BioOne RESEARCH EVOLVED

Molecular Identification and Virulence Evaluation of Fungal Isolates against *Spodoptera exigua* (Hübner) Larvae

Author(s): N. M. Rosas-García, J. M. Villegas-Mendoza and A. Sánchez-Varela

Source: Southwestern Entomologist, 40(4):837-841.
Published By: Society of Southwestern Entomologists
https://doi.org/10.3958/059.040.0414
URL: http://www.bioone.org/doi/full/10.3958/059.040.0414

BioOne (www.bioone.org) is a nonprofit, online aggregation of core research in the biological, ecological, and environmental sciences. BioOne provides a sustainable online platform for over 170 journals and books published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Web site, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/page/terms_of_use.

Usage of BioOne content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

VOL. 40, NO. 4 SOUTHWESTERN ENTOMOLOGIST DEC. 2015
SCIENTIFIC NOTE

Molecular Identification and Virulence Evaluation of Fungal Isolates against *Spodoptera exigua* (Hübner)[1] Larvae

N. M. Rosas-García*, J. M. Villegas-Mendoza, and A. Sánchez-Varela

Laboratorio de Biotecnología Ambiental, Centro de Biotecnología Genómica-IPN, Blvd. del Maestro s/n, Col. Narciso Mendoza, Reynosa Tamp. CP 88710

Beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), has an extensive host range and is a serious insect pest of a variety of crops worldwide. The pest is native to Southeast Asia and was first discovered in North America in 1876 (Capinera 2014). *S. exigua* is an economically important pest in many production regions in Mexico where it attacks grasses, maize (*Zea mays* L.), bean (*Phaseolus vulgaris* L.), tomato (*Solanum lycopersicum* L.), *Yucca* L., sorghum (*Sorghum bicolor* (L.) Moench), and rice (*Oryza sativa* L.) growing areas (Ruiz et al. 2013) as well as cotton (*Gossypium hirsutum* L.) in The Laguna Region (Vargas-Espejel et al. 2004) and chard (*Beta vulgaris* L.), broccoli and cabbage (*Brassica oleracea* L.) at Puebla (Barrientos-Gutiérrez et al. 2013). Damage directly impacts crop yield and quality, significantly decreasing the value of products and by-products of the crops in domestic and export markets (DOF 2002). The pest is resistant to several insecticides (Cook et al. 2004) and many strains of *Bacillus thuringiensis* Berliner (Lasa et al. 2007).

Natural enemies such as bugs, lady beetles, and wasps have been recommended for control (Garza-Urbina and Terán-Vargas 2007), nucleopolyhedroviruses are effective (Caballero et al. 2009, Cai et al. 2010), and entomopathogenic fungi that regulate other lepidopterans might be an alternative control for *S. exigua*. *Beauveria bassiana* (Bals.) Vuill., *Isaria fumosorosea* Wise, and *Metarhizium anisopliae* (Metschnikoff) Sorokin, have been reported to be effective against *S. litura* (Lepidoptera: Noctuidae) (Asi et al. 2013, Han et al. 2014). Although biological activity of entomopathogenic fungal strains varies by different insect pests, fungi are an alternative for insect control.

Fungal isolates were provided by the Laboratorio de Biotecnología Experimental, CBG-IPN, and labeled as B1, C1, J4, K1, M1, O1, and P2 in our laboratory. The fungi were maintained in Petri dishes with YPD medium supplemented with ampicillin (100 mg/ml) at 4°C until used. Isolates were activated in 250-ml Erlenmeyer flasks containing 50 ml PDB enriched with 1% yeast extract. Cultures were incubated at 250 rpm and 30°C for 7 days, 100 µg mycelia of each isolate were placed into a 1.5-ml Eppendorf tube, and DNA was extracted according to the method of Hoffman and Winston (1987). For molecular identification ITS1 and ITS2 intergenic regions and 18S subunit rRNA 5.8S gene were detected using primers: (forward) ITS1 (5′- TCCGTAGGTGAACCTGCGG-3′) and (reverse) ITS4

[1](Lepidoptera: Noctuidae)

*Corresponding author: nrosas@ipn.mx

(5′-TCCTCCGCTTCTTGATATGC-3′) (White et al. 1990). PCR reaction mixtures were prepared from 2 μM of each primer, 2 mM $MgCl_2$, 0.2 mM dNTPs master mix, 1 μl Go *Taq* polymerase enzyme, and 1 μl DNA template in a final volume of 25 μl. PCR was done in a thermal cycler (PelkinElmer 2400, Waltham, MA) with an initial denaturation step at 94°C for 5 minutes, followed by 30 cycles at 94°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute, and a final extension at 72°C for 7 minutes. Amplified products were electrophoresed in a 1% agarose gel and visualized under UV light. A clean scalpel was used to excise linear DNA from the gel and DNA was extracted with a PureLink Quick Gel Extraction kit (Invitrogen, San Francisco, CA). PCR products were cloned using vector pGEM-T easy (Promega, Madison, WI) according to manufacturer instructions. Plasmidic DNA was extracted from colonies of transformed *E. coli* cells according to protocol of Sambrook and Russel (2001). Five clones of each isolate were digested with restriction enzyme *EcoRI* to select those to be sequenced. Sequencing was done in a DNA analyzer (ABI Prism 3730, Applied Biosystems, Foster City, CA). Sequences were analyzed and edited with the programs BioEdit and Lasergene v. 7.0 (DNASTAR, Madison, WI), and aligned with BLASTn in the NCBI database. Sequences were submitted to GenBank. The isolates were inoculated in 250-ml Erlenmeyer flasks containing 50 ml SDB enriched with 1% yeast extract. Cultures were placed in a shaker at 300 rpm and 20-25°C for 24-48 hours. One milliliter from each culture was used to inoculate Petri dishes containing SDA enriched with 1% yeast extract. Plates were incubated for 15-20 days at room temperature.

Conidia were removed from the media surface with 50 ml of distilled water and 0.5% Tween 80. Conidial concentration for each isolate was determined in a Neubauer chamber (Optik Labor, Götlitz, Germany), and adjusted to 10^7 conidia per milliliter. *S. exigua* neonate larvae were bioassayed with 5 ml of artificial diet in 30-ml plastic cups (Rosas-García 2002). Freshly prepared conidia suspension (100 μl) was put on the surface of solidified diet and allowed to dry. One neonate larva was put into each container and closed with a plastic lid. Twenty-five larvae were in each replication, for a total of 75 larvae for each treatment and one check. The percentage of larvae that died was recorded 7 days later, and data were arcsine transformed before ANOVA and mean comparison by Tukey ($P \leq 0.05$). Dead larvae were put into humidity chambers for 7 days to allow fungal growth. Surviving larvae were weighed to determine sublethal effect from feeding inhibition. Data were analyzed by ANOVA and Tukey ($P \leq 0.05$). Fungal isolates B1, C1, J4, K1, M1, and P2 were identified as *Beauveria bassiana* (six isolates) and O1 as *Acremonium implicatum* (one isolate) (Table 1). Significant difference ($F = 6.40$; df = 7,16; $P < 0.01$) was observed in numbers of neonate larvae killed by fungal isolates, each isolate killed more than 31%, and no mycosis was observed in larval cadavers. *B. bassiana* and *Acremonium* sp. are pathogens of several insect orders (ARSEF 2009), but the isolates studied did not cause significant pathogenic effect. Lack of virulence might be attributed to wide-spectrum resistance by *Spodoptera* spp. (Zeng et al. 1998, Cook et al. 2004). Behavior or development of surviving larvae might have been affected and are sublethal effects (Adamczyk et al. 1999, Williams et al. 1999). Sublethal effects by fungal infection differed significantly ($F = 18.82$; df = 7,553; $P < 0.01$) among the isolates. *B. bassiana* C1 was the only isolate that caused a sublethal effect by decreasing feeding with subsequent weight loss of larvae. *A. implicatum* O1 increased larval weight for a positive effect; this might be because the fungus provided nutrients (Cardoza et al. 2003). In-depth analysis is necessary to determine potential of fungi to control *S. exigua* larvae.

Table 1. Molecular Characterization of Fungal Isolates and Virulence Determination in *Spodoptera exigua* Neonate Larvae

Isolate	Expected size (bp)	Fungal identity	Max. identity (%)	GenBank accession number	% ± SE killed[a]	Weight ± SE of surviving larvae (mg)
B1	231	*Beauveria bassiana*	99	GU189514	20.523 ± 5.136bc	21.0 ± 1.0bc
C1	570	*Beauveria bassiana*	99	GU189516	30.113 ± 2.311c	7.0 ± 0.0a
J4	570	*Beauveria bassiana*	99	GU189517	5.053 ± 3.243ab	19.0 ± 1.0bc
K1	570	*Beauveria bassiana*	99	GU189518	11.206 ± 5.331ab	23.0 ± 1.0bc
1	570	*Beauveria bassiana*	99	GU189519	8.296 ± 3.243ab	22.0 ± 1.0bc
O1	587	*Acremonium implicatum*	100	GU189520	5.053 ± 3.243ab	34.0 ± 1.0d
P2	570	*Beauveria bassiana*	99	GU189520	9.926 ± 4.296ab	16.0 ± 1.0b
Check	--	--	--	--	1.810 ± 0.000a	27.0 ± 1.0[c]

Means with the same letter in a column are not significantly different, Tukey $P < 0.01$.
[a]Means were from arcsine transformation of percentages.

Acknowledgment

The authors thank Secretaría de Investigación y Posgrado for financial support for projects SIP20090844, SIP20100032, and COFAA, from Instituto Politécnico Nacional.

References Cited

Adamczyk, J. J. Jr., B. R. Leonard and J. B Graves. 1999. Toxicity of selected insecticides to fall armyworms (Lepidoptera: Noctuidae) in laboratory bioassays studies. Fla. Entomol. 82: 230-236.

ARSEF. 2009. Catalog of isolates. Collection of entomopathogenic fungal cultures. USDA-ARS Plant Protection Research Unit, Ithaca, NY.

Asi, M. R., M. H. Bashir, M. Afzal, K. Zia, and M. Akram. 2013. Potential of entomopathogenic fungi for biocontrol of *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). J. Animal Plant Sci. 23: 913-918.

Barrientos-Gutiérrez, J. E., A. Huerta-de la Peña, J. S. Escobedo-Garrido, and J. F. López-Olguín. 2013. Manejo convencional de *Spodoptera exigua* en cultivos del municipio de Los Reyes de Juárez, Puebla. Rev. Mexicana Ciencias Agrícola 4: 1197-1208.

Caballero, P., R. Murillo, D. Muñoz, and T. Williams. 2009. El nucleopoliedrovirus de *Spodoptera exigua* (Lepidoptera: Noctuidae) como bioplaguicida: análisis de avances recientes en España. Rev. Colombiana Entomol. 35: 105-115.

Cai, Y., Z. Cheng, C. Li, F. Wang, G. Li, and Y. Pang. 2010. Biological activity of recombinant *Spodoptera exigua* multicapsid nucleopolyhedrovirus against *Spodoptera exigua* larvae. Biol. Control 55: 178-185.

Capinera, J. L. 2014. Beet armyworm, *Spodoptera exigua* (Hübner) (Insecta: Lepidoptera: Noctuidae). EENY-105. Featured creatures from the Entomology and Nematology Department, UF/IFAS Extension.

Cardoza, Y. J., C. G. Lait, E. A. Schmelz, J. Huang, and H. Tumlinson. 2003. Fungus-induced biochemical changes in peanut plants and their effect on development of beet armyworm, *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae). Environ. Entomol. 32: 220-228.

Cook, D. R., B. R. Leonard, and J. Gore. 2004. Field and laboratory performance of novel insecticides against armyworms (Lepidoptera: Noctuidae). Fla. Entomol. 87: 433-439.

Diario Oficial de la Federación (DOF). 2002. Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación. Norma Oficial Mexicana NOM-081-FITO-2001. Manejo y eliminación de focos de infestación de plagas, mediante el establecimiento o reordenamiento de fechas de siembra, cosecha y destrucción de residuos.

Garza-Urbina, E., y A. P. Terán-Vargas. 2007. El gusano soldado *Spodoptera exigua* y su manejo en la planicie Huasteca. Instituto Nacional de Investigaciones forestales, acuícolas y pecuarias, San Luis Potosí, México.

Han, J. H., B. R. Jin, J. J. Kim, and S. Y. Lee. 2014. Virulence of entomopathogenic fungi *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* for the microbial control of *Spodoptera exigua*. Mycobiology 42: 385-390.

Hoffman, C. S., and F. Winston. 1987. A ten-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. Gene 57: 267-272.

Lasa, R., I. Pagola, I. Ibañez, J. E. Belda, T. Williams, and P. Caballero. 2007. Eficacy of *Spodoptera exigua* multiple nucleopolyhedrovirus as a biological insecticide for beet armyworm control in green houses of Southern Spain. Biocontrol Sci. Technol. 17: 221-232.

Rosas-García, N. M. 2002. Elaboración de formulados de *Bacillus thuringiensis* var. *kurstaki* y determinación de la actividad tóxica contra larvas de *Diatraea saccharalis* (Fabricius) (Lepidoptera: Pyralidae) en laboratorio y campo. Tesis de Doctorado en Ciencias con especialidad en Biotecnología. F.C.B. División de Estudios de Postgrado, U.A.N.L. Monterrey, N.L. México.

Ruíz C., J. A., E. Bravo M., G. Ramírez O., A. D. Báez G., M. Álvarez C., J. L. Ramos G., U. Nava C., and K. F. Byerly M. 2013. Plagas de importancia económica en México: aspectos de su biología y ecología. Libro Técnico 2. INIFAP-CIRPAC-Campo Experimental Centro Altos de Jalisco, Tepatitlán de Morelos, Jalisco.

Sambrook, J., and D. W. Russel. 2001. Molecular Cloning: A Laboratory Manual, 3rd. ed. Cold Spring Harbor Press, New York.

Vargas-Espejel, M. U., U. Nava-Camberos, M. Ramírez.Gómez, and M. Ramírez-Delgado. 2004. Desarrollo, crecimiento y sobrevivencia del gusano soldado *Spodoptera exigua* (Hübner) en algodón transgénico y convencional en la Comarca Lagunera. Rev. Chapingo Serie Zonas Áridas 3: 81-87.

White, T. J., T. Bruns, S. Lee, and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, pp. 315-322. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], PCR Protocols: A Guide to Methods and Applications. Academic Press, New York.

Williams, W. P., F. M. Davis, J. L. Overman, and P. M. Buckley. 1999. Enhancing inherent fall armyworm (Lepidoptera: Noctuidae) resistance of corn with *Bacillus thuringiensis* genes. Fla. Entomol. 82: 271-277.

Zeng, X., H. Zhang, Z. Yu, and C. Fu. 1998. Bioassay method for evaluation toxicity of *Bacillus thuringiensis* against larvae of *Spodoptera exigua.* Chin. J. Biol. Control 4: 175-172.