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FELLOWSHIP FINAL REPORT

Genomic characterization of six Mexican baculovirus strains with activity against *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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REPORT INFO

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Period of residence in region Centre-Val de Loire: October 12, 2020-August 31, 2021

Keywords :
Baculovirus, Spodoptera frugiperda, phylogeny, bioassay, proteins.

ABSTRACT

The fall armyworm Spodoptera frugiperda is recognized as a polyphagous, voracious, and economically important pest in America and other continents. The control of this pest has been used mainly chemical insecticides, but biological control is an alternative strategy, and different isolates of baculoviruses were evaluated for this control. In this work, the biological activity, in vitro susceptibility, phylogenetic relationship, and protein expression in insect cells of six SfNPV baculoviruses isolated from S. frugiperda were determined. The infection of the cell line Sf9 was permissive to four of the five SfNPVs strains and non-infective to the SfGV strain. SfNPV isolates from Argentina, Honduras and the USA were more virulent than those from Mexico, resulting in up to 12 and 1000 times more effectiveness. The genes lef-8, lef-9 y polh/granulin were partially amplified in five SfNPVs and the SfGV, where nucleotide changes were identified in lef-8 of the SfNPVs and lef-8, lef-9, and granulin from SfGV-RV. The phylogenetic analysis showed that the five strains SfNPVs turn out to be closely related to the others reported SfNPV, just like the strain SfGV-RV and SfGV. The protein expression of host cells in response to SfNPV-Fx identified six proteins differentially expressed. They are involved in changes in the host cell, altering its cellular structure and normal functions. The characterization from these six SfNPV strains has established the basis for exploring the specific mechanisms, evolution, and ecology to evaluate the potential to be used as biological control agents against S. frugiperda.

1- Introduction

Maize (*Zea mays* L. subsp. *mays*) (Poaceae) is a crop of great economic and food importance worldwide (Moya Raygoza et al., 2018), and since several decades ago monocultures have been established in agricultural systems to meet their demand, which has influenced the increase in the presence of various pests (Altieri et al., 2018). The fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) (Smith, 1797) is recognized as a polyphagous, voracious, economically important pest in North and South America (Cokola et al., 2021), affecting a wide variety of plant species such as maize, sorghum, cotton and soybeans (Montezano et al., 2018). The control of fall armyworm mainly relies on chemical insecticides, but resistance against these treatments has risen (Gutierrez et al., 2019). Consequently, biological control is a sustainable strategy. Entomopathogens,

parasitoids, and predators provide promising alternatives since, in addition to their specificity, they do not represent a risk to human health, the environment, or to beneficial insects (Aktar et al., 2009).

Baculoviruses belong to the family Baculoviridae (Harrison et al., 2018), are generally highly selective pathogens of insects of the orders Lepidoptera, Hymenoptera and Diptera (Théze et al., 2018). They are a diverse group of viruses with supercoiling, circular double-strain DNA genomes, ranging in sizes from 80 to 180 kb and encoding between 90 and 180 genes. The two commonly found virion phenotypes in baculovirus are occlusion-derived virions (OVD) and budded virions (BV) (Herniou et al., 2001; Jehle et al., 2006). Occlusion bodies (OBs) are viral particles that allow viruses to survive in the environment and are composed of a crystalline matrix of protein, polyedrin in nucleopolyedroviruses (NPV) and granulin in granuloviruses (GV) that occlude virions. The Baculoviridae family is divided into 4 genera: *Alphabaculovirus* (Lepidoptera specific NPVs; group I and II), *Betabaculovirus* (Lepidoptera specific GVs), *Gammabaculovirus* (Hymenoptera specific NPVs), and *Deltabaculovirus* (Diptera specific NPVs) (Jehle et al., 2006).

Autographa californica multiple NPV (AcMNPV) polyhedrin (*polh*) gene was the first baculovirus gene sequenced (Rohrmann et al., 1981) and used for phylogenetic studies (Cowan et al., 1994). However, as genome sequences became available, some studies showed different genes had different phylogenies, implying that to understand the evolution of baculoviruses, some genes were more appropriate than others. This led to the definition of core genes, and the selection of a set of conserved genes that combined will lead to more robust phylogenetic results (Herniou et al., 2001; Herniou, 2003).

Several baculoviruses with activity towards *S. frugiperda* have been isolated and studied throughout the American continent (Barrera et al., 2011; García et al., 2020; Ordóñez et al., 2020). The Food and Plant Biotechnology laboratory of the Life Sciences Division, Campus Irapuato-Salamanca of the University of Guanajuato, Mexico, has a collection of six previously characterized strains of SfNPVs, with molecular variations and different biological activity to *S. frugiperda* (Núñez et al., 2014; Rios et al., 2011; Ríos et al., 2012).

The objective of this work was to characterize at the biological, molecular and phylogenetic level 6 strains of baculovirus isolated from *Spodoptera frugiperda* in different regions of the American continent. Susceptibility of infection in insect cell lines and proteins expressed during the infection process in this insect were studied.

2- Experimental details

Virus. Were used five SfNPVs strains: SfNPV-An_2 isolated in Coahuila, Mexico; SfNPV-Arg isolated in Argentina; SfNPV-Fx isolated in the United States; SfNPV-Ho isolated in Honduras and SfNPV-Sin isolated in Sinaloa, Mexico, and a granulovirus, SfGV-RV isolated in Coahuila, Mexico, all with activity toward *S. frugiperda.*

Viral propagation. Larvae of *S. frugiperda* were used, which were maintained in a semi-artificial diet (100 mL distilled water; 12.5 g bacteriological agar; 120 g corn; 50 g yeast; 5 g wheat germ; 25 g ground corn spike; 2.5 g sorbic acid; 5 g ascorbic acid; 3.125 g methylparaben; 8.75 g slats mixture; 62.5 g soybean; 3.125 mL formaldehyde 37%; 0.75 mg streptomycin; and 18.75 g Vanderzant Vitamin mixture) under insectarium conditions (60% relative humidity, 26°C and 16:8 hours of light: darkness) in an environmental chamber (PERCIVAL). The infection was carried out on the diet surface, using 500 µl of $1x10^6$ OB/ml and 10 larvae of 2° stage of *S. frugiperda*, individualized with a grid were placed and subsequently incubated for 6-7 days under the previously described conditions.

Biological characterization of SfNPV strains. The LC_{50} was determined in the first instar larvae of *S. frugiperda;* the bioassay was performed using 20 larvae for each dilution, distributing ten larvae per petri dish containing a semiartificial diet. The highest concentration

used for the SfNPV-Sin strain was $4.2x10^8$ OB/ml; for SfNPV-An_2, it was $3.9x10^3$ OB/ml and $3.1x10^4$ SfNPV-RV, and six concentrations were used with a dilution factor between 0.5-0.75. The larval mortality was documented for five days, and the results were analyzed by Probit analysis (Ibarra and Federici, 1987).

DNA extraction. The OBs from infected larvae were purified through sucrose density gradients by ultracentrifugation in an SW-32 rotor (Optia XPN-100, Beckman Coulter) at 24,000 rpm for 1:30 hours; the OBs were treated with a TE buffer solution (0.01 M Tris-HCl, 0.001 M EDTA, pH 7.6) and alkali solution (0.1M Na_2CO_3, 0.1M NaCl, pH 10.8) over 15 minutes in agitation; the virions were purified through sucrose density gradients at 28,000 rpm for 40 minutes, and later the DNA extraction took place with proteinase K buffer (0.01 M Tris, 0.005 M EDTA, 0.5% SDS), incubated during 15 minutes at 60°C and 0.1 µg of proteinase K (Invitrogen) incubated for 30 minutes at 60°C. The DNA extractions were performed with phenol:chloroform:isoamyl alcohol (25:24:1), precipitated with isoamyl alcohol and washed with 70% ethanol, and the pellet was dissolved in sterile distilled water.

Amplification by PCR. *lef-8, lef-9* and *polh* genes were amplified by PCR from DNA extracted from the six baculovirus strains, for which degenerated oligonucleotides were used (Thézé et al., 2018) and the universal oligonucleotides M13 Rev, M13 Fw y BGH Rev for the direct sequencing of the PCR products. The specific oligonucleotides for the gene *lef-8* amplify a 702 bp fragment, the *lef-9* 295 bp, and the *polh* of 540 bp. The fragments amplification of *lef-8* and *lef-9* genes was performed by PCR touchdown (initial denaturalization at 95°C/ 3 min; 15 cycles decreasing the alignment temperature -1°C each cycle 95°C/30 seconds, 55°C/ 30 seconds, 72°C/30 seconds; 20 cycles 95°C/ 30 seconds, 60°C/ 30 seconds, 73°C/ 30seconds; final extension 72°C/ 7 minutes). The *polh* gene was amplify by conventional PCR (initial denaturalization 95°C/ 4 minutes; 95°C/ 30 seconds, 55°C/1minute, 72°C/ 1 minute; final extension 72°C/ 10 minutes). The PCR product was purified with the PureLink PCR purification Kit (Invitrogen), and the amplicons were sequenced on the Illumina platform in the company MACROGEN in Korea.

Phylogenetic analysis. The nucleotides sequences of *lef-8, lef-9* and *polh* were downloaded from the NCBI GenBank from the genomes of SfNPVs 3AP2 (GenBank: EF035042), 19 (GenBank: EU258200), 459 (GenBank: MK503924), ColA (GenBank: KF891883), ArgM (GenBank MW162628), 281 (GenBank: MK503923) and B (GenBank: HM595733) and SfGVs VG008 (GenBank: KM371112), Arg (GenBank MH170055) and VG014 (GenBank: KJ698693, KJ698695 and KJ698691for genes *lef-8, lef*-9 and *polh* respectively) to compare them with the partial sequences of the six *S. frugiperda* baculoviruses used in the current study. For the phylogenetic analysis, were used the amino acid sequences of Lef-8, Lef-9 and Polh proteins from the reference genomes of SfNPVs and SfGVs previously mentioned.

The nucleotides sequences of the sequenced amplicons were assembled in the SeqMan 5.0 program (DNASTAR Inc.). The amino acid alignment was performed in the Mega X program (Kumar et al., 2018) using the Muscle algorithm and fitting to the size of the fragments obtained from the sequencing, to be later concatenated in the Mesquite software (version 3.5.1). The Phylogenetic analysis was executed in the Mega X software using the neighbor-joining method (Saitou and Nei, 1987), and the amino acid substitution model applied was p-distance. Gaps were treated as missing data. Bootstrap analyses (using 1000 replications) were used to assess the confidence in the branching order.

Cell line infection tests. *In vitro* infections were performed to test whether the Sf9 (Thermofisher) cell line is permissive to the six baculoviruses described above. The cell line was maintained in Sf900 culture media (Thermofisher) supplemented with 5% fetal bovine serum. The cell culture flasks (25 cm^2) were cultured 24 hours previous to the infection with $1x10^6$ cells; the infection was performed with ODV for which $2x10^8$ OBs were

resuspended in 300 µL of an alkali solution (0.1M Na_2CO_3, 0.1M NaCl, pH 10.8) and agitation incubated (50 rpm) for 10 minutes and afterward mixed with cell culture media Sf900 and sterilized by filtration (Lynn, 2003).

Comparative protein analysis by two-dimensional gel electrophoresis. Differential protein expression was evaluated at eight hours post infection with the SfNPV-Fx baculovirus in the Sf9 cell line. The cell culture flasks (25 cm^2) were cultured 24 hours before the infection with $1x10^6$ cells and attached overnight. The medium was exchanged with 1 ml of fresh medium alone (control) or with inoculum at a multiplicity of infection (MOI) of 10 (Zhang et al., 2017) for two hours and without removing the inoculum, both infected and mock-infected flasks were replenished with 4 ml fresh media and incubated for eight hours. The recovered culture medium and cells were centrifuged at 2,000 rpm/3 min, and the supernatant was discarded. The cell pellet was washed twice with sterile distilled water, and the proteins were extracted using lysis buffer (8 M urea, 2 M thiourea, 0.5% CHAPS, 1 mM DTT, and 1mM PMSF) (Vierstraete et al., 2003). The sample was incubated at 4°C for 2 hours with vortexing for 1 min every 20 min. Finally, it was centrifuged at 12,000 rpm/15 min/4°C, and the supernatant was recovered.

The protein concentration was determined by the Bradford assay (Kit II Protein assay Bio-Rad). Isoelectric focusing electrophoresis was carried out with 7-cm (pH 3–10) IPG strips (ReadyStrips, Bio-Rad), according to the manufacturer's instructions; 0.2 mg protein was loaded onto the gel, and the strips were rehydrated for 16 h in rehydration solution (Urea 8 M, CHAPS 2%, DTT 1%, IPG buffer 1% (carrier ampholyte mixtures pH 3-10)) in a final volume of 125 µl. The isoelectric focusing (PROTEAN i12 Bio-Rad) was programmed in a gradient mode, which was first focused for 20 min at 250 V, 4,000 V for 1 hour, and 4,000 V until 15 kVh had been achieved. Later, the strips were equilibrated in buffer with 6 M Urea, 50 mM Tris pH 8.8, 30% glycerol, 2% SDS, and 0.002% bromophenol blue, and were subsequently treated by the reduction of dithiothreitol 1% for 15 min and alkylation of iodoacetamide 2.5% for 15 min. Then, the treated strips were transferred onto 12% uniform SDS-polyacrylamide gels, and each was run at 90 Volts for 30 min and 120 Volts until the bromophenol blue dye reached the bottom of the gel (Echeverri et al., 2010). The gel was stained with the Coomassie blue stain method and then scanned (Gel Doc™ EZ Imagen, Bio-Rad).

Three independent biological replicates were conducted for each of the control and baculovirus–host cell treatments. Spot matching, quantitation, and significant differences between treatments were determined using Delta2D ver. 4.8.2 software (DECODON). Six spots were selected to be identified by MS/MS and analyzed by electrospray Ion Trap Mass Spectrometry (ESI-TRAP) in the Proteomics and Gene Expression Laboratory of the Potosino Institute of Research, Science and Technology in the city of San Luis Potosi, San Luis Potosi, Mexico.

3- Results and discussion

Cell line infectivity. The evaluation of susceptibility of the Sf9 cell line was tested with the six baculoviruses in the study. It resulted in the replication permissiveness of four out of the six studied baculoviruses strains, being these the SfNPV-An_2, SfNPV-Arg, SfNPV-Fx, and SfNPV-Ho strains (Fig. 1) which were capable of infecting, replicating and developing OBs within the cell. In constrast, the strains SfNPV-Sin and SfGV-RV were not infectious to this particular cell line.

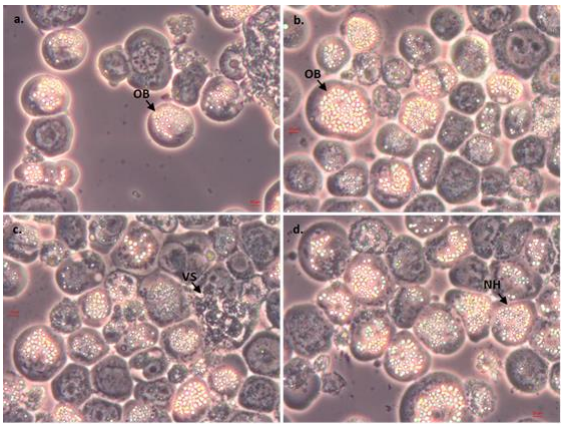

Fig. 1. Sf9 cells at 96 hours post-infection with the baculoviruses a. SfNPV-An_2, b. SfNPV-Arg, c. SfNPV-

Fx, d. SfNPV-Ho. OB: Occlusion bodies; VS: Virogenic stroma; NH: Nuclear hypertrophy. Objective 40X. Scale bar 10 µm

The Sf9 cell line infections with the permissive SfNPV strains were performed using ODV, presenting infection signs in isolated cells at day five post-infection. On the 10th day, the supernatant was collected, which contained the BV, which was used repeatedly to infect a new cell culture, presenting infection signs after the third day. It was detected that a significant number of infected cells with the SfNPV-Fx were lysed between 4-6 post-infection days and the SfNPV-An_2, SfNPV-Arg, and SfNPV-Ho also lysed the cells, however, not with the efficiency of SfNPV-Fx (data not presented).

Biological characterization of SfNPV strains. From the biological characterization of the three strains evaluated in *S. frugiperda* larvae with the Sf baculoviruses (SfNPV-An_2, SfNPV-Sin, and SfGV-RV), the LC_{50} calculated from the SfNPV-Sin strain was 1,619.24 OB/mm^2, for the SfNPV-An^2 strain it was 13.7 OB/mm^2, and for SfGV-RV it was 3,500 OB/mm^2 (Table 1). The LC_{50} of the other three strains involved in the present study (SfNPV-Arg, SfNPV-Fx, and SfNPV-Ho), which had been previously characterized (Núñez et al., 2014), was 1.15 OB/mm^2 for SfNPV-Arg, 3.42 OB/mm^2 for SfNPV-Fx, and for SfNPV-Ho it was 4.36 OB/mm^2 (Table 1). These results demonstrated that the Mexican strains are statistically less virulent to the *S. frugiperda* colony than the strains from other countries. It was shown that the most virulent strain was the SfNPV-Ar strain; the Mexican strain with the highest potential was strain SfNPV-An_2.

Table 1. LC_{50} from SfNPV strains with activity towards *S. frugiperda*.

Viral strain	**n**	**Slope (±SE)**	**LC_{50} (OB/mm^2) (95% Fiducial limits)**	**X^2**
SfNPV-Sin	360	1.36 (±0.13)	1,619.24(1,123.51-2,334.01)**d**	2.23
SfNPV-An_2	360	0.96 (±0.1)	13.7 (21.24-83.32)**c**	1.46
SfGV-RV	360	1.31 (±0.21)	3,500(2,200.10-5,700.43)**e**	1.30
SfNPV-Arg	360	1.72 (±0.19)	1.15 (0.65-2.14)**a**	4.5
SfNPV-Fx	360	1.45 (±0.08)	3.42 (1.85-6.37**b**	4.8
SfNPV-Ho	360	2.06 (±0.14)	4.36 (2.39-7.95)**b**	1.4

n: Number of evaluated larvae; X^2: chi-square

Sequencing of *lef-8, lef-9,* and *polh* genes. The genetic polymorphism was studied with the six baculoviruses, using the sequence from *lef-8*, *lef-9*, and *polh* genes, resulting in amplicons from approximately 702, 295, and 540 bp, respectively. After the sequence editing, the sequence alignements were 613 pb long the gene *lef-8*, 163 for *lef-9*, and 486 for *polh*. The sequences used for the phylogenetic analysis were upload to the GenBank from the NCBI with the access numbers MK501795-MK501800 (*lef-8*) MK507900-MK507905 (*lef-9*) y MK558035-MK558040 (*polh/gran*).

The fragment alignment from the nucleotide sequences of *lef-8*, *lef-9*, and *polh* of the five SfNPVs was compared with the reference strain SfNPV (Access Number: NC 009011). Regarding the *lef-8* alignment, there were differences in nucleotide substitutions but not in the amino acids, turning in identities superiors to the 98%, in the SfNPV-An_2, SfNPV-Arg, SfNPV-Fx, SfNPV-Ho, and SfNP-Sin strains, respectively. The aligned sequences of *lef-9* they did not show changes in nucleotides or amino acids. In *polh*, differences were not present in the nucleotide or amino acid sequences between the five SfNPVs studied concerning the SfNPV.

For the studied *Betabaculovirus*, SfGV-RV was used to reference the SfGV granulovirus from the data bank NCBI (GenBank: KM371112). The sequenced fragments *lef-8*, *lef-9*, and *granulin* have shown nucleotide and amino acid differences and identities higher than 98% for the three sequences. In *lef-8*, there were eight nucleotide substitutions and two amino acid substitutions, while *lef-9* and *granulin* have shown three and nine nucleotide substitutions, respectively, although none amino acid substitution.

Phylogenetic analysis. A total of 499 positions of concatenated amino acid sequences from *lef-8*, *lef-9*, and *polh* of the five nucleopolyhedroviruses SfNPV-Arg, SfNPV-Ho, SfNPV-Fx, SfNPV-An_2, and SfNPV-

Sin also and the granulovirus SfGV-RV isolated from *S. frugiperda*, as well as the SfNPVs and SfGVs used as references and downloaded from the NCBI GenBank, were used for the phylogenetic analysis. The phylogenetic reconstruction (Fig. 2) included the five strains SfNPV-Arg, SfNPV-Fx, SfNPV-Ho, SfNPV-Sin, SfNPV-An$_2$ along with the other SfNPVs strains already reported in the same clade, and the SfGV-RV strain was strongly related to the SfNPV strain reference strain. The NPVs are found gathered in the *Alphabaculovirus* group and the GV within the *Betabaculovirus*.

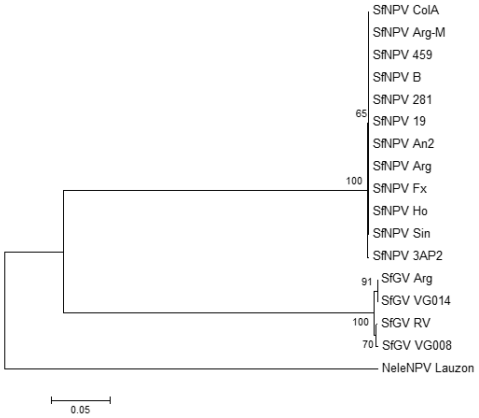

Figure 2. Phylogenetic tree constructed from partial Lef-8, Lef-9 y polh/granulin amino acid sequences of baculovirus isolates by the neighbor-joining analysis method. Bootstrap resampling was done 1000 times, and resulting bootstrap values are shown on the corresponding branches. The NeleNPV virus was used as outgroup. Scale bar indicates the number of substitutions per site.

Comparative analysis by two-dimensional gel electrophoresis. Among the six strains studied, the SfNPV-Fx strain presented the highest virulence towards the Sf-9 cell line. For this reason, only with this strain was analyzed the protein differential expression at eight h.p.i. In the Fig. 3, they have marked out the six more differential spots selected to be identified by MS-MS. Two spots showed in the control condition and absented in the infected condition were spot 1, corresponding at the protein Tubulin beta chain, which changed its expression approximately seven times. Spot 2, corresponding to Eukaryotic translation initiation factor 5A. Likewise, a spot present in the infected condition and not in the control condition (Spot 3) was the protein Eukaryotic translation initiation factor 6, which changed his expression 20 times. The rest of the spots were present in both conditions. They were selected because the expression level was deficient, for example, spot 6, corresponding to the 60S acidic ribosomal protein, or if the differential expression was higher, spot 4, representing the Pyruvate dehydrogenase E1 component subunit alpha mitochondrial.

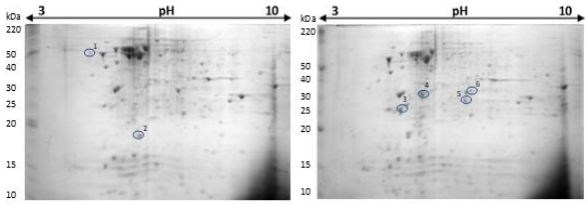

Figure 3. Representative gels showing expression at 8 h p.i. in cell lysates of (A) control Sf9 cells; (B) SfNPV-Fx infected Sf9 cells. Numbers indicate spots that were extracted from gels and subjected to MS/MS analysis.

The present study evaluated the permissiveness in the cell line Sf9 to the infection of five SfNPVs and an SfGV isolated from *S. frugiperda*, turning out to be permissive to four of the tested SfNPVs (SfNPV-An$_2$, SfNPV-Arg, SfNPV-Fx y SfNPV-Ho). Several authors have studied the *in vitro* permissiveness of other SfNPVs in different cell lines. However, only those derived from *S. frugiperda* have turned out permissive (IPLB-SF-21AE, Sf9, IAL-SFD), and some others have reported limited viral replication in cell lines from different hosts (Almeida et al., 2010; Sihler et al., 2018), which denote a high *in vitro* specificity from *Spodoptera* baculovirus, like SeMNPV and SpltMNPV, have turn out to be also susceptible to cell lines derived from their hosts (Chan et al., 2017), and in some cases to cell lines from their same host genera, suggesting that the NPVs strains with activity towards the *Spodoptera* genus are susceptible to infect exclusively cell lines derived from insects of their genus.

The phenotypical diversity is typically observable between NPVs, presenting different biological activities. The biological activity of the strains SfNPV-Sin, SfNPV-An$_2$, and

SfNPV-RV strains was compared with the other baculovirus strains involved in the present study and previously characterized (Núñez et al., 2014). The native strain SfNPV with the highest virulence was SfNPV-An_2 since it presented an LC_{50} of 13.7 OB/mm^2. In contrast, the strain with the lowest virulence was SfNPV-Sin ($1.619x10^3$ OB/mm^2), was 1,600 times less virulent than the Argentine strain SfNPV-Arg (1.15 OB/mm^2) as well as with the other two strains, USA strain SfNPV-Fx (3.42 OB/mm^2) and the Honduran strain SfNPV-Ho (4.36 OB/mm^2) (Rangel-Núñez et al., 2014). It was also 10.79 times less virulent than SfNPV-An_2 and had less potential for the biological control of *S. frugiperda*. The strain SfNPV-RV was 2.47 times less virulent than SfNPV-Sin, a situation that was expected as it is a GV. In this work, a significant variation was observed in the virulence levels between the studied strains, contrasting with those obtained for other authors with a more considerable similarity in the virulence levels of isolated strains of *S. frugiperda* has been observed (Rowley et al., 2010).

Genotypical variants have been identified from SfNPV isolates of different geographic areas within the American continent, like Argentina, Antilles, Mexico, United States, Nicaragua, Colombia, and Honduras (Barrera et al., 2011; Rangel-Núñez et al., 2014; Ríos et al., 2012), and it is well known that the baculovirus genotypical variants generally exhibit phenotypical variations, particularly concerning its pathogenicity and speed of kill (Harrison et al., 2008). The genetic variations persistent in the same species occur due to recombinant events, point mutation, insertion or DNA deletion, host DNA acquisition, and this occurs in different populations of the same NPV species, thus suggesting that its heterogeneity is vital for the survival, adaptation and virus evolution. Consequently, the sequencing of the different strains of SfNPV analyzed in this work should later give the guideline to know and study these variations.

Other tools used to characterize genetic variations in isolates are PCR techniques, sequencing, and phylogenetic inference in highly conserved baculovirus genes. In the studied SfNPVs, minor variations occurred at the nucleotide level in the fragments of the *lef-8* gen, and there was no change in the *lef-9* and *polyhedrin* gene. In SfGV-RV, there was variation presented in the *lef-8, lef-9,* and *granulin* genes fragments. In the United States, part of a 40 *S. frugiperda* NPVs collection was studied phylogenetically from the USDA (Rowley et al., 2010), identifying nucleotide changes exclusively in the gene fragments from *lef-8* and *polyhedrin*. The present study only observed slight changes at the nucleotide level in *lef-8*, but these changes were not reflected at the amino acid level.

The phylogenetic reconstruction grouped all SfNPVs in the same clade, it wasn´t consistent with the localities in where they were isolated, since some geographically distant strains showed significant similarity; SfNPV-Arg, Argentinian isolated and SfNPV-Fx, American isolated, shown to be correlated despite being isolates from different countries (they even had similar virulence levels), and both NPVs isolated in Mexico (SfNPV-Sin and SfNPV-An_2) were grouped in the same clade too, which shows a phylogenetic similarity between NPVs with activity towards the same species of insects but isolated in other places. Furthermore, the enormous difference in virulence presented by both Mexican SfNPV strains indicates very different strains, even and they have been isolated in the same country. Finally, when compared these strains with others previously sequenced, were observed a high similarity regardless of whether they were from separate geographic regions, coinciding with what was reported by Popham et al. (2021) in their most recent study with strains of *S. frugiperda* from different areas.

4- Conclusion

This study matches what was previously reported by other authors regarding the high specificity exhibited by SfNPVs, which opens the interest to a depth study in the virus-host interaction and interpreted the specificity mechanisms of said baculoviruses. The SfNPV-Fx strain shown a higher lytic capacity than the other baculoviruses studied *in vitro*. This is the reason why it would be interesting to do further

research in that matter. Another critical point to highlight is the genotypic and phenotypic variability found between the investigated SfNPV isolates. This highlight the need for sequencing the genomes of several SfNPVs to compared at genomic level genetic variability which could be associated with differences in biological activity. All this would make it possible to determine in the future which of these American strains of SfNPV are the most suitable to be developed for biological control programs against the fall armyworm.

5- Perspectives of future collaborations with the host laboratory

In this research project, a first molecular characterization of six strains of baculovirus with virulence toward *S. frugiperda* was carried out. The strains studied showed a great similarity when analyzing 3 conserved genes, but a great difference was observed at the virulence level between them. It is necessary to continue working with Dr. Herniou's group to complete the assembly and analysis of the six sequenced SfNPV genomes, to be able to establish the evolutionary relationships between the strains and to identify the differences at the level of specific virulence genes, which affect their different pathogenicity levels, in order to be able to select those strains of baculovirus with a greater potential toward the fall armyworm, the most important pest of maize in the world.

6- Articles published in the framework of the fellowship

Ingrid Zanella-Sáenz, Elisabeth A. Herniou, Ilse Alejandra Huerta-Arredondo and Ma. Cristina Del Rincón-Castro· 2021. Biological and genetic characterization of six baculoviruses isolated from *Spodoptera frugiperda* (Lepidoptera: Noctuida e) in its native geographical range. Journal of Economic Entomology.

7- Acknowledgements

We thank Jonatan C. Rangel-Núñez for their technical assistance with all experiments. This work was supported by LE STUDIUM SMART LOIRE VALLEY Fellowships Programme under the Marie Skłodowska-Curie grant agreement (No. 665790), and the Consejo Nacional de Ciencia y Tecnología (CONACYT) with the Grant (No. 140615) and the Ingrid Zanella´s fellowship (No. 495732).

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