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Phylogenetic Analysis of Baculovirus Isolates from Diseased Insects in Southern Vietnam

Nguyen Thi Phuong Thao[1], Nguyen Thi Thuy[1], Nguyen Khac Duy[1], Doan Chinh Chung[2], Do Minh Si[2], Le Thanh Long[1*]

[1]Institute of Tropical Biology, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam
[2]University of Science, Ho Chi Minh City, Vietnam
Email: *lelong2510@gmail.com

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Abstract

The aim of this study was to investigate the molecular identification and assess the genetic relationship of baculovirus isolated from Southern Vietnam. The diseased insect samples were collected from the different fields. The partial sequence of 450 base pairs of lef-8 gene was amplified and sequenced to assess the genetic variations of baculovirus isolates specific for *Spodoptera litura*, *Helicoverpa zea*, and *Helicoverpa armigera*. The sequences alignment demonstrated that *Helicoverpa zea* specific isolates exhibited six single nucleotide polymorphic sites. Whereas, twenty five single polymorphic sites were found in *Spodoptera litura* specific isolates. Thus, *Spodoptera litura* specific isolates were higher polymorphic than *Helicoverpa zea* specific isolates. The genetic distance analyses showed that the distance between Vietnamese baculovirus isolates and Group II Alphabaculovirus isolates was lower than other Baculovirus groups. The phylogeny of Vietnamese isolates in relation to other baculovirus isolates was also determined using partial sequences of lef-8 gene. The phylogenetic tree placed all Vietnamese isolates in Group II Alphabaculovirus, where seven Vietnamese *Helicoverpa zea* specific isolates were most closely related to *Helicoverpa zea* SNPV, fourteen Vietnamese *Spodoptera litura* specific isolates were located with *Spodoptera litura* NPV-G2 in one clade and a Vietnamese *Helicoverpa armigera* isolate was appeared to be closely related to *Helicoverpa armigera* SNPV-NNg1, *Helicoverpa armigera* NPV-C1, *Helicoverpa armigera* NPV-G4.

Keywords

Baculovirus, *Helicoverpa armigera*, *Helicoverpa zea*, Lef-8 Gene, Phylogeny, *Spodoptera litura*

*Corresponding author.

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1. Introduction

Baculoviruses are a very diverse group of viruses with double-stranded, circular, supercoiled genomes, with sizes varying from about 80 to over 180 kb and that encode between 90 and 180 genes [1]. The genome is packaged in rod-shaped nucleocapsids that are 230 - 385 nm in length and 40 - 60 nm in diameter [2] [3]. In the most well characterized baculoviruses, the virions are present as two types, occluded virions and budded virions. Baculoviruses have been reported from a variety of different species of invertebrates. However, the only well documented hosts are Diptera, Hymenoptera, and Lepidoptera. The Baculoviridae family is divided into four genera: Alphabaculovirus (lepidopteran-specific nucleopolyhedroviruses), Betabaculovirus (lepidopteran-specific Granuloviruses), Gammabaculovirus (hymenopteran-specific nucleopolyhedroviruses) and Deltabaculovirus (dipteran-specific nucleopolyhedroviruses) [4]. PCR-base method has been useful tools to identify baculovirus isolates. The late expression factor 8 (lef-8) was a highly conserved DNA region which was used as targets for degenerate PCR primers [5]. The lef-8 gene encodes for a subunit of the baculovirus RNA polymerase, which initiates transcription from late and very late promoters [6]. Lef-8 was previously suitable for studying baculovirus phylogeny [7].

Vietnam is a tropical country with 1,687,000 ha for fruit, treenut and vegetable crops [8]. These are favorable natural conditions for insect specific baculoviruses. However, no study of distribution and genetic variation of baculovirus in Vietnam have been conducted. In present study, partial lef-8 gene amplification and sequencing was applied to identify the distribution and genetic relationship of baculovirus in Southern Vietnam and be compared to the other baculovirus isolates.

2. Materials and Methods

2.1. Insects and Virus Collection

The diseased insects were collected from Ho Chi Minh City, Binh Duong Province, Ninh Thuan Province (**Figure 1**). Fourteen Spodoptera specific isolates were obtained from cabbage. Seven *Helicoverpa zea* specific baculovirus isolates were obtained from tomato and corn. One *Helicoverpa armigera* isolate was obtained from tomato (**Table 1**).

2.2. DNA Extraction from Diseased Insects

DNA extractions of samples of baculovirus infected insects were performed using DNeasy Tissue Kit (69504, Qiagen) according to manuals of the manufacturer. These samples were kept frozen at −20˚C.

2.3. Viral DNA Amplification by PCR

The amplification of partial lef-8 gene was performed using degenerate primers [9] (**Table 2**). PCR was performed in a final volume of 50 µl containing 5 µl 10× PCR Buffer (P2192, Sigma), 1.5 mM $MgCl_2$, 200 µM dNTP (D7295, Sigma), 0.05 units/µl Taq DNA Polymerase (D6677, Sigma), 0.5 µM Forward and Reverse Primer. PCR cycle was performed under the following conditions: one cycle of DNA denaturation at 94˚C in 5 min; 40 cycles at 94˚C in 30 s; annealing at 48˚C in 45 s; extension at 72˚C in 45 s; final extension at 72˚C in 10 min. A single band was visualized following electrophoresis of the reaction product in a 1% agarose gel.

2.4. Phylogenetic Analyses

PCR products were purified using ExoSAP-IT PCR Clean up kit and used as sequencing templates. The nucleotide sequences were determined using the 3730XL DNA Analyzer. The comparison of lef-8 sequences was performed for 22 Vietnamese baculovirus isolates and other isolates from Genbank in **Table 3** [10]. The lef-8 sequences were aligned using CLUSTAL W [11]. Tamura & Nei model was used as a genetic distance model. Neighbor-joining method was applied for phylogenetic construction [12]. Bootstrap analyses (using 1000 replications) were used to assess the confidence in branching order.

3. Results and Discussion

In this study, the amplification of lef-8 gene was successful for 22 isolates which were collected from various

Figure 1. Geographical locations of collecting diseased insects. 1: Ninh Thuan Province, 2: Binh Duong Province, 3: Ho Chi Minh City.

Table 1. Baculovirus isolates with their host insect, host plant and sources.

No.	Isolate	Host insect	Host plant	Collection area
1	Spli-HCM1	*Spodoptera litura*	Cabbage	Ho Chi Minh City
2	Spli-HCM2	*Spodoptera litura*	Cabbage	Ho Chi Minh City
3	Spli-HCM3	*Spodoptera litura*	Cabbage	Ho Chi Minh City
4	Spli-HCM4	*Spodoptera litura*	Cabbage	Ho Chi Minh City
5	Spli-NT1	*Spodoptera litura*	Cabbage	Ninh Thuan Province
6	Spli-NT2	*Spodoptera litura*	Cabbage	Ninh Thuan Province
7	Spli-NT3	*Spodoptera litura*	Cabbage	Ninh Thuan Province
8	Spli-NT4	*Spodoptera litura*	Cabbage	Ninh Thuan Province
9	Spli-NT5	*Spodoptera litura*	Cabbage	Ninh Thuan Province
10	Spli-NT6	*Spodoptera litura*	Cabbage	Ninh Thuan Province
11	Spli-NT7	*Spodoptera litura*	Cabbage	Ninh Thuan Province
12	Spli-BD1	*Spodoptera litura*	Cabbage	Binh Duong Province
13	Spli-BD2	*Spodoptera litura*	Cabbage	Binh Duong Province
14	Spli-BD3	*Spodoptera litura*	Cabbage	Binh Duong Province
15	Hear-BD	*Helicoverpa armigera*	Tomato	Binh Duong Province
16	Hz-HCM1	*Helicoverpa zea*	Tomato	Ho Chi Minh City
17	Hz-HCM2	*Helicoverpa zea*	Tomato	Ho Chi Minh City
18	Hz-HCM3	*Helicoverpa zea*	Tomato	Ho Chi Minh City
19	Hz-BD1	*Helicoverpa zea*	Corn	Binh Duong Province
20	Hz-BD2	*Helicoverpa zea*	Corn	Binh Duong Province
21	Hz-BD3	*Helicoverpa zea*	Corn	Binh Duong Province
22	Hz-BD4	*Helicoverpa zea*	Corn	Binh Duong Province

Table 2. Primer pairs used for amplification.

Primers	Sequence[a]	Size (base pairs)[b]
L8F2	gtaaaacgacggccagtNNNACNRCNGARGAYCC	450
L8R2	aacagctatgaccatgMMNCCYTTYTGNCCRTG	

[a]Degenerate baculovirus primers are in uppercase type. R, A or G; Y, T or C; M, A or C; W, A or T; N, A, C, G, or T; [b]Expected size of the amplification product.

Table 3. Accession numbers of baculovirus isolates.

Genus	Name	Abbreviation	Accession number
Alphabaculovirus Group I	*Antheraea pernyi* NPV-Z	AnpeNPV-Z	NC_008035
	Anticarsia gemmatalis MNPV-2D	AgMNPV-2D	NC_008520
	Choristoneura fumiferana DEF MNPV	CfDEFMNPV	NC_005137
	Choristoneura fumiferana MNPV	CfMNPV	NC_004778
	Epiphyas postvittana NPV	EppoNPV	NC_003083
	Hyphantria cunea NPV	HycuNPV	NC_007767
	Orgyia pseudotsugata MNPV	OpMNPV	NC_001875
	Agrotis ipsilon NPV	AgipNPV	NC_011345
	Agrotis segetum NPV	AgseNPV	NC_007921
	Euproctis pseudoconspersa NPV	EupsNPV	NC_012639
	Helicoverpa armigera NPV-C1	HearNPV-C1	NC_003094
	Helicoverpa armigera NPV-G4	HearNPV-G4	NC_002654
	Helicoverpa armigera MNPV	HearMNPV	NC_011615
	Helicoverpa armigera SNPV-NNg1	HearSNPV-NNg1	NC_011354
	Helicoverpa zea SNPV	HzSNPV	NC_003349
Alphabaculovirus Group II	*Leucania separate* NPV-AH1	LeseNPV-AH1	NC_008348
	Lymantria dispar MNPV	LdMNPV	NC_001973
	Lymantria xylina MNPV	LyxyMNPV	NC_013953
	Mamestra configurata NPV-90-2	MacoNPV-90-2	NC_003529
	Mamestra configurata NPV-B	MacoNPV-B	NC_004117
	Spodoptera exigua MNPV	SeMNPV	NC_002169
	Spodoptera frugiperda MNPV-3AP2	SfMNPV-3AP2	NC_009011
	Spodoptera litura NPV-II	SpliNPV-II	NC_011616
	Spodoptera litura NPV-G2	SpliNPV-G2	NC_003102
Betabaculovirus	*Adoxophyes orana* GV	AdorGV	NC_005038
	Agrotis segetum GV	AgseGV	NC_005839
	Choristoneura occidentalis GV	ChocGV	NC_008168
	Cryptophlebia leucotreta GV	CrleGV	NC_005068
	Cydia pomonella GV	CpGV	NC_002816
	Helicoverpa armigera GV	HearGV	NC_010240
	Phthorimea operculella GV	PhopGV	NC_004062
	Plutella xylostella GV	PlxyGV	NC_002593
	Spodoptera litura GV-K1	SpliGV	NC_009503
	Xestia c-nigrum GV	XnGV	NC_002331
Deltabaculovirus	*Culex nigripalpus* NPV	CuniNPV	NC_003084
Gammabaculovirus	*Neodiprion abietis* NPV	NeabNPV	NC_008252
	Neodiprion lecontei NPV	NeleNPV	NC_005906
	Neodiprion sertifer NPV	NeseNPV	NC_005905

areas of Southern Vietnam. The genetic polymorphisms among 22 baculoviruse isolates were indentified and analyzed using lef-8 gene obtained from degenerated primers. A fragment of 450 base pairs was amplified. However, a nucleotide sequence of 335 base pairs was used for the analysis due to the higher quality and reliability of sequencing after manual edition. These baculoviruse isolates were distributed in 2 main groups including 14 *Spodoptera litura* specific isolates and 7 *Helicoverpa zea* specific isolates. The bases proportion was different between two types of isolates. The base proportion for *Spodoptera litura* specific isolates from Vietnam was 19.2% of thymine, 20.9% of cytosine, 34.6% of adenine and 25.3% of guanine. The base proportion for *Helicoverpa zea* specific isolates from Vietnam was 18.8% of thymine, 23.8% of cytosine, 36.4% of adenine and 21% of guanine.

The lef-8 alignment exhibited six single polymorphic sites among *Helicoverpa zea* specific isolates where the Hz-BD1, Hz-BD2, Hz-BD3, Hz-BD4 isolates differed from the remaining isolates by a G to A transition at position 1993 of lef-8 gene. The Hz-BD1 and Hz-BD3 isolates also exhibited a G to A transition at position 1972 of lef-8 gene. Whereas, the Hz-HCM2 and Hz-BD2 isolates exhibited an A to G transition at position 1908 of lef-8 gene.

The field baculovirus isolates which were collected from various crops and geographic locations commonly expressed genetic variations [13] [14]. In this study, the Vietnamese *Helicoverpa zea* specific isolates from Ho Chi Minh City were genetically different from Binh Duong Province in the number of variable positions of lef-8 gene. The alignment revealed 3 variable positions of Hz-HCM1, Hz-HCM2 and Hz-HCM3 isolates at position 1895, 1896 and 1908 in lef-8 gene. Conversely, the Hz-BD1, Hz-BD2, Hz-BD3 and HzBD4 isolates exhibited 6 variations at position 1989, 1896, 1908, 1972, 1993 and 2033 in lef-8 gene.

In contrast to *Helicoverpa zea* specific isolates, the *Spodoptera litura* specific isolates were highly variable, displaying a total of twenty five single polymorphic sites, suggesting that *Spodoptera litura* specific isolates were high polymorphic than *Helicoverpa zea* specific isolates.

The sequences of *Spodoptera litura* specific isolates and *Helicoverpa zea* specific isolates were compared to SpliNPV-G2 [15] and HzSNPV [9], respectively. The lef-8 gene of fourteen *Spodoptera litura* specific isolates were aligned with SpliNPV-G2 and showed the identity greater than 96%. The lef-8 gene of seven *Helicoverpa zea* specific isolates were aligned with HzSNPV and showed the identity greater than 98%.

The genetic distances with in *Spodoptera litura* specific isolates and *Helicoverpa zea* specific isolates were 0.101 ± 0.065 and 0.053 ± 0.022, respectively, suggesting that the *Helicoverpa zea* specific isolates were lower genetic variable than *Spodoptera litura* specific isolates. The genetic distance between groups analyses also showed that the distance between *Spodoptera litura* specific isolates with Group II Alphabaculovirus were higher than *Helicoverpa zea* (0.234 ± 0.181 and 0.071 ± 0.044, respectively), indicating inter-specific divergence in *Spodoptera litura* specific isolates. Thus, the partial sequence of the lef-8 gene of 335 bp used in this study could be applied to discriminate *Helicoverpa zea* specific isolates and *Spodoptera litura* specific isolates. The genetic distances between Vietnamese isolates were lower than other distances from other groups (**Table 4**). This result supported for a close genetic relationship between Vietnamese isolates and Group II Alphabaculovirus.

The neighbor-joining tree was applied to assess the phylogenetic relationship of Vietnamese isolates in Baculoviridae family using partial lef-8 gene (**Figure 2**). The phylogram was rooted using sequence of NeseNPV, which was clustered with other isolates from Gammabaculovirus in one clade (NeabNPA isolate and NeleNPV isolate). The other major clades included the Betabaculovirus and Group I Alphabaculovirus with 46% bootstrap value and 67% bootstrap value, respectively. The Group I Alphabaculovirus originated as an ancestral Group II Alphabaculovirus [16]. All Vietnamese isolates belonged to Group II Alphabaculovirus which appeared paraphyletic as a sister clade to Group I [14]. The Vietnamese isolates formed several smaller clades within Group II Alphabaculovirus. Seven Vietnamese *Helicoverpa zea* specific isolates were located with HzSNPV isolate. The *Helicoverpa zea* cluster exhibited two groups: one containing 4 isolates from Binh Duong Province (from Hz-BD1 to Hz-BD4) and the other with 3 isolates from Ho Chi Minh City (from Hz-HCM1 to Hz-HCM3). This clade was supported by high bootstrap value of 91% (**Figure 2**). The Vietnamese Hear-BD isolate was found in Group II and appeared to be closely related to HearSNPVNNg1, HearNPV-C1 and HearNPV-G4 with 52% bootstrap value. Fourteen Vietnamese *Spodoptera litura* specific isolates were located with SpliNPV-G2 isolate with 78% bootstrap value, suggesting that these isolates have a close genetic relationship to SpliNPV-G2. There were no subheadings among *Spodoptera litura* specific isolates from Ho Chi Minh City, Ninh Thuan Province and Binh Duong Province.

Table 4. Matrix of Tamura & Nei genetic distance among baculovirus isolates. Lower triangular matrix values were mean genetic distances, upper triangular matrix values were standard errors.

	Spodoptera (Vietnam)	Helicoverpa (Vietnam)	Alpha_1	Alpha_2	Beta	Delta	Gamma
Spodoptera (Vietnam)		0.272	0.352	0.181	0.371	0.712	0.720
Helicoverpa (Vietnam)	0.379		0.236	0.044	0.281	0.767	0.560
Alpha_1	0.472	0.343		0.190	0.360	0.524	0.732
Alpha_2	0.234	0.071	0.261		0.180	0.654	0.596
Beta	0.476	0.399	0.412	0.258		0.687	0.550
Delta	0.873	0.915	0.681	0.760	0.709		1.004
Gamma	0.717	0.639	0.683	0.600	0.503	0.934	

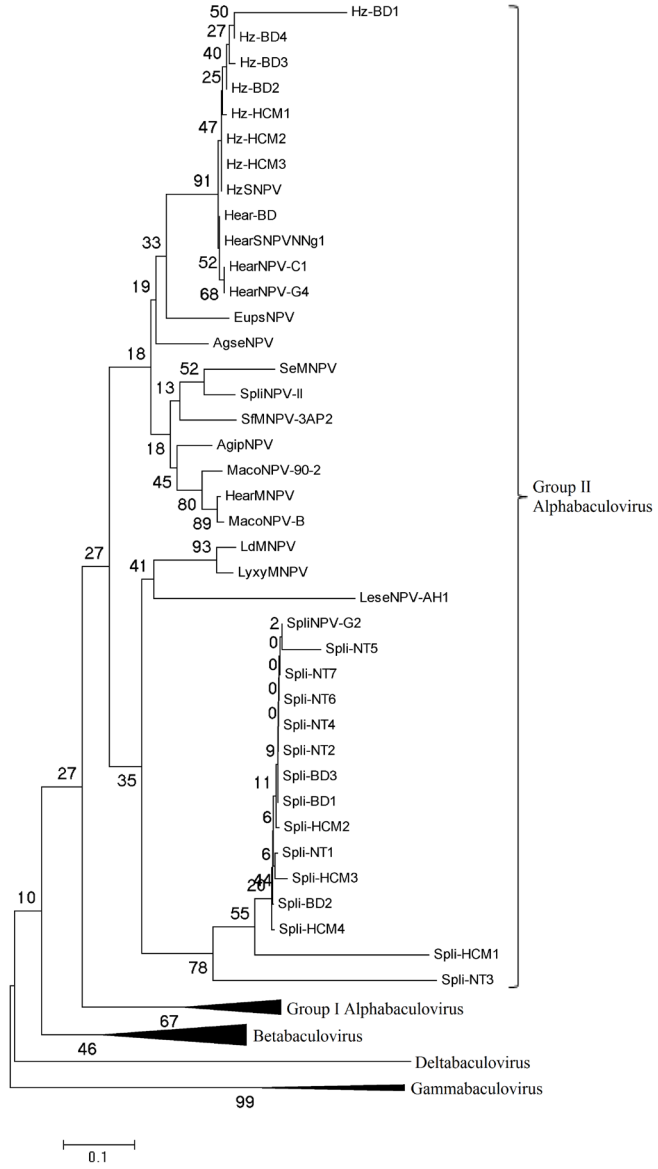

Figure 2. Phylogenetic tree constructed from partial lef-8 sequences of baculovirus isolates by the neighbor-joining analysis method. Bootstrap resampling was done 1000 times, and resulting bootstrap values are shown on the corresponding branches.

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