Journal of Invertebrate Pathology 148 (2017) 152–161

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

The genome sequence of Condylorrhiza vestigialis NPV, a novel baculovirus for the control of the Alamo moth on *Populus* spp. in Brazil

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ARTICLE INFO

Keywords:
Baculovirus
Condylorrhiza vestigialis NPV
Genome sequence
Biological control

ABSTRACT

Condylorrhiza vestigialis (Lepidoptera: Cambridae), commonly known as the Brazilian poplar moth or Alamo moth, is a serious defoliating pest of poplar, a crop of great economic importance for the production of wood, fiber, biofuel and other biomaterials as well as its significant ecological and environmental value. The complete genome sequence of a new alphabaculovirus isolated from *C. vestigialis* was determined and analyzed. Condylorrhiza vestigialis nucleopolyhedrovirus (CoveNPV) has a circular double-stranded DNA genome of 125,767 bp with a GC content of 42.9%. One hundred and thirty-eight putative open reading frames were identified and annotated in the CoveNPV genome, including 38 core genes and 9 *bros*. Four homologous regions (*hrs*), a feature common to most baculoviruses, and 19 perfect and imperfect direct repeats (*drs*) were found. Phylogenetic analysis confirmed that CoveNPV is a Group I *Alphabaculovirus* and is most closely related to *Anticarsia gemmatalis multiple nucleopolyhedrovirus* (AgMNPV) and *Choristoneura fumiferana DEF multiple nucleopolyhedrovirus* CfDEFMNPV. The *gp37* gene was not detected in the CoveNPV genome, although this gene is found in many NPVs. Two other common NPV genes, chitinase (v-*chiA*) and cathepsin (v-*cath*), that are responsible for host insect liquefaction and melanization, were also absent, where phylogenetic analysis suggests that the loss these genes occurred in the common ancestor of AgMNPV, CfDEFMNPV and CoveNPV, with subsequent reacquisition of these genes by CfDEFMNPV. The molecular biology and genetics of CoveNPV was formerly very little known and our expectation is that the findings presented here should accelerate research on this baculovirus, which will facilitate the use of CoveNPV in integrated pest management programs in Poplar crops.

1. Introduction

The *Baculoviridae* is a family of large DNA viruses that infect insects mostly of the order Lepidoptera and is organized into four major genera: *Alphabaculovirus*, lepidopteran-specific nucleopolyhedrovirus, subdivided into Group I or Group II based on the type of fusogenic protein found in the budded virus (BV) envelope; *Betabaculovirus*, lepidopteran-specific granuloviruses, *Gammabaculovirus*, hymenopteran-specific nucleopolyhedrovirus; and *Deltabaculovirus*, dipteran-specific nucleopolyhedrovirus (Herniou et al., 2012). One of the unique characteristics of baculoviruses is their ability to produce two morphologically distinct, but genetically identical, infectious particles during the infection cycle: BV and ODV- occlusion derived virus. The baculoviruses are widely used as biopesticides for controlling agricultural and forestry pests (Moscardi et al., 2002, 2011; Haase et al., 2015a), as expression vectors for the production of recombinant proteins and as viral vectors for gene delivery in mammalian cells (Hüser and Hofmann, 2003; Kost et al., 2005; Kim et al., 2008; Pan et al., 2009, 2010; Haase et al., 2015b).

The baculoviruses contain circular double-stranded DNA genomes of 80–180 kbp, encoding 90–180 putative proteins. Based on the sequencing of baculovirus genomes, the gene repertoire of these viruses has been estimated to be about 900 genes (Ferrelli et al., 2012). A total of 38 predicted ORFs have been identified as core genes that so far are conserved in all fully sequenced baculoviruses and play critical roles in the viral replication cycle (Garavaglia et al., 2012; Javed et al., 2017). To date, 83 sequenced baculovirus genomes, including 56 alphabaculoviruses, 23 betabaculoviruses, three gammabaculoviruses and one deltabaculovirus are available in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov).

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http://dx.doi.org/10.1016/j.jip.2017.06.013
Received 13 March 2017; Received in revised form 22 June 2017; Accepted 27 June 2017
Available online 29 June 2017

In Brazil, poplar (family Salicaceae, genus: *Populus*) has become important for wood production throughout the Southern Region (Parana and Santa Catarina States) and is commonly used for the manufacture of matchsticks and laminates (Otto et al., 2007). The *Populus* genus comprises more than 30 species of trees that provide wood, fiber, biofuel and other biomaterials of economic importance and significant ecological and environmental value. Poplars have also attracted interest due to their potential for landscape and agricultural use worldwide and also as model systems in the study of forest tree biology (FAO, 1979; Balatinecz and Kretschmann, 2001; Jansson and Douglas, 2007). However, poplar cultivation has been hampered by severe attacks by the caterpillar *Condylorrhiza vestigialis* (Guenée, 1854) (Lepidoptera: Crambidae), commonly known as the Brazilian poplar moth or Alamo moth, which is a serious defoliating pest of poplar and has prompted urgent interest for its efficient control. The levels of defoliation caused by the pest can reach 100% in the first two years of plant growth, causing significant losses in stem diameter (Diodatto, 1999).

A virus was isolated from naturally infected larvae of *C. vestigialis* and was identified and classified based on phylogenetic analyses and the structure of its occlusion bodies was revealed by electron microscopy (Castro et al., 2009, 2011). These studies demonstrated that the virus is a Group I *Alphabaculovirus* and appears to be most closely related to *Choristoneura fumiferana DEF multiple nucleopolyhedrovirus* (CfDEFMNPV) and *Anticarsia gemmatalis multiple nucleopolyhedrovirus* (AgMNPV). The occlusion bodies (OBs) containing multiple (M) nucleocapsids per envelope were observed and the virus was designated Condylorrhiza vestigialis nucleopolyhedrovirus (CoveNPV), according to the insect host species from which it was first isolated and the updated taxonomy of the *Baculoviridae* family (Herniou et al., 2012).

Considering the problems caused by the excessive use of chemical pesticides, biological agents have been tested as a control option for integrated pest management (IPM). Biological control strategies have been developed using baculoviruses against *C. vestigialis* and these have shown promising results, where a high virulence of CoveNPV to *C. vestigialis* larvae was observed in the laboratory and in field assays (Machado, 2006). This research on CoveNPV pathogenicity formed a starting point for CoveNPV-based product development and its registration by Brazilian regulatory agencies. This bioinsecticide, under the brand name of '*Baculovirus Alamo*' (wettable powder formulation), is now being used to control *C. vestigialis*, in poplar plantations in Brazil (Machado, 2006; Bráulio Santos, 2013 - personal communication, Haase et al., 2015a). However, very little is so far known about the molecular biology and genetics of this baculovirus.

In the present work, the complete genome sequence of Condylorrhiza vestigialis nucleopolyhedrovirus (isolate CoveNPV-PR.2002) was obtained, analyzed and compared to other baculovirus genomes already sequenced. The research presented here should add to the understanding of the evolution of this family of viruses and contribute to more efficient use of this virus as a bioinsecticide.

2. Materials and methods

2.1. Insects and viral isolates

Infected *C. vestigialis* larvae were collected from poplar plantations in 2002 in Parana State (Brazil) and generously donated by Edilene B. Machado (Swedish Match do Brasil S/A – Curitiba, PR) and Bráulio Santos (Universidade Federal do Paraná-UFPR). Occlusion bodies (OBs) were purified from infected larvae according to procedures described by Maruniak (1986), with modifications previously described by Castro et al. (2009). CoveNPV samples were deposited in the Invertebrate Virus Collection at Embrapa Genetic Resources and Biotechnology and the isolate (CoveNPV-PR.2002) is detailed in the Brazilian Alelo-Micro Information System under accession code BRM 005097.

2.2. DNA purification

Viral genomic DNA was purified from a field isolate of CoveNPV, hereafter named CoveNPV-PR.2002, using a standard method (O'Reilly et al., 1992). Virions were released from polyhedra by dissolving 1×10^9 OB/ml in an alkaline solution (0.1 M Na_2CO_3) at 37 °C for 1 h. Viral DNA was extracted by overnight incubation at 37 °C with 1% SDS and 0.5 mg/ml proteinase K, followed by extraction with phenol-chloroform: isoamyl alcohol (25:24:1). DNA was then precipitated using 0.1 vol of 3 M sodium acetate (pH 5.2) and 2 volumes of absolute ethanol, recovered by centrifugation at 14,000*g* for 15 min and washed with 70% (v/v) ethanol. The dried DNA was resuspended in TE buffer and stored at 4 °C until use. DNA concentration and quality were assessed by 1% agarose gel electrophoresis and a Qubit v. 2.0 Fluorometer and dsDNA BR Assay Kit (Invitrogen), according to the manufacturer's instructions.

2.3. Sequencing, assembly and annotation of the CoveNPV genome

The genome of CoveNPV was sequenced at the Distrito Federal High Performance Genome Center (Genomics DF) using a shotgun approach on the Roche 454 GS FLX Titanium platform. The resulting reads (95,156 reads) were trimmed to remove sequencing adaptors and low quality regions ($Q \geq 20$) using Geneious 6.0.4 software (Biomatters Limited). A total of 94,335 reads were used for genome assembly with the *De Novo* Assembly tool implemented in Geneious 6.0.4 software and Mira Sequence Assembler (Chevreux et al., 1999). The genome coverage was approximately 30×. The so-called homologous regions (*hrs*) and direct repeats (*drs*) were located and analyzed using SelfDotplot and EMBOSS tools (Rice et al., 2000). The smaller repeat unit of the *hr* sequence was identified using BLASTn-2seq and a search for this repeat unit was performed in the complete CoveNPV genome. The *hrs* imperfect palindromic consensus logo was generated using the Weblogo3 webserver (http://weblogo.berkeley.edu) (Crooks et al., 2004) from all the *hr* sequences. Whole-genome multiple alignments and a dot-plot analysis were performed comparing the CoveNPV genome with the AgMNPV [DQ813662], CfDEFMNPV [AY327402] and AcMNPV [L22858] genomes, using the dotmatcher (EMBOSS) tool implemented in Geneious 6.0.4.

The open reading frames (ORFs) were predicted with the *Find ORF* tool (Geneious 6.0.4). According to convention, an ORF was called with a size of at least 150 nt (50 aa) with minimal overlap and the polyhedrin gene (*polh*) was designated ORF1 (Vlak and Smith, 1982; Possee and Rohrmann, 1997). The identified ORFs were compared with NCBI database entries using BLASTx for annotation and verification of the genes and determination of the correct frame size.

2.4. CoveNPV phylogenetic reconstruction

A phylogenetic tree was inferred using sequence data from a concatenated data set of 38 core genes extracted from 83 complete baculovirus genomes (Table A.1), aligned by MAFFT (Katoh et al., 2002). The maximum likelihood (ML) tree was inferred using PhyML v3.0 (Guindon and Gascuel, 2003), with the GTR + I + Γ substitution model, as selected by jModelTest (Darriba et al., 2012). Branch support was estimated by non-parametric bootstrap with 100 replicates. The trees were edited in FigTree (Rambaut, 2008). Kimura 2-parameter pairwise distances were calculated using MEGA 7 (Kumar et al., 2016).

2.5. Analysis of the status of the baculovirus v-cath and chiA genes

Previous studies in our laboratory, involving PCR screens and Southern blotting, suggested that the *v-cath* and *chiA* genes were not present in the CoveNPV genome (data not shown). To confirm the absence or presence of the CoveNPV *v-cath* and *chiA* genes, the trimmed CoveNPV reads were searched for homology with the *v-cath/chiA* genes