

**Biological and molecular identification and characterization of entomopathogenic viruses of Baculoviridae and studying the interactions between different baculoviruses and ectoparasitoids on some Noctuidae species**

- Specialization at the laboratory of “Agricultural Entomology and Pathology of Insects”, University of Navarra, Pamplona, Spain, January-July, 2006.

Studies performing in this laboratory:

- biological and molecular identification and chracterization of some baculoviruses and *Bacillus thuringiensis*.
- Studies on interactions between different baculoviruses and ecto- and endoparasitoids.

- Molecular methods for identification and characterization of SfMNPV, SeMNPV and HaMNPV – spanish isolates and the MbMNPV– bulgarian isolate

#### 1. Purification of occlusion bodies (OBs)

- homogenizing the cadavers
- filtration of the viral suspension through cheesecloth
- adding of SDS
- centrifugation
- resuspension of the pellet in 1 ml bidistilled water
- storing the viral suspension at 4<sup>0</sup> C

## 2. Extraction of viral genomic DNA

- adding of buffer with pH 10,5
- incubation with proteinase K
- two procedures with phenol
- a procedure with chloroform
- adding of sodium acetate and absolute ethanol
- adding of 70 % ethanol
- dissolution of DNA in buffer

## 3. Restriction endonuclease (REN) analysis of genomic viral DNA

- Incubation with enzyme and buffer at 37 °C for 4-12 h.

## 4. Gel electrophoresis

## 5. Polymerase chain reaction (PCR)

Solutions required:  $\text{NH}_4$ ,  $\text{MgCl}_2$ , primer F, primer R, dNTPs, Taq polymerase, DNA and  $\text{H}_2\text{O}$ .

- denaturation at 94 °C, 30 sek.
- annealing at 50 °C, 1 min.
- extension at 72 °C, 1 min.

- Biological characterization on the MbMNPV and HaMNPV:
  - titration of occlusion body particles
  - “*In vivo*” passaging of the viruses
  - determination the median lethal concentration, dose and time of the baculoviruses

Methods for rearing of the ectoparasitoid *Euplectrus plathypenae* (Hymenoptera: Eulophyidae) and the endoparasitoid *Chelonus insularis* (Hymenoptera: Braconidae) on *S. frugiperda* larvae (Lepidoptera: Noctuidae).



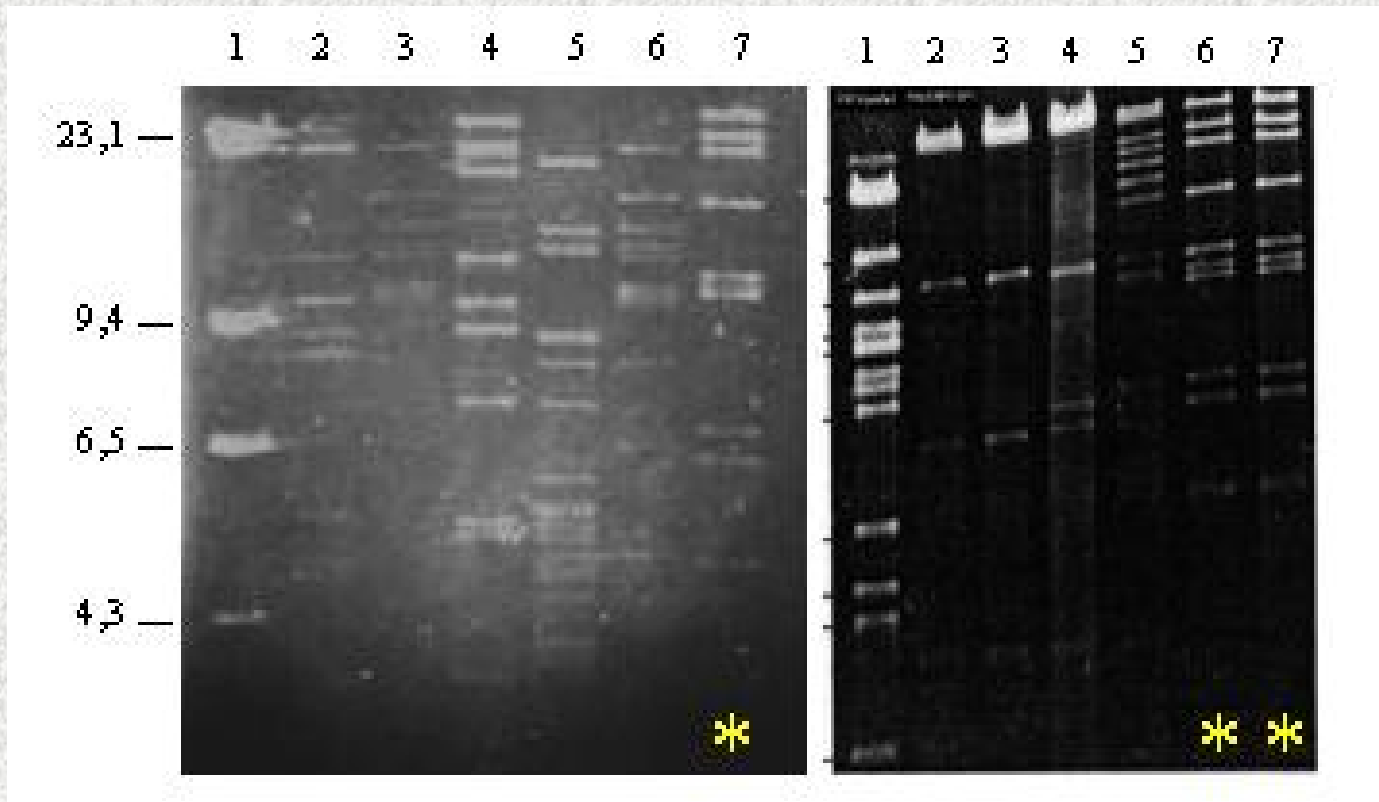
*E. plathypenae*



*S. frugiperda*



*C. insularis*



**Fig. 1:** (a) REN digestion of the viral DNA of AcMNPV-C6 (lanes 2-4) and MbMNPV-Bul (lanes 5-7) digested with HindIII, EcoRI and PstI. A molecular size marker is shown in lane 1 and the sizes of its fragments are indicated to the left of the figure. (b) Figure obtained from Figueiredo et al. (1999) showing the PstI analysis of the genomic DNA of different isolates of *Spodoptera exigua* and *Helicoverpa armigera* NPVs (lanes 1-4), and two different isolates of MbMPNV from Poland (lane 6) and France, which corresponds to the active compound of Mamestrin® (lane 7). Numbers indicate lanes that show identical REN profiles between the two figures.

- Studying the interactions between HaMNPV, SeMNPV and SfMNPV and the ectoparasitoid *Euplectrus plathypenae* (Hymenoptera: Eulophidae) and their phytophagous hosts.
  - effect of the baculovirus on the longevity and productivity *E. plathypenae*
  - determination of the most appropriate larval instar of *S. exigua*, *S. frugiperda* and *H. armigera* for the host-parasitoid-entomopathogen interactions
  - determination the period between the infection with the virus and parazitation of the host
  - studing the ovipositional discrimination of the parasitoid between healthy and virus-infected larvae
  - studing the possiblity of the parasitoid to transmit the viral infection from virus-infected to healthy larvae
  - effect of parasititsm on the genetic composition of a nucleopolyhedrovirus in host competition conditions

- **Conclusions:**

- I. The methods for biological and molecular identification and chracterization has been aquired.
- II. The methods for rearing the ecto- and endoparasitoids *E. plathypenae* and *C. insularis* has been aquired.
- III. Host-parasitoid-entomopathogen interactions:

1. The virus does not adverse the longevity of the parasitoid.
2. The 4th instar is the most appropriate larval instar for *S. exigua*, *S. frugiperda* and *H. armigera*.



3. The period between parasitation and virus-infection is:
  - *S. exigua* – 48 h.
  - *H. armigera* – 24 h.
  - *S. frugiperda* – at the same time



*S. exigua*



*H. armigera*



*S. frugiperda*

4. The parasitoid distinguishes healthy from virus-infected larvae.
5. The parasitoid transmits the virus from infected larvae to 24 % *S. frugiperda* healthy larvae and 32 % to *H. armigera* healthy larvae.