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IN VITRO AND *IN VIVO* HOST RANGE OF *ANTICARSIA GEMMATALIS* MULTIPLE NUCLEAR POLYHEDROSIS VIRUS[1]

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SUMMARY

A clone of the wild type (wt) *Anticarsia gemmatalis* multiple nuclear polyhedrosis virus AgMNPV, derived from a geographical isolate (Hondrina, Brazil) and designated AgMNPV-CL4-3A1, was used to determine the host range of this virus in six established lepidopteran cell lines: *Anticarsia gemmatalis* (BCIRL-AG-AM1), *Helicoverpa zea* (BCIRL-HZ-AM1), *Heliothis virescens* (BCIRL-HV-AM1), *Helicoverpa armigera* (BCIRL-HA-AM1), *Trichoplusia ni* (TN-CL1), *Bombyx mori* (BMN), and a coleopteran cell line *Anthonomus grandis* (BRL-AG-1). In addition, the *in vivo* host range of this clone was also assayed in larvae of *Helicoverpa zea, Heliothis virescens, Trichoplusia ni,* and the homologous species *Anticarsia gemmatalis* by probit analysis. On the basis of temporal studies of $TCID_{50}$ values, BCIRL-HV-AM1 cells gave the highest extracellular virus (ECV) titer (9.7×10^6 $TCID_{50}$/ml) followed by BCIRL-HA-AM1 cells (8.3×10^5 $TCID_{50}$/ml) and BCIRL-AG-AM1 cells (3.2×10^5 $TCID_{50}$/ml). In addition, a low ECV titer of 1.37×10^3 $TCID_{50}$/ml was detected from TN-CL1 cells 96 h postinoculation, while BRL-AG-1, BMN, and BCIRL-HZ-AM1 cells were nonpermissive to AgMNPV-CL4-3A1 on the basis of $TCID_{50}$ results. AgMNPV-CL4-3A1 and the wild type AgMNPV had similar restriction profiles that were different from wild type AcMNPV. The LC_{50} values were 96.9, 564.6, 733.3, and 1.1×10^4 occlusion bodies/cm^2 of diet for *A. gemmatalis, Helicoverpa zea, Heliothis virescens,* and *T. ni,* respectively.

Key words: Anticarsia gemmatalis; velvetbean caterpillar; nuclear polyhedrosis virus; host range; Lepidoptera; Coleoptera; insect cell lines; *in vivo*; *in vitro*.

INTRODUCTION

The *Anticarsia gemmatalis* multiple nuclear polyhedrosis virus (AgMNPV) is an important biological pesticide extensively used to control populations of the velvetbean caterpillar (VBC), *Anticarsia gemmatalis* (Hübner), a major lepidopteran defoliator of leguminous crops in Brazil (15,16). *A. gemmatalis* is also an important pest of soybean in the southeastern U.S. (24), particularly in Florida (23) and Louisiana (25). The AgMNPV has shown promise in the control of VBC by reducing significantly (7–11 d after application of AgMNPV treatments) the number of caterpillars in soybean fields (18).

Several studies have been conducted to elucidate the *in vivo* host range of the VBC virus. For example, Carner et al. (2) reported that velvetbean caterpillar larvae were highly susceptible to crude preparations of polyhedral inclusion bodies (PIB). They also observed that of seven other noctuid species tested, only larvae of the tobacco budworm *Heliothis virescens* (F.) were highly susceptible, whereas higher dosages were required to kill the corn earworm *Helicoverpa zea* Boddie, the cabbage looper *Trichoplusia ni* Hübner, the soybean looper *Pseudoplusia includens* Walker, and the yellowstriped armyworm *Spodoptera ornithogalli* Guenée. In another study, Pavan et al. (17) found that larvae of *H. zea, T. ni,* and the beet armyworm *Spodoptera exigua* (Hübner) were susceptible to this virus at high concentrations (12.5×10^6 PIB/larva), but that only 24% and 4% of treated *P. includens* and the fall armyworm *Spodoptera frugiperda* (J. E. Smith) larvae, respectively, were killed.

In vitro studies using genotypic variants of AgMNPV have shown different degrees of replication in TN368 (*T. ni*), IAL-PiD (*Plodia interpunctella*), Hz-AM (*H. zea*), and IPLB-Sf21 (*S. frugiperda*) cell lines with IPLB-Sf21 showing one of the highest replication titers (8). Four other established cell lines, UFL-AG-286 derived from embryos of *A. gemmatalis*, IPLB-SF-21AE derived from ovarian tissue of *S. frugiperda*, and IAL-TND1 and IAL-SFD1 both derived from imaginal disks of *T. ni* and *S. frugiperda*, respectively, were found to be susceptible to AgMNPV (19). Our study reports on the susceptibility of other insect cell lines not previously tested as well as the infectivity of this baculovirus for four laboratory reared lepidopteran species.

MATERIALS AND METHODS

Cell lines and media. The cell lines and media used in this study are described in Table 1. All cell lines were identified by DAF (DNA Amplification Fingerprinting)-PCR (10).

Insects. Insect larvae used in all of the bioassays were obtained from established laboratory colonies maintained at the Biological Control of Insects Research Laboratory, Columbia, Missouri.

Virus. An occlusion body (OB) sample of wt AgMNPV Hondrina isolate (Hondrina, Brazil) was obtained from Dr. Drion Boucias, University of Florida,

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TABLE 1

INSECT CELL LINES USED IN THE STUDY

Species	Cell line designation	Source of cell line	Passage number	Growth medium[a]	Reference
Helicoverpa zea	BCIRL-HZ-AM1	pupal ovaries	254	TC199-MK[b]	11
Helicoverpa armigera	BCIRL-HA-AM1	pupal ovaries	252	TC199-MK	13
Heliothis virescens	BCIRL-HV-AM1	pupal ovaries	471	TC199-MK	9
Anticarsia gemmatalis	BCIRL-AG-AM1	ovarian tissue	8	TC199-MK	12
Bombyx mori	BMN	unknown	50	TC-100[c]	6
Trichoplusia ni	TN-CL1	ovarian tissue	330	Ex-Cell 400[d]	14
Anthonomus grandis	BRL-AG-1	embryonic	174	Ex-Cell 400	22

[a]All media were supplemented with 10% fetal bovine serum, 50 μg of streptomycin per ml, and 50 units of penicillin per ml.
[b]TC-199MK is an insect cell culture medium (Sigma Chemical Co., St. Louis, MO).
[c]TC-100 is an insect cell culture medium (Sigma Chemical Co., St. Louis, MO).
[d]Ex-Cell 400 is a serum-free insect cell culture medium (JRH Biosciences, Lenexa, KS).

TABLE 2

MEAN VALUES OF AgMNPV-CL4-3A1 EXTRACELLULAR VIRUS (ECV) IN INSECT CELL LINES[a]

p.i. (h)	Cell line						
	BCIRL-AG-AM1	BCIRL-HV-AM1	BCIRL-HA-AM1	TN-CL1	BRL-AG-1	BMN	BCIRL-HZ-AM1
6	NT	ND	NT	NT	NT	NT	NT
12	NT	ND	NT	NT	NT	NT	NT
18	NT	0.065 ± 0.019	NT	NT	NT	NT	NT
24	NT	0.07 ± 0.071	0.024 ± 0.017	ND	ND	ND	ND
48	ND	2.91 ± 0.065	0.34 ± 0.094	ND	NT	ND	NT
72	0.046 ± 0.001	3.3 ± 1.13	0.493 ± 0.016	LT	NT	ND	NT
96	0.066 ± 0.017	5.9 ± 2.08	0.83 ± 0.246	LT	NT	ND	NT
120	0.32 ± 0.122	9.7 ± 1.17	NT	NT	ND	NT	ND

[a]Mean $TCID_{50} \times 10^6$ ml ± S.E. values based on three replications titered in BCIRL-HV-AM1; p.i. = postinoculation; NT = not tested; ND = not detected ($< 3.0 \times 10^3$ $TCID_{50}$/ml); LT = low titer: at 72 p.i. $4.56 \times 10^3 \pm 0.054$ $TCID_{50}$/ml; at 96 p.i. $1.37 \times 10^3 \pm 0.053$ $TCID_{50}$/ml.

Gainesville, Florida. This isolate had never been previously passed in cell culture. Consequently, to obtain an inoculum of this isolate for *in vitro* studies, 0.1 ml of a 1:10 dilution of the original OB sample was applied to each well (7.7 cm^2) of ten 50-well plastic trays containing semisynthetic insect diet (3). Second-instar *H. virescens* larvae were placed in each well, and trays were incubated at 28° C for 5 d. The OB from these larvae were harvested as previously described (11) and stored at 4° C for later use. Hemolymph from these larvae was collected on ice 5 d after exposure to virus, and approximately 5 ml passed through a 0.45-μm Costar filter. A T-25 cm^2 flask containing 5 ml of 2×10^5 cells/ml of BCIRL-HV-AM1 was inoculated with 0.1 ml of infectious hemolymph and incubated at 28° C for 5 d. We harvested cells by pipetting the medium back and forth on the cell sheet, and the cell suspension was centrifuged at 3000 ×g for 15 min in a Beckman Model TJ-6 tabletop centrifuge at 4° C. The titer of the supernatant containing extracellular virus was determined with BCIRL-HV-AM1 as the indicator cell line according to a previously described method (12). Dilution endpoint assays were performed on the indicator cell line in duplicate and results recorded after 7 d of incubation at 28° C. A computer program written in BASIC employing the Spearman–Karber method was used to calculate the $TCID_{50}$ values (5). A clone, designated AgMNPV-CL4-3A1, was isolated from the wild type parent from infected BCIRL-HV-AM1 cells by plaque purification three times on the same cell line.

Occluded and extracellular virus production in cell lines. To determine host range, OB and extracellular virus (ECV) production of the virus clone, each cell line, was seeded at 1×10^5 cells/ml in three T-25 cm^2 flasks each containing 5 ml growth medium and incubated overnight at 28° C. Medium was then removed and cells were inoculated at a multiplicity of infection (MOI) of 1.0 and placed on a rocker platform for 2 h. The inoculum was removed and cells were washed twice with 5 ml of Hanks' balanced salt solution to remove any residual inoculum. Fresh medium was then added and cells were incubated at 28° C for various time periods. A 0.4-ml sample of the supernatant fluid from inoculated cultures was collected from each of the three replicate flasks/cell line used for each time period (Table 2). To obtain duplicate $TCID_{50}$ values from each replicate, we used duplicate 96-well Falcon plates and BCIRL-HV-AM1 as the indicator cell line. A study was also conducted to determine OB production of AgMNPV-CL4-3A1 in BCIRL-AG-AM1 and BCIRL-HV-AM1. Three T-25 cm^2 flasks were seeded with 1×10^5 cells/ml, inoculated with virus (MOI = 1.0), and treated as previously described. BCIRL-AG-AM1 cells were incubated for 72 h and 120 h at 28° C in three replicate flasks used for each time period, while BCIRL-HV-AM1 cells were incubated for 72 h at 28° C. Cells from the latter were removed from the flask by washing with 0.5 ml CMF/PBS (1×) followed by 0.5 ml trypsin-EDTA (1×) treatment (Intergen, Purchase, NY) for 1 min, and pipetting back and forth with 5 ml fresh media, whereas BCIRL-AG-AM1 cells, which were loosely attached, were harvested without trypsin by pipetting back and forth with 5 ml fresh media. Cells were then pelleted by centrifugation at 1500 ×g for 10 min with a Beckman Model TJ-6 centrifuge. The pellet was resuspended in 5 ml deionized water and sonicated at 50 W for 5 s. Occlusion bodies were enumerated with a hemacytometer.

In vivo *infectivity studies.* Infectious hemolymph was collected from 50 second-instar *H. virescens* and filter sterilized as previously described. Each of four T-75 cm^2 flasks containing 5 ml of 1×10^5 BCIRL-HV-AM1 cells/ml was infected with 0.5 ml of infectious hemolymph containing ECV and incubated for 5 d at 28° C. Occlusion bodies were released from cells when samples were sonicated at 50 W for 5 s with a Sonifier Cell Disrupter (Heat Systems Ultrasonics, Inc., Plainview, NY). Three samples were taken from this preparation and OB were enumerated with a hemacytometer. Initially, one to two preliminary tests were conducted with serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}) of this OB preparation indistilled water to determine the optimum OB concentrations needed to obtain a range of larval mortalities between approximately 0% to 100% in each of four insects: *Heliothis virescens, Helicoverpa zea, T. ni,* and *A. gemmatalis.* These selected OB concen-

TABLE 3

PROBIT ANALYSIS RESULTS OF LARVAL MORTALITIES OF THE FOUR LEPIDOPTERAN SPECIES

Species	Regression line	Adjusted r^2	OB/cm² of diet[a]		
			LC_{50}	L_1	L_2
Helicoverpa zea	$Y = 2.76 \pm 0.811\ X$	0.881	564.6 yz	105.8	3313.2
A. gemmatalis	$Y = 0.314 + 2.36\ X$	0.775	96.9 y	27.3	845.5
T. ni	$Y = 0.019 + 1.24\ X$	0.900	1.1×10^4 z	2327.0	5.4×10^4
Heliothis virescens	$Y = 2.47 + 0.88\ X$	0.850	733.3 yz	105.9	7787.2

[a]L_1 and L_2 are the lower and upper 95% confidence limits of the LC_{50}s. LC_{50} values with the same letter are not significantly different at the 95% confidence interval based on the *t*-distribution ($t_{\alpha=0.05,\ df=n-2}$).

trations were again prepared with distilled water and used in all further bioassays. To each well of a 50-well plastic tray containing semisynthetic insect diet was added 0.1 ml of OB at a given concentration along with one first-instar larva. A total of 75 larvae were used for each dilution of occlusion bodies in each of three separate replications. Trays were checked daily for dead larvae for 7 d and percentage mortality was recorded. Bioassay data for control response were corrected with Abbott's formula (1). Percentage mortalities were then converted to probits, plotted against the log dose of OB, and LC_{50} determined from linear regression analysis. We determined significant differences in LC_{50} among the four species by using 95% confidence intervals based on the *t*-distribution (20).

Viral DNA extraction and restriction endonuclease digestion of wt AgMNPV, AgMNPV-CL4-3A1, and wt Autographa californica *MNPV (AcMNPV).* Viral DNA was extracted from occluded virus (OV) by alkali treatment of *in vitro*-produced OB. The latter was produced by infection of three T 75 cm² flasks (15 ml medium/flask) seeded with 1×10^5 BCIRL-HV-AM1 cells/ml with AgMNPV-CL4-3A1 at an MOI of 1.0 and incubated at 28° C for 72 h. To establish the identity of AgMNPV-CL4-3A1, the restriction endonuclease pattern (REN) of DNA from this viral clone was compared to the DNA of wt AgMNPV as well as to a wt AcMNPV (12).DNA was extracted from AcMNPV OV produced in TN-CL1 cells. Equal concentrations of AgMNPV-CL4-3A1, wt AgMNPV, and AcMNPV DNA were digested according to a previously published report (11) with the following restriction enzymes: *Hin*dIII, *Eco*RI,*Bam*HI, *Bgl*II and *Pst*I (Boehringer Mannheim Corp., Indianapolis, IN). Lambda DNA (Boehringer Mannheim) digested with *Hin*dIII was used as our standard molecular size marker. Gel electrophoresis was performed on the DNA digests with a 0.7% agarose gel according to a previous report (11). An average migration distance calculated for each fragment was based on three separate gel runs. The molecular size of DNA fragments in kilobase pairs (kb) was determined with the method of Southern (21).

RESULTS

Extracellular virus and occlusion body production. AgMNPV-CL4-3A1 ECV titers in the cell lines as measured by $TCID_{50}$s are indicated in Table 2. BCIRL-HV-AM1 gave the highest ECV titer at 9.7×10^6 $TCID_{50}$/ml followed by 8.31×10^5 $TCID_{50}$/ml in BCIRL-Ha-AM1 and 3.2×10^5 $TCID_{50}$/ml in the homologous cell line BCIRL-AG-AM1. However, ECV was not observed in the homologous cell line until 72 h postinoculation, while the ECV titer in BCIRL-HV-AM1 at 18 h postinoculation was already at 6.5×10^4 $TCID_{50}$/ml. In addition, ECV production was higher in the heterologous cell line BCIRL-HA-AM1 (8.3×10^5 $TCID_{50}$/ml) than in BCIRL-AG-AM1 (6.6×10^4 $TCID_{50}$/ml) after 96 h postinoculation. TN-CL1 was the only other cell line to show some ECV production (1.37×10^3 $TCID_{50}$/ml) at 96 h postinoculation. The remaining three cell lines (BRL-AG-1, BMN, BCIRL-HZ-AM1) were refractile to AgMNPV-CL4-3A1 on the basis of $TCID_{50}$ analyses.

Of the four cell lines producing observable ECV titers, only the heterologous BCIRL-HV-AM1 cell line and the homologous cell line BCIRL-AG-AM1 produced OB. Occlusion body production was first observed in BCIRL-AG-AM1 following inoculation with AgMNPV-CL4-3A1 at 120 h postinoculation and produced an average of $1.42 \pm 0.30 \times 10^6$ OB/ml, whereas BCIRL-HV-AM1 at 72 h postinoculation produced $7.5 \pm 0.92 \times 10^6$ OB/ml.

Bioassay tests. The homologous species *A.gemmatalis* was the most susceptible of the four lepidopteran insects tested to the AgMNPV-CL4-3A1 clone with a LC_{50} of 96.9 OB/cm² of diet (Table 3). *T. ni* was the least susceptible to AgMNPV-CL4-3A1 with a LC_{50} of 1.08×10^4 OB/cm². The LC_{50} for *T. ni* was also significantly higher than that of *A. gemmatalis*($P < 0.05$). On the basis of confidence intervals, no significant differences in LC_{50} were found between *Heliothis virescens, T. ni,* and *Helicoverpa zea*($P > 0.05$).

Restriction endonuclease analysis. A comparison of the *Hin*dIII restriction pattern between wt AgMNPV and AgMNPV-CL4-3A1 revealed almost similar profiles except for additional bands at the 21.3, 9.1, and 3.0 kb regions of the wt AgMNPV and the presence of a unique fragment at the 6.5 kb region of the clone (Fig. 1). The *Eco*RI profile of the wt AgMNPV genome contained several fragments at the 6.5 kb region which were absent in AgMNPV-CL4-3A1. In addition, AgMNPV-CL4-3A1 contained a singular *Eco*RI restriction fragment at the 22.8 kb region which was missing in wt AgMNPV. The *Bgl*II fragmentation profiles between wt AgMNPV and AgMNPV-CL4-3A1 were similar except for two additional DNA fragments found in wt AgMNPV at the 22.8 and 4.3 kb regions. AgMNPV-CL4-3A1 had an additional DNA fragment found near the 6.5 kb region in an otherwise almost identical *Pst*I restriction profile to wt AgMNPV. In contrast, the overall restriction profiles between wt AcMNPV and AgMNPV-CL4-3A1 were completely different although the two (REN) profiles still shared some fragments of similar molecular size (Fig. 2).

DISCUSSION

Our investigation provides more information on the *in vitro* host range of the VBC MNPV and susceptibility of additional cell lines BCIRL-HA-AM1, BCIRL-HV-AM1, and BMN. In addition, this study provides quantitative data on ECV and OB production. Viral replication in BCIRL-HV-AM1 and BCIRL-HA-AM1 cells was confirmed by $TCID_{50}$ time course analysis.Published information is somewhat limited concerning the production of occlusion bodies in cell lines by AgMNPV and our study presents some of the few quantitative data. It must be emphasized that this is the first report that a *Heliothis virescens* (BCIRL-HV-AM1) cell line was found susceptible to AgMNPV producing substantial numbers of both ECV and OB. Infected BCIRL-HV-AM1 cells (1×10^5 cells/ml) produced

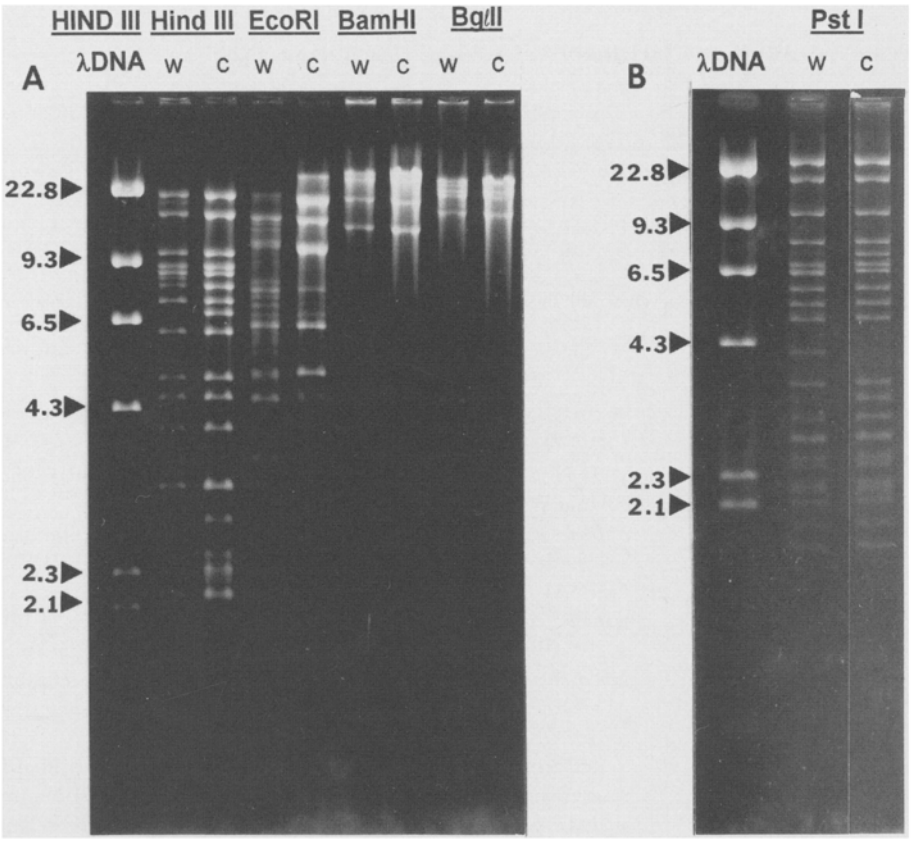

FIG. 1. Comparison of *Hin*dIII, *Eco*RI, *Bam*HI, *Bgl*II and *Pst*I restriction patterns between wild type (wt) AgMNPV and AgMNPV-CL4-3A1 clone (c). λ DNA was used as the molecular weight standard and the sizes (kb) of individual bands are indicated.

approximately 8×10^6 OB/ml which is within the reported range for the production of OB by other baculoviruses. In addition, BCIRL-HV-AM1 produced a maximum ECV titer of 9.7×10^6 $TCID_{50}$/ml or an approximately 45-fold increase compared with that in the homologous cell line. With regard to ECV production, measurable replication in the homologous BCIRL-AG-AM1 cell line by AgMNPV-CL4-3A1 did not occur until 72 h postinoculation (4.6×10^4 $TCID_{50}$/ml), which is substantially different from what Sieburth and Maruniak (19) reported in another VBC cell line (UFL-AG-286) grown in TC-100 + 10% fetal bovine serum and infected with their prototype virus AgMNPV-2. They found that after 24 h postinfection, the AgMNPV-2 titer already reached 1.99×10^5 $TCID_{50}$/ml and peaked at 72 h postinfection (1.07×10^6 $TCID_{50}$/ml), whereas AgMNPV-CL4-3A1 did not reach that level in BCIRL-AG-AM1 even after 120 h postinoculation (3.2×10^5 $TCID_{50}$/ml). Differences in these results may be attributed to differences in cell lines, virus isolates, and experimental conditions. Also their higher initial postinfection titers may be due to residual inoculum. In our study, the $TCID_{50}$ time course analysis showed no detectable level of AgMNPV-CL4-3A1 replication in BCIRL-HZ-AM1 cells. In contrast, Maruniak et al. (8) reported that six plaque-purified AgMNPV isolates replicated in the Hz-AM cell line though no quantitative data were provided.

In our study, probit analysis of larval mortality data revealed that the four species (*Heliothis virescens, Helicoverpa zea, T. ni, A. gemmatalis*) showed a wide range of susceptibility to the AgMNPV-CL4-3A1 clone with the lowest LC_{50} for *A. gemmatalis* (96.9 OB/cm^2) and the highest for *T. ni* (1.1×10^4 OB/cm^2).Carner et al. (2) used either crude or purified preparations of VBC virus to determine the susceptibility of various insect species to VBC virus. However, it is difficult to compare the LC_{50} values in our study with theirs because different experimental conditions and expression of virus concentration were used. Nevertheless, data from both studies show a general trend in larval susceptibility to the VBC virus: *A. gemmatalis* > *Helicoverpa zea* > *Heliothis virescens* > *T. ni*.

*Hin*dIII, *Bam*HI *Bgl*II and *Pst*I restriction patterns of wt AgMNPV and AgMNPV-CL4-3A1 were similar but with the addition of several minor fragments occurring in*Eco*RI and *Hin*dIII digest, and a single additional fragment in the *Bgl*II profile of wt AgMNPV. These observations may be attributable to: (1) presence of other baculoviruses in the original sample of wt AgMNPV Hondrina isolate, (2) a mixture of genotypic variants of AgMNPV with different numbers of *Hin*dIII, *Eco*RI, and *Bgl*II cleavage sites, or (3) both of the above two possibilities.

Major bands at the 6.5 kbp region in both the *Hin*dIII and *Pst*I fragmentation profiles are present in AgMNPV-CL4-3A1 which are absent in wt AgMNPV. In our study, the enzymes *Bam*HI, *Bgl*II, *Hin*dIII, *Eco*RI, and *Pst*I were found to cleave the AgMNPV-CL4-3A1 genome into 4,5, 18, 18, and 21 fragments, respectively. These fragment numbers are different from those reported by Johnson and Maruniak (4) for *Bam*HI (four fragments), *Bgl*II, (seven fragments), EcoRI (10 fragments), and *Hin*dIII (24 fragments) restriction profiles of AgMNPV-2 and from what Maruniak (7) reported in six other plaqued, purified AgMNPV isolates (mean number *Hin*dIII fragments 24.8; mean number *Pst*I fragments 23.7). Inherent variation in the number of restriction fragments and fragment sizes could probably

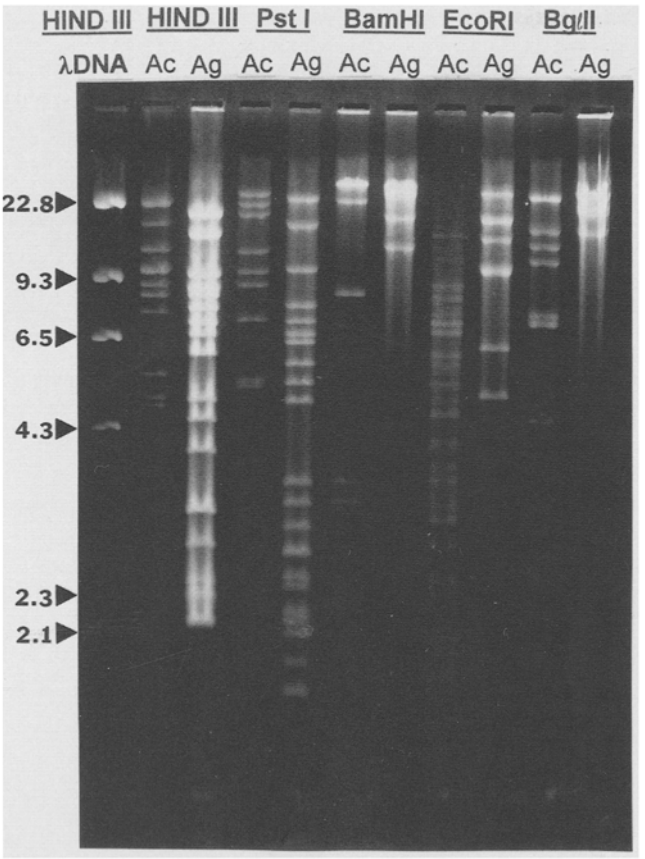

FIG. 2. Comparison of *Hin*dIII, *Eco*RI, *Bam*HI, *Bgl*II, and *Pst*I restriction patterns between wild type AcMNPV (Ac) and AgMNPV-CL4-3A1 (Ag). λ DNA was used as the molecular weight standard and the sizes (kb) of individual bands are indicated.

be a reflection of virus sources (geographical isolates) as well as cell culture passage. AgMNPV isolates have also been shown to be different from each other as a result of insertions or deletions in the viral DNA (7) and this certainly could account for the discrepancies.

The results of these studies show BCIRL-HV-AM1 to be the best producer of both ECV and OB, indicating that this cell line would be the most suitable for the commercial *in vitro* production of AgMNPV. Because there has been some question as to the true identity of AgMNPV, we have shown its REN pattern is different from AcMNPV. Furthermore, DNA hybridization studies (Grasela, unpublished) confirm the difference between these two baculoviruses.

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REFERENCES

1. Abbott, W. S. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18:265–267; 1925.
2. Carner, G. R.; Hudson, J. S.; Barnett, O. W. The infectivity of a nuclear polyhedrosis virus of the velvetbean caterpillar for eight noctuid hosts. J. Invertebr. Pathol. 33:211–216; 1979.
3. Ignoffo, C. M. The nuclear polyhedrosis virus of *Heliothiszea* (Boddie) and *Heliothis virescens* (Fabricius) II. Biology and propagation of diet-reared *Heliothis*. J. Invertebr. Pathol. 7:217–226; 1965.
4. Johnson, D. W.; Maruniak, J. E. Physical map of *Anticarsia gemmatalis* nuclear polyhedrosis virus (AgMNPV-2) DNA. J. Gen. Virol. 70:1877–1883; 1989.
5. Lynn, D. E. A BASIC computer program for analyzing endpoint assays. Biotechniques 12:880–881; 1992.
6. Maeda, S.; Mukohara, Y.; Kondo, A. Characteristically distinct isolates of the nuclear polyhedrosis virus from *Spodoptera litura*. J. Gen. Virol. 71:2631–2639; 1990.
7. Maruniak, J. E. Molecular biology of *Anticarsia gemmatalis* baculovirus. Mem. Inst. Oswaldo Cruz, Rio de Janeiro 84:107–111; 1989.
8. Maruniak, J. E.; Johnson, D.; Gowan, D. AgNPV replication in cell culture and physical map of its DNA. J. Cell. Biochem. (Supplement) 10c:49; 1986.
9. McIntosh, A. H.; Andrews, P. A.; Ignoffo, C. M. Establishment of a continuous cell line of *Heliothis virescens* (F) (Lepidoptera: Noctuidae). In Vitro 17:649–650; 1981.
10. McIntosh, A. H.; Grasela, J. J.; Matteri, R. L. Identification of insect cell lines by DNA amplification fingerprinting (DAF). Insect Mol. Biol. 5:187–195; 1996.
11. McIntosh, A. H.; Ignoffo, C. M. Characterization of five cell lines established from species of *Heliothis*. Appl. Entomol. Zool. 18:262–269; 1983.
12. McIntosh, A. H.; Ignoffo, C. M. Replication of *Autographa californica* nuclear polyhedrosis virus in five lepidopteran cell lines. J. Invertebr. Pathol. 54:97–102; 1989.
13. McIntosh, A. H.; Ignoffo, C. M.; Chen, Q., et al. Establishment of a cell line from *Heliothis armigera* (Hbn) (Lepidoptera: Noctuidae). In Vitro 19:589–590; 1983.
14. McIntosh, A. H.; Rechtoris, C. Insect cells: colony formation in agar medium. In Vitro 10:1–5; 1974.
15. Moscardi, F. Use of viruses for pest control in Brazil: the case of the nuclear polyhedrosis virus of the soybean caterpillar, *Anticarsia gemmatalis*. Mem. Inst. Oswaldo Cruz 84, Suppl. 3, 51–56; 1989.
16. Moscardi, F.; Correa-Furriery, B. S. Biological control of soybean caterpillar. In: Shibles, R., ed. World Soybean Research Conference III: Proceedings. Boulder and London: Westview Press; 1985:703–711.
17. Pavan, O. H.; Boucias, D. G.; Pendland, J. C. The effects of serial passage of a nucleopolyhedrosis virus through an alternate host system. Entomophaga 26:99–108; 1981.
18. Richter, A. R.; Fuxa, J. R. Timing, formulation, and persistence of a nuclear polyhedrosis virus and a microsporidium for control of the velvetbean caterpillar (Lepidoptera: Noctuidae) in soybeans. J. Econ. Entomol. 77:1299–1306; 1984.
19. Sieburth, P. J.; Maruniak, J. E. Susceptibility of an established cell line of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) to three nuclear polyhedrosis viruses. J. Invertebr. Pathol. 52:453–458; 1988.
20. Sokal, R. R.; Rohlf, F. K. Biometry. 2nd ed. San Francisco, CA: W. H. Freeman Press; 1981.
21. Southern, E. M. Measurement of DNA length by gel electrophoresis. Anal. Biochem. 100:319–323; 1979.
22. Stiles, B.; McDonald, I. C.; Gerst, J. W. Initiation and characterization of five embryonic cell lines from the cotton boll weevil *Anthonomous grandis* in a commercial serum-free medium. In Vitro Cell. Dev. Biol. 28A:355–363; 1992.
23. Strayer, J. R.; Greene, G. L. Soybean insect management. Fla. Coop. Ext. Serv. Circ. 395:1–15; 1974.
24. Turnipseed, S. G. Insects. In: Caldwell, B. E., ed. Soybeans: improvements, production, and uses. Amer. Soc. of Agronomics, Madison, WI; 1973:545–572.
25. Wuensche, A. L. Relative abundance of seven species and three predaceous genera in three soybean ecosystems in Louisiana. M.S. thesis. Louisiana State University, Baton Rouge; 1976.