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Short Communication

Characterization of baculoviruses from the Martignoni collection

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ABSTRACT

47 samples from the Martignoni baculovirus collection were characterized by PCR amplification of the *lef-8* gene. This led to the identification of sequences from viruses that either were not present in the database, or had been identified, but not further characterized. These included an NPV and a GV from *Pseudoletia* (*Mythimna*) *unipuncta*, and NPVs from *Coloradia pandora*, the oak and hemlock looper (probably *Lambdina* sp.), *Peridroma* sp., the pine butterfly (probably *Neophasia* sp.), *Hemileuca* sp., *Orgyia vetusta*, and several *Choristoneura* sp. A phylogenetic tree was constructed relating these viruses to their closest relatives in the database.

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1. Introduction

The US Forest Service Laboratory in Corvallis, Oregon developed a biological control program directed against forest insects during the 1960s. Dr. Mauro Martignoni and others collected a variety of diseased insects mostly from the Western US (Arif, 2010). Upon Dr. Martignoni's retirement, his collection of virus and diseased insects were given to the author.

With the advent of PCR amplification, sequence databases, and DNA sequence analysis programs, it has become possible to relatively easily evaluate the novelty of uncharacterized viruses. In this report, PCR amplification of a portion of the *lef-8* gene was used to determine if there were novel viruses present in this collection that would justify sequencing of their complete genomes.

2. Materials and methods

2.1. Virus samples

The samples were supplied by Mauro Martignoni when he retired in 1986 from the USDA Forest Service Laboratory in Corvallis, OR and included upwards of 200 samples of infected insects and partially purified occlusion bodies collected between 1960 and 1980. They were stored at 4° and arbitrarily assigned numbers (Table 1) for use in this study. When multiple samples with identical sequences were identified, only one was further characterized and included in this study.

2.2. Documentation

In addition to the information in Table 1, the following lists samples that were identical or very similar to each other in the collection, or to other viruses in the database and shows the documentation available.

2.2.1. *Pseudoletia* (*Mythimna*) sp. GV #2, 3, 8, 9, 166, and 170. #2 was collected on August 10, 1963.

2.2.2. *Coloradia pandora* (Pandora pine moth) NPV #6, 19, 152, 157. #19 was from Inyo National Forest, CA, June 1981.

2.2.3. *Pseudoletia* (*Mythimna*) NPV #7 and 28. #7 was dated 12/13/65.

2.2.4. Pine Butterfly #11 from Missoula MT, July 1972.

2.2.5. *Hemerocampa* (*Orgyia*) *vetusta* #13 (9/3/69). This virus was not characterized because it was similar to the *O. pseudotsugata* MNPV (Ahrens et al., 1997).

2.2.6. Oak and hemlock looper (#15 [8/62] and #171 [1970]), respectively.

2.2.7. #17 *Heliothis* NPV. Collected in Chile (2/29/74), South America. This isolate was not characterized because the sequence data was similar to that from *Helicoverpa zea* SNPV (NP_542661) (Chen et al., 2001).

2.2.8. *Malacosoma* sp. NPV #18. Tualatin, OR (10/64).

2.2.9. *Choristoneura viridis* GV #22 from Gearhart Mt., OR 5/15/79.

2.2.10. *Choristoneura murinana* NPV #28 described in (Rohrmann et al., 1982).

2.2.11. #164. *Malocosoma* NPV. 5/1/1968

2.2.12. *Pseudohazis* (*Hemileuca*) NPV #165 (probably *Hemileuca eglanterina* – Western sheep moth) Saturniidae. This virus was likely described in (Hughes, 1978).

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Table 1
Selected viruses characterized from the Martignoni collection.

#	Sample name (abbrev)	Likely host	Accession	Closest sequence[a]
2	*Pseudoletia* (*Mythimia*) sp. (MyspGV)	*Mythimia* sp.	KC798388	SfGV (76%)
7	*Pseudoletia* (*Mythimia*) sp. (MyspNPV)	*Mythimia* sp.	KC798389	MacoNPV-A (68%)
11	Pine butterfly NPV	*Neophasia* sp.	KC798390	CfdefMNPV (96%)
15	Oak looper NPV	*Lambdina* sp.	KC798391	OrleNPV (63%
18	*Malacosoma* sp. (MaspNPV)	*Malacosoma* sp.	KC798392	MacaNPV (100%)
19	*Coloradia pandora* (CopaNPV)	*C. Pandora*	KC798393	CopaNPV (99%)
22	*Choristoneura viridis* (ChviGV)	*C. viridis*	KC798394	ChocGV (100%)
26	*C. murinana* (ChmuNPV)	*C. murinana*	KC798395	ArroNPV (100%)
164	*Malacosoma* sp. (MaspNPV)	*Malacosoma* sp.	KC798396	MaamNPV (99%)
165	*Hemileuca* sp. (HespNPV)	*Hemileuca* sp.	KC798397	EudiNPV (61%)
167	*Peridroma* sp. (PespNPV)	*Peridroma* sp.	KC798398	PemaNPV (100%)

[a] Shows % amino acid sequence identity in brackets.

2.2.13. *Peridroma* sp. #167 and 168. NPV. 11/26/63 and 6/5/80, respectively.

2.3. DNA template production/purification

The samples consisted of either desiccated or decayed insect carcasses in suspension, or purified or partially purified PIB. The latter samples were alkali dissolved by adding 20–50 μl of the sample to 450 μl H_2O with 50 μl of 1 M Na_2CO_3, 0.5 M NaCl and incubating at 42° for 10 min. The samples were then microcentrofuged for 30 min at room temperature to pellet the virions and resuspended in 50 μl TE and PCR amplified (see below). If no PCR product was evident, the virion sample was extracted using a Qiagen DNAeasy Blood and Tissue Kit and the DNA was then used in a PCR reaction as described below.

For samples that were composed of diseased insect carcasses, the polyhedron inclusion bodies (PIB) were partially purified by suspending the material in 0.5% SDS in TE (10 mM Tris, 1 mM EDTA, pH 8.0) and squeezing them through 4 layers of gauze in a funnel into a 15 ml tube. For analysis, samples of this material were then microfuged briefly and resuspended in TE and then alkali dissolved and processed as described above.

2.4. Polymerase chain reaction (PCR)

For PCR, two degenerate primers complementary to the baculovirus *lef-8* gene were used (Jehle et al., 2006). LEF-8 is a subunit of the viral RNA polymerase found in all baculoviruses. The primers were fused to a M13 reverse, or T7 promoter primer (underlined below) to allow sequencing of the PCR product. The primers and genome location are as follows:

prL8-2	
41,373–41,389	M13
reverse	
<u>CAGGAAACAGCTATGACC</u>AYRTASGGRTCYTCSGC	
prL8-1B	
42,075–42,088	T7
<u>TAATACGACTCACTATAGGG</u>CAYGGHGARATGAC	

A sample was usually diluted 1:200 in TE and 1 μl was used in a 20 μl PCR reaction using a BioRad iQ SYBER Green supermix kit with 1 μl of a 1 μM mixture of the two primers. In this protocol, the heat from the PCR reaction denatures the virion proteins and DNA and makes the DNA template accessible to the Taq polymerase. The PCR reaction was a two step procedure using two different annealing temperatures. 95° 5 min, 10 cycles of 94°, 1 min; 47°, 1 min; 72°, 1 min; and then 37 cycles of 94°, 20 s; 52°, 45 s; 72°, 45 s and then ending with 72° for 10 min. After amplification, 5 μl was examined on a 1% agarose gel to determine if a product of the predicted size was produced. If a product was detected, the remainder of the PCR product (15 μl) was purified using a Qiagen QIAquick pcr purification kit and used for DNA sequencing.

2.5. DNA sequencing and analysis

DNA sequencing was done at the Center for Genome Research and Biocomputing at Oregon State University. The instrumentation and technology employed ABI Prism® 3730 Genetic Analyzer, 3730 Data Collection Software v. 3.0, and DNA Sequencing Analysis Software v. 5 and employed BigDye® Terminator v. 3.1 Cycle Sequencing Kit chemistry. Sequence data was analyzed using the MacVector suite of software programs and all comparisons are based on predicted amino acid sequences.

3. Results and discussion

For this investigation, 47 samples were processed and resulted in the production of 32 PCR products of the appropriate size that were sequenced. Some samples appeared to be mixtures. Readable sequence was produced from 26 samples, some of which were identical to one another. When multiple sequences were identical, a single sample was sequenced from the opposite direction. The virus samples that were characterized are listed in Table 1. The likely species name is indicated and the closest related sequence in Genbank is indicated along with the percent identity. These sequences were subjected to clustalw analysis and a phylogenetic tree was produced (Fig. 1). Three categories of related baculovirus sequences were identified and used for further analysis. These included, (i) identical sequences from baculovirus genomes that had been completely sequenced, (ii) identical sequences from isolates whose genome had not been sequenced, and (iii) samples for which there were no identical sequences.

From this analysis, the following was evident.

3.1. GV sequences

#2 *Pseudoletia* (*Mythimna*) sp. GV was more closely related to the *Spodoptera frugiperda* GV (76%), than to the *Pseudoletia* (*Mythimna*) *unipuncta* GV sequence (72%) present in the database. #22 *Choristoneura viridis* GV is identical to the *C. occidentalis* GV sequence.

3.2. NPV sequences, Group I

The pine butterfly sequence (#11), was closest to CfdefNPV (96%). The *C. murinana* NPV (ChmuNPV) (#26) was identical to *Archips rosanus* NPV sequence. The closest related sequenced genome was for CfNPV (93%).

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