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Spodoptera frugiperda multiple nucleopolyhedrovirus as a potential biological insecticide: Genetic and phenotypic comparison of field isolates from Colombia

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ABSTRACT

Thirty-eight isolates of *Spodoptera frugiperda* multiple nucleopolyhedrovirus (SfMNPV), collected from infected larvae on pastures, maize, and sorghum plants in three different geographical regions of Colombia, were subjected to molecular characterization and were compared with a previously characterized Nicaraguan isolate (SfNIC). Restriction endonuclease analysis (REN) using six different enzymes showed two different patterns among Colombian isolates, one profile was particularly frequent (92%) and was named SfCOL. The physical map of SfCOL was constructed and the genome was estimated to be 133.9 kb, with few differences in terms of number and position of restriction sites between the genomes of SfNIC and SfCOL. The *Pst*I-K and *Pst*I-M fragments were characteristic of SfCOL. These fragments were sequenced to reveal the presence of seven complete and two partial ORFs. This region was collinear with SfMNPV *sf20–sf27*. However, two ORFs (4 and 5) had no homologies with SfMNPV ORFs, but were homologous with *Spodoptera exigua* MNPV (*se21* and *se22*/*se23*) and *Spodoptera litura* NPV (*splt20* and *splt21*). Biological characterization was performed against two different colonies of *S. frugiperda*, one originating from Colombia and one from Mexico. Occlusion bodies (OBs) of the SfCOL isolate were as potent (in terms of concentration–mortality metrics) as SfNIC OBs towards the Mexican insect colony. However, SfCOL OBs were 12 times more potent for the Colombian colony than SfNIC OBs and three times more potent for the Colombian colony than for the Mexican colony. SfCOL and SfNIC showed a slower speed of kill (by ~50 h) in insects from the Colombian colony compared to the Mexican colony, which was correlated with a higher production of OBs/larvae. SfCOL is a new strain of SfMNPV that presents pathogenic characteristics that favor its development as the basis for a biopesticide product in Colombia.

1. Introduction

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is an important and widely distributed pest in tropical and sub-tropical areas of the Americas. This species can cause severe yield losses in maize, rice, and sorghum (Murua and Virla, 2004; Sparks, 1979). Chemical control is widely employed against this pest but can have an adverse impact on populations of non-target organisms and excessive use can favor the development of pest resistance (Chandler and Summer, 1991). The multinucleocapsid nucleopolyhedrovirus (NPV) of *S. frugiperda* (SfMNPV) has been isolated from fall armyworm populations in North, Central, and South America (Berretta et al., 1998; Escribano et al., 1999; Loh et al., 1982; Shapiro et al., 1991). Some isolates have been evaluated under field conditions as potential biopesticides to control *S. frugiperda* on maize (Cisneros et al., 2002; Moscardi, 1999) resulting in important levels of larval mortality combined with significant mortality from natural parasitism (Armenta et al., 2003; Castillejos et al., 2002).

Three SfMNPV isolates from Nicaragua, United States, and Brazil have been biologically and genetically characterized and their genomes have been completely sequenced (Harrison et al., 2008; Simón et al., 2004, 2011; Wolff et al., 2008). With the aim of selecting a candidate SfMNPV isolate for use in biocontrol experiments in Mexico and Honduras, four SfMNPV isolates were collected from geographically distant populations (United States, Nicaragua, and Argentina) and were characterized by restriction endonuclease (REN) analysis (Escribano et al., 1999). The Nicaraguan isolate (SfNIC) was selected for formulation and field trials in Honduras and Mexico as it showed the highest insecticidal activity against a Mexican population of *S. frugiperda*, as measured by lethal concentration metrics (Williams et al., 1999).

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The population genetic structure and genomic organization of the SfNIC isolate has been the subject of intensive study. Following plaque purification, the SfNIC population was found to comprise nine genotypes (named SfNIC-A to I). The principal variation between the genotypes was located in a single region of the genome, between map units 14.8 and 26.8. This region includes *Pst*I-F, -L, -K fragments (Simón et al., 2004; Simón et al., 2005b). These genetic differences were reflected in important differences in the insecticidal activity, speed of kill, occlusion body (OB) production or number of virions within OBs of each of the genotypes (Simón et al., 2004, 2008).

In Colombia, *S. frugiperda* causes losses of up 60% in maize and the need for sustainable control measures against this pest is well recognized in this region (ICA, 2008; Quintero et al., 2004). The aim of this study was to select a SfMNPV isolate for use against *S. frugiperda* in a control program currently under development in Colombia. To achieve this, the natural diversity of SfMNPV isolates collected in Colombia was evaluated by molecular and biological characterization and compared with the Nicaraguan isolate that has been extensively characterized.

2. Materials and methods

2.1. Insects – source and rearing

Larvae of *S. frugiperda* were obtained from two laboratory colonies. One colony was established at the Universidad Pública de Navarra (UPNA) with pupae received from ECOSUR, Tapachula and Southern Mexico. This colony was refreshed periodically with pupae from a colony maintained in ECOSUR over a period of approximately 5 years. The second colony was established in the Biological Control Laboratory of the Colombian Corporation of Agricultural Research (CORPOICA) using larvae collected from maize fields close to Bogota, Colombia. Both colonies were maintained at 25 °C, 75% RH (relative humidity) and 16 h light–8 h dark photoperiod on a wheat germ-based semi-synthetic diet (18% corn flour, 3.4% brewer's yeast, 3.2% wheat germ, 1.5% casein, 0.45% ascorbic acid, 0.11% nipagin, 0.05% formaldehyde, 0.13% benzoic acid and 1.5% agar) described by Greene et al. (1976).

2.2. Virus isolates and amplification

Thirty-eight *S. frugiperda* larvae with typical signs and symptoms of baculovirus infection were collected in pastures, maize and sorghum crops located in three departments of Colombia: Córdoba, Tolima and Meta. OB suspension obtained from the field collected samples were amplified in a single passage in *S. frugiperda* fourth instars from the Colombian colony by the droplet feeding method (Hughes and Wood, 1981). For this, 25 starved larvae were inoculated orally with an OB suspension and reared individually until death. The OBs from cadavers were purified as described by Caballero et al. (1992). The OB pellet was resuspended in two volumes of milli-Q water and the concentration was determined using an improved Neubauer hemocytometer (Hawksley Ltd., Lancing, UK) under phase contrast microscopy at 400×. Purified OBs were stored at 4 °C.

2.3. Viral DNA extraction and REN analysis

Virions were released from OBs by mixing 100 μl of purified OB suspension (~10^9 OBs/ml), with 100 μl of 0.5 M Na_2CO_3, 50 μl of 10% sodium dodecyl sulphate (SDS) in a final volume of 500 μl and incubating for 10 min at 60 °C. Undissolved OBs and other debris were removed by low speed centrifugation (3800 × *g*, 5 min). The supernatant containing the virions was treated with 25 μl proteinase K (20 mg/ml) for 45 min at 50 °C. Viral DNA was extracted twice with saturated phenol and once with chloroform and isolated from the aqueous phase by alcohol precipitation. The pellet was resuspended in 50–100 μl of 0.1× TE (Tris–EDTA) buffer for 10 min at 60 °C. DNA concentration was estimated by spectrophotometric absorbance at 260 nm wavelength. For REN analysis the 38 Colombian isolates were compared with reference to SfNIC (Simón et al., 2004). A sample of 2 μg of viral DNA was mixed with 10 U of one of the following restriction enzymes *Pst*I, *Bam*HI, *Hin*dIII, *Eco*RI, *Bgl*II and *Sma*I (Takara, Shiga, Japan) and incubated for 4–12 h at 37 °C. Reactions were stopped by mixing with 4 μl of loading buffer solution (0.25% w/v bromophenol blue, 40% w/v sucrose). Electrophoresis was performed using horizontal 1% agarose gels in TAE buffer (0.04 M Tris–acetate, 0.001 M EDTA, pH 8.0) at 20 V for 10–24 h. DNA fragments were stained with ethidium bromide and visualized on a UV transilluminator (Chemi-Doc, BioRad, California, USA). An isolate showing the predominant REN profile was named SfCOL and was selected for further molecular and biological characterization.

2.4. Physical mapping

The physical map of SfCOL was achieved by comparison with the complete genome sequence and the physical map of the Nicaraguan isolate (Simón et al., 2004, 2005a, 2011). For this, restriction fragments obtained after digestion of SfCOL DNA with different enzymes were compared with those of Nicaraguan isolate and co-migrating fragments and isolate specific fragments were identified. Fragments absent in the SfCOL profiles were eliminated from the physical maps and the isolate-specific fragments were included. On this basis, preliminary physical maps were constructed. Physical maps were confirmed by terminal sequencing of SfCOL isolate-specific *Pst*I fragments.

Two differential bands that were found to be characteristic in the SfCOL *Pst*I profile, namely *Pst*I-K (4.9 kb) and *Pst*I-M (3.6 kb) fragments, were used for cloning and sequencing. The selected fragments were cloned into pUC19 plasmid using low melt agarose ligation. The ligation reaction was prepared by mixing 50 ng of purified vector pUC19, 10× ligase buffer, 2 U of ligase enzyme and 15 μl of *Pst*I fragments obtained after melting excised pieces of agarose, incubated at 16 °C overnight and used to transform One Shot *Escherichia coli* cells (Invitrogen). The cells were plated on LB-ampicillin (100 mg/ml) plates containing IPTG (0.1 mM) and X-gal (40 μg/ml). White colonies that included the DNA fragments were amplified in liquid LB containing ampicillin (100 mg/ml) at 37 °C overnight. DNA was then extracted by alkaline lysis, digested with the relevant endonuclease and separated on 1% agarose and compared with SfCOL total genomic DNA digests.

The *Pst*I-K (4.9 kb) and *Pst*I-M (3.6 kb) fragments were completely sequenced by the primer walking method and assembled. Sequence information was analyzed for the presence of open reading frames (ORFs) using open reading frame finder (NCBI). Homology searches were performed at the nucleotide and amino acid levels using all putative ORFs. DNA and protein comparisons with entries in the updated GenBank/EMBL, SWISS PROT and PIR databases were performed using BLAST (NCBI) (Altschul et al., 1990; Pearson, 1990).

2.5. Biological characterization

The insecticidal activity of the SfCOL isolate was compared with that of SfNIC strain. The median lethal concentration (LC_{50}) and mean time to death (MTD) were determined in *S. frugiperda* larvae originating from both the Mexican and the Colombian laboratory colonies. For this, second-instars were starved for 8–12 h at 26 °C and then allowed to drink from an aqueous suspension containing

10% (w/v) sucrose, 0.001% (w/v) Fluorella blue, and OBs at one of the following five concentrations for each isolate: 1.92×10^3, 9.60×10^3, 4.80×10^4, 2.40×10^5 and 1.20×10^6 OBs/ml. This range of concentrations was previously determined to kill between 5% and 95% of experimental insects from the Mexican colony (Simón et al., 2004), based on an average volume of 0.077 µl consumed by each larva (Simón et al., unpublished data). Control larvae were treated identically but drank from a sucrose and dye solution that did not contain OBs. Larvae that ingested the suspension within 10 min were transferred to individual wells of a 24-well tissue culture plate with a piece of semisynthetic diet. Bioassays were performed using 24 larvae per virus concentration and 24 control larvae. Insects were reared at 25 °C, and mortality was recorded every 8 h until death or pupation. The bioassay was performed on three occasions. Virus induced mortality was subjected to probit analysis using the PoloPlus program (LeOra-Software, 1987).

Time mortality data were subjected to Weibull survival analysis using the Generalized Linear Interactive Modeling (GLIM) program (Crawley, 1993). Equivalent effective OB concentrations for time mortality assays were based on ~90% mortality estimates from the concentration–mortality bioassay. Groups of 24 overnight starved second instars from the Mexican colony were inoculated with 2.5×10^6 OBs/ml of SfNIC OBs and 1.5×10^6 OBs/ml for SfCOL, using the droplet feeding method, reared on semisynthetic diet at 25 °C, and checked at intervals of 8 h until death. Similarly, insects from the Colombian colony were inoculated with 1.2×10^9 OBs/ml for SfNIC, and 2.2×10^6 OBs/ml for SfCOL. The assay was performed on three occasions.

OB production of SfNIC and SfCOL isolates was determined in cohorts of 24 overnight-starved second instars inoculated with an OB concentration that resulted in ~90% mortality and reared on semisynthetic diet at 25 °C until death. The assay was performed three times. All the larvae that died from NPV disease (at least 20 for each virus treatment per replicate, a total of ~60 larvae per virus treatment) were individually collected and stored at −20 °C. For OB counting, each larva was homogenized in 100 µl of distilled water and counted in triplicate in a Neubauer hemocytometer. The results were analyzed by Kruskal–Wallis and Mann–Whitney nonparametric statistics using the SPSS program (SPSS version 10.0). Values of *P* were subjected to false discovery rate adjustment for multiple pairwise comparisons (Benjamini and Hochberg, 1995).

3. Results

3.1. REN patterns and genome size of Colombian NPV isolates

Two different REN profiles were identified in the 38 isolates from Colombia following treatment with six restriction endonucleases. Of these, 35 isolates (92.1%) were identical in all six REN profiles. This predominant profile was named SfCOL, and was selected for further molecular and biological characterization. The selected isolate originated from pasture in Monteria, Córdoba Department.

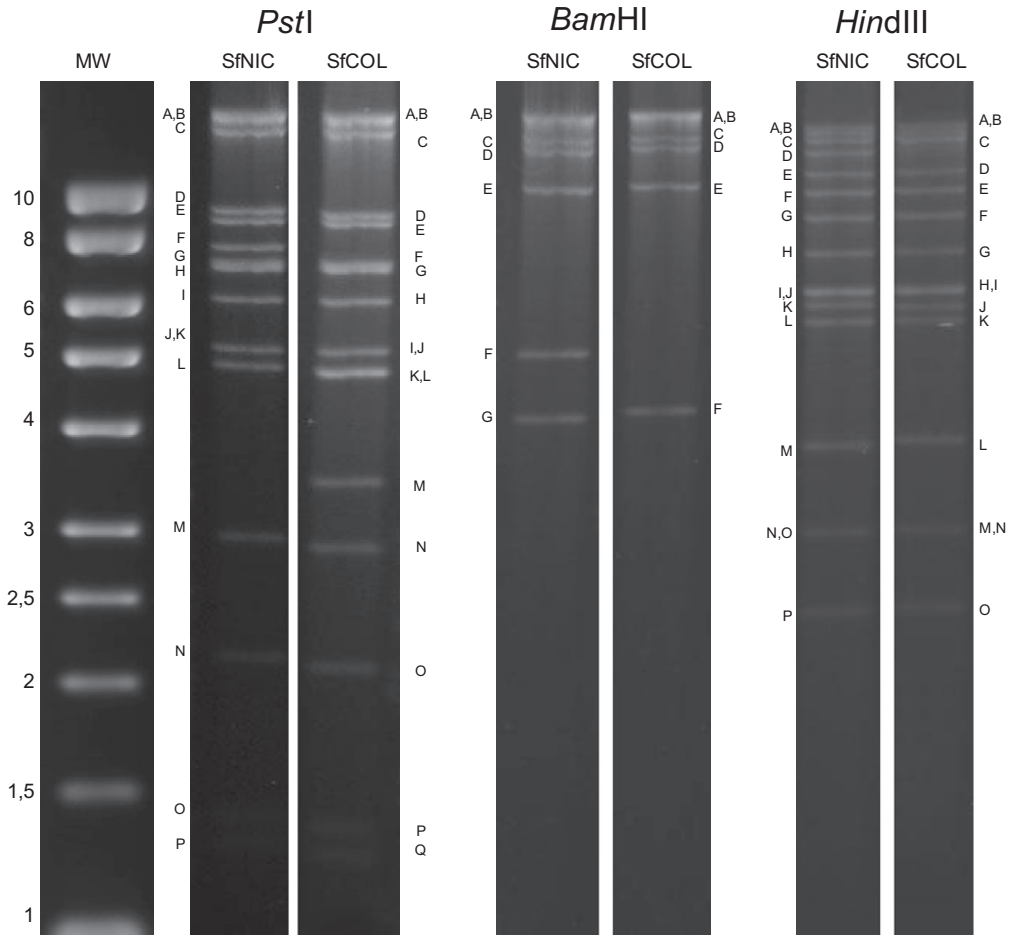

Fig. 1. REN patterns of Colombian SfMNPV isolate DNA (SfCOL) digested with *Pst*I, *Bam*HI and *Hin*dIII as indicated, generated by electrophoresis in 1% agarose gel. The REN patterns were compared with those of Nicaraguan isolate (SfNIC) generated with the same enzymes. All DNA fragments are marked with a letter corresponding to their sizes. The first lane indicated the molecular weight (MW) that corresponds to a 1 kb DNA marker (Stratagene).

Table 1
Molecular sizes of *Pst*I, *Bam*HI and *Hind*III restriction endonuclease fragments of SfMNPV genomic DNA from Nicaraguan (SfNIC) and Colombian (SfCOL) isolates.

Fragment	Restriction size fragments (bp)					
	*Pst*I		*Bam*HI		*Hind*III	
	SfNIC	SfCOL	SfNIC	SfCOL	SfNIC	SfCOL
A	28,084	28,084	53,872	54,655	17,385	28,843
B	24,926	24,926	28,992	28,992	16,151	17,385
C	12,465	12,465	16,158	19,460	14,826	16,151
D	8864	8864	14,179	16,158	13,072	11,008
E	8481	8481	10,26	10,260	11,008	8452
F	7517	7079	5119	4216	8452	7498
G	7079	6932	4216	158	7498	7182
H	6932	6124	158		7182	6231
I	6124	5182			6231	6175
J	5182	4899			6175	5886
K	4899	4887			5886	5552
L	4769	4769			5552	3775
M	2953	3575			3775	2988
N	2112	2953			2988	2428
O	1339	2112			2428	2131
P	1228	1339			2131	930
Q		1228			930	676
R					676	608
S					608	
Total	132,954	133,899	132,954	133,899	132,954	133,899

The three other isolates presented identical REN profiles that differed only slightly from that of SfCOL (data not shown).

The REN profiles of the SfCOL DNA obtained after digestion with *Pst*I, *Bam*HI and *Hind*III were very similar to the corresponding SfNIC profiles and digestion fragments were designated alphabetically (Fig. 1). Co-migrating fragments were detected by stain intensity in *Hind*III (H-I and M-N), the *Pst*I (fragments A-B, I-J and K-L) and in the *Bam*HI REN patterns (fragments A-B).

The REN profile of SfCOL isolate showed two fragments, *Pst*I-K (4.9 kb) and *Pst*I-M (3.6 kb), that were absent in SfNIC whereas the SfCOL isolate lacked a 7.5 kb fragment present in SfNIC (*Pst*I-F) (Fig. 1, Table 1). The *Bam*HI profile of the Colombian isolate was also similar to that of SfNIC except that *Bam*HI-F fragment (5.1 kb) present in SfNIC was absent in SfCOL. *Hind*III profiles were also similar, except that *Hind*III-C and -D fragments (14.8 and 13.1 kb) present in the SfNIC profile were absent in SfCOL, whereas an additional fragment *Hind*III-A (28.8 kb) was present in SfCOL. The remaining fragments co-migrated in SfNIC and SfCOL profiles, suggesting that those fragments were similar.

The sizes of the SfCOL REN fragments were estimated by comparison with the known sizes of SfNIC fragments and the complete genome sequence (Simón et al., 2005a, 2011) (Fig. 1, Table 1). The SfCOL genome was estimated to be 133.9 kb in length, calculated from the average sum of restriction fragment sizes across the three endonuclease digests, which was similar to that of SfNIC (132.9 kb).

3.2. Physical maps of the SfCOL genome

The physical map was constructed based on the map of SfNIC (Simón et al., 2005a, 2011). The *Pst*I-F (7.5 kb) fragment present in SfNIC was absent in SfCOL, whereas two additional fragments *Pst*I-K (4.9 kb) and *Pst*I-M (3.6 kb) were apparent in SfCOL (Fig. 1). The sum of the sizes of these two additional fragments (8.5 kb) was similar to that of the SfNIC *Pst*I-F fragment, suggesting that the SfCOL genome likely presents an insertion of ~1.0 kb in this region. No other differences were observed. These observations led us to believe that the SfCOL *Pst*I-K and *Pst*I-M fragments were the result of an additional restriction site, not present in the *Pst*I-F region of the SfNIC genome. To confirm this, the *Pst*I-K and *Pst*I-M fragments were sequenced. This region was 8462 bp in length encompassing SfCOL *Pst*I-K (4887 bp) and *Pst*I-M (3575 bp) fragments (Fig. 2), that correspond to the genomic region between m.u. 13.9 and 20.0 of the SfCOL genome. These sequences were homologous and collinear to SfNIC *Pst*I-F that correspond to the genomic region between nucleotides 18,515 and 26,031 (Simón et al., 2005a, 2011). Seven complete ORFs were identified and two partial ORFs located at the ends of the *Pst*I-K and *Pst*I-M regions; these were named ORF1–ORF9. The partial ORF1 in SfCOL *Pst*I-K fragment corresponds to the 5′ end of SfNIC *Pst*I-F fragment, whereas the other partial ORF (ORF9) corresponds to the 3′ end of SfNIC *Pst*I-F fragment. A homologous repeat region (44 bp) with imperfect palindrome was identified between ORF1 and ORF2. ORF1 corresponds with *cathepsin*, ORF2 with *late expression factor 7* (*lef7*), ORF3 with *chitinase*, ORF4 and ORF5 with proteins of unknown function, ORF6 with *gp37*, ORF7 with *protein tyrosine phosphatase-2* (*ptp-2*), ORF8 with *ecdysteroid UDP-glucosyltransferase* (*egt*) and ORF9 with an unknown function protein homologous to *sf27* (Fig. 2B).

The SfCOL deduced amino acid sequences were compared with SfNIC (Simón et al., 2011), SfMNPV from United States (Sf3AP2) (Harrison et al., 2008), *Spodoptera exigua* MNPV (SeMNPV) (Ijkel et al., 1999) and *Spodoptera litura* NPV (SpltNPV) (Pang et al., 2001) genomes (Table 2). The percentage of amino acid sequence identity (Id) and similarity (Sim) revealed that five complete ORFs (2, 3, 6, 7 and 8) and two partial ORFs (1 and 9) presented homology with SfMNPV genomes (Id 94–100%; Sim 96–100%), however, the ORF4 and ORF5 did not present homologies with SfNIC or Sf3AP2. These ORFs shared homology with *splt20* and *splt21* ORFs of the SpltNPV genome respectively (Id 96–98%; Sim 98%) and with *se21* and *se22* + *se23* ORFs of the SeMNPV genome (Id 70–79%; Sim 79–86%). In contrast, the sequenced region of SfCOL lacked ORF *sf23* present in the other SfMNPV genomes. The genomic organization of SfCOL is more closely related to that of Sf3AP2, SfNIC and SeMNPV than to SpltNPV, although orthologs of *splt20* and *splt21* were detected in SfCOL with a high degree of similarity (Table 2).

The sequence analysis of SfCOL *Pst*I-K and *Pst*I-M fragments also revealed the absence of the recognition sites *Hind*III and *Bam*HI enzymes that were present in the SfNIC genome. Using this information, physical maps were constructed for *Bam*HI and *Hind*III (Fig. 3). The SfCOL genome was cleaved into 6 and 18 visible fragments with *Hind*III and *Bam*HI enzymes, respectively, whereas the SfNIC genome was digested to produce 7 and 19 fragments by each of these enzymes. The SfCOL REN profile lacked the SfNIC *Hind*III-D fragment (13.0 kb) due to the lack of a *Hind*III site in the SfCOL genome. Consequently, the SfCOL *Hind*III-A fragment (28.8 kb) is the result of the sum of the SfNIC *Hind*III-C (14.8 kb) and -D (13.0 kb) fragments (Fig. 3). Treatment of SfCOL DNA with *Bam*HI revealed that the 5.1 kb fragment present in the SfNIC profile (*Bam*HI-F) was absent and had been incorporated in the *Bam*HI-C fragment (19.5 kb), that resulted from the sum of the SfNIC *Bam*HI-D (14.1 kb) and -F (5.1 kb) fragments (Fig. 3).

3.3. Biological characterization

The biological activity of SfCOL OBs was compared with that of SfNIC OBs using laboratory colonies of *S. frugiperda* from Mexico and Colombia (Table 3). The LC_{50} values of the SfNIC and SfCOL OBs were very similar in larvae from the Mexican colony. In contrast, SfNIC OBs (LC_{50} 5.37×10^5 OBs/ml) were approximately 12-fold less pathogenic than SfCOL OBs (LC_{50} 4.57×10^4 OBs/ml) to the Colombian colony. Overall, SfCOL OBs were approximately threefold more potent to the Colombian colony larvae than to the Mexican colony larvae. However, the SfNIC isolate was fivefold less potent to the Colombian colony than to the Mexican colony. No mortality was observed in the controls.

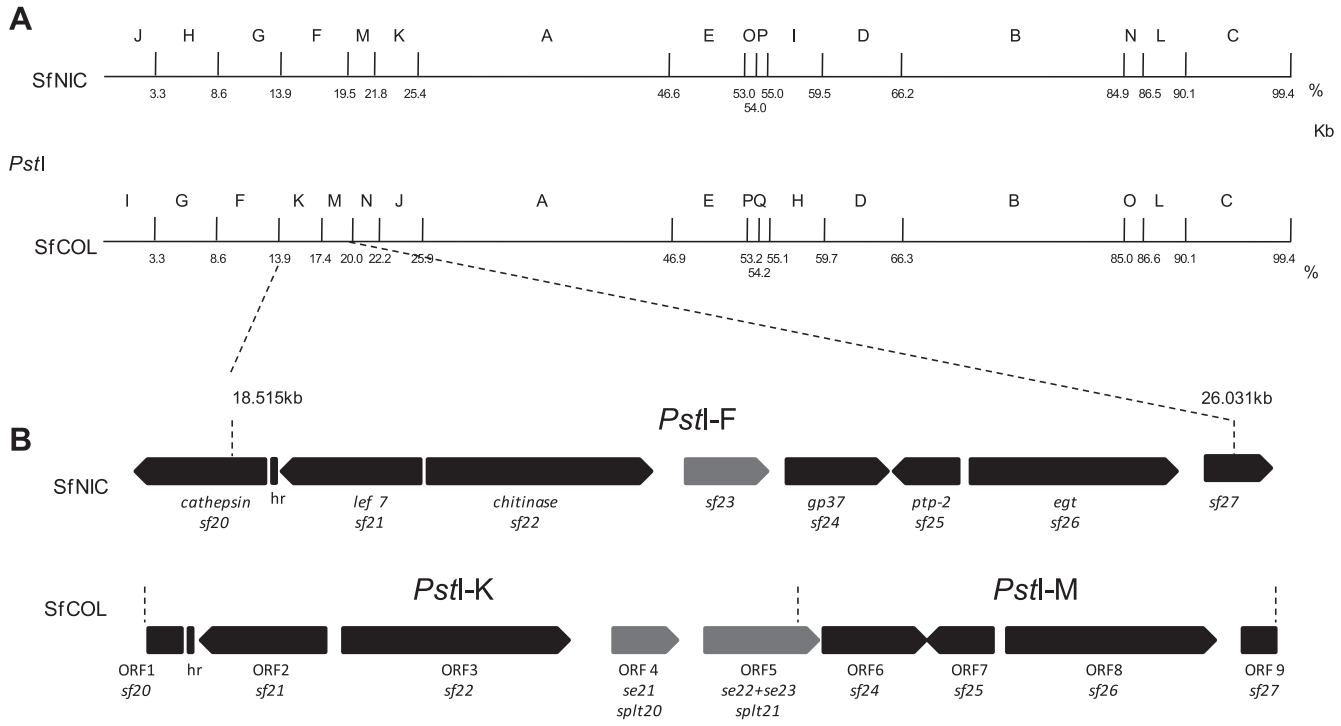

Fig. 2. (A) Comparison of *Pst*I physical maps of SfNIC and SfCOL genomes. (B) ORFs present in the *Pst*I-F fragment of the SfNIC genome, and partial ORFs found in the 5′ end of the *Pst*I-K and 3′ end of the *Pst*I-M fragments of SfCOL profile. The homologous genes are indicated below the arrows.

Table 2
Percentage of deduced amino acid sequence identity and similarity of the proteins encoded by SfCOL ORFs to their orthologues in the SfNIC (Simón et al., 2011), Sf3AP2 (Harrison et al., 2008), SeMNPV (Ijkel et al., 1999) and SpltNPV (Pang et al., 2001) genomes.

SfCOL ORFs	Gene family	Size aa	ORF number/% identity (similarity)			
			SfNIC	Sf3AP2	SeMNPV	SpltMNPV
1	*cathepsin*	85	*sf20*/97(97)	*sf20*/97(97)	*se16*/90(91)	*splt17*/90(98)
2	*lef-7*	320	*sf21*/98(98)	*sf21*/98(98)	*se17 + se18*/56(67)	*
3	*chitinase*	573	*sf22*/98(99)	*sf22*/99(99)	*se19*/85(90)	*splt18*/83(90)
4	Unknown	162	*	*	*se21*/70(79)	*splt20*/96(98)
5	Unknown	286	*	*	*se22 + se23*/78(86)	*splt21*/98(98)
6	*gp37*	262	*sf24* /94(96)	*sf24*/94(96)	*se25*/83(88)	*splt22*/88(94)
7	*ptp-2*	167	*sf25*/99(100)	*sf25*/99(100)	*se26*/68(83)	*splt23*/63(83)
8	*egt*	525	*sf26*/99(100)	*sf26*/96(96)	*se27*/76(91)	*splt24*/78(91)
9	Unknown	77	*sf27*/100(100)	*sf27*/100(100)	*se28*/64(71)	*splt25*/82(89)
TOTAL	% Ident (sim)		98 (99)	98 (98)	74 (83)	85 (93)

* No homologies with SfCOL.

MTD values were estimated for virus concentrations that resulted in ~90% larval mortality (Table 3). SfNIC and SfCOL presented similar MTD values for each of the colonies analyzed. With a MTD of 160 h and 168 h for SfNIC and SfCOL, respectively, these viruses were significantly slower to kill Colombian colony insects than Mexican insects (with a MTD of 125 h and 119 h for SfNIC and SfCOL, respectively). The slower speed of kill was correlated with a higher OB production in the Colombian colony insects compared to those from the Mexican colony.

OB production of the different isolates was determined in second instars that had consumed a ~90% lethal concentration of OBs. The results were not normally distributed and were subjected to non-parametric analysis and are shown as medians with the corresponding interquartile ranges (Fig. 4). No differences were observed in OB production among the SfCOL and SfNIC isolates in each of the two insect colonies tested. Both isolates produced significantly greater numbers of OBs in insects from the Colombian colony, with approximately threefold more OBs/larva (range 6.4×10^7–7.5×10^7 OBs/larva) than observed in the Mexican colony insects (range 1.8×10^7–2.1×10^7 OBs/larva).

4. Discussion

The aim of this study was to select a native SfMNPV isolate that could be used as the basis for a biological insecticide product to control *S. frugiperda* in Colombia. Field NPV isolates were collected from different host plants in various regions in Colombia. Thirty-eight isolates were examined by REN analysis. Baculovirus REN profiles from the same lepidopteran host species in different geographic regions frequently show variation in the number and position of restriction sites. This simple technique has been used to reveal the occurrence of distinct strains in different geographical isolates of SfMNPV (Berretta et al., 1998; Escribano et al., 1999; Loh et al., 1982; Shapiro et al., 1991). In this study, 35 out of the 38 isolates displayed identical REN profiles with six different

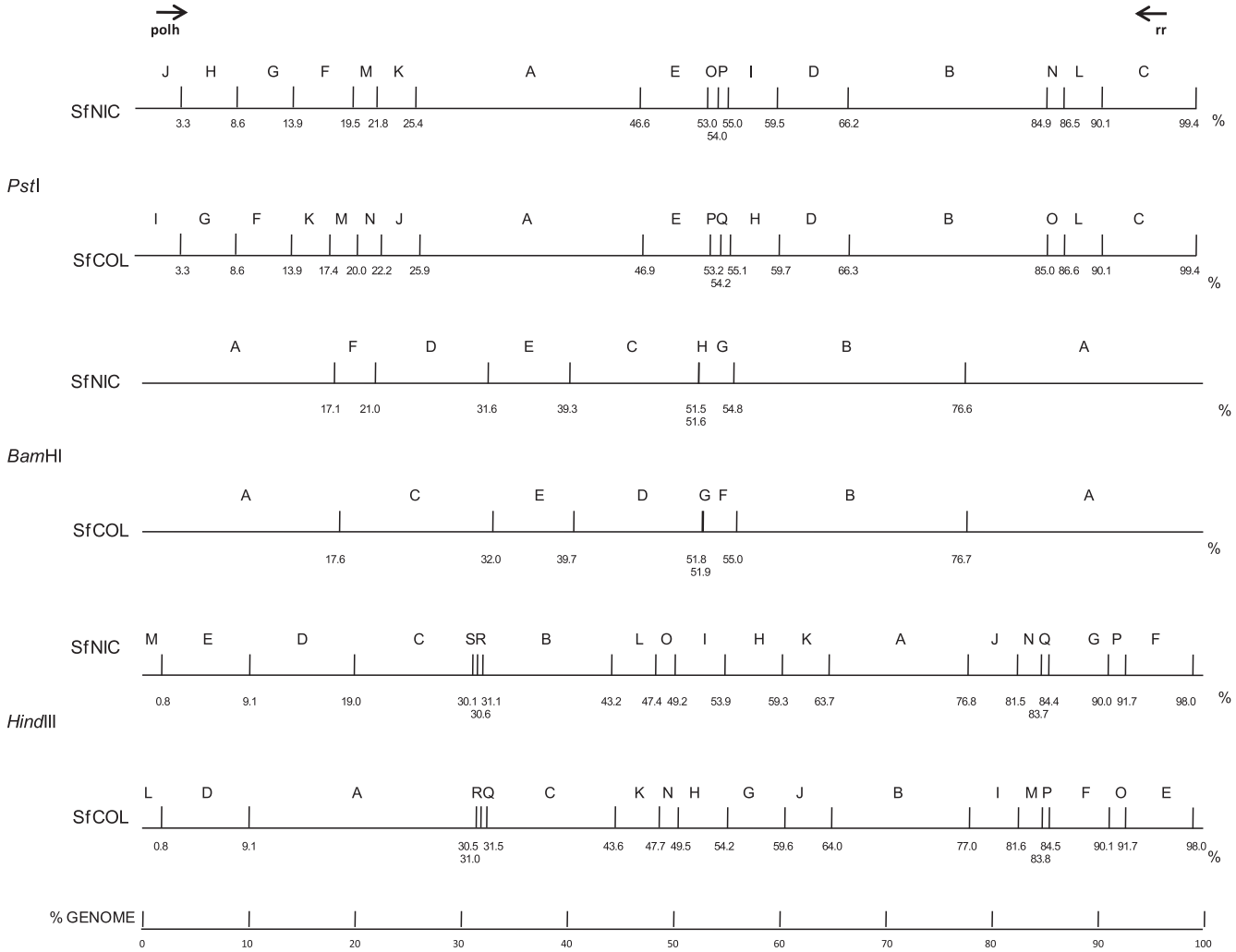

Fig. 3. Physical maps of the SfCOL genome compared with those of SfNIC. Restriction maps for *Pst*I, *Bam*HI and *Hind*III are shown. The circular DNA of each genome is represented in linear form. The first nucleotide of the genome corresponds to the *polyhedrin* gene. Numbers of map units (m.u.) representing restriction sites are indicating below the maps. Scales of m.u. are indicated at the bottom. The SfCOL genome size was estimated to be 133.7 kb.

Table 3
Estimated LC_{50} values, relative potencies and mean time to death (MTD) values of SfNIC and SfCOL isolates in *S. frugiperda* second instars from two different populations originating from Mexico and Colombia.

Origin insect culture	Virus	LC_{50} (OBs/ml)	Relative potency	Fiducial limits (95%)		MTD (h)	Fiducial limits (95%)	
				Low	High		Low	High
Mexico	SfNIC	1.52×10^5	1.0	–	–	125b	121	129
	SfCOL	1.14×10^5	1.3	0.84	2.11	119b	115	123
Colombia	SfNIC	5.37×10^5	0.2	0.16	0.48	160a	154	166
	SfCOL	4.57×10^4	3.3	2.14	5.52	168a	162	174

Probit regressions were fitted using the PoloPlus program with a common slope of 1.143 ± 0.103 (mean ± standard error). A test for non-parallelism was not significant ($\chi^2 = 4.98$, df = 3, $p > 0.05$). Relative potencies were calculated as the ratio of effective concentrations relative to the SfNIC isolate. MTD were estimated by Weibull analysis. MTD values labeled with different letters were significantly different.

enzymes, suggesting that all 35 isolates are the same virus strain. An isolate from Monteria (Córdoba, Colombia) was selected as being representative of the predominant isolates and named SfCOL. Few differences in the presence and distribution of REN cleavage sites were observed between the SfCOL and the SfNIC genomes (Simón et al., 2004, 2011). This is a common feature between distinct geographical NPV isolates that differ minimally in their REN profiles and physical maps, normally due to point mutations and small deletions or insertions (Chen et al., 2002; Zhang et al., 2005).

Although the REN profiles of Colombian isolates appear to be unique, the variations relative to SfNIC occurred in a region of the SfMNPV genome that commonly differs between strains (Harrison et al., 2008; Simón et al., 2004, 2005a; Wolff et al., 2008). Variability in this region was also observed among genotypes of SeMNPV that is highly collinear with the SfMNPV genome (Muñoz et al., 1998; Simón et al., 2005a). Localized regions of variation are also a common feature in other baculoviruses; strains of *Autographa californica* MNPV (AcMNPV) or SeMNPV differ in specific genomic regions that tend to comprise a high prevalence of

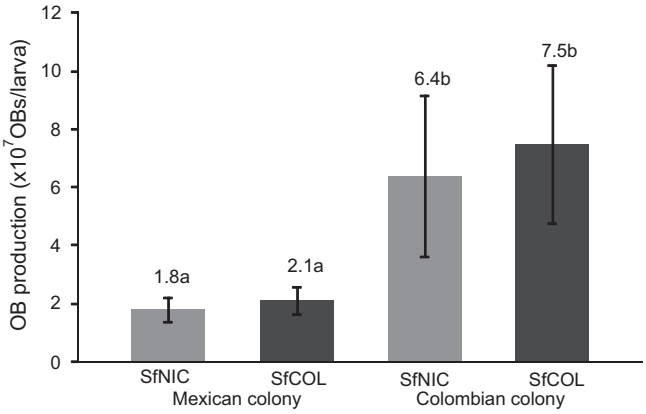

Fig. 4. Median OB production per larva of SfNIC and SfCOL isolates using larvae from the Mexican and Colombian colonies. Values followed by identical letters did not differ significantly for pairwise comparisons (Mann–Whitney U-test followed by Benjamini and Hochberg (1995) false discovery rate adjustment of *P* values for multiple comparisons). Vertical lines indicate the interquartile range.

auxiliary genes (Dai et al., 2000; Erlandson et al., 2007; Stiles and Himmerich, 1998). Auxiliary genes such as *cathepsin* and *egt* have been identified within this SfMNPV genomic region (Flipsen et al., 1995; Slack et al., 1995). In addition, a homologous region (*hr*) was also detected. *Hrs* are implicated in important functions such as origins of DNA replication and transcriptional enhancement (Pearson and Rohrmann, 1995) and have been found also near to regions of variability in others baculovirus genomes (de Jong et al., 2005; Hayakawa et al., 2000; Li et al., 2002). Sequencing of this SfCOL variable region including *Pst*I-K and *Pst*I-M fragments, revealed an insertion of ~1.0 kb compared to SfNIC. This region was collinear with that of SfNIC or Sf3AP2, however within SfCOL two ORFs that do not have their orthologues in SfNIC or Sf3AP2 genomes were identified. These two ORFs were closely related to *splt20* (96%) and *splt21* (98%), but less closely related to *se21* (70%) and *se22* + *se23* (78%) (Ijkel et al., 1999; Pang et al., 2001). In contrast, SfCOL lacked the *sf23* ORF of unknown function which is found in SfMNPV genomes (Harrison et al., 2008; Simón et al., 2011). The heterogeneity found at the genome level between SfNIC and SfCOL isolates could be attributed to exchanges of genetic material. Heterologous recombination has been shown to occur in cell culture (Hajós et al., 2000) and has been suggested to occur between *Rachiplusia ou* MNPV (RoMNPV) and AcMNPV in natural populations (Croizier et al., 1988). In addition, recombination has been postulated as possible major cause of genetic heterogeneity in NPV wild populations (Crozier and Riberio, 1992). Genomes of different baculoviruses can co-infect the same cell and closely related viruses that normally infect different host species can be forced to co-infect the same cell (Herniou et al., 2003). In this case, the geographic distribution of the natural host of SfMNPV, *S. frugiperda* and that of SpltNPV, *S. litura*, do not overlap. While *S. frugiperda* is distributed across tropical and subtropical regions of the Americas (Sparks, 1979), *S. litura* is found in Asia and Oceania (Takatsuka et al., 2003) and no infestations of *S. litura* have been reported in the Americas. However, SfCOL virus may have acquired these two ORFs by recombination with a SpltNPV virus when both viruses coinfected a susceptible host with a geographical distribution that overlapped the distribution of each virus. The range of hosts that both SfMNPV and SpltNPV infect in nature has not been characterized in detail. It may be possible, therefore, that a moth species infected by SpltNPV migrated into the natural distribution area of SfMNPV and its host(s). If common, this behavior could increase the likelihood of recombination between these viruses and the generation of novel variants.

Differences in gene content between SfCOL and SfNIC are likely to have effects on the phenotypic characteristics of the isolates. Baculoviruses isolated from the same species at different sites frequently vary in their pathogenicity and virulence (Cory and Myers, 2003; Erlandson et al., 2007). Differences in biological activity are common among virus isolates from distinct geographical regions (Erlandson, 2009; Fuxa, 2004) or among cloned variants derived from a single wild-type virus obtained by *in vitro* (Lynn et al., 1993; Ribeiro et al., 1997; Simón et al., 2004a) and *in vivo* cloning techniques (Muñoz et al., 2000). The selection of an isolate as a biocontrol agent requires geographical isolates to be tested against insect populations from the locality of the program. Compared to exotic isolates, native baculovirus isolates might also be easier to register as the basis for a bioinsecticide product in pesticide regulatory systems. The analysis of baculovirus phenotype using long term laboratory cultures of insects may be hindered if the colony susceptibility does not accurately reflect the range of susceptibilities of naturally occurring populations of the pest in question. To address this, two different *S. frugiperda* populations were tested; a long standing laboratory culture from Mexico and a recently established colony from field collected insects in Colombia. The Colombian and Nicaraguan isolates presented similar lethal concentration values when tested against the Mexican colony insects. However, the SfCOL isolate was twelve-fold more pathogenic than SfNIC isolate against larvae from Colombian colony. In contrast, SfCOL showed a slower speed of kill in insects from the Colombian colony compared to the Mexican colony, which was correlated with a higher production of OBs/larvae. The extended infection period might increase production of viral progeny (Wilson et al., 2000). This is a common phenomenon within baculoviruses as reported previously for other baculoviruses (Muñoz et al., 2000; Simón et al., 2004, 2008). As virus productivity is a crucial component of virus fitness, increases in yield should be highly beneficial to the virus for transmission and persistence (Cory and Myers, 2003).

Given the high activity of SfCOL OBs towards the Colombian colony insects, SfCOL is likely better suited for the development as a biological insecticide for the control of *S. frugiperda* populations in Colombia than the SfNIC isolate. Similarly, insects from a Honduran *S. frugiperda* population were more susceptible to the neighboring SfNIC isolate than to geographically distant isolates (Escribano et al., 1999) and local isolates of *Trichoplusia ni* SNPV (TnSNPV) or AcMNPV were more infective towards local pest populations than those from geographically distant origins (Erlandson et al., 2007). Clearly, there is an adaptive advantage for the virus to retain high infectivity toward the local host population, presumably as a result of continuous host-pathogen coevolution. In conclusion, the SfCOL isolate showed improved insecticidal characteristics to the insects from a local colony compared to an isolate from Nicaragua. The SfCOL isolate may prove suitable as the basis for a biopesticide for control of *S. frugiperda* infestations in Colombia.

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