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
pestilent pests in US. The ability of WCR to develop resistance to chemical
insecticides, Bt crops and rotation calls for the discovery of new insecticides.

The peritrophic matrix (PM) 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 WCR insects,
which include protection from mechanical abrasion, compartmentalization of digestive
enzymes and/or digestion products, transport of ingested toxic bases, and prevention
of parasitic, bacterial and possibly viral infections (Ngayoga et al., 2008). The
major proteins of this chitinacious, digestion-resistant structure are chitin-degrading-like
and muco-proteins, the latter have multiple chitin-binding domains that provide
a barrier against abrasive food particles and parasites. A study in Tribolium castaneum
showed that most of the PM proteins had catalytic activity, binding activity and
transport function (Campbell et al., 2009). In Tribolium castaneum, at least 11 relative
peritrophic matrix proteins (PMP) genes have been identified and found to be
differentially expressed in different parts of the gut, suggesting that they have
diverse roles in nutritional absorption (Kawamura et al., 2010). In addition, the
localization of some of these genes result in the depletion of the fat body, growth
arrest, mating 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 gene suppressor (most common targets or novel transcriptome).
In this study, 5 peritrophic proteins (muco1) and two chitin synthesis genes from PM were
identified and investigated for their potential use as WCR pest management.

Materials and Method

Insects: Non-diapause WCR eggs, 3rd instar larvae and adults were purchased from
Crop Characteristics, Inc. (Farmington, NC). Adults were kept in rearing cages with
artificial diet in a growth chamber at 21°C ± 1°C and 75 ± 5% RH. Eggs were kept in
an incubator at 21°C ± 1°C and 75 ± 5% RH and hatching. Neonates and 3rd instar
larvae were kept in petri dishes containing artificial diet.

Identification of target genes: Using nuclease and chitin synthesis genes from Tribolium as
query, a Blast search was conducted to search potential orthologous of these
genes in the WCR transcriptome (Evans et al., 2018). The results were further
amplified, sequenced and searched in the NCBI database for accuracy.

RNA extraction, qDNA synthesis and qPCR: Total RNAs were extracted from
pooling samples or tissues with RNeasy Mini Kit (Qiagen). The quantity and quality
of RNA samples were evaluated on 0.2% agarose gel and NanoDrop (Thermo
Scientific). cDNA was synthesized with QuantTect Reverse Transcription Kit (Qiagen).
qPCR were carried out on Applied Biosystems 7500 Fast Real-Time PCR System (Applied
Biosystems). The (ACT) primer (Jenkins et al., 2007) were used to observe the qPCR
expression of target genes using actin as housekeeping gene.

ddRNAi Exposure: ddRNAi was synthesized using Megaprep™ Transcription Kit
(Invitrogen). Laroni extracts were performed using 7N cell culture plates with
artificial diet coated with 350 ng/mg disteen of the target gene. A reporter
GFP was used as control. Laroni were exposed to ddRNAi every other day for
6 days (8 nematodes per well x 3 replications per treatment). Adult larvae
were performed in plastic containers with 10 plasmid of artificial diet. Food.
Plates were coated with 300 µg/mL of the target gene. GFP-GFP or GFP-T of all
plasms. Treated food plates were replaced every other day for a total of 3 times.

Results and Discussion

Search of our WCR transcriptome database returned 5 nucleic (peritrophic protein) genes
and two chitin synthesis genes with an identity of at least 40% with T.
castaneum orthologs (Table 1). Chitin synthesis 14 (CS14) was almost exclusively
expressed in the gut of 3rd instar larvae and adults (Figure 2A), while chitin
synthesis 13 (CS13) was expressed in both tissues excluding the gut of 3rd instar
larvae and adults (Figure 15). CS13 expression was at least 2.2 times higher in gut
in tissues in adults as compared to larvae (Figure 2A).

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-GFP and
CS14 (300 ng/mg) after 8 hours exposure.

Figure 4. Visual difference in size of larvae on artificial diet coated with water, GFP-GFP and
CS14 ddRNAi (500 ng/mg) after 8 hours exposure.

Figure 5. Larval growth inhibition after 8 days exposure to CS14, CS15, Muco1 and CS15 ddRNAi at
300 ng/mg. A-SK for CS14, CS15, Muco1 and CS15 ddRNAi and B. Average weight is based on
3 nps of 10 larvae each.

Adults treated with CS14 ddRNAi showed decreased diet consumption compared to the
control treatments (Figure 2). Treatment of larvae with CS14, CS15, Muco1 and CS15 ddRNAi led to
apparent knockdown of genes (Figure 4A) and generated evident differences in larval size
(Figure 4B). Weight loss was observed in larva treated with CS14 and CS15 ddRNAi (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 orthology of 2 chitin synthesis genes and five muco genes were identified in WCR.
- Expression of CS14, Muco1, Muco1 and Muco1 are specific to the gut of larva and adult
- White expression of CS15 is not gut specific.
- Six days of exposure to CS14 at a concentration of 350 ng/mg was capable up to 50% knockdown
of CS14 and muco gene. ddRNAi of CS14 and CS15 decreased the levels of their respective transcripts
but not the other CS and Muco genes (data not shown).
- WCR adults treated with CS14 ddRNAi exhibited reduced feeding rates after 6 days treatment.
- Growth inhibition was observed in larvae treated with CS14 and CS15 ddRNAi. Both gene
expression progressively to 80% mortality after 15 days of treatment.
- The apparent lack of inhibition of growth in larvae treated with the Muco1 and Muco1
ddRNAi might indicate that these proteins are formed more slowly or inhibition of one gene
might rescue the phenotype through increased synthesis of other PM genes.
- 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.

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D3243 Characterization of ATP binding cassette subfamily C transporters in Reticulitermes flavipes. Swapna Priya Rajarapu (prajarapu@purdue.edu), Jesse Cate 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 (ana.cooper@kscu.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 (ybjung88@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 Yoon, Chungnam National Univ., Daejeon, South Korea

D3247 PBAN/DH/Pyrokinin signalling system in Anamalia. Russell Jurenka (jurenka@iesiate.uno.edu), Iowa State Univ., Ames, IA

D3248 Cry1Ac toxin mode of action in heliothines. Heba Abdelgaffar (habdelga@utk.edu)1, Cris Oppert1, Jessica Monserrate2 and Juan L. Jurat-Fuentes1, 1Univ. of Tennessee, Knoxville, TN, 2Bayer CropScience, Morrisville, NC

D3249 Discovery of the first Chelicera pyrokinin receptor from the southern cattle tick, Rhipicephalus microplus. Yunlong Yang1, Ronald Nachman2 and Patricia V. Pietrantoni (p-pietrantoni@tamu.edu)2, 1Texas A&M Univ., College Station, TX, 2USDA - ARS, College Station, TX

D3250 Application of RNAi to control of tobacco whitefly, Bemisia tabaci (Hemiptera: Aleyrodidae), and target gene selection. Jeong Hee Kim (wjdgm133@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 Yoon, Chungnam National Univ., Daejeon, South Korea

D3251 Comparative analyses of selected genes possibly involved in cellular uptake of dsRNA between Diabrotica virgifera and Ostrinia nubilalis. Young Ho Kim (yjkim@kscu.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.g @ars.usda.gov) and Dawn E. Gunderson-tidnal, USDA - ARS, Beltsville, MD

D3253 Peritrophic matrix genes on western corn rootworm, Diabrotica virgifera virgifera Le Conte. Newton Carneiro (newton800@gmail.com), Haichuan Wang1, Ana Velaz1 and Blair Siegfried2, 1Univ. of Nebraska, Lincoln, NE, 2Univ. of Florida, Gainesville, FL

D3254 Genome-wide survey of vacuolar-ATPase genes in the yellow fever mosquito, Aedes aegypti [Diptera: Culicidae]. Basak Coskun (basak@kscu.edu), Moustapha Soumaila Isha, 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 (haz78@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 (yd21@ucr.edu) and Michael E. Adams, Univ. of California, Riverside, CA

D3257 Barriers to RNAI response in stink bugs. Elaine Filevichile (EFishlevich@dow.com), Megan 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. Bindyia Sachdev (bindyia.sachdev@gmail.com), Patricia Pelegri1, Saad Moussa1, S Sivakumar1, Naresh Arora2, Didgo Martins-de-Sa2, Wagner Lucena2, Sonia Freitas3, Maria Grossi-de-Sa4 and Raj Bhattacharya1, 1International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India, 2Embrapa-Genetic Resources and Biotechnology, Brasilia-DF, Brazil, 3Univ. 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 dysynchronous diapause development. George Yocom (george.yocom@ars.usda.gov), Ariane Benetti1, Joseph Krell1, William Renn1, Theresa Pitts-Singer1 and Julia Bowsher1, 1USDA - ARS, Fargo, ND, 2USDA - ARS, Logan, UT, 3North Dakota State Univ, Fargo, ND

D3261 The fate of oral uptake and injected ammonia as the stable isotope 15N15Cl 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 Horton, Ethan Hartman and Nina Zagvazdina, USDA - ARS, Gainesville, FL

D3263 Disrupting the vibrational mating behavior of Diaphorina citri. Sylvia Lujo (slyuyouf@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, Rhysomatus nigriceps (Coleoptera: Curculionidae). Elsy Delgado-Garcia (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. Kyong Yeol Lee (icky@knu.ac.kr), Jae-Kyoung Shim, Bong Gi Choi, Jimmo Koo and Duc-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 Jamiocre 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-woon Lee and Wook Hyun Cha (whcha17@gmail.com), Kyungsung Univ., Busan, South Korea