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MICROBIOLOGY

New nucleopolyhedrovirus isolate in the management of *Spodoptera eridania*

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Abstract: *Spodoptera eridania* is considered a polyphagous pest, as it attacks several crops. The aim of this study was to identify a virus isolate present in symptomatic *S. eridania* caterpillars. The virus sample was extracted and OBs purification performed. Viral DNA was extracted using the PureLink Viral RNA/DNA Mini Kit (ThermoFisher) and primers prl8-1 and prl8-1B were used for amplification of the gene fragment. The isolate was identified showing high genetic similarity with the isolate "*Spodoptera eridania* nucleopolyhedrovirus isolate CNPSo-165", characterizing itself as a novelty in the scenario and as promising, adding the tactics available for the integrated management of *S. eridania*.

Key words: Baculovirus, Integrated pest management, Virus identification, Pathogenicity.

INTRODUCTION

Spodoptera eridania (Cramer) (Lepidoptera: Noctuidae) is a widespread native species in the tropical regions of the Americas (Pogue 2002). The management of this pest is carried out exclusively through synthetic insecticides and most of the time they are executed incorrectly, caused by the indiscriminate use of these products and their inefficiency, in addition to the environmental impacts caused by their use (Favetti et al. 2015).

Control methods that use biocontrol agents, such as insect viruses, are important options. Baculoviruses (Baculoviridae) are the most studied and used to control agricultural pests (Sosa-Gómez et al. 2020). Viral infection begins when the host feeds on substrates contaminated with occlusion bodies (OBs). At the end of the infection, the larvae have a weakened and melanized integument and an internal anatomy, which has been largely liquefied (Jehle et al. 2006).

Given the above, the objective of this study was to identify the virus isolate present in dead larvae with symptoms of baculovirus infection and to evaluate the effectiveness of the isolate in the management of *S. eridania*.

MATERIALS AND METHODS

Virus sampling and purification of occlusion bodies (OBs)

Five dead larvae of *S. eridania* with symptoms of baculovirus infection (flaccid caterpillars hanging from plants in an inverted V shape) were collected in greenhouse tomato cultivation areas in the municipality of Santa Maria de Jetibá (ES), which has a highland tropical climate with mild temperatures (annual average of 19°C). The samples were sent to the entomology laboratory of the Center for Scientific and Technological

Development in Phytosanitary Management of Pests and Diseases (NUDEMAFI – Núcleo De Desenvolvimento Científico E Tecnológico Em Manejo Fitossanitário) and kept at -20°C for 2 months until purification of the occlusion bodies (OBs).

The cadavers were macerated in a porcelain mortar with a 1% SDS buffer solution (Sodium Dodecyl Sulfate). The liquid resulting from the maceration was filtered through voile fabric, to remove the fatty tissues and the coarsest parts of the caterpillars. The filtered viral suspension was centrifuged three times in a 1% SDS buffer solution at 6,000 rpm for 20 minutes. The pellet from the last centrifugation was resuspended in sterilized distilled water, with the aid of a vortex shaker, and stored at -20°C (Hashimoto et al. 2000). 50 µl of the resuspended pellet was removed and placed in the center of the "Neubauer chamber" and then the presence of baculovirus crystals was visualized under a light microscope.

DNA extraction

Total purified DNA was extracted using the PureLink Viral RNA/DNA Mini Kit (ThermoFisher) according to the manufacturer's manual.

PCR and sequencing

The extracted DNA was used as a template for PCR using primers prl8-1 (CAGGAAACAGCTATG ACCCAYGGHGARATGAC) and prl8-1B (TAATACGACTCAC TATAGGGCAYGGHGARATGAC) designed by Lange et al. (2004), who amplify a fragment of the late expression factor 8 gene (LEF-8).

Amplicons from positive samples were purified and sent for sequencing and compared using the Basic Local Alignment Search Tool (BLASTN) algorithm.

Efficiency of the isolate on *Spodoptera eridania*

50 third-instar *S. eridania* caterpillars were individualized and left without food for four hours. Subsequently, the caterpillars were fed an artificial diet impregnated with 20 µl of polyhedral suspension at a concentration of $1x10^8$ polyhedra/ml. The concentrations were previously determined by counting in a Neubauer chamber. Caterpillars that completely consumed the artificial diet disks within a period of 48 hours were transferred to containers with pathogen-free artificial diet. In the control treatment, the diet disks were treated only with sterile water. Mortality was recorded after 8 days.

Specificity of the new isolate

10 third-instar *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae), *Diaphania hyalinata* (Linnaeus, 1767) (Lepidoptera, Crambidae) and *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae), caterpillars were individualized and *S. frugiperda* caterpillars were fed an artificial diet impregnated with 20 µl of polyhedron suspension at a concentration of $1x10^8$ polyhedra/ml. *D. hyalinata* and *P. xylostella* were fed with discs of pumpkin and cabbage leaves, respectively. Caterpillars of *S. frugiperda* that completely consumed the artificial diet discs within a period of 48 hours were transferred to containers with pathogen-free artificial diet and caterpillars of *D. hyalinata* and *P. xylostella* were transferred to containers containing leaf discs of pumpkin and cabbage. Mortality was assessed after 8 days.

RESULTS

PCR and sequencing

The baculovirus isolate collected from *S. eridania* showed 97.57% similarity with the *Spodoptera eridania* isolate "nucleopolyhedrovirus isolate CNPSo-165" (GenBank MT040195).

Efficiency of the isolate on *Spodoptera eridania*

The *S. eridania* isolate "nucleopolyhedrovirus isolate Se04" killed 100% of the caterpillars, proving to be efficient in managing this pest (Table I).

Specificity of the new isolate

The *S. eridania* isolate "nucleopolyhedrovirus isolate Se04" did not kill caterpillars of *S. frugiperda, D. hyalinata* and *P. xylostella*, presenting itself as specific for *S. eridania* (Table II).

DISCUSSION

S. eridania isolate "nucleopolyhedrovirus isolate Se04" was able to kill *S. eridania* larvae, but not *S. frugiperda* larvae. A study found that SperNPV-CNPSo-165 was also not pathogenic to *S. frugiperda* larvae, but was pathogenic to *S. albula* (Rodrigues et al. 2020), possibly due to the close relationship between these species (Le Ru et al. 2018).

In general, the baculovirus host range can vary according to the viral species isolated, and in some cases, an isolate can be infectious for more than 20 hosts, for example, AcMNPV (Salem et al. 2012), and others are specific and appear as infectious only in a single host (Wang et al. 2008).

Table I. Mortality of *Spodoptera eridania* caterpillars fed an artificial diet impregnated with 20 µl of polyhedra suspension at a concentration of 1x10^8 polyhedra/ml.

Treatment	Mortality (%)
Control	0,0±0,0 a
Nucleopolyhedrovirus isolate Se04	100,0±0,0 b
p-value	<0,05

***Means followed by the same letter do not differ from each other at the 5% probability level using the Tukey test.**

Table II. Mortality of *Spodoptera frugiperda, Diaphania hyalinata* and *Plutella xylostella* caterpillars fed an artificial diet impregnated with 20 µl of polyhedron suspension at a concentration of 1x10^8 polyhedra/ml of the *S. eridania* isolate "nucleopolyhedrovirus isolate Se04".

Treatment	Mortality (%)
Spodoptera frugiperda	0,0±0,0 a
Diaphania hyalinata	0,0±0,0 a
Plutella xylostella	0,0±0,0 a
p-value	>0,05

*** Means followed by the same letter do not differ from each other at the 5% probability level using the Tukey test.**

Therefore, the baculovirus specificity varies to the detriment of the isolate (Clem & Passarelli 2013). However, by presenting specificity, as in the case of the isolate identified in this study, it makes it, with greater potential to be used in the biological control of insect pests of forest and agricultural crops, as they present greater safety for humans and other animals and a viable alternative to chemical insecticides (Sun & Peng 2007).

CONCLUSIONS

In this work, a new baculovirus isolated from *S. eridania* "nucleopolyhedrovirus isolate Se04" was reported. The virus was found to be lethal to *S. eridania* larvae and unable to kill *S. frugiperda, D. hyalinata* and *P. xylostella.*

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