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Partial venom gland transcriptome of a *Drosophila* parasitoid wasp, *Leptopilina heterotoma*, reveals novel and shared bioactive profiles with stinging Hymenoptera

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ABSTRACT

Analysis of natural host-parasite relationships reveals the evolutionary forces that shape the delicate and unique specificity characteristic of such interactions. The accessory long gland-reservoir complex of the wasp *Leptopilina heterotoma* (Figitidae) produces venom with virus-like particles. Upon delivery, venom components delay host larval development and completely block host immune responses. The host range of this *Drosophila* endoparasitoid notably includes the highly-studied model organism, *Drosophila melanogaster*. Categorization of 827 unigenes, using similarity as an indicator of putative homology, reveals that approximately 25% are novel or classified as hypothetical proteins. Most of the remaining unigenes are related to processes involved in signaling, cell cycle, and cell physiology including detoxification, protein biogenesis, and hormone production. Analysis of *L. heterotoma*'s predicted venom gland proteins demonstrates conservation among endo- and ectoparasitoids within the Apocrita (e.g., this wasp and the jewel wasp *Nasonia vitripennis*) and stinging aculeates (e.g., the honey bee and ants). Enzyme and KEGG pathway profiling predicts that kinases, esterases, and hydrolases may contribute to venom activity in this unique wasp. To our knowledge, this investigation is among the first functional genomic studies for a natural parasitic wasp of *Drosophila*. Our findings will help explain how *L. heterotoma* shuts down its hosts' immunity and shed light on the molecular basis of a natural arms race between these insects.

1. Introduction

The order Hymenoptera comprises approximately 130,000 insect species, with as many as 20% of these estimated to be parasitoid wasps in the Apocrita (Pennacchio and Strand, 2006). The reproductive strategies within this group target host development and viability, and contribute to community structure and ecology. Venom protein bioactivity has been studied since the early twentieth

Abbreviations: A, adenosine; Acph/ACPH, acid phosphatase; APIME, *Apis mellifera*; Asp–Tyr–Asp, aspartate–tyrosine–aspartate; bp, base pair; C, cytidine; CBP, chemosensory-binding protein; CDD, Conserved Domain Database; cGMP, cyclic guanosine monophosphate; CO_2, carbon dioxide; cDNA, complementary deoxyribonucleic acid; CUNY, City University of New York; dbEST, Expressed Sequence Tags database; dir, direct; DNA, deoxyribonucleic acid; EBI, European Bioinformatics Institute; EC, Enzyme Commission; EST, expressed sequence tag; FA, farnesoic acid; *for*, *foraging* gene; G, guanosine; GEF, guanine nucleotide exchange factor; Glu–Cys, glutamate–cysteine; h, hours; IgE, immunoglobulin E; JAK-STAT, Janus kinase-signal transducer and activator of transcription; JH, juvenile hormone; KAAS, KEGG Automatic Annotation Server; kD, kiloDalton; KEGG, Kyoto Encyclopedia of Genes and Genomes; *Lh*, *Leptopilina heterotoma*; MAP, mitogen-activated protein; mer, repeating unit; ml, milliliter; MUSCLE, Multiple Sequence Comparison by Log-Expectation; MRJP, major royal jelly protein; N, any nucleotide; NCBI, National Center for Biotechnology Information; NF-kappa B, nuclear factor kappa-light-chain-enhancer of activated B cells; ng, nanogram; NIH, National Institutes of Health; NSF, National Science Foundation; nr, non-redundant; NRI, National Research Initiative; OBP, odorant-binding protein; ORF, open reading frame; PBP_BOBP, pheromone-binding protein/general odorant-binding protein; PCR, polymerase chain reaction; PDV, polyDNA virus; PF, Pfam accession number; PKG, cyclic guanosine monophosphate-dependent protein kinase; PLB, phospholipase B; polyA, poly adenosine monophosphate; PRIAM, Profils pour l'Identification Automatique du Métabolisme; PSI, Position-Specific Iterated; PTM, post-translation modification; RISE, Research Initiative for Scientific Enhancement; RNA, ribonucleic acid; RRM, RNA Recognition Motif; Ser/Thr, serine/threonine; SMART, Simple Modular Architecture Research Tool; spp., *species pluralis*; STKc-PKA, Serine/Threonine Kinase, cAMP-dependent Protein Kinase; T, thymidine; TCA, tricarboxylic acid; µg, microgram; USDA, United States Department of Agriculture; rev, reverse; VLP, virus-like particle; *y*, *yellow*; *w*, *white*.

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century, when the first snake (Noguchi, 1909) and scorpion venoms were investigated (Todd, 1909). The venom studies for pain-inflicting social insects such as bees, bumblebees, yellow jackets, and ants, have clarified the ontology of venom proteins and provided treatment applications (deGraaf et al., 2009; Hoffman, 1977; Peiren et al., 2005). In contrast to social insects, parasitoid wasps must apprehend and physiologically control their hosts to assure the success of their offspring. Early indications suggest that the venom pharmacopeia of these insects will prove to be richer (Danneels et al., 2010), paralleling the specific demands of host–parasite interactions.

Venom factors provide the armament for success in the host/parasitoid arms race. Venom proteins target host physiology and development to provide the developing parasitoid with a secure and nutrient-rich environment that will optimize its consumption of host resources (Rivers and Denlinger, 1994, 1995). Hosts often are subdued through neuro-active venom components that may cause prolonged paralysis, particularly in ectoparasitic wasp attack (Rivers et al., 2002). Additionally, parasitic wasps protect their progeny either by passively evading the host immune system (e.g., *Asobara tabida*, (Prevost et al., 2009)) or by actively suppressing host immunity (e.g., *Leptopilina* spp. (Dubuffet et al., 2009; Lee et al., 2009)). Many studies in *D. melanogaster* have found that the cellular and humoral responses are predominantly under the control of Toll/NF-kappa B and JAK-STAT signaling pathways. Melanization of wasp egg also contributes to the host defense response (Govind, 2008; Lemaitre and Hoffmann, 2007; Schlenke et al., 2007). These molecular mechanisms appear to be active in other insects as well (Bitra et al., 2012), and are targets of inhibitors arising from venoms, polydnavirus gene expression, and calyx fluid (Nappi et al., 2009; Strand and Burke, 2012).

Leptopilina heterotoma (*Lh*), a member of a moderately sized genus (Allemand et al., 2002; Schilthuizen et al., 1998), successfully parasitizes most *Drosophila* species tested (Carton et al., 1986; Schlenke et al., 2007). It has been known for over fifty years that *Lh* strains must produce venom factors (Walker, 1959). The majority of the virulence activity is attributed to the action of virus-like particles (VLPs) that are produced and assembled in the long gland-reservoir complex (Chiu et al., 2006; Ferrarese et al., 2009; Morales et al., 2005; Rizki and Rizki, 1992). The long gland is a simple cylindrical organ lined peripherally with large, polyploid secretory cells. Internal and concentric to this cell layer is a single-celled layer of intimal cells, which lines the long gland lumen. A supracellular canal system of individual secretory units, one per secretory cell, feeds into the long gland lumen (Ferrarese et al., 2009). Antibody staining experiments have revealed that some VLP proteins are produced in the secretory cells; they enter the long gland lumen via secretory units and appear associated with small membranous structures. These structures undergo morphogenesis and assemble 3–6 spikes to assume unique stellate morphologies. Stellate VLPs and their constituent proteins block hemocyte-mediated wasp egg encapsulation by inducing cell lysis and apoptosis (Chiu and Govind, 2002; Chiu et al., 2006; Ferrarese et al., 2009; Morales et al., 2005; Rizki and Rizki, 1992).

L. heterotoma attack delays larval host development (Schlenke et al., 2007). The biological activities of venom components that contribute to the alteration of *Drosophila* development and immunity are largely unknown. We are interested in understanding not only the nature of bioactive molecules in the venom and those associated with VLPs, but also the process of VLP assembly and morphogenesis that occurs in the unique long gland-reservoir environment. We also want to know if the venom factors can contribute to immune suppression via an activating or adjuvant-type role, and whether VLPs have a viral origin.

To address these questions, we have initiated a cDNA-based transcriptome analysis of the venom gland. The enzymatic profile and KEGG terms of our Blast-based protein predictions suggest that in addition to conserved signaling, cell cycle, and housekeeping proteins, the *Lh* venom gland expresses hypothetical and unknown proteins that may help maintain the glandular environments for VLP and venom activities. Many enzymes with predicted biological activities that have been reported in studies of other parasitoid wasps, and in the stinging Aculeata, also appear to be utilized by *Lh*. Given the conservation among immune pathways in insects, of which *Drosophila* has been the classic model (Cherry and Silverman, 2006; Govind, 2008; Schmid-Hempel, 2005; Tanji and Ip, 2005), we predict that *Lh* venom factors with inhibitory functions in the *D. melanogaster* host will also modulate immune physiologies of other *Drosophila* species. A comprehensive understanding of the molecular strategies underlying the success of this natural *Drosophilia* parasitoid can potentially be used to target economically significant insect pests and pathogens.

2. Methods

2.1. Insect stocks

L. heterotoma strain New York USA (Chiu et al., 2006; Schlenke et al., 2007) were raised in house at 25 °C on the *y w* strain of *D. mela*nogaster on standard corn meal and yeast diet.

2.2. Transcriptome library preparation and sequencing

500 *Lh* females were anesthetized by CO_2 and washed with 70% alcohol. Their long gland-reservoir-ovipositor complexes (called venom glands here), were removed simply by pulling the ovipositor, and frozen at −70 °C. Eight micrograms of total RNA were extracted and used to prepare a standard cDNA library (Evrogen) in the pAL17.3 vector using the SMART approach (Zhu et al., 2001). The library was amplified by PCR.

SMART-Sfi1A oligonucleotide 5′-AAGCAGTGGTATCAACGCAGAGTG GCCATTACGGCCrGrGrG-3′
CDS-Sfi1B primer 5′-AAGCAGTGGTATCAACGCAGAGTGGCCGAGGCG GCCd(T)20-3′
SMART PCR primer 5′-AAGCAGTGGTATCAACGCAGAGT-3′
pAL 17 dir primer 5′-CCAGGGTTTTCCCAGTCACGA-3′
pAL 17 rev primer 5′-CACAGGAAACAGCTATGACCA-3′
More than 950 randomly selected clones in ten 96-well plates were sequenced by Sanger method (Genewiz, New Jersey).

2.3. Sequencing confirmation

A dozen clones were re-sequenced. Transformed *Escherichia coli* were grown for 12 h at 37 °C in 5 ml of Luria Broth-ampicillin cultures. Approximately 500 ng of the associated pAL 17.3 plasmids were obtained from 1 ml Luria Broth-ampicillin cultures grown for 12 h at 37 °C. QIAprep Spin Miniprep Kit (http://www.qiagen.com) procedure was followed to obtain the cloned inserts that were then sequenced using a T7 sequencing primer (Genewiz, New Jersey).

T7 Universal 20mer Primer: 5′-TAA TAC GAC TCA CTA TAG GG-3′

The sequences were compared to the originals using EBI (http://www.ebi.ac.uk/Tools/) Needleman pairwise alignment (Needleman and Wunsch, 1970). The average percent identity of the nucleotide sequences was 98.8%, calculated as the number of indels and mismatches.

2.4. Raw EST processing

The raw Sanger nucleotide sequences were processed with the standard methodologies of (1) phred/phrap/consed (Ewing and Green, 1998; Ewing et al., 1998) and (2) Cap3 (Huang and Madan, 1999). For phredphrap, base calls and quality assignments were