Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *84*, Supl. III, 63-65, 1989

SELECTION OF A BACULOVIRUS STRAIN WITH A BIVALENT INSETICIDAL ACTIVITY

OCTAVIO HENRIQUE O. PAVAN & HELENA C. T. RIBEIRO (+)

Laboratório de Virologia de Insetos, Departamento de Genética, IB, UNICAMP, 13081 Campinas, SP, Brasil

Baculoviruses are considered to be specific and are highly virulent in their host insects. The question of *in vivo* specificity, however seems to be in some cases dose-related as shown by Pavan et al. (1981) in the case of *Anticarsia gemmatalis* Nucleopolyhedrosis Virus (AgNPV). The inoculation method also may play an important role (Pavan & Boucias, 1981).

The importance of specificity and virulence of Baculoviruses increases as they are extensively being used as biological insecticides. These viruses being simply produced as they come from natural populations tend ro reflect a classical coevolution model involving the pathogen and its host and tending to a moderate pathogenicity in an equilibrium situation. This type of situation would lead to an inneficient system and problems of the same type as found in chemical insecticides.

Recently however, several authors have shown that, the at least 600 different types of Baculoviruses are a very complex biological system represented by a single covalently closed, superhelical double stranded DNA molecule ranging in molecular weight from 60 to 110 million daltons representing 90 to 175 thousand base pairs (Kelly, 1977; Harrap et al., 1977; Bud & Kelly, 1977; Knudson & Tinsley, 1978; Smith & Summers, 1978; Mathews, 1982). Recently naturally occurring genomic variants have been detected in several of these virus by restriction enzyme analysis of viral DNA (Lee & Miller, 1978; Smith & Summers, 1978). In some cases significant differences in virulence are found between variants (Vail et al., 1982). Hughes et al. (1983) were able to show a correlation between virulence and DNA restriction enzyme patterns. This type of result provides the bases to investigate molecular alterations involved in the change of virulence. Although there are a number of studies documenting the host range and virulence of baculovirus, the mechanisms involved in virulence have not been determined. On the other side however, these studies clearly indicate a system of genetic variability in these baculovirus populations therefore susceptible to genetic selection processes.

Pavan et al. (1981) and Carner et al. (1979) have shown that the AgNPV is capable of infecting several species of lepidoptera as alternate hosts. One of these species, *Diatraea saccharalis* the sugarcane borer (SCB), can be infected with high dosages of the AgNPV and is able to produce normal polyhedral inclusion bodies (PIB) exhibiting a typical infection.

The fact that an alternate host is susceptible to a high dosage of a virus and the observation by Maruniak, J. E. (personal communication) of the presence of a large number of genomic variants suggests very clearly the possibility of selecting a more virulent isolate of the virus.

Laboratory populations *D. saccharalis* are very well stablished by methodology described by Degaspari et al. (1981) and large amounts of homogenous and pathogen-free larvae were furnished through a cooperative research program by the Entomology Section of the IAA/PLANALSUCAR (Araras, SP, Brazil).

Natural populations of the SCB in Brazil do not exhibit any endemic viral pathogen. A granulosis virus was introduced by Pavan et al. (1983). Using laboratory reared third instar larvae (11 days old) a study was conducted to determine the possibility to obtain a more virulent isolate of the AgNPV to *D. saccharalis*. The larvae were maintained at 28 ^{o}C ($\pm$ 1 ^{o}C) in 12 hour photophase.

In order to select the virus the serial passage procedure was used. Virus at a high multiplicity

This work was supported by CNPq, MIC/STI and under a cooperative agreement with the Entomology Section of IAA/PLANALSUCAR.

(+) Doctoral fellow – CNPq.

of infection was orally inoculated to 3rd instar SCB larvae. Virus isolated from larvae exhibiting typical signs of infection and homogeneous polyhedra were used as the inoculum for the next generation. With this procedure it is expected that the more virulent genomic variants to *Diatraea* would be replicated resulting in a selected and more efficient pathogen to the insect species.

The AgNPV was obtained from *A. gemmatalis* infected larvae with a strain originally isolated from diseased larvae collected in Campinas, Brazil, in 1972.

For the inoculation, the virus was purified by homogenizing infected larvae in a .1% solution contaning phenylthiourea to prevent melanization. Polyhedral inclusion bodies were extracted using alternate cycles of low (500 g for 2 min) and high (8000 g for 20 min) speed centrifugation steps. The concentration of PIB in the solutions were determined using a hemacytometer.

Groups of 3rd instar larvae were fed diet discs treated with 5 different doses of PIB. The larvae that completely consumed the discs within a 24 h period were then transfered to containers with uncontaminated diet. Control groups fed discs treated with water. Mortality was checked daily. Infection by NPV was confirmed by phase microscopy of representative tissue smears.

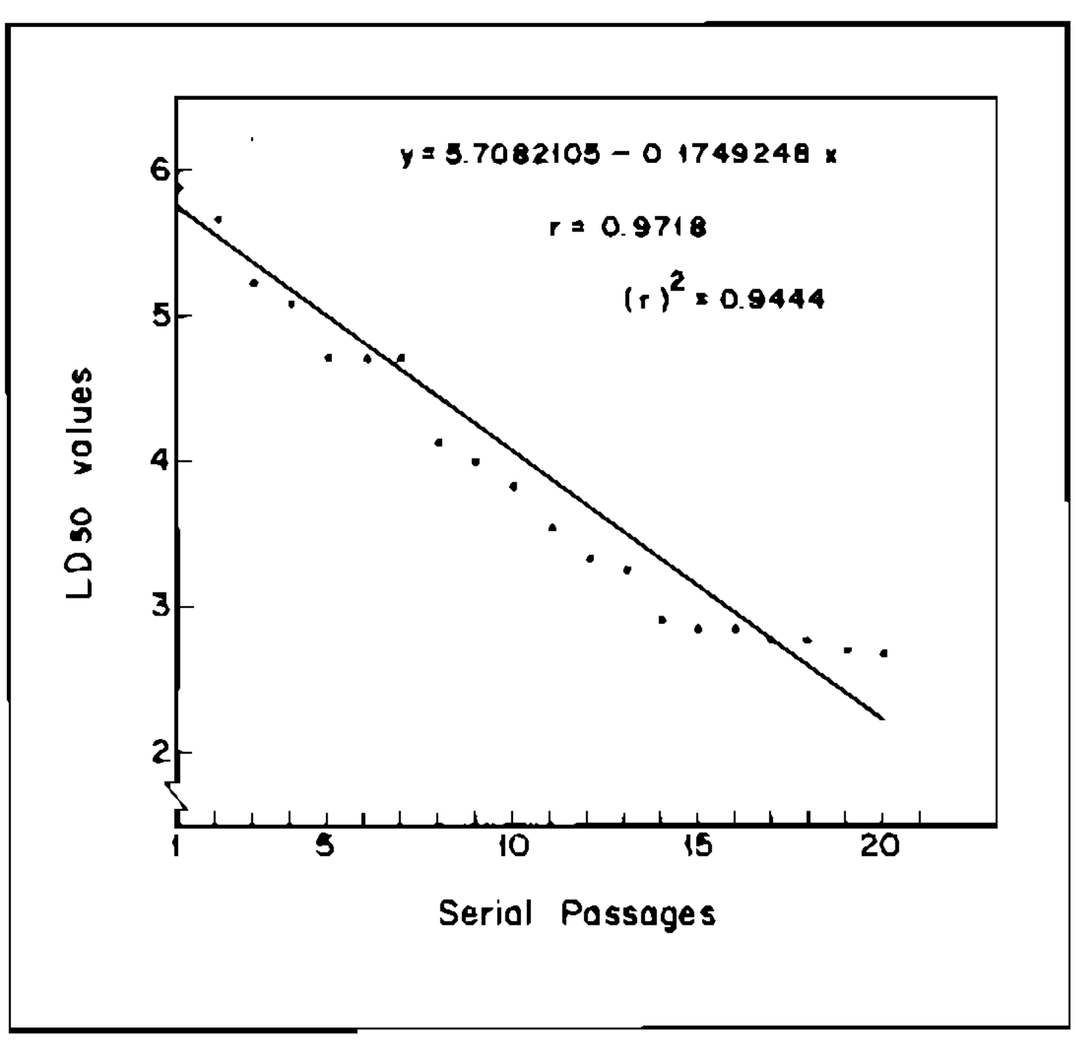
In the Table the LD_{50} values for 3rd instar *D. saccharalis* are presented for each passage. The result for the first passage represents the inoculum obtained from *A. gemmatalis* larvae inoculated into SCB larvae. The results show a 1500 fold reduction in the value of LD_{50} over a series of 20 serial passages which represents a unique result when compared to data from literature. The LD_{50} value for the last isolate ($n = 20$) is in the same range observed for the original virus of *D. saccharalis*, the DsGV (Pavan et al., 1983).

The gradual reduction of the LD value which represents the increase in virulence can be clearly seen in the Figure. The value of the LD_{50}, in log, is inversely proportional to the number of passages, showing a linear correlation with high regression coeficient values (r and r^2).

TABLE

Lethal dose 50% (LD_{50}) values for the AgNPV passed serially in *Diatraea saccharalis*. Values for 3rd instar larvae (11 days old) reared at 28 °C (± 1 °C) in 12 hours photophase

Serial passage number	Number of tested individuals	LD_{50} values (PIB/larvae)
1	508	7.89×10^5
2	514	4.51×10^5
3	480	1.63×10^5
4	566	1.20×10^5
5	500	5.02×10^4
6	490	4.89×10^4
7	500	4.77×10^4
8	482	1.28×10^4
9	500	9.45×10^3
10	500	6.64×10^3
11	595	3.26×10^3
12	568	2.04×10^3
13	540	1.83×10^3
14	563	7.49×10^2
15	600	7.17×10^2
16	594	6.92×10^2
17	560	6.19×10^2
18	600	5.84×10^2
19	510	5.39×10^2
20	592	5.27×10^2



LD_{50} values (log) for each generation of the serial passage of the AgNPV in *Diatraea saccharalis*. Values for 3rd instar larvae (11 days old) maintained at 28 °C (± 1 °C) and 12 hours photophase.

The type of response to the selective pressure observed readly discards the possibility of simple selection of the most infective genomic variant. This corroborates the preliminary results of SDS – PAGE of viral proteins showing no differences between the isolates.

An unexpected result however, was the fact that the isolate from passage number 20(F20) still retains the virulence to the original host *A. gemmatalis in vivo* and *in vitro* (Pavan & Maruniak, unpublished).

These results indicate either a very slow selective increase of a type of variant or a very steady alteration of the genome caused by the replication in a different cellular environment. The later model would implicate in the insertion or deletion of small DNA fragments. A similar situation observed by Croizier et al. (1985) indicated that the alteration of virulence would be related to the insertion of host derived copia-like transposon.

More recent data from our laboratory indicates that a very small alteration in the variable region of the genome is responsible for the great biological change.

The importance of the results lies in the fact that a deep transformation of the basic characteristics of this virus, that is specificity and pathogenicity, can be manipulated by a safe and simple method.

This isolate is being used today as a bivalent insecticide against two pests of great importance being the *A. gemmatalis* in soybean and *D. saccharalis* in sugarcane. The application for its registration as a comercial bioinsecticide has been forwarded.

This isolate is the main subject of a cooperative research project being carried out between our laboratory and Dr J. E. Maruniak in the University of Florida dealing with the identification of the molecular changes involved in this process of selection.

REFERENCES

BUD, H. M. & KELLY, D. C., 1977. The DNA contained by nuclear polyhedrosis virus isolated from four Spodoptera spp (Lepidoptera, Noctuidae); genome size and configuration assessed by electron microscopy. I. *Gen. Virol.*, *37:* 135-143.

CARNER, G. E.; HUDSON, J. S. & BERNETT, O. W., 1979. The infectivity of a nucleopolyhedrosis virus for control of the velvetbean caterpillar for eight other noctuid hosts. *J. Invertebr. Pathol.*, *33:* 211-216.

CROIZIER, G.; CROIZIER, L.; BIACHE, G. & CHAUFAUX, J., 1985. Evolution de la composition genetique et du pouvoir infectieux du baculovirus de *Mamestra brassicae* L.: Au cours de 25 multiplications sucessives sur les larves de la noctuelle du chou. *Entomophaga*, *30:* 365-374.

DEGASPARI, N.; BOTELHO, P. S. M. & MACEDO, N., 1981. Controle químico de *Diatraea saccharalis* em cana-de-açúcar na região Centro Sul do Brasil. *Bol. Tec. IAA/PLANALSUCAR*, *3:* 1-16.

HARRAP, K. A.; PAYNE, C. C. & ROBERTSON, J. S., 1977. The properties of three baculoviruses from closely related hosts. *Virology*, *79:* 14-31.

HUGHES, P. R.; GETTIG, R. R. & McCARTY, W. J., 1983. Comparison of the time-mortality response of *Heliothis zea* nuclear polyhedrosis virus. *J. Invertebr. Pathol.*, *41:* 256-261.

KELLY, D. C., 1977. The DNA contained by nuclear polyhedrosis viruses isolated from four *Spodoptera* sp. (Lepidoptera, Noctuidae); Genome size and homology assessed by DNA reassociation kinetics. *Virology*, *76:* 468-471.

KNUDSON, D. L. & TINSLEY, T. W., 1978. Replication of a nuclear polyhedrosis virus in a continuous cell line of *Spodoptera frugiperda:* Partial characterization of the viral DNA, comparative DNA-DNA hybridization, and patterns of DNA synthesis. *Virology*, *87:* 42-57.

LEE, H. H. & MILLER, L. K., 1978. Isolation of genotypic variants of *Autographa californica* nuclear polyhedrosis virus. *J. Virol.*, *27:* 754-767.

MATHEWS, R. E. F., 1982. *Classification and Nomenclature of Viruses.* p. 1-199, Karger, Basel.

PAVAN, O. H. O. & BOUCIAS, D. G., 1981. Vírus de Poliedrose Nuclear de *Anticarsia gemmatalis:* Métodos de inoculação e especificidade. In Seminário de Pesq. de Soja 2º Anais. EMBRAPA, p. 180-186.

PAVAN, O. H. O.; BOUCIAS, D. G.; ALMEIDA, L. C.; GASPAR, J. O.; BOTELHO, P. S. M. & DEGASPARI, N., 1983. Granulosis Virus of *Diatraea Saccharalis* (DsGV): Pathogenicity, replication and ultrastructure. In: International Cong. ISSCT, 18. La Habana, Cuba. Proceedings Vol. II, tomo 2: 644-659.

PAVAN, O. H. O.; BOUCIAS, D. G. & PENDLAND, J. C., 1981. The effects of serial passage of a nucleopolyhedrosis virus through an alternate host system. *Entomophaga*, *28:* 99-108.

SMITH, G. E. & SUMMERS, M. D., 1978. Analysis of baculovirus genomes with restriction endonucleases. *Virology*, *89:* 517-527.

VAIL, P. V.; KNELL, J. D.; SUMMERS, M. D. & COWAN, D. K., 1982. *In vivo* infectivity of baculovirus isolates, variants and natural recombinants in alternate host. *Environ. Entomol.*, *11:* 1187-1192.