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Conserved baculoviral ORFs 10 and 14 from *Bombyx mori* multiple nucleopolyhedrovirus

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ABSTRACT. ORFs 10 and 14 from *Bombyx mori* multiple nucleopolyhedrovirus (BmMNPV) were amplified, cloned and sequenced. Nucleotide analysis of these genes and those of other baculoviruses showed that these genes are highly conserved. The p10 protein from BmMNPV ORF10 has 70 amino acid residues similar to that of the four other known BmNPV strains. The BmMNPV ORF14 alignment showed a higher identity with the nucleopolyhedrovirus ORF14 from the baculovirus BmNPV and from *Autographa californica* multiple nucleopolyhedrovirus. The BmMNPV ORF14 protein has a putative transmembrane domain in the C-terminal region, which is similar to that of other baculoviruses. A phylogenetic analysis showed that BmMNPV ORF14 protein has higher similarity with BmNPV ORF14 and ORF23 of *A. californica* multicapsid nucleopolyhedrovirus (Ac23). We conclude that proteins produced by ORFs 10 and 14 from BmNPV and BmMNPV are highly conserved in NPVs and MNPVs. The high degree of conservation among members of these genera indicates the importance of these proteins, which could mean an important function that is active throughout the infection cycle.

Key words: Baculovirus; BmMNPV; ORF10; BmMNP; ORF14 *Bombyx mori* multiple nucleopolyhedrovirus subgroup

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INTRODUCTION

Baculoviridae are a diverse virus family that is pathogenic to the Lepidoptera order. This family is subdivided into two genera, *Nucleopolyhedrovirus* (NPV - nuclear polyhedrosis virus) and *Granulovirus* (granulosis virus), which are differentiated by the morphology of occlusion bodies. The viruses from *Nucleopolyhedrovirus* genera may have only a single nucleocapsid per virion (single nuclear polyhedrosis virus) or more than one nucleocapsid per virion (multiple nuclear polyhedrosis virus - MNPV) (Blissard, 1996).

The baculovirus genome is composed of a circular double-strand DNA that is surrounded by a protein capsid (Hayakawa et al., 2000). Thus, this shape consists of the infective form of the virus (nucleocapsid). The virions, during the infection cycle, can assume two different forms: one of them is responsible for the systemic infection, cell to cell, inside of the insect body, referred to as the budded virus; the second form, called the polyhedra-derived virus, that has occluded multiple nucleocapsids in a crystalline matrix composed mainly of polyhedrin, responsible for the horizontal transmission of the disease, from insect to insect (Gomi et al., 1999; Acharya et al., 2002; Rahman and Gopinathan, 2003). These two different phenotypic forms have diverse functions in the infection cycle.

The infection caused by nucleopolyhedrovirus is the main reason of economic losses in sericulture, a very important agroindustrial activity in Brazil and in the world. The isolation of a *Bombyx mori* multiple nucleopolyhedrovirus (BmMNPV) subgroup in Brazil was described by Brancalhão (2002) in silk larvae collected in Paraná State, Brazil. This type of virus is highly virulent, since several nucleocapsids per virion can be found (Torquato et al., 2006). Contaminated food (*Morus alba* L.) is the main contamination source for silkworms (Watanabe, 2002).

When the virus reaches the middle intestine of the insect, it is submitted to a higher alkaline pH (approximately 11), which dissolves polyhedrin, releasing the virions in the digestive lumen. In the first hour of infection, the virions pass into the middle intestine epithelial cells via membrane fusion (Horton and Burand, 1993), transferred to the nucleus, where they lose the protein envelope and release the DNA, during the period of 1 h after infection.

This virus is easily spread by hemolymph, feces, and larval regurgitation, and its transmission could occur also by vertical transmission by its progeny (Khurad et al., 2004). The baculovirus produces some proteins that help in the infective process, such as GP64 (Garry and Garry, 2008). There are also other proteins that are produced during the late phase of infection, such as chitinase and cysteine protease, which probably act in the insect's tissue dissolution, disrupting the larval cuticle, and release the polyhedra (Hawtin et al., 1997).

Nucleopolyhedrovirus infection has become one of the most important economic issues for sericulture in tropical regions. Until now, there is no therapeutic agent available to control virus infection, and prevention against insect infection is the only current possible solution.

Recent studies on *Bombyx mori* have been carried out in search of pure strains and resistant or tolerant hybrids to nucleopolyhedrovirus, since the infected insects demonstrate a series of morphological and behavior changes, and consequently, they end up dying after a few days (Rahman and Gopinathan, 2003; Yao et al., 2003).

The aim of this study was to investigate if BmMNPV open reading frames (ORFs) 10 and 14 could be correlated to this baculovirus phenotype. This study was carried out by gene sequencing and analysis of structural parameters of their respective proteins.

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MATERIAL AND METHODS

Biological material and baculovirus infection

The experiments were carried out with *Bombyx mori* hybrids from Fiação de Seda BRATAC S/A, which produces larvae for economical purposes in Paraná State, Brazil. One hundred third-instar larvae were maintained in the laboratory with the same management in relation to feed (fresh mulberry leaves) and under controlled environmental conditions (temperature of 25 ± 2°C, relative humidity of 75 ± 10% and white light with photoperiod of 14:10 h).

The BmMNPV inoculum was obtained from *B. mori* larvae isolated in Paraná State, Brazil (Brancalhão, 2002). The viral polyhedron suspension to be administered was quantified using a Neubauer counting chamber. The concentration was adjusted to 1.8 x 10^6 OBs/mL (polyhedral occlusion bodies/mL).

When the larvae reached the 5th instar, the inoculations were carried out 24 h after ecdysis. The larvae were fed mulberry leaf disks (2 cm in diameter), previously pulverized with 30 µL BmMNPV viral suspension, and the control group was fed leaf disks containing filtered water. After feeding, the larvae were transferred to boxes properly identified, where they received daily mulberry leaves without BmMNPV.

Seven days after inoculation, the larval silk glands were dissected and stored in absolute isopropanol at -20°C.

Silk gland DNA extraction

The individual DNA samples were extracted, and since the silk glands were stored in isopropanol at -20°C, they were washed with 0.7% NaCl. The protocol was based on that described by Monesi et al. (1998) with some modifications. Briefly, the silk glands were cut up with scissors, lysed with 1 mL lysis buffer (50 mM Tris-HCl, pH 8.0; 50 mM EDTA, pH 8.0; 1.5% sarcosyl; 10 mM NaCl, and 1 mg/mL proteinase K), at 60°C for 3 h and extracted with phenol/chloroform as described by Sambrook and Russell (2001).

Baculovirus DNA extraction

DNA extraction of BmMNPV was carried out according to the protocol proposed by Yang et al. (1997), with some modifications. Infected larvae (45) were macerated in distilled water, and the solution was filtered several times until it was a milky white liquid. Subsequently, 1 mL of this solution was homogenized with 10% TESP buffer (50 mM Tris-HCl, pH 8.5; 10 mM EDTA, pH 8.0; 100 mM NaCl; 1 mM PMSF), and then centrifuged at 6500 *g* for 10 min at 4°C. The pellet was resuspended in two volumes of TESP, containing 1% Triton X-100, and centrifuged again. The pellet was resuspended with TESP, followed by centrifugation, and then resuspended in TMP buffer (100 mM Tris-HCl, pH 7.5; 10 mM $MgCl_2$; 1 mM PMSF) and treated with DNAse and RNAse. The solution was incubated at 37°C for 15 min. Next, 30 mL TESP buffer was added to this solution and another centrifuged step was carried out. The final pellet was resuspended in 1 mL TESP. In this first step, BmMNPV nucleocapsids were obtained, free of *B. mori* RNA and DNA. In order to purify the viral DNA, a solution containing nucleocapsids was lysed with 2 mL GTE buffer (6 M guanidine; 50 mM Tris-HCl;

10 mM EDTA, pH 7.0, which was mixed and centrifuged). The pellet was resuspended in 3 mL TE buffer (1.0 mM Tris-HCl, pH 7.4, and 0.1 mM EDTA, pH 8.0) also containing 0.5% SDS and 0.5 mg/mL proteinase K, incubated overnight at 55°C and extracted using the phenol/chloroform standard procedure.

The viral DNA was obtained by precipitation with 0.2 M NaCl and 7 volumes of isopropanol and resupended in 0.1X TE.

BmMNPV ORF10 and ORF14 amplification, cloning and sequencing

BmMNPV ORF10 and ORF14 were amplified using primers (synthesized by Alpha DNA) with known sequences of BmNPV virus (Gomi et al., 1999) (Table 1). The amplification was carried out with a final volume of 15 µL, with 1X Taq polymerase buffer (Invitrogen®), 0.75 mM $MgCl_2$, 200 µM dNTPs and 0.8 µM of each primer, 1 U Taq DNA polymerase (Invitrogen®) and 20 ng genomic DNA. The amplification reaction was performed in a Mastercycler gradient thermocycler (Eppendorf®) using a denaturation step of 94°C for 60 s, followed by 35 cycles of 94°C for 60 s, 63°C for 60 s and 72°C for 60 s. A final extension step at 72°C for 30 min was then used. The amplification reactions were repeated at least twice.

Table 1. Primers used in this study.

Primers	Primer sequence (5' → 3')
ORF10 Forward	GCATTTGAGGATGCCGGGAC
ORF10 Reverse	ACTGCGTTTACCACGACGAG
ORF14 Forward (1)	ATGGACGGTGTAAAGTTGCTGG
ORF14 Reverse (1)	TCAAAATCAACGCCGTCGTC
ORF14 Forward (2)	GAAGACAGCATTTCAAGCAACG
ORF14 Reverse (2)	GCAATTTGTACGCTTGCGAC
ORF14 Forward (3)	ACAACCAAAACCGCATGTGG
ORF14 Reverse (3)	ACGGACACGTTCGTGTTTGG
ORF14 Forward (4)	TCGTGTACGGCATGTGCGAC
ORF14 Reverse (4)	TGTCGTCTTGCGAGTCTTCG
ORF14 Forward (5)	GATTACACATCGGCGTCTCAG
ORF14 Reverse (5)	AGGCCCAGCCAGCGGTTTAC

The specific band of 402 nucleotides obtained by viral genomic DNA amplification with primers relating to ORF10 was recovered on a 1.5% agarose gel, using the MinElute Gel Extraction Kit (Qiagen®) according to manufacturer instructions and cloned with pGEM-T-Easy vector.

The ORF14 amplified products of 441, 520, 520, 513, and 595 bp, which correspond to the first, second, third, fourth, and fifth primer pairs, respectively, were individually cloned with pGEM-T-Easy vector. The recombinant DNA of these clones are called BmORF10, BmORF14-1, BmORF14-2, BmORF14-3, BmORF14-4, and BmORF14-5. The transformation was carried out with *Escherichia coli* strain DH5α bacteria by thermal shock (Sambrook and Russell, 2001). The clones were purified using the QIAprep Spin Miniprep Kit (Qiagen®).

The sequencing reaction consisted of the use of the DYEnamic ET Dye Terminator Kit (Amersham-GE®) with 400-500 ng plasmid. Sequencing was performed on automatic sequencer MegaBACE 1000 (GE Healthcare®). The sequences were analyzed in relation to homology and identity in biological databanks, such as EMBL (EBI) (http://www.ebi.ac.uk/embl/), ExPASy

Proteomics (http://www.expasy.ch/) and GenBank (http://www.ncbi.nlm.nih.gov/).

The amino acid sequence alignment of ORF10 of BmMNPV was carried out using the ClustalW2 program (Thompson et al., 1997) and the sequences from BmNPV isolate K4 (AF247684), BmNPV isolate T3 (L33180), BmNPV Zhenjiang strain (AF533973), BmNPV Xuwen strain (AF536206), BmNPV 10-kDa protein (S76783), BmNPV isolate K3 (AF247683), BmNPV isolate K1 (AF247681), and *Autographa californica* NPV p10 gene (M10023).

Similarly, an alignment of sequences referring to ORF14 from BmMNPV was performed with BmNPV isolate T3 (L33180), *A. californica* MNPV clone C6 (AcMNPV, L22858), *Rachiplusia or* MNPV (RoMNPV, AY145471), *Plutella xylostella* MNPV isolate CL3 (PxMNPV, DQ457003), and *Maruca vitrata* MNPV (MvMNPV, EF125867). The amino acid composition was calculated using Pep-Info (www.ebi.ac.uk/servicestmp/.html) and the ProtParam (http://www.expasy.ch/tools/protparam.html) software. Hydropathy plots were obtained using the Bioedit program on the Hopp and Woods (1981) scale. In order to find the possible transmembrane domains in the BmMNPV ORF14 protein, the TMHMM server 2.0 tool (http://www.cbs.dtu.dk/services/TMHMM-2.0) was used, and to verify the presence of N-terminal signal peptides the SignalP 2.0 prediction server (Nielsen et al., 1997) was used (http://www.cbs.dtu.dk/services/SignalP).

RESULTS AND DISCUSSION

BmMNPV DNA

To verify that the isolated BmMNPV DNA fraction was free from *B. mori* DNA, the DNA isolated from infected and control *B. mori* silk glands and baculovirus isolated DNA were amplified with the BmORF14-1 primer pair and *B. mori* actin gene (Figure 1). In the BmMNPV DNA, only the 441-bp fraction from ORF14-1 primer pair is detected (Figure 1, lane 4), and the fraction consisting of ~800 bp from the *B. mori* actin gene is not detected (Figure 1, lane 1), which confirms that our baculovirus fraction was not contaminated with the *B. mori* DNA. The silk gland DNA from infected (Figure 1, lanes 2 and 5) and control *B. mori* (Figure 1, lanes 3 and 6) confirms that the BmORF14-1 primer pair is exclusive for the baculovirus genome.

ORF14 sequence analysis

Pereira et al. (2008) had previously detected, from silk gland DNA from infected *B. mori* larva, a partial sequence of BmMNPV ORF14, which was amplified by RAPD primer in studies related to resistance and susceptibility of *B. mori* to this nucleopolyhedrovirus. In order to confirm this sequence, which was detected with an RAPD primer, specific primers were designed for ORF14 through a known sequence from BmNPV genome (Gomi et al., 1999).

The amplified products were cloned and sequenced. The analysis of ORF14 nucleotide sequence revealed that the sequence of MNPV subgroup of *B. mori* (BmMNPV) had identity equivalent to 99, 94, 93, 89, 79%, respectively, with: BmNPV genome, isolate T3 (Gomi et al., 1999); AcMNPV genome, clone C6 (Ayres et al., 1994); PxMNPV genome, isolate CL3 (Harrison and Lynn, 2007); RoMNPV genome (Harrison and Bonning, 2003), and MvMNPV genome (Chen et al., 2008).

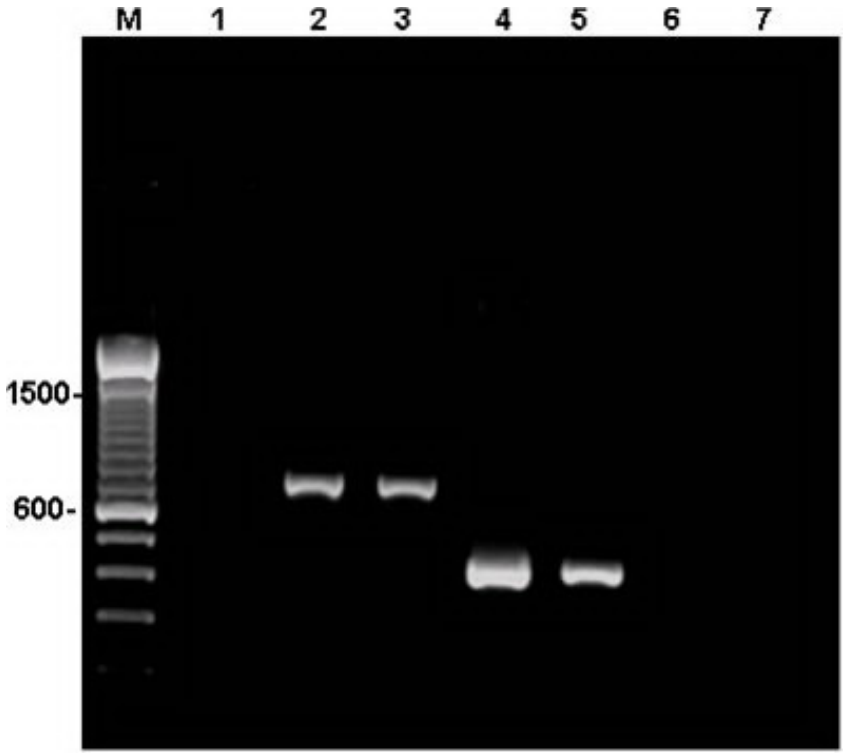

Figure 1. *Bombyx mori* multiple nucleopolyhedrovirus (BmMNPV) DNA qualitative analysis. To test the purified viral DNA, partial amplifications of the *B. mori* Actin A3 gene (*lanes 1* to *3*) and of ORF14 (BmORF14-1, *lanes 4* to *6*) were done. BmMNPV DNA (*lanes 1* and *4*), nucleopolyhedrovirus inoculated *B. mori* DNA (*lanes 2* and *5*) and control *B. mori* DNA (*lanes 3* and *6*). *Lane 7*, PCR negative control. 1.5% agarose gel. M = 100 bp; Invitrogen.

The ORF14 sequence was shown to be conserved in BmMNPV when compared to the BmNPV sequence. This fact indicates that the differences demonstrated by the sequencing of the polymorphic band found by RAPD primer in studies carried out by Pereira et al. (2008), could be due to errors induced by the RAPD low stringency amplification protocol.

BmMNPV ORF10 and ORF14 amino acid sequences

The analysis of BmMNPV ORF10 protein sequence showed no difference in the amino acid sequence of the BmNPV isolate K4 (Hong et al., 2000), BmNPV isolate T3 (Gomi et al., 1999), BmNPV Zhenjiang strain (Jiang et al., 2008), and BmNPV Xuwen strain (Jiang et al., 2008). The number of coded amino acids for p10 gene could vary among different isolates of nucleopolyhedrovirus and in different species of Lepidoptera. There are some isolates that show 70 amino acid residues, whereas others show 94 (Figure 2; Hong et al., 2000).

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BmMNPV              MSKPNVLTRILDAIAETNTKVDSVQTQLNGLEESFQPLDGLPAQLTDFNTKISEIQSILT 60
BmNPV               MSKPNVLTRILDAIAETNTKVDSVQTQLNGLEESFQPLDGLPAQLTDFNTKISEIQSILT 60
BmNPV-K4            MSKPNVLTRILDAIAETNTKVDSVQTQLNGLEESFQPLDGLPAQLTDFNTKISEIQSILT 60
BmNPV-Zhenjiang     MSKPNVLTRILDAIAETNTKVDSVQTQLNGLEESFQPLDGLPAQLTDFNTKISEIQSILT 60
BmNPV-Xuwen         MSKPNVLTRVLDAIAETNTKVDSVQTQLNGLEESFQPLDGLPAQLTDFNTKISEIQSILT 60
BmNPV-10 kDa        MSKPTVLTPILDAIAETNTKVDSVQTQLNGLEESFQPLDGLPAQLTDFNTKMSEIQSILT 60
BmNPV-K3            MSKPNVLTQILDAVTETNTKVDSVQTQLNGLEESFQLLDGLPAQLTDLNTKISEIQSILT 60
BmNPV-K1            MSKPNVLTQILDAVTETNTKVDSVQTQLNGLEESFQLLDGLPAQLTDLNTKISEIQSILT 60
AcNPV-P10           MSKPNVLTQILDAVTETNTKVDSVQTQLNGLEESFQLLDGLPAQLTDLNTKISEIQSILT 60
                    ****.*** :***::********************* **********:***:********

BmMNPV              GDTAPDPPDS---------------------- 70
BmNPV               GDTAPDPPDS---------------------- 70
BmNPV-K4            GDTAPDPPDS---------------------- 70
BmNPV-Zhenjiang     GDTAPDPPES---------------------- 70
BmNPV-Xuwen         GDTAPDPPES---------------------- 70
BmNPV-10 kDa        GDTGPDLPESLKPNLKSQAFEFDSDARLGKRSSK 94
BmNPV-K3            GDIVPDLPDSLKPKLKSQAFELDSDARRGKRSSK 94
BmNPV-K1            GDIVPDLPDSLKPKLKSQAFELDSDARRGKRSSK 94
AcNPV-P10           GDIVPDLPDSLKPKLKSQAFELDSDARRGKRSSK 94
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Figure 2. *Bombyx mori* multiple nucleopolyhedrovirus (BmMNPV) ORF10 protein sequence. Alignment of amino acid sequences of ORF10 from multiple nucleopolyhedrovirus subgroup of *B. mori* using the ClustalW2 software (EMBL-EBI). The sequences were: *B. mori* nucleopolyhedrovirus isolate K4 (AF247684); isolate T3 (L33180); Zhenjiang strain (AF533973); Xuwen strain (AF536206); 10-kDa protein (S76783); isolate K3 (AF247683); isolate K1 (AF247681), and *Autographa californica* nucleopolyhedrovirus p10 gene (M10023).

The BmNPV ORF10 sequence has been previously analyzed by Hu et al. (1994). This sequence codes for a protein of 70 amino acids that is not essential for viral replication. A study by Williams et al. (1989), which involved infected cells with AcMNPV mutated in p10 protein, suggests that this protein may be related to infected cell lysis or more precisely to nucleus disintegration (Acharya and Gopinathan, 2001).

The BmMNPV ORF14 codes for the same sequence of amino acids from BmNPV, but displays amino acid modifications (Figure 3). The sequence that belongs to the ORF14 from BmMNPV had an identity of 99% with ORF23 of AcMNPV, which is related to viral folding protein (Gomi et al., 1999). It also had an identity of 88% with folding protein or F protein of PxMNPV (Harrison and Lynn, 2007), and with a folding protein of *A. californica* as well (Ayres et al., 1994). However, an identity of 84% was observed with a fusion protein of RoMNPV (Harrison and Bonning, 2003), whereas a 65% identity to MvMNPV was detected (Chen et al., 2008).

The AcMNPV ORF23 protein is not essential for viral replication (Lung et al., 2003), but is a very important structural protein, since it provides the virus entrance into the host cell. In AcMNPV, this protein is found in budded virus, which is responsible for host systemic infection. Hence, the evolution and sequence alteration of these folding proteins could dramatically affect the process of virus entrance.

Mutations in ORF23 of the AcMNPV alter the number of nucleocapsids per virion, increasing the presence of the single nucleocapsid, where the death of the insect occurs more slowly (Yu et al., 2009). Packing of multiple nucleocapsids suggests a selective advantage of baculoviruses that are MNPV in relation to the single nucleocapsid per virion (Washburn et al., 2003).

This type of protein is present in the family Baculoviridae from the *Nucleopolyhedrovirus* genera. However, protein F is present in the *Granulovirus* genera and has a similar function as viral protein GP64 (Garry and Garry, 2008).

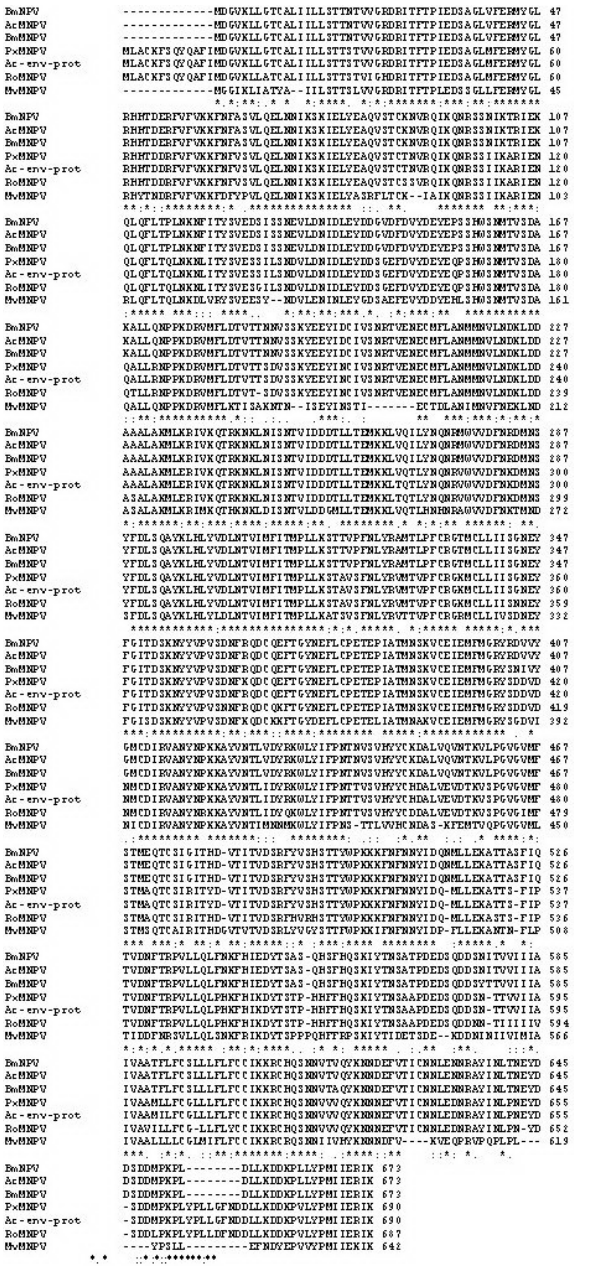

Figure 3. *Bombyx mori* multiple nucleopolyhedrovirus (BmMNPV) ORF14 protein sequence. Alignment of BmMNPV ORF14 amino acid sequences using the ClustalW2 software (EMBL-EBI) (e-value corresponding to 0.0). The sequences were: *B. mori* nucleopolyhedrovirus, BmNPV (L33180); *Autographa californica* multiple nucleopolyhedrovirus C6 clone, AcMNPV (NP047428); *Plutella xylostella* multiple nucleopolyhedrovirus, PxMNPV (YP758489); *A. californica* proteic envelope, Ac-env-prot (NP054052); *Rachiplusia or* multiple nucleopolyhedrovirus, RoMNPV (NP703013), and *Maruca vitrata* multiple nucleopolyhedrovirus, MvMNPV (YP950744).

Viral fusion proteins are present in viruses such as human immunodeficiency virus (HIV) and flu virus (*Influenza* spp) as well (Jing et al., 2008). These proteins allow the entrance of folded viruses, which have a coat based on a lipid bilayer, into the cells that they infect. The exposure of fusion proteins from flu virus or HIV to low pH and membrane receptors of targeted cells, respectively, reveals hydrophobic regions that are called fusion peptides, which are inserted directly into the hydrophobic center of the lipid bilayer of the targeted membrane (Jing et al., 2008).

Another mechanism of virus entrance into the host cell often studied is that of Rhabdovirus, which affects a broad range of animal hosts (such as insects, fishes, mammals, and humans) but also the plant kingdom. The viruses of this genus possess a glycoprotein G, which has in its sequence two to three regions with consecutive repetitions of seven hydrophobic amino acids, followed by positively charged amino acids (Carneiro et al., 2002). All these repeated sequences of hydrophobic amino acids are related to viral fusion in cell membranes. Part of this glycoprotein G (residues from 421 to 461), which includes ectodomain and transmembrane regions, could potentiate the fusion activities between the membranes (Jeetendra et al., 2003).

Similar sequences were found in the amino acid residues from BmMNPV ORF14 (amino acids 212-225, 228-239, 306-316, and 580-607; Figure 3). If the entrance of nucleopolyhedrovirus into *B. mori* cells occurs as with glycoprotein G in Rhabdovirus, further analysis should be done to verify this point.

The genetic distance related to the protein produced by ORF14 of BmMNPV, when compared to proteins of higher identity (Figure 3), was graphically represented (Figure 4) in a dendogram (Saitou and Nei, 1987), which revealed a closer correlation between BmNPV and AcMNPV proteins. The proteins are referred to as F proteins and have a similar role as GP64, which plays a role in the process of membrane fusion and is present in budded viruses, which enter the host cells via endocytosis (Garry and Garry, 2008).

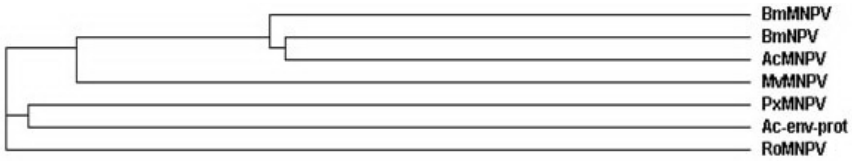

Figure 4. Phylogenetic tree using neighbor-joining method to *Bombyx mori* multiple nucleopolyhedrovirus (BmMNPV) ORF14 protein. For abbreviations, see legend to Figure 3.

BmMNPV and BmNPV ORF14

The BmMNPV and BmNPV ORF14 protein sequences were analyzed *in silico* in relation to their molecular mass, isoelectric point and signaling peptide. The gene attributed to ORF14 consists of 2022 nucleotides that code for a protein of 673 amino acids. Using the ProtParam program (http://www.expasy.ch/tools/protparam.html), the molecular mass for BmMNPV and BmNPV was estimated at 77,905 and 77,952, respectively, a probable isoelectric point of 5.47 was determined for both, as well as a signaling peptide of 24 residues was found. BmMNPV and BmNPV ORF14 were analyzed by the ProtFun 2.2 software, and the data related to protein localization and their differences were predicted (Jiang et al., 2005). This analysis (Table 2) demonstrated that both BmNPV and BmMNPV ORF14 probably code for a membrane protein that could be involved in fusion or the endocytosis process, providing the virus entrance into the

host cell. When the amino acid sequence was analyzed in order to predict the gene category, it was assumed that it could be involved with a stress response (Table 3). This could happen at the moment when the production of this protein is essential for the *Nucleopolyhedrovirus* life cycle.

Table 2. Comparison between the protein of ORF14 from *Bombyx mori* multiple nucleopolyhedrovirus (BmMNPV) and *B. mori* nucleopolyhedrovirus (BmNPV) in agreement with the probable function.

Functional category	BmMNPV		BmNPV	
	Prob	Odds	Prob	Odds
Amino acid biosynthesis	0.014	0.623	0.014	0.625
Biosynthesis of cofactors	0.073	1.011	0.069	0.962
Cell envelope*	0.804	13.186	0.804	13.186
Cellular processes	0.031	0.420	0.031	0.420
Central intermediary metabolism	0.219	3.471	0.213	3.381
Energy metabolism	0.030	0.328	0.030	0.328
Fatty acid metabolism	0.016	0.265	0.016	1.265
Purines and pyrimidines	0.569	2.341	0.568	2.339
Regulatory functions	0.013	0.080	0.013	0.080
Replication and transcription	0.019	0.073	0.019	0.073
Translaction	0.036	0.816	0.036	0.816
Transport and binding	0.834	2.033	0.833	2.032

*Indicates the probable functional category of the protein.

Table 3. Comparison of the protein of ORF14 from *Bombyx mori* multiple nucleopolyhedrovirus (BmMNPV) and *B. mori* nucleopolyhedrovirus (BmNPV) in agreement with the probable gene category.

Gene category	BmMNPV		BmNPV	
	Prob	Odds	Prob	Odds
Signal transducer	0.167	0.780	0.172	0.802
Receptor	0.156	0.916	0.163	0.958
Hormone	0.001	0.206	0.001	0.206
Structural protein	0.011	0.411	0.013	0.458
Transporter	0.024	0.217	0.024	0.217
Ion channel	0.008	0.148	0.008	0.148
Voltage gated ion channel	0.002	0.097	0.002	0.099
Cation channel	0.010	0.215	0.010	0.215
Transcription	0.030	0.235	0.030	0.236
Transcription regulation	0.021	0.166	0.021	0.165
Stress response*	0.156	1.770	0.171	1.940
Immune response	0.063	0.741	0.045	0.530
Growth factor	0.043	3.052	0.043	3.052
Metal ion transport	0.012	0.026	0.012	0.025

*Indicates the probable functional category of the protein.

Similar predicting gene structure analyses were carried out in several studies involving human proteins from the MHC complex (Jiang et al., 2005) and baculovirus GP64 protein as well (Garry and Garry, 2008).

Upon analysis of the primary protein structure in relation to cellular membrane, in which it is probably inserted, a transmembrane helix that was conserved in all baculoviral proteins was predicted (Figure 5). The transmembrane helix region was located between amino acids 580 and 602, where it is considered to be a sequence rich in hydrophobic amino acids that are close to C-terminal region of the protein.

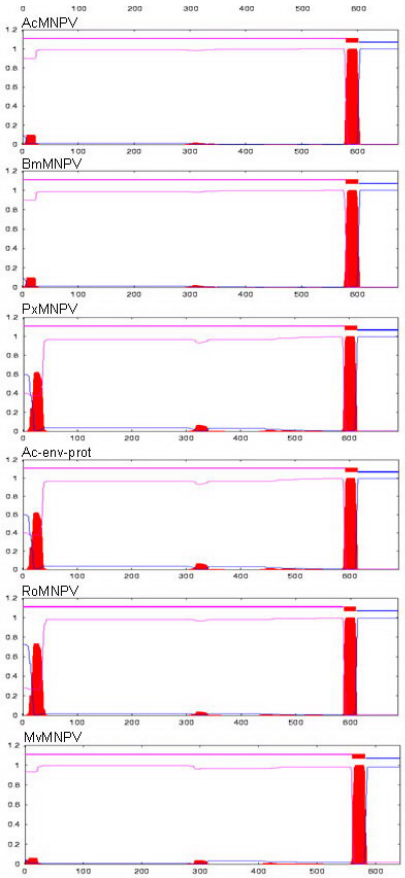

Figure 5. Baculoviral ORF14 potential transmembrane domains. The amino acid residue numbers are positioned on the x-axis and the existence probability of transmembrane domains are on the y-axis. For abbreviations, see legend to Figure 3.

The transmembrane helix analysis was conducted by Garry and Garry (2008) in order to describe GP64, a baculoviral protein that has a C-terminal transmembrane region rich in hydrophobic amino acids, similar to the one found in our analysis.

Likely, the vesicular stomatitis virus has a region, called glycoprotein G, that acts as a receptor, promoting the viral envelop fusion with the host cell membrane through a low pH, followed by the endocytosis process (Jeetendra et al., 2003). This region is also rich in hydrophobic amino acids, but when mutations (insertions and deletions) are detected, there is also a reduction in host cell fusion activity. The inactivation of viruses occurs with diethylpyrocarbonate, where histidine residues present in protein G are modified (Stauffer et al., 2007).

Regions with hydrophobic amino acids that are located in the transmembrane domain of virus proteins, are usually involved in the process of fusion between cellular membranes. These highly hydrophobic regions that encompass membrane-spanning regions are present in other baculoviral proteins (Figure 5), where there is little difference in hydrophobicity between the compared sequences.

In the region related to the amino acid in position 400, there is a hydrophobicity variation among the sequences, in which BmNPV exhibits residues more hydrophobic, but shares similarity with proteins from other baculoviruses (Figure 6). The C-terminal region of the protein also shows variations. Hydrophobicity parameters are important because they affect the interactions between molecules and their linkages. Not only hydrophobicity, but also factors such as pH and transmembrane do-

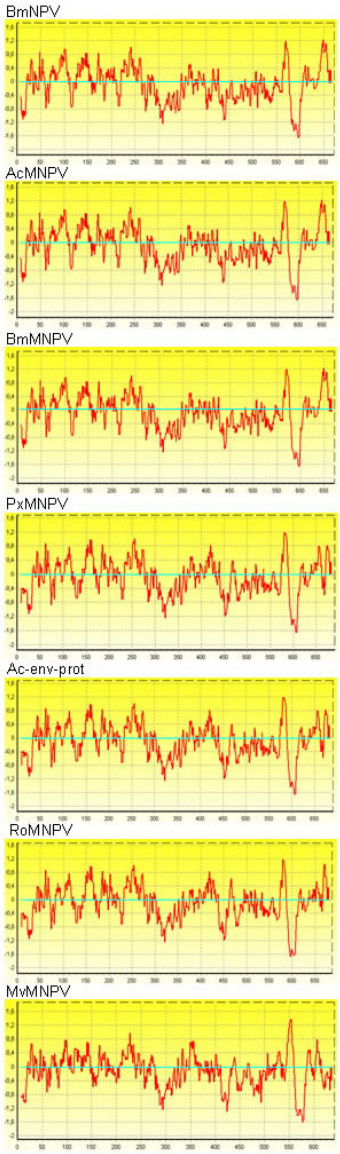

Figure 6. Comparison of the BmMNPV ORF14 hydrophilicity plots with BmNPV, AcMNPV, PxMNPV, Ac-env-prot, RoMNPV, and MvMNPV. Positive values indicate hydrophilic areas, whereas negative values indicate hydrophobic areas. Data obtained from the Bioedit program on the Hoop-Woods scale. For abbreviations, see legend to Figure 3.

mains are involved in the process of cellular adhesion. In addition, coronaviruses (Kanjanahaluethai et al., 2007) show a transmembrane structure that has a sequence of hydrophobic amino acids that works as in vesicular stomatitis virus. Thus, at low pH, it activates the fusion between the cell membranes.

Several studies have been carried out with baculoviruses with the objective of characterizing genes and identifying their functions. The data presented in this paper provide evidence that proteins produced by ORFs 10 and 14 from BmNPV and BmMNPV are highly conserved proteins in NPVs and MNPVs. The high degree of conservation, between members of these genera, indicates the importance of these proteins, and could translate to a possibly significant function, which is active throughout the infection cycle.

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