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PROGRAM BOOK

Peritrophic matrix genes in *Diabrotica virgifera virgifera* (Le Conte)

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Introduction

Diabrotica virgifera virgifera (LeConte) (Western Corn Rootworm, WCR) is one of the most devastating pests in US. The ability of WCR to develop resistance to chemical insecticides, Bt crops and crop rotation calls for the discovery of new biopesticides.

The peritrophic matrix (PM) it is an extracellular structure in the gut epithelium of most insects and consists primarily of chitin and PM associated proteins. Several physiological functions have been proposed for the function of PM in insects, which include protection from mechanical abrasion, compartmentalization of digestive enzymes and/or digestion products, neutralization of ingested toxins, and prevention of parasitic, bacterial and possibly viral infections (Hegedus et al., 2009). The major proteins of this cohesive, digestion-resistant structure are chitin deacetylase-like and mucin-like proteins, the latter has multiple chitin-binding domains that provide a barrier against abrasive food particles and parasites. A study in *Helicoverpa armigera* showed that most of the PM proteins had catalytic activity, binding activity and transport function (Campbell et al., 2008). In *Tribolium castaneum* at least 11 putative peritrophic matrix protein (PMP) genes had been identified and found to be differentially expressed in different parts of the gut, suggesting that they have different roles in midgut physiology (Jasrapuria et al., 2010). In addition, the knockdown of some of these genes resulted in the depletion of the fat body, growth arrest, molting defects and mortality (Agrawal et al. 2014).

RNAi has been shown to reduce transcription levels of target genes in WCR. Inhibition of PM proteins using RNAi opens an opportunity to determine their functions and potential as targets for novel insecticides. In this study, 5 peritrophic proteins (mucins) and two chitin synthesis genes from WCR were identified and investigated for their potential use for WCR pest management.

Materials and Method

Insects: Non-diapause WCR eggs, 3rd instar larvae and adults were purchased from Crop Characteristics, Inc. (Farmington, MN). Adults were kept in rearing cages with artificial diet in a growth chamber at 23±1° C and 75±5 % RH. Eggs were kept in an incubator at 27±1° C and 75±5 % RH until hatching. Neonates and 3rd instar larvae were kept in petri dishes containing artificial diet.

Identification of target genes: Using mucin genes and chitin synthesis genes from *Tribolium* as query, a Blast search was conducted to search potential orthologs of these genes in a WCR transcriptome database (Eyun et al. 2014). Orthologs were further amplified, sequenced and searched in the NCBI database for accuracy.

RNA extraction, cDNA synthesis and qRT-PCR: Total RNAs were extracted from pooled samples or tissues with RNeasy Mini Kit (Qiagen). The quality and quantity of RNA samples were evaluated on 1% agarose gels and NanoDrop-1000 respectively. cDNA was synthesized with QuantiTect Reverse Transcription (QIAGEN). qRT-PCR were carried out on Applied Biosystems® 7500 Real-Time PCR Systems (Applied Biosystems). The ΔΔCt method (Livak et al. 2001) was used to calculate the relative expression of target genes using actin as housekeeping gene.

dsRNA Exposure: dsRNA was synthesized using MegascriptT7 Transcription Kit (Applied BioSystems). Larval bioassays were performed using 24 well cell culture plates with artificial diet coated with 500 ng/cm² dsRNA of the target gene and GFP dsRNA and water were used as controls. Larvae were exposed to dsRNA every other day for 6 days (8 neonates per well x 3 replications per treatment). Adult bioassays were performed in plastic containers containing 10 plugs of artificial diet. Food plugs were coated with 500 ng of dsRNA of the target gene, GFP dsRNA or 3 µl of water. Treated food plugs were replaced every other day for a total of 3 times.

Results and Discussion

Search of our WCR transcriptome database returned 5 mucins (peritrophic protein) genes and two chitin synthesis genes with an identity of at least 41% with *T. castaneum* orthologs (**Table 1**). Chitin synthase 14 (CS14) was almost exclusively expressed in the gut of both 3rd instar larvae and adults (**Figure 1A**), while chitin synthase 15 (CS15) was expressed in body tissue excluding the gut of 3rd instar larvae and adults (**Figure 1B**). CS14 expression was at least 2.24 times higher in gut tissue in adults as compared to larvae (**Figure 2A**).

Results and Discussion

Table 1. Two chitin synthase genes (CS14 and CS15) and five mucins (31, 41, 43, 71, 83) were identified on WCR transcriptome database (163,871 contigs) at 1E-15 with similarity to *T. castaneum* at amino acid level.

Genes identified in *D. v. virgifera*	Reference Species	GenBank accession number	Percentage identity to *D. v. virgifera* (Blastx)
Mucin71	*T. castaneum*	ACY95487.1	41%
Mucin82	*T. castaneum*	XP_008192415.1	42%
Mucin43	*T. castaneum*	EFA02771.1	42%
Mucin41	*T. castaneum*	NP_001161929.1	50%
Mucin31	*T. castaneum*	NP_001161929.1	40%
CS15	*T. castaneum*	NM_001039402.1	54%
CS14	*T. castaneum*	AY293879.1	85%

All three Mucin genes (Mucin31, 41 and 71) tested were highly expressed in 3rd instar larvae gut tissue as compared to body tissues without gut (**Figure 1C-E**). Relatively, in the gut of 3rd larvae, the Mucin41 was the highly expressed followed by Mucins71 and 31. As compared to other two Mucins, the expression of Mucin41 was at least 1.8 and 2.6 times higher than that of Mucin71 and Mucin31 respectively (**Figure 2B**).

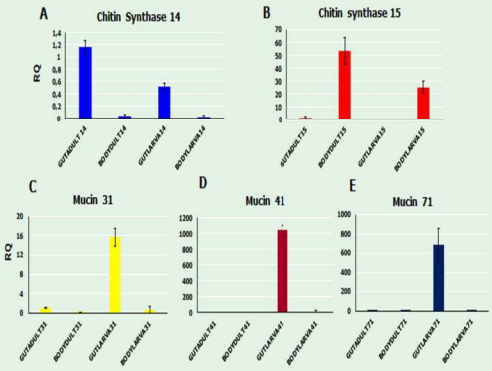

Figure 1. Baseline expression of 5 peritrophic genes in gut and tissues without gut in 3rd instar larvae and adults.

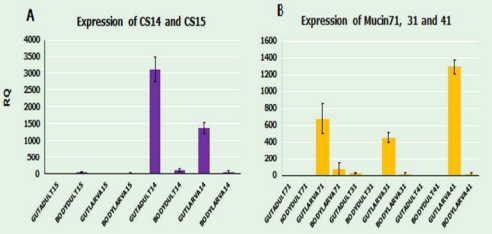

Figure 2. Relative expression of 6 PM genes in gut and tissues without gut from first, second and third instar larvae, and adult stages.

Figure 3. Visual difference in consumption of artificial diet in adults containing water, GFP and CS14 (500ng/cm²) after 8 days exposure.

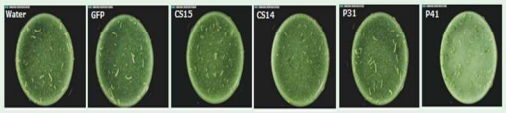

Figure 4. Visual difference in size of larvae on artificial diet coated with water, GFP dsRNA and CS14 dsRNA (500 ng/cm²) after 8 days exposure.

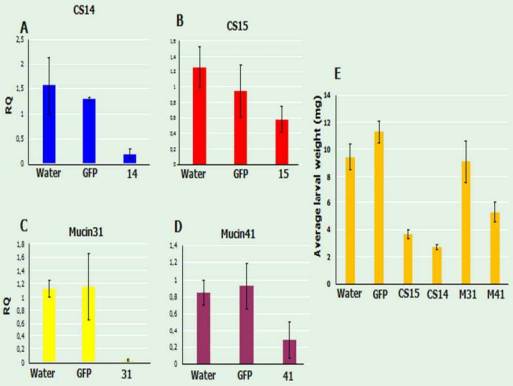

Figure 5. Larval growth inhibition after 8 days exposure to CS14, CS15, Mucin31, and Mucin41 dsRNA at 500 ng/cm². **A-D:** RQ for CS14, CS15, Mucin31 and Mucin41; **E:** Average weight is based on 3 reps of 10 larvae each.

Adults treated with CS14 dsRNA showed decreased diet consumption compared to the control treatments (**Figure 3**). Treatment of larvae with CS14, CS15, Mucin31 and Mucin41 dsRNAs led to apparent knockdown of gene (**Figure 5A-D**) and generated evident differences in larval size (**Figure 4**). Weight loss was observed in larvae treated with CS14 and CS15 dsRNA (**Figure 5E**). These results suggest that CS14 and CS15 might play an important role in food consumption and/or development in both larvae and adults.

Conclusions

- The orthologs of 2 chitin synthase genes and five mucin genes were identified in WCR.
- Expression of CS14, Mucin31, Mucin41 and Mucin71 are specific to the gut of larvae and adult WCR. While expression of CS15 is not gut specific.
- Six days of exposure to dsRNA at a concentration of 500 ng/cm² was capable of up to 90% knockdown of CS and mucin genes. dsRNA CS14, CS15 and Mucin31 and Mucin41 decreased the levels of their respective transcripts but not the other CS and Mucin genes (data not shown)
- WCR adults treated with CS14 dsRNA exhibited reduced feeding rates after 6 days treatment.
- Growth inhibition was observed in larvae treated with CS14 and CS15 dsRNA. Both genes generated up to 90% mortality after 10 days of treatment.
- The apparent lack of inhibition of growth in larvae treated with the Mucin31 and Mucin41 dsRNA might indicate that these proteins are turned over more slowly or inhibition of one gene can rescue the phenotype through increased synthesis of other PM proteins
- These results suggest an important role for the peritrophic membrane in gut physiology and offers a possible target site for RNAi based pest management approaches and for exploiting as a possible target site.

Acknowledgments

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D3242 A metatranscriptomic approach aimed at understanding bacterial roles in the termite holobiont. **Brittany Peterson** (peter137@purdue.edu) and Michael Scharf, Purdue Univ., West Lafayette, IN

D3243 Characterization of ATP binding cassette subfamily C transporters in *Reticulitermes flavipes*. **Swapna Priya Rajarapu** (prajarapu@purdue.edu), Jesse Hoteling and Michael Scharf, Purdue Univ., West Lafayette, IN

D3244 Comparative studies on the lethal giant larvae gene in *Ostrinia nubilalis* (Lepidoptera: Pyralidae) and *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). **Anastasia Cooper** (anacooper@ksu.edu), Young Ho Kim and Kun Yan Zhu, Kansas State Univ., Manhattan, KS

D3245 Presentation withdrawn

D3246 cDNA library construction of the Asian ladybird beetle using gateway cloning system. **Yu-Bin Jung** (ybjung89@naver.com), Jung Kyu Kim, Chan-yeong Kang, Jeong Hee Kim, Ji Hyun Min, Il Hyun Byun, Hyun Ju Jang, Min Gyu Cho, Tae-Hee Ryu, Yong-Man Yu and Young-Nam Youn, Chungnam National Univ., Daejeon, South Korea

D3247 PBAN/DH/Pyrokinin signalling system in Animalia. **Russell Jurenka** (rjurenka@iastate.edu), Iowa State Univ., Ames, IA

D3248 Cry1Ac toxin mode of action in heliothines. **Heba Abdelgaffar** (habdelga@utk.edu)[1], Cris Oppert[2], Jessica Monserrate[2] and Juan L. Jurat-Fuentes[1], [1]Univ. of Tennessee, Knoxville, TN, [2]Bayer CropScience, Morrisville, NC

D3249 Discovery of the first Chelicerata pyrokinin receptor from the southern cattle tick, *Rhipicephalus microplus*. Yunlong Yang[1], Ronald Nachman[2] and **Patricia V. Pietrantonio** (p-pietrantonio@tamu.edu)[1], [1]Texas A&M Univ., College Station, TX, [2]USDA - ARS, College Station, TX

D3250 Application of RNAi to control of tobacco whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae), and target gene selection. **Jeong Hee Kim** (wjdgml133@naver.com), Jung-Kyu Kim, Chan-yeong Kang, Yu-Bin Jung, Ji Hyun Min, Il Hyun Byun, Hyun Ju Jang, Min Gyu Cho, Hyoun-Sub Lim, Yong-Man Yu and Young-Nam Youn, Chungnam National Univ., Daejeon, South Korea

D3251 Comparative analyses of selected genes possibly involved in cellular uptake of dsRNA between *Diabrotica verigifera* and *Ostrinia nubilalis*. **Young Ho Kim** (yhkim@ksu.edu) and Kun Yan Zhu, Kansas State Univ., Manhattan, KS

D3252 RNA interference using double stranded RNAs as molecular biopesticide to regulate the invasive insect pest brown marmorated stink bug (BMSB). **Saikat Kumar Ghosh** (Saikat.Ghosh@ars.usda.gov) and Dawn E. Gundersen-Rindal, USDA - ARS, Beltsville, MD

D3253 Peritrophic matrix genes on western corn rootworm, *Diabrotica virgifera virgifera* Le Conte. **Newton Carneiro** (newtonc800@gmail.com)[1], Haichuan Wang[1], Ana Velez[1] and Blair Siegfried[2], [1]Univ. of Nebraska, Lincoln, NE, [2]Univ. of Florida, Gainesville, FL

D3254 Genome-wide survey of vacuolar-ATPase genes in the yellow fever mosquito, *Aedes aegypti* (Diptera: Culicidae). **Basak Coskun** (basak@ksu.edu), Moustapha Soumaila Issa, Young Ho Kim and Kun Yan Zhu, Kansas State Univ., Manhattan, KS

D3255 Characterization of bursicon homodimers' role in innate immune responses in *Aedes aegypti*. **Hongwei Zhang** (hzz78@mail.missouri.edu) and Qisheng Song, Univ. of Missouri, Columbia, MO

D3256 Ecdysis triggering hormone, a multifunctional peptide regulating reproduction of *Aedes aegypti*. **Yike Ding** (yding005@ucr.edu) and Michael E. Adams, Univ. of California, Riverside, CA

D3257 Barriers to RNAi response in stink bugs. **Elane Fishilevich** (EFishilevich@dow.com), Meghan Frey, Wendy Lo, Premchand Gandra, Murugesan Rangasamy, Justin Lira and Kenneth Narva, Dow AgroSciences, Indianapolis, IN

D3258 Functional analysis of cadherin as a receptor to Cry1Ac toxin in the polyphagous insect pest, *Helicoverpa armigera*. **Bindiya Sachdev** (bindiya.sachdev@gmail.com)[1], Patricia Pelegrini[2], Saad Moussa[1], S Sivakumar[1], Naresh Arora[1], Diogo Martins-de-Sa[2], Wagner Lucena[2], Sonia Freitas[3], Maria Grossi-de-Sa[2] and Raj Bhatnagar[1], [1]International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India, [2]Embrapa-Genetic Resources and Biotechnology, Brasilia-DF, Brazil, [3]Univ. of Brasilia, Brasilia-DF, Brazil

D3259 Airway immunity in the Madagascar hissing cockroach, *Gromphadorhina portentosa*. **Austin Espe** (austin.espe.1@ndsu.edu), Nathan Fisher and Kendra Greenlee, North Dakota State Univ., Fargo, ND

D3260 Variations in thermal history lead to dyssynchronous diapause development. **George Yocum** (george.yocum@ars.usda.gov)[1], Anna Bennett[1], Joseph Rinehart[1], William Kemp[1], Theresa Pitts-Singer[2] and Julia Bowsher[3], [1]USDA - ARS, Fargo, ND, [2]USDA - ARS, Logan, UT, [3]North Dakota State Univ., Fargo, ND

D3261 The fate of oral uptake and injected ammonia as the stable isotope $^{15}NH_4Cl$ on nitrogen metabolism in the American cockroach, *Periplaneta americana* L. **Donald E. Mullins** (mullinsd@vt.edu), Benjamin Gill, Mark Hanigan and Sandra E. Gabbert, Virginia Polytechnic Institute and State Univ., Blacksburg, VA

D3262 A new "walker" tool for efficient placement of *Diaphorina citri* (Hemiptera: Liviidae) on trees in mating behavior bioassays. **Emily Pregmon** (epregmon@ufl.edu), Richard W. Mankin, Sylvia Lujo, Kayla Norton, Ethan Hartman and Nina Zagvazdina, USDA - ARS, Gainesville, FL

D3263 Disrupting the vibrational mating behavior of *Diaphorina citri*. **Sylvia Lujo** (lujosyv@ufl.edu), Ethan Hartman, Kayla Norton, Nina Zagvazdina, Emily Pregmon and Richard Wendell Mankin, USDA - ARS, Gainesville, FL

D3264 Long term storage of bee semen: A six month assessment of cryopreserved semen quality using motility as an index. **Arun Rajamohan** (arun.rajamohan@ars.usda.gov) and Joseph P. Rinehart, USDA - ARS, Fargo, ND

D3265 Antennal sensilla of the Mexican soybean weevil, *Rhyssomatus nigerrimus* (Coleoptera: Curculionidae). **Elsy Delgado-García** (elsydelgado@gmail.com), Colegio de Postgraduados, Montecillo, Mexico

D3266 Effects of oral ingestion of heat shock protein 70 dsRNA on the thermal tolerance of the sweetpotato whitefly, *Bemisia tabaci*. **Kyeong-Yeoll Lee** (leeky@knu.ac.kr), Jae-Kyoung Shim, Bong-Gi Choi, JInmo Koo and Duck-Oung Jung, Kyungpook National Univ., Daegu, South Korea

D3267 The contribution of fatty acid-derived volatiles to aphid resistance in tomato. **Fiona Goggin** (fgoggin@uark.edu) and Jiamei Li, Univ. of Arkansas, Fayetteville, AR

D3268 Involvement of glycerol-3-phosphate dehydrogenase and glycerol 3-phosphatase in rapid cold hardening of the oriental tobacco budworm, *Helicoverpa assulta*. Dae-weon Lee and **Wook Hyun Cha** (whcha17@gmail.com), Kyungsung Univ., Busan, South Korea