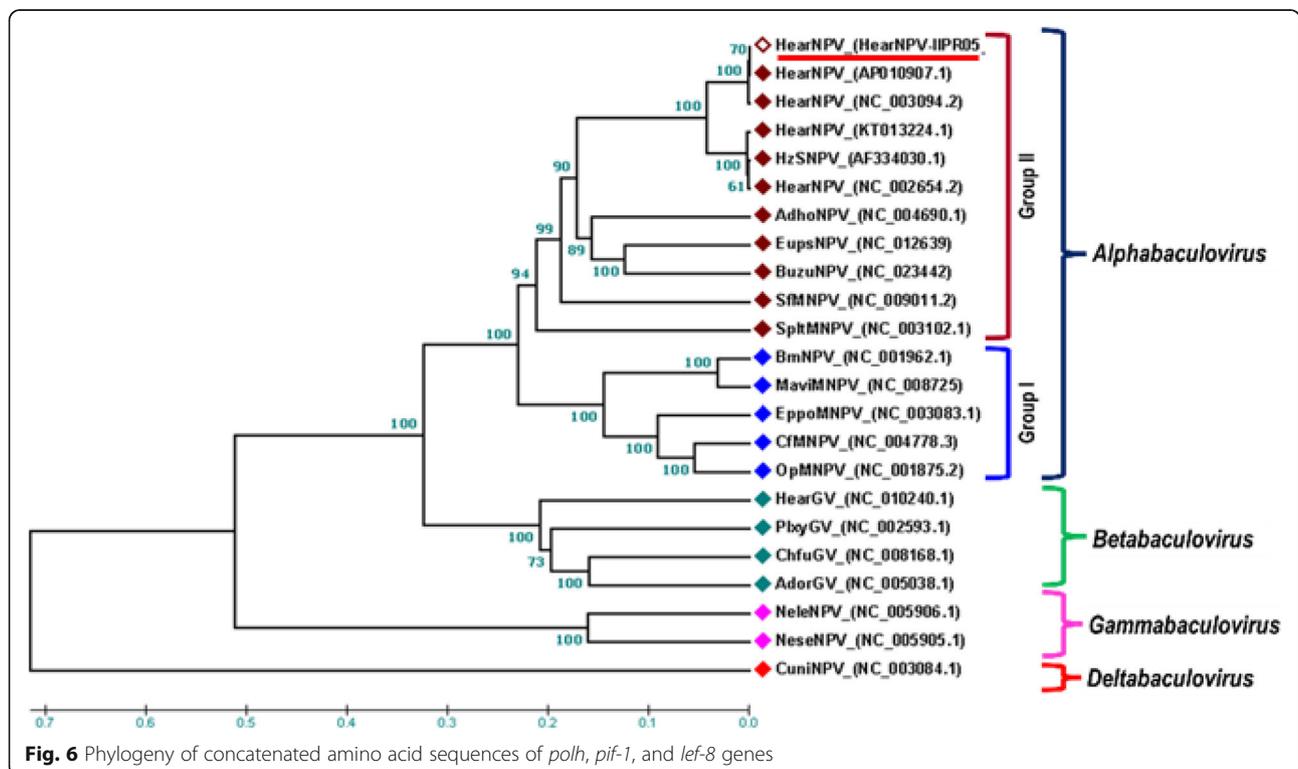
The genetic relatedness of the isolate was determined by phylogenetic analysis of concatenated amino acid sequences from 3 core genes (*polh*, *pif-1*, and *lef-8*) of baculoviruses, following UPGMA analysis (Fig. 6). The phylogenetic tree of representative ICTV recognized baculovirus species and our isolate formed 4 distinct clades, viz., *Alphabaculovirus*, *Betabaculovirus*, *Gammabaculovirus*, and *Deltabaculovirus*, with high bootstrap support and two sub-groups (group I and II NPVs) within *Alphabaculovirus*. The UPGMA tree of concatenated sequences placed our isolate in a clade with ICTV recognized NPV species belonging to the genus *Alphabaculovirus* and appeared to be closely related with members of alphabaculovirus. Phylogenetic analysis of sequences further positioned the isolate among the species of group II NPVs. Within this group, the obtained isolate clustered with two NPV species

infecting *H. armigera* belonging to lepidopteran family Noctuidae (with bootstrap support of 100%).

Discussion

The virus isolate established lethal infection in the healthy larvae of *H. armigera* when fed with purified OB contaminated semi-synthetic diet. The diseased larvae manifested typical signs of baculovirus infection. Similar symptomatology was documented by Whitlock (1974) and Rowley et al. (2011) in NPV infected *H. armigera* larvae.

Survival time and proportion of response in treated insects are critical parameters to assess the effectiveness of the virus isolate (van Beek et al. 1988). The average incubation period of virus in 3rd instar larvae was about 4 to 6 dpi. Whitlock (1974) stated 6–7 or rarely 4–9 days of incubation period in *Heliothis* NPV. The speed of killing a host insect is eventually an estimate of the virulence of virus. Hughes et al. (1983) noted mean survival time ranging from 77.8 to 116.6 h for *H. zea* infected with NPV of different country of origin. The survival time and survival function of *H. armigera* larvae were decreased at the higher OB inoculums. Previous studies reported a decrease in larval median survival time of *Heliothis zea* (van Beek et al. 1988) and *H. armigera* (Dasgupta et al. 2016) with increase in concentration of virus inoculums. The small slope value of concentration-response line for isolate indicated a great variability in response of larvae



within the population in the rate of disease progression (Hughes et al. 1983; Teakle et al. 1986). Differences in biological activity were earlier reported among the baculovirus isolates from distinct geographical regions; however, isolates had overlapping fiducial limits of LC_{50} (Rabindra 1992; Somasekhar et al. 1993; Geetha and Rabindra 1999). Shapiro and Ignoffo (1970) noticed 56-fold differences in concentration-mortality response among the 34 *Heliothis* NPV isolates. Hence, the local geographical strain of baculovirus is always preferred due to its adaptability to local environmental conditions and efficacy under a given set of climatic conditions in an agroecosystem. It is well recognized that the geographic origins of both virus and its host insect can affect their interactions particularly the characteristics of concentration-response and survival time of infected host insect (Milks 1997). The difference in virulence among the strains of baculoviruses was reported to be attributed to structure of the virus and its genetic composition (Escribano et al. 1999).

In the present study, the irregularly spherical OBs and their variable diameter confirmed to be of NPVs rather than GVs. Previous investigations on OB morphology had revealed the similar reports. Hamm and Young (1974) noticed the variation in size and shape of polyhedral inclusion bodies of NPV from *Heliothis zea*. The OBs in the *Baculoviridae* family measured about 0.15–5 μm (Harrison et al. 2018). The OBs from viroed *H. armigera* were observed irregular, and diameter ranged between 0.5 and 2.5 μm (Kumar et al. 2011) with a mean diameter of 0.89 μm (Jacob and Subramanian 1972) and 1.35 μm (Sudhakar and Mathavan 1999).

The *polh*, *pif-1*, and *lef-8* phylogeny strongly suggested that this isolate was found to be a species of group II *Alphabaculovirus*. The present results are in concordance with published reports, wherein the viruses infecting caterpillars of Noctuidae family were placed in group II NPVs on the basis of *lef-8*, *lef-9*, and *polh* gene phylogeny (Herniou et al. 2004; Lange et al. 2004; Jehle et al. 2006a, b). The amino acid-based phylogenetic analysis of baculovirus core genes is reported to be more consistent and robust than individual genes (Harrison et al. 2008). The *polyhedrin* was the first baculovirus gene ever sequenced and one of the most conserved genes of lepidopteran-specific baculoviruses, and sequence data for this gene are available for a large number of baculoviruses hence widely used in phylogenetic analysis (Lange et al. 2004). *Pif-1* gene was also reported to be conserved in baculoviruses (Ohkawa et al. 2005). Similarly, *lef-8* gene was identified in all sequenced baculoviruses (Herniou et al. 2003). Baculovirus genomics provided an approach for rapid and reliable identification of virus isolates (Herniou et al. 2001). Degenerate nucleotides from comparative analysis of lepidopteran-

specific baculovirus genome sequences especially at gene levels such as *lef-8*, *lef-9*, and *polh* were well utilized to demarcate baculovirus species (Jehle et al. 2006a, b) and to classify the baculoviruses.

Conclusion

In the present study, an indigenous alphabaculovirus—HearNPV-IIPR05, specific to *H. armigera*, was isolated and identified. The molecular phylogeny of baculovirus core genes (*polh*, *pif-1*, and *lef-8*) indicated that the isolate belongs to the genus *Alphabaculovirus* of *Baculoviridae*. This isolate could be further utilized for the development of bio-pesticide for managing the *H. armigera* in legumes and other important host crops.

Abbreviations

NPV: *Nucleopolyhedrovirus*; OBs: Occlusion bodies; GV: Granulovirus; LC: Lethal concentration; ST: Survival time; HearNPV: *Helicoverpa armigera* NPV; *Polh*: *Polyhedrin*; *Pif*: *Per os infectivity factor*; *Lef*: *Late expression factor*; ICAR: Indian Council of Agricultural Research; IIPR: Indian Institute of Pulses Research; SDS: Sodium dodecyl sulfate; SEM: Scanning electron microscope; TEM: Transmission electron microscope; dpi: Day post-inoculation; PCR: Polymerase chain reaction; NCBI: National Center for Biotechnology Information; BLAST: Basic Local Alignment Search Tool; ICTV: International Committee on Taxonomy of Viruses

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Authors' contributions

SMB designed the study, conducted the experiments, and prepared the manuscript. PSS, LK, and R contributed in conducting the experiments and preparation of manuscript. All the authors read and approved the final manuscript for publication.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹ICAR-Indian Institute of Pulses Research, Kanpur 208024, India. ²ICAR-Indian Institute of Farming Systems Research, Modipuram 250110, India.

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