

REPLICATION AND INCLUSION BODY CHARACTERISTICS OF TWO *LYMANTRIA DISPAR* NUCLEAR POLYHEDROSIS VIRUS PLAQUE VARIANTS.

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ABSTRACT

Propagation of *Autographa californica* nuclear polyhedrosis virus in cell culture results in the generation of a mutant virus, termed few polyhedra. This plaque variant is characterized by a high budded virus titer, the formation of few polyhedral inclusion bodies (PIBs), and the production of PIBs exhibiting a low potency against its natural host. The isolation and study of a few polyhedra variant of *Lymantria dispar* nuclear polyhedrosis virus (LdNPV) could lead to a means of enhancing viral potency against the gypsy moth.

The replication characteristics of two *Lymantria dispar* nuclear polyhedrosis virus (LdNPV) plaque variants were studied in cell culture and *in-vivo*. In addition, the PIBs produced by the variants were characterized with respect to virion number, size, and morphology. The variants, clonal isolates (CI) A2-1 and 5-6, were found to exhibit differences in the number of PIBs generated, the titer of nonoccluded virus (NOV) produced *in-vitro*, the number of virions present within PIBs, and the potency of PIBs against the gypsy moth.

Infection of 652Y cells with CI A2-1 resulted in cells with an average of 51 PIBs per cell (ranging in number from approximately 3 to greater than 50 PIBs/cell), and with the variant CI 5-6, cells with an average of 4.4 PIBs per cell (ranging from approximately 1 to 12 PIBs/cell). The production of NOV in 652Y cells by the plaque variants was markedly different; CIs A2-1 and 5-6 exhibited TCID₅₀s six days after infection of 4.1×10^{-4} and 1.6×10^{-7} , respectively.

The number of virions present, and size and morphology of PIBs generated by CIs A2-1 and 5-6, were analyzed through electron microscopic examination. CI A2-1 PIBs generated *in-vivo* contained an average of 11.6 virions per square micrometer of PIB surface area, in comparison to 0.09 virions/ μm^2 of PIB surface area within CI 5-6 polyhedra. In addition, larvae infected with clonal isolate A2-1 produced an average of 2.1×10^{-9} PIBs per larvae, ranging in size from 1.2 to 2.8 μm in diameter, with an average of 2.0 μm , while CI 5-6 generated an average of 8.6×10^{-7} PIBs per larvae, ranging from 1.9 to 5.8 μm in diameter, with an average of 2.8 μm . The shape of CI 5-6 and A2-1 PIBs produced *in-vivo* and of CI A2-1 produced *in-vitro* were round. In contrast, CI 5-6 PIBs generated *in-vitro* were primarily triangular, rectangular, or trapezoidal in shape. The potency of the variants against second instar gypsy moth larvae was investigated through bioassay. Preliminary data indicate that the LC-50 of CI A2-1 is approximately 1×10^{-3} PIBs per ml of diet. In contrast, the LC-50 of CI 5-6 is greater than 1×10^{-6} PIBs/ml of diet.

The traits of CI 5-6: a high NOV titer, production of few PIBs, and the low potency of the PIBs are consistent with the classification of this virus as a few polyhedra variant. In contrast, the characteristics of CI A2-1: a lower NOV titer, production of many PIBs, and a wild type potency level are consistent with it being classified a many polyhedra wild type virus. The molecular basis for the few polyhedra phenotype of CI 5-6 is currently under investigation. There appears to be a correlation between potency and the number of virions present within PIBs. If such a relationship does exist these studies may yield insights into the process of virion occlusion and present a means of enhancing viral potency.