Molecular Characterization of a Nucleopolyhedrovirus Newly Isolated from *Ophiusa disjungens* in China

Tong Lin · Li Liu · Runlei Chang ·
Guojun Lang · Wen Xu

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Abstract *Ophiusa disjungens* nucleopolyhedrovirus (OpdiNPV) was newly found in Guangdong Province, China. Using *Bam*HI, *Eco*RI, *Hind*III, *Pst*I, *Xho*I, *Xba*I digestion, the size of the OpdiNPV genome was estimated to be 92,000 base pair. The *pst*I-G genomic fragment of OpdiNPV was cloned and sequenced. Searches of databases identified at least four open reading frames (ORFs) within this fragment. These ORFs encode *odv-e66* (EU 623602), *p87/vp80* (EU 732665), *odv-ec43* (EU617337) and *ac108* gene (EU 732666) respectively. The phylogenetic tree of NPVs based on the combined sequences of *odv-ec43* and *ac108* indicated that OpdiNPV was most closely related to *Mamestra configurata* NPV-A and *Mamestra configurata* NPV-B. The characterization of OpdiNPV in this paper would provide better understanding molecular properties of this virus and be helpful in the development of the newly isolated virus as a biopesticide or an engineered pesticide to control more species of insect pests.

Keywords *Ophiusa disjungens* · Nucleopolyhedrovirus · Physical map · *odc-ec43* · *odv-e66* · *p87/vp80* · *ac108*

Anua indiscriminata is an important pest of forests and fruit trees in South China. Serious damage to the eucalyptus caused by the moth infestation has occurred these years in Guangdong and Guangxi Province of China. The nucleopolyhedrosis virus of *A. indiscriminata*, which was named as AiNPV by Li [1], was found when the larvae were bred in laboratory. A transmission electron micrograph revealed that the occlusion bodies (OB) contained several virions with multiple envelope packaged within a single viral envelope. We found that *A. indiscriminata* is a junior synonym for *Ophiusa disjungens*, so AiNPV is renamed as OpdiNPV by us. Bioassays on lethal concentration and lethal time were conducted by Li [1]. To achieve a further understanding of this virus, the molecular properties of the virus were observed and analyzed in this study.

An OpdiNPV isolate (Guangdong strain, from South China Agricultural University) was obtained from the diseased larvae, *O. disjungens*, with nucleopolyhedrosis symptoms. The OBs were purified according to the procedure described by Peter [4] and Lin [2]. The cadavers were homogenized in approximately 10 times their own volume of 1 % SDS and left overnight at room temperature, then homogenate was filtered through four layers of muslin. The filtrate was centrifuged at 3,000×g for 15 min at 4 °C for three times and at 3,000×g for 30 min at last. In the end, pellet was suspended with ddH₂O and stored at −70 °C for future use.

The OBs were dealt with dissolution buffer (0.3 M Na₂CO₃, 0.03 M EDTA, 0.51 M NaCl) at 37 °C for 1 h, and proteinase K at 56 °C for overnight. Then DNA was extracted by phenol/chloroform treatment. The virus DNA was dissolved in 20 µl TE buffer.

T. Lin (✉) · W. Xu
College of Forestry, South China Agricultural University,
Guangzhou 510642, China
e-mail: lintong@scau.edu.cn

L. Liu
The Department of Applied Engineering, Zhejiang Economic
and Trade Polytechnic, Hangzhou 310018, China

R. Chang
China Eucalypt Research Centre, Zhanjiang 524022, China

G. Lang
Institute of Insect Sciences, Zhejiang University,
Hangzhou 310029, China

Fig. 1 Restriction endonuclease fragment profiles of *Ophiusa disjunctus* NPV digested with: *Bam*HI (lane2), *Eco*RI (lane3), *Hind*III (lane4), *Pst*I (lane5), *Xho*I (lane6), *Xba*I (lane7), *Bam*HI (lane9), *Eco*RI (lane10), *Hind*III (lane11), *Pst*I (lane12), *Xho*I (lane13) or *Xba*I (lane14) and electrophoresed on a 0.7 % agarose gel at 50 V to separate the fragments. λ DNA/*Hind*III (lane1), 1,000 bp Ladder (lane8) and λ -*Eco*RT14 (lane15) were used as size standards

Table 1 Size of restriction endonuclease fragments (kbp) of *Ophiusa disjunctus* NPV

Fragment	Restriction enzyme					
	<i>Bam</i> HI	<i>Eco</i> RI	<i>Hind</i> III	<i>Pst</i> I	<i>Xho</i> I	<i>Xba</i> I
A	31.5	18.2	17.8	9.8	15.8	35
B	26.2	16.0	14.8	8.1	14.2	24
C	23.2	9.1	12.0	8.0	9.5	13.8
D	5.6	8.3	10.2	7.0	7.2	11.2
E	4.6	7.2	9.5	7.0	6.8	8.1
F		6.8	7.9	6.5	5.4	
G		6.0	6.5	5.0	5.0	
H		5.1	5.9	5.0	4.0	
I		4.8	3.0	4.7	3.5	
J		4.5	2.9	4.5	3.3	
K		2.6	1.9	3.6	3.3	
L		2.2		3.5	2.5	
M		2.0		3.2	2.2	
N		1.5		3.0	1.9	
O				2.6	1.7	
P				2.5	1.4	
Q				1.9	1.4	
R				1.6	1.3	
S				1.4	0.9	
T				1.2	0.7	
U				1.0		
V				0.8		
Total	91.1	92.0	92.4	91.9	92.1	92.1

Virus DNA was digested with *Bam*HI, *Eco*RI, *Hind*III, *Pst*I, *Xho*I, *Xba*I (TaKaRa) at 37 °C for 2–3 h, then electrophoresed on 0.7 % agarose gels at 50 V for 15–22 h to separate the fragments. The sizes of viral DNA fragments were estimated using GelScan XL software (Pharmacia LKB).

The *pst* I-G fragment of OpdiNPV DNA was cloned into pUC18 (TAKARA) plasmid vector according to the methods of Sambrook [6]. The recombinant plasmids were transformed into *E. coli* DH 5 α cells (TIANGEN), screened for *lacZ* complementation, recovered by using DNA Product Purification Kit (TIANGEN) from agarose gels and purified by precipitation with glass milk. The termini of the fragment was sequenced by using the universal M13 primers at first, and further sequenced by primer walking using specific primers (Sangon).

Baculovirus gene comparison was performed with sequences from GenBank and published papers. Phylogenetic trees for *odv-ec43* (EU617337) and *ac108* genes were constructed by the N-J method (MEGA4) with the default settings for random break tie and the distance option for mean character difference. Statistical support for each node was evaluated by bootstrap analysis with 1,000 replicates [3, 8].

Electrophoresis patterns of the OpdiNPV genome digested with restriction endonuclease are shown in Fig 1. The sizes of the *Bam*HI, *Eco*RI, *Hind*III, *Pst*I, *Xho*I and *Xba*I fragments of the OpdiNPV genome are shown in Table 1. The fragments were designated alphabetically starting with A for the largest fragment for each enzyme digest as proposed by Vlak and Smith [7]. The mean size estimates for the complete OpdiNPV genome ranged from 91.1 to 92.4 kbp. These estimates were determined comparing restriction endonuclease fragment mobilities with those of λ DNA/*Hind*III, 1,000 bp Ladder and/or λ -*Eco*RT14 molecular mass DNA standards. The size of the entire genome was calculated to be 92 kbp, which is much smaller than certain of the better studied NPVs including the AcMNPV (133 kbp) and the *Mamestra brassicae* MNPV (MbMNPV) genome of 150 kbp [5].

pst I-G fragment of OpdiNPV genome is composed of 5,056 bp. This fragment contained four ORFs which were *odv-e66* (EU 623602), *p87/vp80* (EU 732665), *odv-ec43* (EU617337) and *ac108* gene (EU 732666) respectively. The ORFs encoding ODV-Ec43 and Ac108 are complete, and the ORFs encoding ODV-E66 and P87/VP80 are partial.

The amino acid sequences of two conserved baculovirus genes, including *odv-ec43* and *ac108*, were used for analysis of the phylogenetic relationship between OpdiNPV and other NPVs. The phylogenetic tree (Fig. 2) of NPVs based on the combined sequences of *odv-ec43* and *ac108* indicated that OpdiNPV was most closely related to SeMNPV, SINPV, SfMNPV, AsNPV and MacoNPV. It also appeared that the relationships between SeMNPV, SINPV, SfMNPV, AsNPV, MacoNPV, ChChNPV and

Fig. 2 A phylogenetic tree for nucleopolyhedroviruses based on the amino acid sequences of *odv-ec43* (EU617337) and *ac108* joint genes. Statistical support (%) for each node was evaluated by bootstrap analysis with 1,000 replicates. The GenBank accession numbers for *odv-43* and *Ac108* protein are as follows: AdhoNPV: *Adoxophyes honmai* NPV (NP_818729.1, NP_818730.1); AsNPV: *Agrotis segetum* NPV (YP_529736.1, YP_529735.1); ApCiNPV: *Apocheima cinerarium* NPV (ADB_84433.1, ADB_84434.1); AcNPV: *Autographa californica* NPV (NP_054139.1, NP_054138.1); BmNPV: *Bombyx mori* NPV (NP_047509.1, NP_047508.1); ChChNPV: *Chrysodeixis chalcites* NPV (YP_249703.1, YP_249704.1); EcobSNPV: *Ecotropis obliqua* NPV (YP_874278.1, YP_874279.1); EpNPV: *Euproctis pseudoconspersa* NPV (NP_203264.1, YP_002854704.1); HaNPV: *Helicoverpa armigera* NPV (NP_075163.1, YP_002332607.1); HzSNPV: *Helicoverpa zea* SNPV (AF334030_28, NP_542721.1); LdMNPV: *Lymantria dispar* MNPV (NP_047744.1, NP_047745.1); LxMNPV: *Lymantria xylinia* MNPV (YP_003517843.1, YP_003517844.1); MacoNPV-A: *Mamestra configurata* NPV-A (AAQ_11099.1, NP_613162.1); MacoNPV-B: *Mamestra configurata* NPV-B (NP_689254.1, NP_689253.1); MvMNPV: *Maruca vitrata* MNPV (YP_950815.1, YP_950814.1); OINPV: *Orgyia leucostigma* NPV (YP_001651003.1, YP_001651004.1); OpMNPV: *Orgyia pseudotsugata* MNPV (NP_046265.1, NP_046264.1); SeMNPV: *Spodoptera exigua* MNPV (NP_037819.1, NP_037818.1); SfMNPV: *Spodoptera frugiperda* MNPV (YP_001036351.1, YP_001036350.1); SINPV: *Spodoptera litura* NPV (NP_258364.1, NP_258365.1); TrmiNPV: *Trichoplusia ni* SNPV (YP_308983.1, YP_308984.1)

TrmiNPV were closer. It is more distantly related to other NPVs, such as LdMNPV, LxMNPV, LsNPV, AcMNPV, BmNPV, EpNPV, EcobSNPV and ApCiNPV.

In conclusion, OpdiNPV was newly found nucleopolyhedrovirus in China. Restriction endonuclease analysis showed that the size of the entire genome of OpdiNPV was 92 kbp, which is smaller than most baculoviruses whose genomes have been completely sequenced so far. OpdiNPV isolated from *O. disjunctus* is being developed as a biopesticide for the control of *O. disjunctus*. The aforementioned characterization of the newly isolated virus would provide better understanding molecular properties of this virus and be helpful in the development of OpdiNPV as a biopesticide or an engineered pesticide to control more species of insect pests.

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