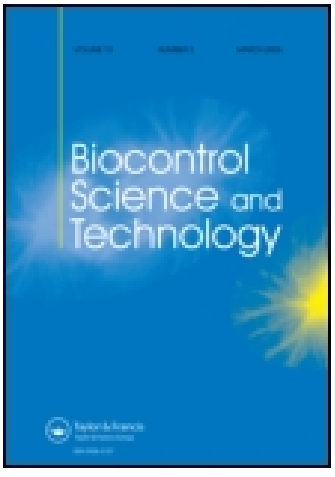


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Yan Chen [a], Bing Li [b], Weide Shen [b], Shanying Zhu [a], Yan Wu [a] & Wenbing Wang [a]

[a] Institute of Life Sciences, Jiangsu University, Zhenjiang, P.R. China

[b] School of Life Sciences, Soochow University, Suzhou, P.R. China

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## SHORT COMMUNICATION

# Analysis of oral infection and *helicase* gene of the nucleopolyhedroviruses isolated from *Philosamia cynthia ricini* and *Antheraea pernyi*

Yan Chen[a], Bing Li[b], Weide Shen[b], Shanying Zhu[a], Yan Wu[a] and Wenbing Wang[a]*

[a]*Institute of Life Sciences, Jiangsu University, Zhenjiang, P.R. China;* [b]*School of Life Sciences, Soochow University, Suzhou, P.R. China*

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*Philosamia cynthia ricini* is an important commercial silkworm in Asia. In this report, a nucleopolyhedrovirus isolated from *P. cynthia ricini* (PhcyNPV) larva was purified and compared with *Antheraea pernyi* nucleopolyhedrovirus (AnpeNPV), a pathogen of *A. pernyi*, another commercial silkworm in China. The two viruses had similar polyhedral morphology and shared high sequence homologue of viral fragments including the *p143* gene. However, the restriction fragments, digested with *Sal*I, *Xho*I, *Hin*dIII and *Pst*I, respectively, were different. The cross-infectivity of the two viruses was also tested. AnpeNPV caused 57% mortality in larvae of *P. cynthia rici*, whereas PcrNPV did not kill larvae of *A. pernyi*. Results indicated that PhcyNPV and AnpeNPV had closed relatedness, and that PhcyNPV might be a variant of AnpeNPV.

**Keywords:** *Philosamia cynthia ricini*; *Antheraea pernyi*; nucleopolyhedrovirus; helicase gene; host range; insect



Baculoviridae is a family of large, enveloped viruses characterized by rod-shaped virions and covalently closed double-stranded circular DNA genomes ranging in size from 80 to 180 kb. Most NPVs generally exhibit a narrow host range (Lu 1998). Lethal infection is commonly restricted to insect species from which the NPVs are originally isolated and to closely related insect species (Ishikawa, Ikeda, Alves, Thiem, and Kobayashi 2004). Several viral factors have been identified to affect NPV host range properties in cell culture systems (Miller 1997). These include the host cell factor 1 (*hcf*-1) of *Autographa californica* multicapsid NPV (AcMNPV) (Lu and Miller 1996), the putative *helicase* (*p143*) of *Bombyx mori* NPV (BmNPV) (Croizier, Croizier, Argaud, and Poudevigne 1994; Kamita and Maeda 1997) and the host range factor 1 (*hrf*-1) of *Lymantria dispar* multicapsid NPV (LdMNPV) (Thiem, Du, Quentin, and Berner 1996). The *p143* gene plays an important role in host range determination, and much work focuses on the AcNPV and BmNPV, which are ideal for the study of host range determination mechanisms of baculoviruses because of their high DNA homology (allowing recombination events to occur efficiently) and nonoverlapping host range characteristics (Kamita and Maeda 1997). The Helicase protein containing seven conserved motifs (I, Ia, II–VI) were essential for the replication function (Ahrens and Rohrmann 1996; Liu and Carstens 1999). The amino acid changes at position 564 and 577 of AcNPV *p143*

*Corresponding author. Email: wenbingwang@ujs.edu.cn

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products are required to kill *B. mori* larvae (Argaud, Croizier, Lopez-Ferber, and Croizier 1998).

*Philosamia cynthia ricini*, the Indian eri silkworm, contributes significantly to the production of commercial silk in India and China (Vijayan et al. 2006). The disease caused by *P. cynthia ricini* NPV (PhcyNPV) is a major threat to the silk-spinning industry. PhcyNPV and *Antheraea pernyi* nucleopolyhedrovirus (AnpeNPV) were described as different NPVs at present, although it has been reported that AnpeNPV is a virulent pathogen of *P. cynthia ricini* (Su 1994). The full length genomic DNA of AnpeNPV has been reported (Nie et al. 2007), and only a few fragments and genes have been primarily studied (Wang, Zhu, Wang, Yu, and Shen 2005; Shi, Pan, and Lu 2007), however, molecular information of PhcyNPV is still unknown. In this report, in order to compare the characteristics between PhcyNPV and AnpeNPV, the cross infectivity was tested, and restriction fragments of the two viral DNA were compared. It indicated that the two viruses are closed related.

The occluded viruses were purified from *P. cynthia ricini* larval cadavers, which were provided by the Institute of Guangxi Sericultural Research and Development in south China. The AnpeNPV was kindly obtained from Professor Qin Li of Shenyang Agricultural University in north China. The larvae of *P. cynthia ricini* were provided by the Sericultural Research Institute, Chinese Academy of Agricultural Sciences, and the larvae of *A. pernyi* were collected from a silkworm cultural field in Henan Province, China. The larvae were cultured with fresh leaves at 25°C.

Fourth-instar larvae of *A. pernyi* and *P. cynthia ricini* were starved for 24 h and then fed on a diet plug contaminated with one of the two viruses with a concentration of $10^4$ occlusion bodies (OBs) per milliliter and reared separately for cross infection test.



### Digestion of viral DNA and cloning of the *p143* genes

The procedures for both OBs and viral DNA preparation were as described by Cheng, Carnerb, Langec, Jehlec, and Arif (2005). Two g DNA were digested with appropriate restriction endonucleases (*Sal*I, *Xho*I, *Hin*dIII or *Pst*I, respectively) overnight.

To compare the *p143* gene, two specific primers were designed to amplify the *p143* gene open reading frame (ORF) of AnpeNPV and PhcyNPV by using a PCR (primer forward: 5′-caa cgc cac tag caa agc tat c-3′; primer reverse: 5′-gaa cgg tta cac ggt tgt caa c-3′), according to the *p143* sequence of AnpeNPV in GenBank (ABF50298). The fragments were cloned into T-vector and were sequenced. The nucleotide sequences were compared with the GenBank database using the BLASTN and BLASTP alignments at the NCBI web site (www. ncbi.nlm.nih.gov/).

### Identification of the two NPVs

The polyhedral morphology of PhcyNPV and AnpeNPV was observed under an electron microscope. The particles were triangular in shape and varied in size from 1 to 3 μm (Figure 1A,B). Hence, it is difficult to distinguish between the type viruses by observation using a microscope.

To compare the DNA sequence of the two viruses, a fragment library of PhcyNPV was constructed and the restriction fragments of the two viruses were compared. Some insert sequences of PhcyNPV (published in GenBank, EU143371 and EF576874) had a very high level of similarity to that of AnpeNPV. To compare the DNA structure, the restriction fragments of the two viruses were compared using digestion of *Sal*I, *Xho*I, *Hin*dIII and

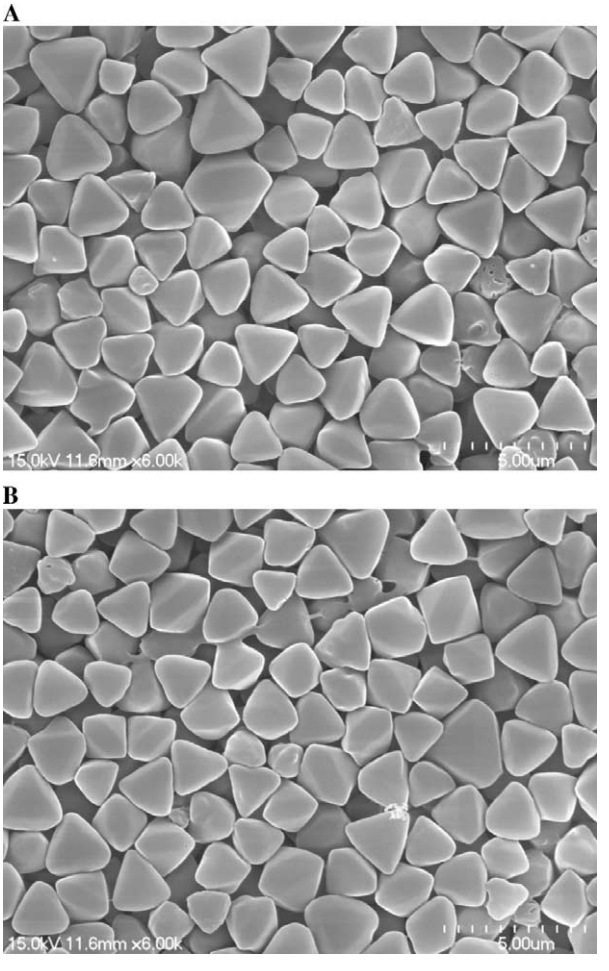


Figure 1. The polyhedral morphology of PhcyNPV and AnpeNPV was observed under electron microscope. The OBs were triangular in shape and varied in size from 1 to 3 μm. (A) AnpeNPV; (B) PhcyNPV.

*Pst*I, respectively. The results showed very clear restriction fragment length polymorphisms between them (Figure 2A). The most similar fragments were of the *Pst*I digestion profiles, although clear differences were observed in the 6.5–13-kbp range.

To confirm the infection of the two viruses, the cross infectivity of PhcyNPV and AnpeNPV for *A. pernyi* and *P. cynthia ricini* larvae, respectively, were tested. Infectivity assays showed that the AnpeNPV produced a fatal infection in its native host, while the PhcyNPV produced no infection in *A. pernyi* (Figure 3A,B). The heterologous larvae remained healthy, even after oral inoculation with a high concentration of PhcyNPV, and no OBs of PhcyNPV were visualized in the haemocoel of inoculated heterologous hosts. In all cases, the infected larvae showed the typical symptoms of NPV disease. Both AnpeNPV and PhcyNPV produced a fatal infection in the larvae of *P. cynthia ricini*, and the average infectivity were 57 and 79%, respectively (Figure 3B). The DNA extracted from *P. cynthia rici* infected with AnpeNPV or PhcyNPV was digested by *Sal*I, *Xho*I, *Hin*dIII and *Pst*I, respectively, to confirm that which NPV killed the larvae (Figure 2B). The results indicated

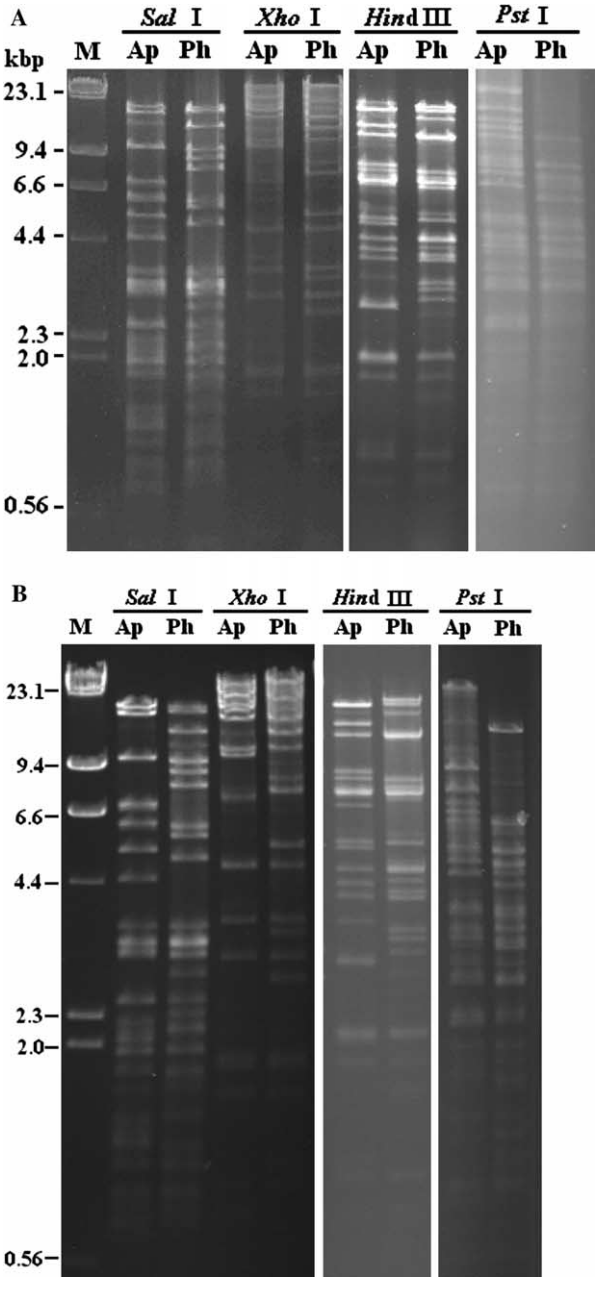


Figure 2. Comparison of restriction enzyme digestion profiles of AnpeNPV and PhcyNPV. Viral DNA of the two OBs was isolated from *Antheraea pernyi* (AnpeNPV) and *Philosamia cynthia ricini* (PhcyNPV). AnpeNPV and PhcyNPV DNA were digested with *Sal* I, *Xho* I, *Hind* III and *Pst* I, respectively, and analyzed by elecrophoresis in an ethidium bromide stained 1% agarose gel. M, the marker of Lambda DNA fragments with *Hin*d III digestion; Ap, AnpeNPV; Ph, PhcyNPV. (A) The DNA from primary polyhedra of the two viruses; (B) the DNA from the polyhedra after infection of the *P. cynthia ricini* larvae.

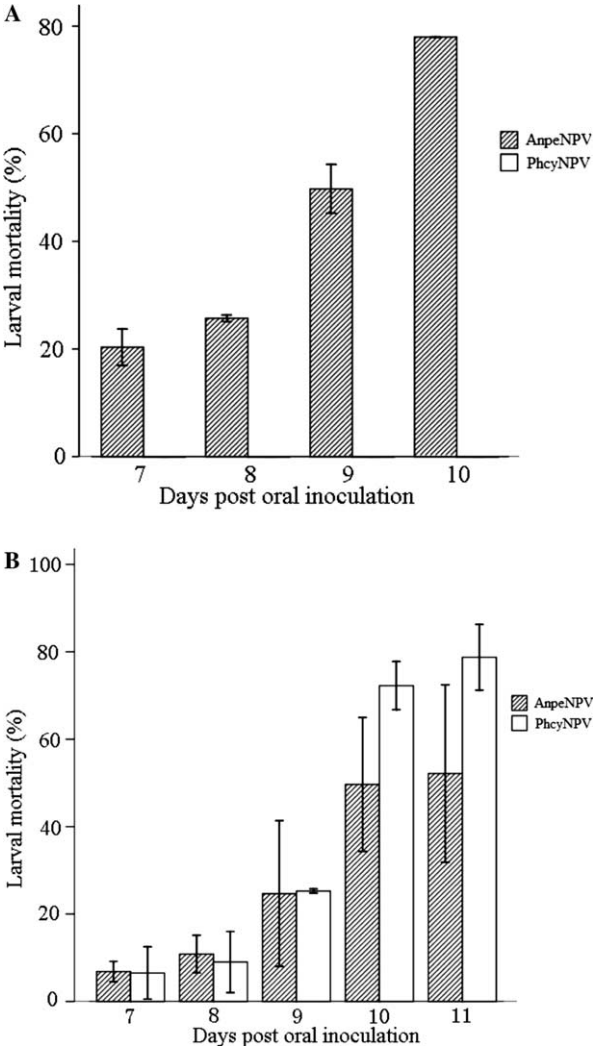


Figure 3. Cross infectivity analysis of PhcyNPV and AnpeNPV for *A. pernyi* and *P. cynthia ricini* larvae, respectively. (A) Indicates the mortality of the *A. pernyi* larvae infected with PhcyNPV and AnpeNPV, respectively. The PhcyNPV can not *per os* infect the larvae of *A. pernyi*. (B) Indicated the mortality of the *P. cynthia ricini* larvae infected with PhcyNPV and AnpeNPV, respectively. Both of the two viruses can *per os* infect the larvae of *P. cynthia ricini*, while the mortality caused by AnpeNPV (57 %) was lower than that caused by PhcyNPV (79 %).

that AnpeNPV can infect both *A. pernyi* and *P. cynthia rici* larvae, however, PhcyNPV can infect *P. cynthia rici*, and can not kill the larvae of *A. pernyi.*

**Analysis of *p143* gene**

According to the sequencing results of PhcyNPV and AnpeNPV *p143* genes, both the ORFs of *p143* genes contained 3639 base pairs, which encoded 1212 amino acids. The nucleotide and amino acid sequence analysis showed that the PhcyNPV *p143* ORF shared the 98.8% nucleotide similarity with that of AnpeNPV, and 45 nucleotides were different

between the DNA align of the two viruses, which generated 18 amino acids substitutions (published in GenBank, EU143371 and EU195295).

Previous studies indicated that viral gene replication and expression were critical steps in determination of host specificity of baculoviruses (Chen, Quentin, Brennan, Kukel, and Thiem 1998; Shirata 1999). We inferred some other viral factors may play a role in the difference between the two viruses, such as the *hcf* gene of *Rachiplusia ou* MNPV (RoMNPV) and AcMNPV (RoMNPV was the variant of AcMNPV) (Harrison and Bonning 2003). In the Helicase sequences of RoMNPV and AcMNPV, none of the domains determining the host range region was different except for one amino acid position 1149 where the III motif was nearby, whereas the amino acid sequence identity of HCF was only 84.1% between the AcMNPV and RoMNPV homologues. Studies suggested that replacement in the AcNPV genome of three AcNPV-specific amino acids by the three corresponding BmNPV-specific amino acids at positions 556, 564, and 577 of the Helicase protein extended the AcNPV host range to *B. mori* larvae, even only two adjacent nucleotides (G and C in AcNPV to A and T in the expanded host range mutant), which generated a single amino acid substitution (564 Ser in AcNPV compared with Asp in BmNPV), was sufficient to expand the host range of AcNPV (Croizier et al. 1994; Argaud et al. 1998). Accordingly, we inferred *p143* might not be the main cause for determining the host range, although the differences can be found at position 905–1200 in the C-terminal region, and also, a demonstrated host range motif, a putative nuclear localization signal (NLS) and a putative DNA binding motif characterized by a helix-turn-helix structure at the position (956–970).

In conclusion, despite the restriction fragments and infection characteristics being different between PhcyNPV and AnpeNPV, the results indicated that the two viruses had close relatedness, and the PhcyNPV might be a variant of AnpeNPV.



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