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Short Communication

CHANGE IN THE VIRULENCE OF THE *LYMANTRIA DISPAR* NUCLEOPOLYHEDROVIRUS DURING PASSAGE IN THE INSECT HOST

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ABSTRACT

The virulence of the Asian and North American strains of the gypsy moth nucleopolyhedrovirus was studied. Viruses were passaged in gypsy moth larvae, and high-performance sequencing of viral genomes was carried out. It was shown that the virulence of the Asian strain, which was initially 100-fold lower than the North American strain, increased to the level of the latter after passaging. However, the deletion of virulence-associated vef-1 gene, revealed in the genome of the original Asian strain, indicated that it did not recover after passaging. Thus, the increase in virulence was likely determined by other changes in the genome.

Keywords: *Lymantria dispar*; Nucleopolyhedrovirus; Strains; Sequencing; Virulence

INTRODUCTION

Lymantria dipar (L.) is one of the most biologically and economically significant phyllophagous insects of the forest. It has an extensive habitat and is widespread in the territories of Eurasia, North America and northern Africa. Occasionally, gypsy moth outbreaks become pandemic, leading to damaged or dead trees on millions of hectares (Marshall, 1981). In Russia, *Lymantria dipar* is the most common species among the phyllophagous insects: the area of forests with its dense, defoliating populations amounts to an average of 1 million hectares annually (data from the Republican Forest Protection Center). In the populations of the gypsy moth, the nucleopolyhedrovirus (NPV) is widespread, which can be an important factor in the population dynamics of the insect (Elkinton, 1990, Hoch et al., 2001). Products based on gypsy moth NPV have been developed in several countries and have been used as biological control agents against the pest. The NPV strains isolated from various geographic populations of the host insect differ significantly in their biological activity (Shapiro et al., 1984; Martemyanov et al., 2015, Martemyanov et al., 2017). One of the most significant reasons for these differences is most likely the genotypic heterogeneity of baculovirus strains (Martemyanov et al., 2015; Martemyanov et al., 2017). In the process of in vivo passage, the virulence changes due to the accumulation of non-synonymous nucleotide substitutions in the genome (leading to the replacement of the amino acids in the protein products of certain virus genes), or large-scale mutations, such as deletions of the whole genes (Krell, 1996; McLachlin et al., 2001). Thus, the present work was an attempt to reveal the molecular determinants of the virulence of the *Lymantria dipar* nucleopolyhedrovirus during its passaging in larvae of the host insect.

MATERIALS AND METHODS

In a series of four passages, two strains of the virus - Asian (LdMNPV-27/0) and North American (LdMNPV-45/0) from the collection of IASE of the SB RAS were used. For passaging of viruses, larvae from a laboratory culture of gypsy moth, free of covert baculovirus infection, were used. Larvae were cultured on an artificial diet as described previously (Ilyinykh et al., 2004). During the passaging of viruses, fourth instar larvae were used, which were the progeny of one female (i.e. from same family). The insects were infected with the virus in a concentration that caused the death of about 100% of individuals. To do this, the surface of the artificial diet in the Petri dishes was treated with a virus and 10 larvae were placed in each dish. The total number of infected larvae was 100 individuals in each passage. The insects that died from NPV were collected daily, frozen at -200C and stored in a freezer. For sequencing and annotation of the strains (see below), viral material from 15 larvae which died from NPV was used, and the remainder of the dead insects were used for the next passage and determination of the virulence of the strains. Before the next passage and the determination of virulence, the virus from the dead larvae was isolated by centrifugation (Kavabarata and Matsumoto, 1973) and the titer of polyhedra in Goryaev's chamber (analogous to Thoma chamber) was counted. After each passage, the virulence of the compared

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Table 1: Biological activity (LC_{50}) of North American and Asian strains of NPV *Lymantria dispar* (L.) in passage series.

Assay no.	Virus strain	Passage	LC_{50} (95CL)	95% fiducial limits		Slope ± SE	Heterogeneity (χ^2/n)
				lower	upper		
1	North American LdMNPV-45/0	Zeroth	1.5×10^3	0.75×10^3	2.8×10^3	0.003	0.701 ± 0.076
2		First	0.28×10^3	0.0001	3.3×10^3	0.017	0.4893 ± 0.1309
3		Second	0.4×10^3	0.1×10^3	1×10^3	0.011	0.5022 ± 0.0722
4		Third	0.7×10^3	0.0231	0.9×10^4	0.02	0.4606 ± 0.119
5		Fourth	0.68×10^3	0.1443	7.4×10^3	0.008	0.498 ± 0.124
6	Asian LdMNPV-27/0	Zeroth	1×10^6	0.6×10^5	0.18×10^6	0.008	0.8984 ± 0.0868
7		First	0.9×10^6	0.1×10^3	0.6×10^6	0.06	0.598 ± 0.1549
8		Second	0.35×10^4	0.8078	0.1×10^6	0.05	0.5202 ± 0.1436
9		Third	0.8×10^3	0.24×10^3	0.22×10^4	0.001	0.4337 ± 0.0657
10		Fourth	0.4×10^3	0.072	5×10^3	0.007	0.4795 ± 0.1175

strains was determined using second instar larvae, which were kept in Petri dishes (10 insects/dish). Virulence was assessed by the lethal concentration (LC50), determined from a series of five to six tenfold dilutions of the virus aligned with the polyhedra titer. To infect the insects, petri dishes containing artificial nutrient medium were sprayed with appropriate dilutions of the virus; distilled water was used as a control. For each virus concentration and control, 100 larvae were used. Mortality was assessed daily and, if necessary, insects killed by NPV were removed from Petri dishes. The total mortality of insects from NPV was taken into account on the 17th day of treatment with the virus. The number of individuals which died from the NPV in the experiment ranged from 22% to 100%; in the control group death was not noted. For statistical data processing, probit analysis was used (Probit Analyze StatPlus v5 b). With the help of high-performance sequencing on the MiSeq (Illumina) platform, complete genomes of the original strains and also those resulting from four consecutive passages, were assembled de novo and annotated. Sequencing and annotation of strains of the NPV of the *Lymantria dipar* was carried out in the SB RAS Genomics core facility (ICBFM SB RAS, Novosibirsk) according to the methods we described earlier (Kabilov et al., 2015; Martemyanov et al., 2017).

RESULTS AND DISCUSSION

Comparison of the LC50 values showed that the virulence of the original North American strain LdMNPV-45/0 was higher than that of the Asian LdMNPV-27/0 strain by more than two orders of magnitude. Thus, the LC50 values of the compared strains were 1.5×10^3 and 1×10^6 polyhedra /ml, respectively (Table). Using high-throughput sequencing on the MiSeq platform, we collected and annotated the complete genomes of the strains LdMNPV-27/0 (Martemyanov et al., 2017) and LdMNPV-45/0 (Martemyanov et al., 2015). Comparison of the genomes revealed two genes, vef-1 (virus increasing factor 1) and bro-p (baculovirus repeated orf p), which were absent in the majority of the virions of initial strain LdMNPV-27/0. The vef-1 gene is directly related to the virulence of the NPV (Popham et al., 2001; Slavicek, Popham, 2005). As for the bro-p gene, no information on its effect on the virulence of baculovirus has been found in the literature. Initially, with the absence of vef-1, we attributed a lower biological activity of the original Asian strain of SNP NS compared to the American strain (Martemyanov et al., 2017).

However, in the process of passaging, the LC50 value of the North American strain varied slightly, while in the Asian one it decreased by more than 2 orders of magnitude. After the 2nd passage, the difference in the biological activity between strains became statistically insignificant (Table). The LT50 value of the Asian strain also decreased in the process of passage and in the 4th passage the differences between these values in the compared strains became statistically insignificant (data not shown). Thus, as a result of the passage, the virulence of the original North American strain remained practically at the same level, and the Asian strain increased significantly. From these results it appears that changes in virulence are associated with changes in the genomes of the strains being compared.

Apparently, the relatively low virulence of the original Asian strain is not just associated with the deletion of the vef-1 gene. It might be assumed that in the process of passaging in artificial selection, there could be an increase in viruses without deletion in the vef-1 gene, which are initially present in the original strain LdMNPV-27/0 in a very small amount. However, the sequencing of the genomes of strain LdMNPV-27 after 2 and 4 steps of passage did not reveal such an increase, which means that other mechanisms influencing virulence are involved. Further bioinformation analysis will reveal genes and genetic differences influencing the virulence of the strains being compared.

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