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EVIDENCE FOR TWO SMALL VIRUSES PERSISTENTLY INFECTING ESTABLISHED CELL LINES OF *PHTHORIMAEA OPERCULELLA*, DERIVING FROM EMBRYOS OF THE POTATO TUBER MOTH

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SUMMARY

Two small viruses were isolated from established cell lines of *P. operculella* deriving from embryos. The first one probably related to the Nodaviridae family, is a 30 nm in diameter icosahedral virus, with a bisegmented RNA genome and a single polypeptide of 39 kilodaltons. The second one related to the Parvoviridae family, is a 25 nm in diameter icosahedral virus with a DNA genome and a capsid constituted of 4 polypeptides of respectively, 90,000; 64,000; 56,000 and 43,500 daltons. The two viruses probably chronically infect the cell lines and may be consider latent viruses.

KEY WORDS: *Phthorimaea operculella*, Lepidoptera, cell line, Nodavirus, Densovirus, latent virus

In the field of viral biological controls, only one group of viruses can be used at present, the baculoviruses (polyhedrosis viruses and granulosis viruses), which have been homologated to be used as biological agents (Tinsley *et al.*, 1980). However, several other viruses can potentially, be used, for their virulence, pathogenicity and/or facility to be multiplied, such as some small free viruses (Fédière *et al.*, 1990). Unfortunately, for several

reasons, they were not accepted for direct use in biological programs. Nevertheless, an alternative may exist. Persistent viruses, with a multiplication rate staying at a low level, well tolerated by the cells, are regularly mentioned in the literature (Friesen *et al.*, 1980; Oldstone, 1989). These types of viruses could probably always be detected on larvae coming from the field or reared in the laboratory, if systematic researches were conducted (Zeddam *et al.*, 1994). These viruses, generally called latent viruses, chronically infecting larvae, could be stimulated under particular conditions, to induce important mortality (Kelly *et al.*, 1981; Léry *et al.*, 1997). The study of such viruses, focusing in a first step on their identification, the knowledge of the mechanisms by which they can be tolerated and their mode of persistence, and in a second step, on the factors or agents conditioning their reactivation could become of great interest. Such studies are generally difficult to conduct because of the low levels of virus detected *in vivo*. Therefore, *in vitro* models must reflect the chronic natural infections (Jousset *et al.*, 1993; Léry *et al.*, 1997).

The *in vitro* studies conducted in our laboratory, as part of the biological program against the main potato crop pest in warm areas, the potato tuber moth (PTM) *Phthorimaea operculella*, allowed us to obtain such models. During the establishment of multiple cell lines from embryonic PTM cells (Léry *et al.*, 1994, Léry *et al.* 1995b) and while assays were performed to multiply the granulosis virus isolated from this insect (PTMGV) by infection and transfection of these cell lines, two new viruses, chronically infecting the cells, were identified.

The first virus was isolated from cells experimentally transfected with DNA extracted from the PTMGV Tunisia isolate. 2 x 10^6 cells from ORS-Pop-93 cell line at the 30th passage (Léry *et al.*, 1995b) were seeded in 25 cm^2 culture flasks and cultivated in modified Grace's medium (Léry and Fédière, 1990) containing 10% fetal calf serum (FBS) for 24 h at 27°C. 4 μg of purified DNA in 0.05 *M* Tris, pH 7.5, mixed with 20 μl DOTAP (Boehringer) in the presence of modified

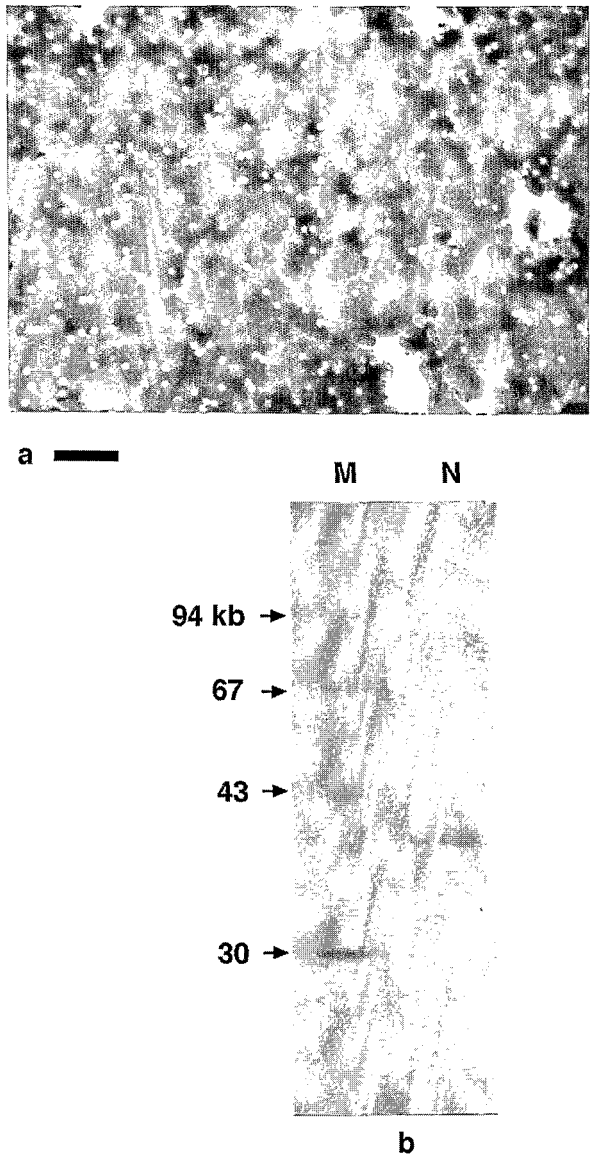

FIGURE 1 - Characterization of the Nodavirus isolated from the ORS-Pop-93 cell line established from embryos of the potato tuber moth *Phthorimaea operculella*.
a: Electron micrograph of purified viral particles. Bar represents 200 nm.
b: Electrophoretic profile of purified viral particles on a 10% polyacrylamide gel. M: Marker, N: Nodavirus.

Grace's medium without FBS were used for the transfections (Léry *et al.*, 1995a). After 2 weeks post-transfection, a cytopathogenic effect was clearly observed. The cells and supernatants were collected for the detection of both granules and virions. The cells were

Potter-homogenized and ultrasonicated, and the suspension was centrifuged at 15,000g for 10 min. After ultracentrifugation of the supernatant for 2 h at 100,000g, the pellet resuspended in 0.05 *M* Tris, pH 7.5 was kept overnight, then deposited on a 15% to 45% (W/W) sucrose gradient and ultracentrifuged for 2 h 30 at 120,000g. A small virus was then isolated. The band containing this virus was collected and concentrated as above. The 500 µl purified suspension, with an optical density of 1.0 at 260 nm measured on a spectrophotometer, consisted of 30 nm icosahedral particles measured by electron microscopic study (Fig. 1a). The electrophoresis of genome purified by the method of Hilz *et al.* (1975) showed the RNA nature of the nucleic acid. The 2 bands observed revealed its bisegmentation. The analysis of the proteins separated by electrophoresis in 10% polyacrylamide gel, according to Laemmli (1970), revealed one polypeptide of 39 kilodaltons (Fig. 1b). All these characteristics indicate that this virus might be a member of the Nodaviridae family. The purified virus was used for *in vivo* and *in vitro* multiplicating attempts on a PTM model, but no multiplication could be noticed, revealing the low pathogenicity of the virus and confirming the presence of small amounts of virus in the non experimentally infected cell line. Further assays to increase the quantity of virus, using other *in vivo* and *in vitro* models (*Galleria mellonella*, *Spodoptera littoralis*) gave the same negative results. The selection during the passages of non chronically infected cells (having an advantage compared to the infected ones), as well as a contamination of the DNA used for the transfections cannot be excluded. Several systematic attempts were thus made to recover the virus *in vitro* from early passages of new PTM cell cultures from which the ORS-Pop-94A cell line (PopA) derived (Léry *et al.*, 1994).

From the non experimentally infected cells of PopA, cultivated in the same conditions as the ORS-Pop-93 cell line and regularly harvested between the 4th and the 7th passage, another virus could be isolated. This new virus, purified and characterized as above, is probably related to the Densovirus group (Parvoviridae). It was a 25 nm in diameter icosahedric virus, with a DNA genome, and a capsid consisting of 4 polypeptides of 90,000; 64,000; 56,000 and 43,500 daltons respectively, the last as a major band (Fig. 2). As observed for the Nodavirus, it was impossible to duplicate the phenomenon *in vitro* or to multiply the purified virus *in vivo*, revealing its low pathogenicity and that the virus probably chronically infects the cells. This second result also confirms the great difficulty in multiplying these viruses in order to characterize them completely.

The isolation of these two viruses from different *P. operculella* cell cultures, raises the problem of the origin of these two viruses. Several possibilities exist. First, a contamination with the viruses multiplied in our laboratory can be excluded because no Nodavirus had been isolated or used previously. The cell lines were tested regularly against the viruses commonly utilized in our laboratory and the *P. operculella* cell lines were not permissive. Second, a natural contamination of

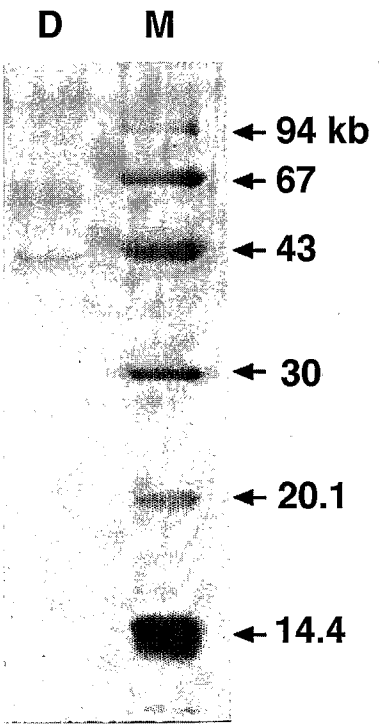

FIGURE 2 - Electrophoretic profile of purified viral particles of the Densovirus persistently infecting the cells of the ORS-Pop-94A cell line, established from embryos of the potato tuber moth *Phthorimaea operculella*, on a 10% polyacrylamide gel. M: Marker, D: Densovirus.

the inoculum used to reveal the virus is always possible, but the Nodavirus which has a RNA genome was reaveled after transfection with DNA and the Densovirus was found on non-experimentally infected cells. Third, the viruses are chronically infecting the cell lines. In this case, the cell lines could contain a small number of progeny cells that have spontaneously become permissive for virus replication, but the negative results obtained to multiply the purified virus, both *in vitro* and *in vivo*, indicate that more probably, the viruses could be considered latent viruses, staying at a low concentration. When the heterologous infection or transfection induced the multiplication of the persistent virus, probably due to a derepression of the cellular system, too much virus is present in the cells to stop it. But when the persistent virus is used as inoculum, the cellular system can block its multiplication.

Nevertheless, the *in vitro* isolation, of these two viruses from cells of embryonic origin, probably reveals a transovarian phenomenon of transmission and the presence in adults and larvae of these viruses can be admitted. Systematic studies, conducted on larvae collected in the field over several years, showed the presence of small viruses in electron microscopy, at a very low level, and with the same sizes as the two viruses described in this paper (Zeddam *et al.*, 1994). So, serological and genetic studies could be attempted to establish the origin of the viruses revealed *in vitro*. These chronic PTM infections by small persistent viruses raises the problem of the interpretation of epidemiological results which could be obtained on the impact of the PTMGV. It has to be borne in mind that not only the PTMGV, but may be other viruses can induce the epizooties observed in the field and in stored potatoes.

The multiplication of these viruses was at least demonstrated *in vitro* with the observed cytopathogenic effects. So, when the necessary conditions for their reactivation are controlled (Kelly *et al.*, 1981) *in vitro* and *in vivo*, the latent viruses could be considered potential new agents for PTM biological control.

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