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Occurrence and characterization of a tetrahedral nucleopolyhedrovirus from *Spilarctia obliqua* (Walker)

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ABSTRACT

Spilarctia obliqua Walker (Lepidoptera: Arctiidae) is a polyphagous insect pest damaging pulses, oil seeds, cereals, vegetables and medicinal and aromatic plants in India. The pest also infests turmeric and ginger sporadically in Kerala. We observed an epizootic caused by a nucleopolyhedrovirus (NPV) in field populations of the insects in December 2013. The NPV was purified and characterized. The isolate was tetrahedral in shape and belonged to multicapsid NPV. The REN profile of the SpobNPV genome with *Pst* I, *Xho* I and *Hin*dIII enzymes showed a genome size of 99.1 ± 3.9 kbp. Partial*polh*, *lef-8* and *lef-9* gene sequences of the isolate showed a close relationship with HycuNPV and SpphNPV. Phylogram and K-2-P distances between similar isolates suggested inclusion of the present SpobNPV isolate to group I NPV. The biological activity of the isolate was tested under laboratory conditions against third instar larvae of *S. obliqua* and the LC_{50} was 4.37×10^3 OBs/ml occlusion bodies (OBs) per ml. The median survival time (ST_{50}) was 181 h at a dose of 1×10^6 OBs/ml and 167 h at a dose of 1×10^8 OBs/ml. SpobNPV merits further field evaluation as a potential biological control agent of *S. obliqua*, a serious pest of many agriculturally important crops in the Oriental region.

1. Introduction

Spilarctia obliqua (Walker) (Arctiidae: Lepidoptera), is a sporadic and polyphagous insect pest widely distributed throughout the Oriental region (CPC, 2004). It was reported to attack 126 plant species belonging to 25 families, including pulses, oilseeds, cereals, vegetables, mulberry, turmeric, medicinal and aromatic plants, causing heavy economic loss (Gupta and Bhattacharya, 2008; Senthil Kumar et al., 2011). The caterpillars feed gregariously during the early (first to third instar) larval stages and solitarily in the late (fourth to fifth instar) larval stages. Chemical control of this pest is difficult and uneconomical because the pest feeds on several weed plants (Gupta and Bhattacharya, 2008). In a survey for natural enemies of the pest, we observed a nucleopolyhedrovirus (NPV) (Family: Baculoviridae) causing epizootic of the insect in Kozhikode, Kerala, India.

Baculoviruses comprise of the most diverse family of double stranded DNA viruses and more than 90% of the 600 reported baculoviruses are from the insect Order Lepidoptera (Martignoni and Iwai, 1981). The persistence of bacuoloviruses in the environment for horizontal transmission (Miller, 1997) along with their high pathogenicity to host insects and narrow host range make them highly potential biocontrol agents against lepidoptern pests (Jakubowska et al., 2005). The NPVs that infect lepidopteran insects are further classified into taxonomic group I or group II (Herniou et al., 2001). The NPVs that infect the same host insect in different geographical regions may differ. Thus, the isolates of AgseNPV infecting *Agrotis segetum* (Denis & Schiffermüller) in France and Poland were distinct (Allaway and Payne, 1983). It has also been shown that the large collections of NPV isolates of *Spodoptera littoralis* (Boisduval), SlNPV from Israel belonged to two distinct genotypes, SlNPV-A and SlNPV-B (Cherry and Summers, 1985).

In this paper, we report a new tetrahedral shaped multicapsid NPV infecting *S. obliqua* from India. The virus was characterized based on nucleocapsid packaging, electron microscope (EM) studies, restriction enzyme analysis of the genome, and by sequence analyses of the partial *polh*, *lef-8* and *lef-9* genes. The biological activity of this NPV against *S. obliqua* and its utility in biological control is also discussed.

2. Materials and methods

2.1. Insects

Field collected *S. obliqua* first instar larvae were reared in the laboratory (at 26 °C, RH 70%) to adult moths on turmeric (*Curcuma*

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longa L.) leaves in plastic containers (4 l) secured with nylon mesh. The eggs laid by the moths were used to propagate healthy populations of the insect in the laboratory for experiments.

2.2. Virus source and propagation

The virus was isolated from dead *S. obliqua* larvae found hanging on plants with characteristic viral infection symptoms (Fig. 1) at the farm of ICAR-Indian Institute of Spices Research, Kozhikode (11°17′59.81″N, 75°50′30.76″E), Kerala during an epizootic in December 2013. The cadavers were individually transferred to sterile microfuge tubes and preserved at −20 °C until further use. A crude homogenate of the virus was prepared by grinding a single larval cadaver in distilled water. The homogenate was filtered through four layers of cheese cloth to remove larval debris. The number of occlusion bodies (OBs) in the filtrate was adjusted to 1×10^6 OBs/ml using a Neubauer haemocytometer in aqueous solution of 0.05% Tween 20 (v/v). Multiplication of the virus was done in fourth instar *S. obliqua* reared in the laboratory. For this, 1 ml of viral suspension was spread uniformly on a fresh turmeric leaf of 20 × 10 cm on both sides and air dried in a laminar flow hood. Ten fourth instar larvae starved for 2 h were allowed to feed on the contaminated leaf for 24 h in a plastic jar (4 l). The insects were transferred to a fresh diet afterwards in a clean container and maintained at 25 °C till death. The setup was replicated three times.

2.2.1. Extraction of OBs

OBs were extracted from dead larvae by homogenizing the cadavers in water and purified by filtration and differential centrifugation (O'Reilly et al., 1992). Briefly, a larval cadaver was homogenized in 3 ml of 0.1% aqueous sodium dodecyl sulfate (SDS) (w/v) and the crude suspension was filtered through four layers of cheese cloth. The filtrate was centrifuged at 29×*g* for 30 s and the supernatant was further centrifuged at 2935×*g* for 5 min at 4 °C. The pellet was serially washed at 2935×*g* at 4 °C, once with 3 ml each of SDS (0.1%), 0.5 M aqueous sodium chloride and distilled water. The semi-purified virus was further purified by sucrose gradient (40–65% w/w) centrifugation at 96,000×*g*. The pellet containing OBs was finally suspended in double distilled water. The OB concentration was determined under phase contrast microscopy at 400× and stored at −20 °C for further use. The isolate was deposited in the Entomopathogenic virus repository of Indian Institute of Spices Research (IISR) with accession number IISR-NPV-02.

Fig. 1. Dead *S. obliqua* larva showing characteristic signs of NPV infection.

2.2.2. Nucleocapsid packaging

Nucleocapsid packaging of the isolate was studied following Bernal et al. (2013). Occlusion derived virions (ODVs) were released from purified OBs of 1×10^{10} OBs/ml by exposure to alkaline buffer (0.1 M Na_2CO_3; pH: 10.5) for 30 min at 28 °C. Polyhedrin and other debris were removed by low-speed centrifugation (2500×*g*, 2 min). The ODV containing supernatant was banded by density equilibrium centrifugation (30,000×*g*, 1 h at 4 °C) on 30–60% (w/w) continuous sucrose gradient. The banding pattern was visually inspected and photographed.

2.3. Extraction of viral DNA

Viral DNA was extracted following Bernal et al. (2013) with slight modifications. Briefly, virions were released from OBs by incubating a mixture of 100 µl of OB extract containing 10^9 OBs/ml, 100 µl 0.5 M Na_2CO_3, 50 µl 10% (w/v) SDS and 250 µl sterile distilled water for 10 min at 60 °C. Undissolved OBs and other debris were removed by low speed centrifugation (3800×*g*, 5 min). The supernatant containing the virions was treated with 25 µl proteinase K (20 mg/ml) for 1 h at 50 °C. Viral DNA was extracted by adding an equal volume of a mixture of phenol/chloroform/isoamyl alcohol (25:24:1) followed by high speed centrifugation at 16,627×*g* for 10 min at 4 °C. The DNA was precipitated from the supernatant by adding 0.1× of 3 M sodium acetate, pH 5.2 and 2.5 × of absolute ethanol and the contents were kept on ice for 15 min followed by centrifugation at 14,167×*g* for 15 min at 4 °C. The pellet containing the DNA was washed with 70% cold ethanol, suspended in Tris-EDTA (TE) buffer and stored at 4 °C.

2.3.1. REN analysis

Two µg of viral DNA was digested with *Pst* I, *Xho* I and *Hin*dIII (New England BioLabs) for 5–7 h at 37 °C under the conditions recommended by the supplier. Digested fragments were loaded together with λ DNA digested with *Hin*dIII and 1 kb ladder and electrophoresis was performed on 0.7% gel, stained with ethidium bromide and visualized on a UV trans-illuminator (BioRad) and photographed. The molecular size of the fragments was determined by the graphical method (Southern, 1979).

2.3.2. PCR amplification, sequencing of partial polh, lef-8 and lef-9 genes and phylogenetic analysis

PCRs were performed to amplify the partial *polh, lef-8* and *lef-9* genes with degenerate primer pairs (prPH-1, prPH-2, prL8-1, prL8-2 and prL9-1, prL9-2) as described by Lange et al. (2004) with slight modifications. Amplification reactions were performed in a 25 µl reaction under the following PCR conditions: for *polh* gene an initial denaturation at 95 °C for 3 min, followed by 36 cycles of 95 °C for 30 s, 53 °C for 1 min, 72 °C for 1 min, and a final extension step of 72 °C for 10 min; for partial *lef-8* gene, an initial denaturation step of 94 °C for 5 min and 35 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min, and a final extension step of 72 °C for 10 min; for partial *lef-9* gene, an initial denaturation step of 94 °C for 5 min and 35 cycles of 94 °C for 1 min, 65 °C for 1 min, 72 °C for 30 s, and a final extension step of 72 °C for 10 min.

The amplified bands were excised, purified using Sigma GelElute™ Gel Extraction Kit (Sigma, India) as per manufacturer's instructions and outsourced to SciGenom Labs Private Ltd., India for sequencing. Sequencing was carried out with the same set of primers used for PCR amplification and the gene sequences were deposited in GenBank. DNA sequences were subjected to BLAST (Basic Local Alignment Search Tool) search to identify sequences deposited in GenBank that had significant homology. A phylogenetic tree was constructed by maximum parsimony (MP) and neighbor joining (NJ) methods inferred from the concatenated amino acid sequences of *polh, lef-8* and *lef-9* using MEGA 5.0 (Tamura et al., 2011). The gaps in alignment were treated as missing data and the phylogeny was tested by 1000 bootstrap replicates. The MP tree was searched by a max-mini branch-and-bound method and Dayhoff model (gamma shape parameter $\alpha = 2.25$) was used for the neighbor joining tree analysis. Group II NPVs (SeMNPV and SpltNPV) were used as outgroups. Distance matrices from separate and concatenated nucleotides of *polh, lef-8* and *lef-9* nucleotides were determined by using the Pairwise Distance calculation of MEGA version 5.0 applying the Kimura 2-parameter model (Tamura et al., 2011).

2.4. Bioassays

Median lethal concentration response (LC_{50}) and survival time analysis (ST_{50}) of SpobNPV to third instar larvae were carried out by leaf disc bioassay method. In LC_{50} studies, viral suspensions of 10^2, 10^4, 10^6 and 10^8 OBs/ml were prepared in aqueous 0.05% Tween 20 (v/v). Ten µl of viral suspension was spread on turmeric leaf discs (1.5 cm diameter); air dried and individually placed inside 12-well tissue culture plates (2.3 cm dia). Each plate constituted an independent replication and six plates were used per viral suspension constituting 72 insects per treatment. Third instar *S. obliqua* larvae starved for about 6 h was released individually into the wells and secured with a lid. Larvae which consumed the entire diet within 12 h were transferred to plastic containers (4 l) replication-wise and maintained on fresh turmeric leaves at 26 ± 2 °C, 60–70% relative humidity and a 12:12 h day:night photoperiod. For ST_{50} studies, virus suspensions of 10^6 and 10^8 OBs/ml were used. The other experimental details were as mentioned earlier. In control, larvae fed on leaves treated with aqueous 0.05% Tween 20. Observations on mortality were recorded at daily intervals for concentration-response studies and mortality was recorded at every 12 h interval for survival time assays. Mortality due to infection was recorded up to 10 days post inoculation, after which no mortality was observed. No mortality was observed in control. The bioassays were repeated two times to confirm the results.

2.5. Electron microscopy

For scanning electron microscopy (SEM) studies, the purified virus was dehydrated through a graded ethanol series and dried with hexamethyldisilane (Martins et al., 2005). The pellet was spread on carbon adhesive stubs, gold sputtered for 20 s and directly viewed under a Scanning Electron Microscope (Hitachi SU6600).

For transmission electron microscopy (TEM) studies, purified polyhedra were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 h at 4 °C, post-fixed in 1% osmium tetroxide in the same buffer for 1 h and dehydrated in acetone, and embedded in epoxy resin. Blocs were sectioned in a Leica Ultracut UC7 ultramicrotome, double stained with uranyl acetate and lead citrate, and viewed under TECNAI 200 Kv HR-TEM (Fei Electron Optics).

2.6. Statistical analyses

Median lethal concentration was calculated using probit analysis as described by Finney (1962) using SAS® 9.3 software for statistical analysis (SAS, 2011). Median survival time (ST_{50}) and 95% confidence intervals for insects receiving each treatment were calculated based on Kaplan-Meier survival distribution functions using the web-based program OASIS (Yang et al., 2011).

3. Results

3.1. Nucleocapsid packaging studies

The OBs formed a single band in the sucrose gradient centrifugation. When ODVs were subjected to sucrose gradient centrifugation, multiple bands were observed. At least, six clearly distinguishable, high intensity bands were observed, indicating that the isolated NPV belongs to multiple nucleopolyhedrovirus (Fig. 2). The banding pattern was stable when confirmed from three samples.

3.2. Restriction endonuclease analysis

REN profiles of the genomic DNA of the SpobNPV with restriction enzymes, *Pst* I, *Xho* I and *Hin*dIII are shown in Fig. 3. There were 20, 12 and 16 recognition sites for *Pst* I, *Xho* I and *Hin*dIII respectively in the genome of SpobNPV (Fig. 3). The estimated genome size was between 92.6 and 105.9 kbp with an average size of about 99.1 ± 3.9 kbp.

3.3. Sequencing of partial polh, lef-8 and lef-9 genes and phylogenetic analysis

The sequence data generated for the three conserved genes (*polh, lef-8* and *lef-9*) of the present NPV was deposited in GenBank

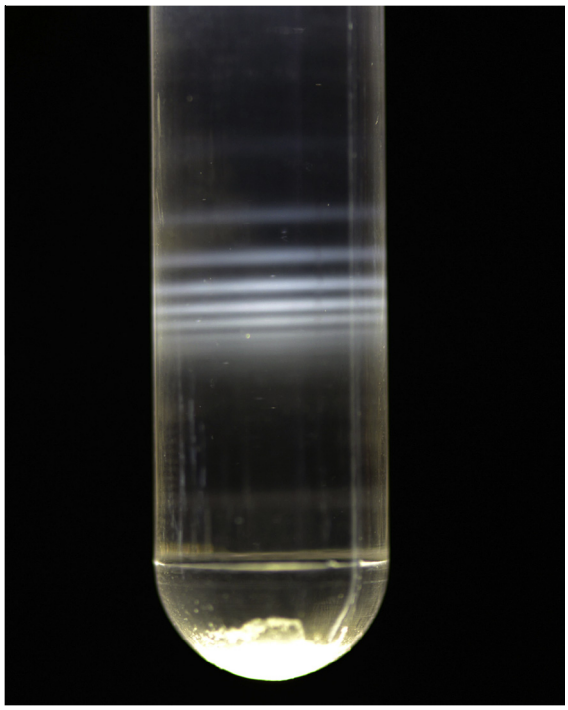

Fig. 2. ODV banding pattern of SpobNPV showing multiple bands after continuous sucrose gradient (30–60% w/w) separation.

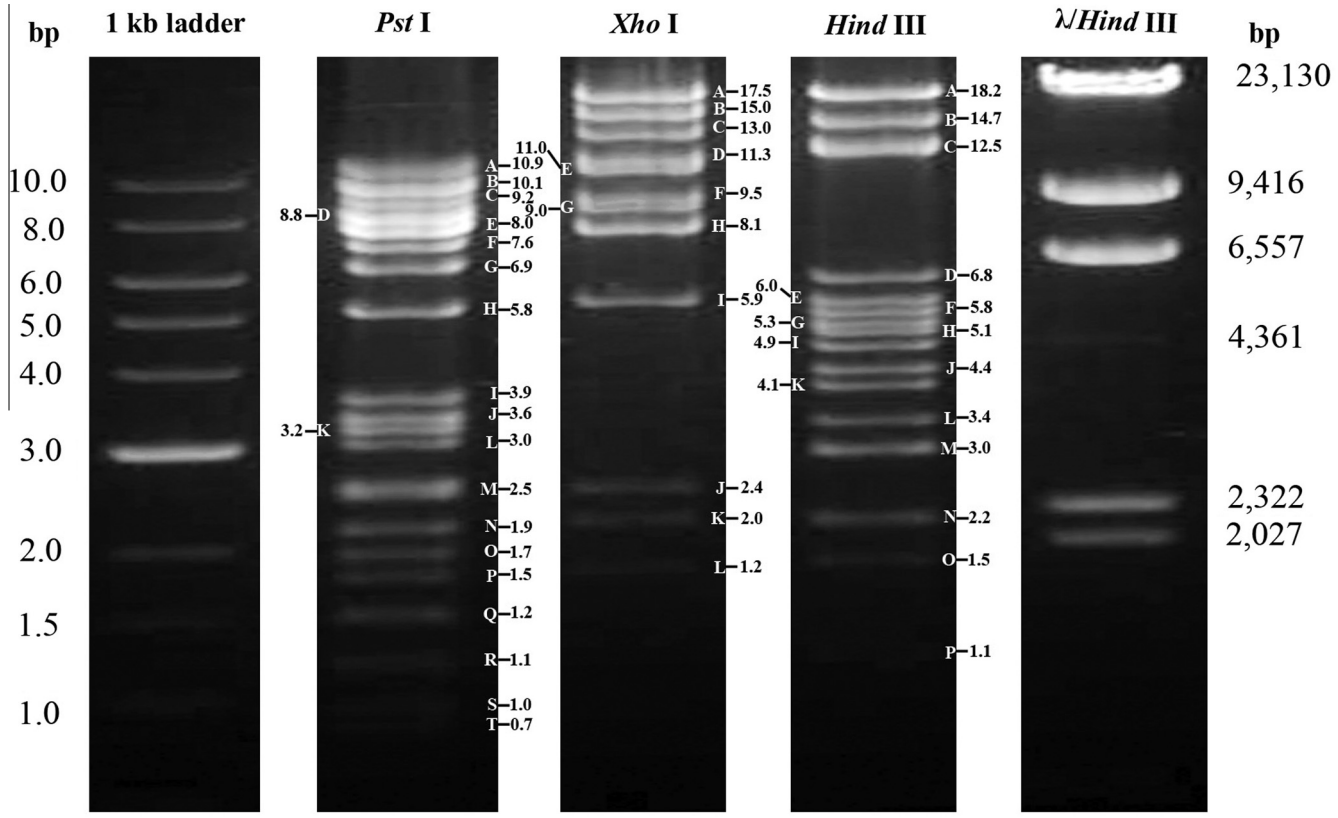

Fig. 3. Restriction enzyme digestion profile of *S. obliqua* NPV DNA using the restriction endonucleases *Pst* I, *Xho* I and *Hin*dIII in 0.7% agarose gel. 1 kb DNA ladder and λ-*Hin*dIII marker were used as molecular size markers. Fragment size is in kbp.

with accession numbers, KP172301, KT149869 and KP172302. BLAST similarity search of polyhedrin gene sequence of the isolated SpobNPV showed 97% and 93% similarity with HycuNPV (AY706700) and SpphNPV (AY706684) respectively, both infecting members of Arctiidae family. *Lef-8* and *lef-9* homology search also showed a similar relationship indicating the closeness of this NPV with HycuNPV (AY706560, AY706625) (95, 97%) and SpphNPV (AY706536, AY706601) (89, 96%). Phylogenetic analysis with concatenated amino acid sequences of other NPVs retrieved from GenBank indicated that SpobNPV is closely related to HycuNPV and SpphNPV (Fig. 4). The K-2-P distances between SpobNPV and that of HycuNPV and SpphNPV were 0.030, 0.076; 0.047, 0.121 and 0.029, 0.038 for *polh, lef-8* and *lef-9* sequences and 0.037 and 0.091 for concatenated *polh, lef-8* and *lef-9* nucleotide sequences, respectively. Based on the similarities in phylogram and K-2-P distances between the aligned single and concatenated gene sequences, it was clearly evident that SpobNPV was a new addition to group I NPV.

3.4. Bioassays

Bioassay studies showed larval mortality at low doses of SpobNPV. The LC_{50} value against third instar *S. obliqua* was 4.37×10^3 OBs/ml by leaf disc bioassay. The 95% fiducial limits ranged from 1.93×10^3 to 9.41×10^3 OBs/ml. Survival time analysis at two doses indicated that the median lethal time for third instar larvae were 181.01 h (±2.05) (95% fiducial limits: 176.99–185.04 h) at a dose of 1×10^6 OBs/ml and 166.99 h (±2.40) (95% fiducial limits: 162.28–171.69 h) at a dose of 1×10^8 OBs/ml, respectively (Table 1).

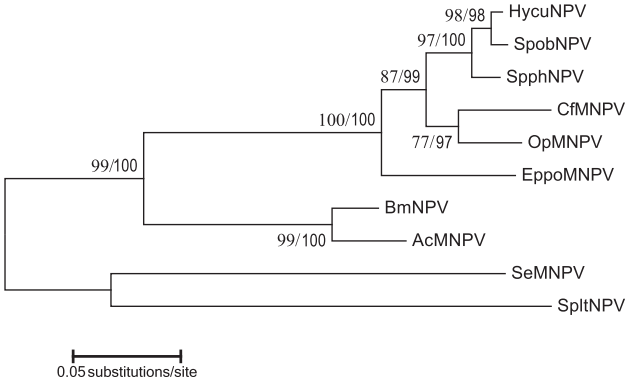

Fig. 4. Neighbor-joining tree based on concatenated amino acid sequences of partial *polh, lef-8* and *lef-9* analysis of different group I NPVs, including *Hyphantria cunea* NPV, HycuNPV (AY706700, AY706560 and AY706625); *Choristoneura fumiferana* MNPV, CfMNPV (U40833 and AF512031); *Orgyia pseudotsugata* MNPV, OpMNPV (M14885 and U75930); *Epiphyas postvittana* NPV, EppoNPV (AY043265); *Bombyx mori* NPV, BmNPV (DQ231336-38); *Autographa californica* MNPV, AcMNPV (AY706681, AY706528 and AY706593); *Spilosoma phasma* NPV, SpphNPV (AY706684, AY706536 and AY706601); *Spilarctia obliqua* NPV, SpobNPV (KP172301, KT149869 and KP172302). Numbers above or below the nodes indicate bootstrap values generated after 1000 replications of maximum parsimony (MP) and neighbor joining (NJ) analyses, respectively (=MP/NJ). *Spodoptera exigua* MNPV, SeMNPV (GQ392064 and AF169823) and *Spodoptera litura* NPV, SpltNPV (AY706714, AY706580 and AY706645) belonging to group II NPVs were used as outgroups.

3.5. Electron microscopy

Under SEM, the OBs appeared roughly tetrahedral in shape and the size of the polyhedra ranged from 1.1 to 2.0 μm with an

Table 1
Survival time analysis of third instar *S. obliqua* treated at different doses of SpobNPV.

Dose	N	ST_{50} (h)[a]	SE	95% C.I.
1×10^8	71	166.99a	2.40	162.28–171.69
1×10^6	71	181.01b	2.05	176.99–185.04

[a] Survival time values with different letters are significantly different at $p < 0.05$ by log-rank test.

average size of about 1.5 ± 0.1 μm (n = 20) (Fig. 5a). Some OBs were irregular in shape with bulbous protrusion. Abnormal OBs with pits were also observed (Fig 5b), which indicates incomplete occlusion of virions inside the polyhedra.

TEM studies confirmed the present SpobNPV isolate as multiple nucleocapsid NPV. OBs were found to contain many virions and several multiple rod shaped nucleocapsids were found packaged within a viral envelope (Fig. 6). The number of nucleocapsids per virion ranged from 1 to 11 and more commonly, 2–8 nucleocapsids per virion were observed inside the polyhedra. The size of the virion with envelope was approximately 0.27 ± 0.01 × 0.11 ± 0.004 μm (n = 15).

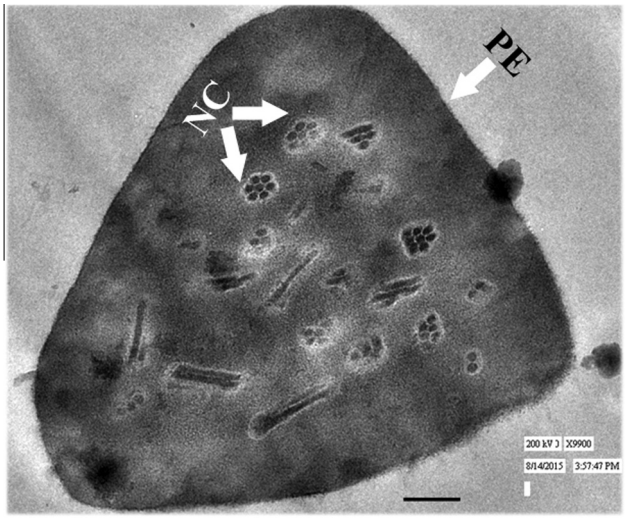

Fig. 6. Transmission electron micrograph of purified tetrahedral shaped SpobNPV polyhedra. Solid arrows indicate multiple nucleocapsids (NC) in a virion surrounded by a single membrane and the polyhedral envelope (PE). Bar = 0.2 μm.

4. Discussion

The development of baculoviruses as effective bio-control agents against insect pests depends on the identification of a virulent isolate. The geographical influence on the development of isolates has been demonstrated for several NPVs and the isolates vary in their biological activities (Takatsuka et al., 2003; Rowley et al., 2011). Isolates of NPV collected from the same geographical regions (Barrera et al., 2011) and variants with minor genetic mutations (Simón et al., 2012) can also differ in their pathogenicity. Phenotypic and molecular characterization of an isolate is therefore important in the development of NPV based biological control programmes.

An epizootic in *S. obliqua* caused by NPV was observed in the field during December, 2013. The diseased caterpillars were restless, climbed to elevated positions and found hanging upside down with their abdominal prolegs showing typical symptoms of baculovirus infection. Nucleocapsid packaging studies revealed multiple bands (6) in sucrose gradient indicating that the isolate belongs to multiple nucleocapsid NPV, which was confirmed by TEM. The number of bands formed by an NPV depends on the maximum number of nucleocapsids per envelope (Kawanishi and Paschke, 1970) and the number of nucleocapsids per virion may range from 1 to 29 (Tanada and Kaya, 1993). REN analysis of the SpobNPV genome with *Pst* I, *Xho* I and *Hin*dIII enzymes showed that the genome size varied between 92.6 and 105.9 kbp. The average genome size was approximately 99 kbp, which is very well within the range of the genome size of baculoviruses that varies from 81 to 160 kbp (Blissard and Rohrmann, 1990).

PCR amplification of the highly conserved baculovirus genes *polh, lef-8* and *lef-9* combined with molecular phylogenetic analysis provide a powerful tool to identify lepidopteran-specific baculoviruses (Jehle et al., 2006). The *polh, lef-8* and *lef-9* of SpobNPV were sequenced and BLAST searches of the sequences showed >90% similarity with HycuNPV and SpphNPV, all infecting members of the family Arctiidae. In phylogenetic analysis, the gene sequences of HycuNPV and SpphNPV retrieved from the GenBank clustered together with the SpobNPV gene sequences. The

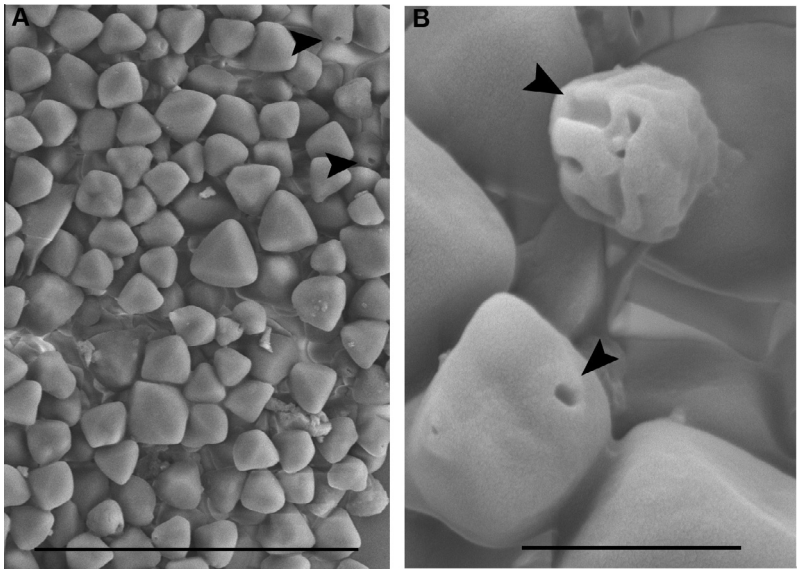

Fig. 5. Scanning electron micrograph showing, (A) occlusion bodies of SpobNPV. (B) Close-up of abnormal polyhedra with pits. Arrows indicate "pits" in the OBs which lost virions during preparation. Bar = 10 μm in (A) and 1 μm in (B).

relatedness of two NPVs can be measured based on their K-2-P values (Jehle et al., 2006). In the present study, the values between these NPVs were more than 0.015, indicating SpobNPV as a new addition to group I NPVs.

The OBs were tetrahedral in shape and the and the size of the polyhedra ranged from 1.1 to 2.0 μm with an average size of about 1.5 μm. Earlier reported NPV of *S. obliqua* from India was hexahedral in shape and the size ranged from 0.42 to 0.67 μm (Kumar et al., 2000) which is less than half the size of the present isolate. The prominent differences in size and shape of the OBs clearly indicated that this isolate of SpobNPV was not reported earlier. Tetrahedral shaped NPVs are very rare in occurrence (Cheng and Carner, 2000) and have been isolated only from a limited number of host insects such as *Hyphantria cunea* (Drury) (Smith, 1976), *Rachiplusia nu* (Guenée), *Lymantria monacha* (L.) (Young and Yearian, 1983), and *Thysanoplusia orichalcea* (L.) (Cheng and Carner, 2000). Earlier studies demonstrated the role of polyhedrin protein sequence in determining the shape of the polyhedra (Carstens et al., 1986; Cheng et al., 1998). We also observed some abnormal OBs with bulbous protrusion and pits on the surface. Similar abnormality was also observed by Cheng and Carner (2000) on NPV of *T. orichalcea*, which indicated incomplete occlusion of virions inside the polyhedra. Mutation in genes, in addition to *polyhedrin* and *fp25k* (Slavicek et al., 1998) and including *p26*, *p10* and *p74* genes (Wang et al., 2009) might lead to such abnormalities in occlusion.

The present SpobNPV isolate was highly virulent against third instar *S. obliqua* as evidenced by a lower LC_{50} value of 4.37×10^3 OBs/ml in leaf disc bioassays, which is less than the earlier reported LC_{50} values of 2.5×10^4 and 3.7×10^4 OBs/ml against *S. obliqua* and *Porthesia xanthorrhoea* Kollar respectively by a SpobNPV isolate (Varatharajan et al., 2006). However, Kumar et al. (2000) had reported a hexahedron shaped isolate of SpobNPV with LC_{50} value of 0.1485×10^4 OBs/ml. The survival times were found to decrease with increasing viral dose and this was reported earlier in other pests (Varatharajan et al., 2006; Kouassi et al., 2009). As the current trends in the use of insect pathogenic viruses for crop pest management emphasize the selection of isolates with higher virulence (Senthil Kumar et al., 2006), isolation of a SpobNPV isolate with high virulence holds promise as a microbial insecticide against this notorious pest which causes severe damage to many economically important crops in the Oriental region.

In conclusion, we report a tetrahedral shaped SpobNPV isolate. Based on the polyhedral morphology and gene sequence data comparisons of *polh*, *lef-8* and *lef-9*, we conclude that this isolate belongs to the group I NPVs (de Zanotto et al., 1993). The differences in host range, size of the genome based on REN analysis, and the K-2-P distances between single and concatenated nucleotides indicates that this isolate is distinct from its close relatives. Thus, the present isolate of *Spilarctia obliqua nucleopolyhedrovirus* belong to a new species in the genus *Alphabaculovirus* of family *Baculoviridae*. Further studies should aim at field testing of this pathogen for inclusion in IPM schedules and for studying its cross infectivity against other arctiid pests.

Acknowledgments

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