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Full Length Article

Isolation and Characterization of Immune Suppressive Genes through Bioinformatic Analysis of Venom Glands Transcriptome of *Bracon hebetor* (Hymenoptera: Braconidae)

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Abstract

Female parasitoid injects in its host a blend of reproductive secretions during oviposition containing mainly ovarian fluids and venom to circumvent the host immune response. *In vitro* studies have revealed that venom inhibits the motility and aggregation of the host larval hemocytes. Bioactive genes with immunosuppressive activity were investigated by screening of the venom gland Transcriptome of the wasp *Bracon hebetor* by using manual BLAST X analysis and functional annotation of the selected contigs. The 5' ends of the selected genes were obtained using RACER kit (Invitrogen) and full length genes were isolated. Cloning and sequencing of the genes were performed and further characterized using bioinformatic tools *i.e.*, Expasy Translate Tool, Clustal Omega and SignalP. Deduced amino acid sequences of the isolated genes (Venom acid phosphatase, Tryptase-2 and CTD nuclear envelope phosphatase) showed significant homologies with other Braconid species, whereas Tryptase-2 and CTD nuclear envelope phosphatase showed good secretory role. Further, in depth studies are required for the expression of isolated genes and their functional analysis. This is the first report of the characterization of immune suppressive genes from the venom gland of *B. hebetor* which may be useful for developing benign control tactics for insect pests of agricultural crops. © 2019 Friends Science Publishers

Keywords: Cloning; Immunosuppression; RACER; Sequence alignments; Transcriptome analysis; Venom genes

Introduction

Host-parasitoid interactions in insects provide a remarkable opportunity to identify novel genes and their products responsible for the major host modifications, which may be exploited further for the development of new insect pest control strategies. A number of antagonistic associations are poorly known in insects which provide a source for the identification of novel natural compounds. These bioactive compounds can disrupt growth, development, reproduction and immune system of insect pests that may be of interest for developing new bio insecticides (Webb and Strand, 2005).

Insects have effective inborn defense responses to contend with foreign intruders. Several immune response signaling pathways, specifically Toll and IMD pathways are activated in the host as a result of the attacks by foreign invaders (Sackton *et al*., 2007) and prompt the expression of immune-response genes (Ferrandon *et al.*, 2007). Insect immune systems consist of physical barriers as well as humoral and cellular defense responses (Hoffmann, 2003). Cellular immunity consists of direct interactions amid intruders and hemocytes. These interactions start directly after an attack is detected and comprise phagocytosis, nodulation and encapsulation in the case of large intruders.

Oviposition into a host hemocoel triggers host defense responses. Non-tolerant hosts successfully encapsulate and kill the parasitic wasp's eggs. But, sophisticated relationships have been established between hymenopteran wasps and their hosts. Parasitic wasps produce toxic components that act to damage or avoid host immunity and thereby enable pre-imaginal growth inside the host (Pennacchio and Strand, 2006). These components include polydnaviruses (PDVs), virus-like particles (VLPs), venoms, teratocytes and ovarian fluids (Beckage and Gelman, 2004).

Despite the impact on cellular immunity, venom proteins can also target the humoral arm of the host immune system. For example, parasitization causes inhibition of melanization in host hemolymph which is usually due to venom proteins. *Pteromalus puparum* venom decreased the expression of antimicrobial peptides *i.e.*, cecropin, attacin, lebocin, lysozyme and proline-rich AMP in fat bodies and hemocytes of *Pieris*

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rapae larvae (Fang *et al.,* 2010). Furthermore, the genes responsible for proPO (prophenoloxidases) activation cascade, *i.e.,* PAPs, were under-expressed.

Different genes or proteins having immunosuppressive function have been identified hitherto from various insect parasitoids *i.e.,* Serpins or group of proteins known as serine protease inhibitors have been identified in *Manduca sexta* and *Drosophila Melanogaster* which are shown to reduce PO activity in its host. Similarly, in an endoparasitic wasp, *Pimpla hypochondriaca*, a reprolysin-type zinc metalloprotease was observed and it was proposed to act as stimulator of host immune repression (Kanost, 1990). Three genes known as VPr1, VPr2 and VPr3 were identified from *P. hypochondriaca* which also interfere PO cascade (Dani and Richards, 2009). Calreticulin and acid phosphatases have been identified from *Nasonia vitripennis*, *Camponotus floridanus* and *Anisopteromalus calandrae* which play a role in suppression of encapsulation and dephosphorylation of immune proteins, respectively (Perkin *et al.,* 2015). Transcriptome analysis of *A. calandrae* venom gland shows a set of immunosuppressive proteins like small serine proteinase inhibitor-like venom protein, Cysteine-rich pacifastin venom protein 1 and 2, Serpin 5, Kazal type serine protease inhibitor-like venom protein 2, Chymotrypsin inhibitor-like, Venom protease- like (Perkin *et al.,* 2015).

Our experimental model organism is *Bracon hebetor*, a larval ectoparasitoid of *Galleria melonella*, commonly known as Greater wax moth. *B. hebetor* is a larval ectoparasitic wasp that parasitizes numerous pyralids of order Lepidoptera (Dweck *et al.,* 2010). It also exploits larvae of many Lepidoptera infesting stored grain commodities (Richards and Thomson, 1932) and other host insects in the meadow crops (Harakly, 1968). The extensive host range, high fertility percentage and less generation time makes *B. hebetor* an exceptional nominee for natural control of many insect pests of family, Pyralidae, together with our tested host, *G. mellonella*. Venom of *B. hebetor* is a rich source of the toxic/virulent genes responsible for host paralysis and mortality. A number of toxins (BrhI and BrhII) with potential insecticidal activity have been identified from the parasitoid, *B. hebetor* which are considered for having novel mode of action *i.e.,* neurotoxic effect on insect pest with low mammalian toxicity (Johnson *et al.,* 1999).

Therefore, this study was conducted to isolate and characterize the bioactive genes from the venom glands of *B. hebetor* which could have a predictable role in the suppression of its host immune function. The characterized genes may be exploited for further in depth molecular studies, which may lead towards the development of sustainable insect control strategies.

Materials and Methods

Current research work was carried out jointly in three collaborating labs. *i.e.,* Insect Molecular Biology Laboratory, Department of Entomology, Molecular Biochemistry Laboratory, Department of Biochemistry, University of Agriculture, Faisalabad (UAF) and Department of Entomology, University of Arizona, Tucson, USA.

Experimental Insects

For laboratory production of parasitic wasp *B. hebetor,* 5th instar larvae of the pyralid host, *G. mellonella* were provided as a food source by following the methodology outlined by Manzoor *et al.* (2011). For rearing of the host insect, raw wax containing host larvae was collected from the mango orchards located in the main campus of UAF, Pakistan and transferred into plastic boxes (40 × 70 cm). For parasitoid rearing, the vials (2 × 10 cm) containing a host larvae with pair of parasitoids (male and female)/each vial were kept under controlled conditions (27–30 ± 1°C, 65 ± 5% relative humidity (RH) and 18 h light/6 h dark photoperiod). Cotton swabs/pads soaked in 50% honey solution were provided as food source for *B. hebetor* adult, whereas the raw wax was replaced occasionally for host larvae feeding.

Analysis of Venom Glands Transcriptome Assembly

Venom glands Transcriptome assembly of *B. hebetor* had already been generated by Manzoor *et al.* (2016) in which, 22425 contigs were reported. To identify the immunosuppressive genes the contigs data was subjected to Blast X manually and then annotation by using Blast 2 Go software *i.e*., Blast2GO PRO (v2.5.0) (Gotz *et al.,* 2008). and three partial genes or contigs with immunosuppressive activity were selected. The 5’ ends of the selected genes were isolated first using gene racer protocol.

RNA Extraction and Purification

Total RNA of the female wasp, *B. hebetor* was extracted by using TRIzol® reagent (Invitrogen) following manufacturer’s protocol. DNase I treatment was carried out to remove residual genomic DNA from extracted RNA using Ambion DNA-free™ Kit. RNA pellets were suspended in DEPC treated water and quantified by Nanodrop, ND-2000 spectrophotometer (Thermo Scientific, USA).

5' end cDNAs Amplification

One microgram (1 μg) of purified RNA was used for gene racer protocol using Gene Racer Core Kit (Invitrogen). Primers were designed to isolate the 5’ end of the selected genes. Two gene specific primers were designed for each one of gene (Table 1). First PCR was performed using gene specific primer and 5’ Racer primer and second PCR was performed using first PCR products as a template with gene specific nested primer and 5’ Racer nested primer.

Table 1: Primers list used for amplification of 5' end genes (Racer products) and full length genes of *B. hebetor*

	Primers used for 5' end genes amplification	
Name	Sequence (5'-3')	Description /Gene Id.
G 1	CGCGGTTTTGTACGATCTGA	Venom acid phosphatase
G1n	CGTCCCTTATTAGTTAATTGTCCCC	gene specific primers (R)
G2	CGACGAGTTGTGCGAAGACTGT	Tryptase-2 gene specific
G2n	CGAATACGTCTTTACCCCATCCGC	primers (R)
G3	CGCTTGTGGACGAAAAATCTGACC	Nuclear envelope phosphatase
G3 n	GCTCCATCGTGATGTGAGTGTATC	gene specific primers (R)
Racer5	CGACTGGAGCACGAGGACACTGA	Racer primers specific for
R5 nest	GGACACTGACATGGACTGAAGGAGT	RNA oligo adapter (F)
	Primersusedfor full length genes amplification	
g1 F	TTAAATACTGGAAGGAACAATG	
g1 R	GGATGACATAATTCGGATCAG	Venom acid phosphatase
g2 F	AAGCACATCAGGTTTTACAATG	
g2R	TTATGGCACATAACTAGTCGT	Tryptase-2
g3F	GGTTAATATTGTCATAGACATG	Nuclear envelope phosphatase

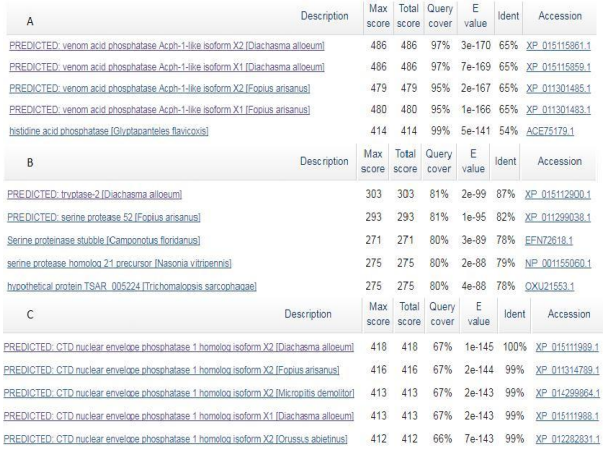
Touchdown PCR protocol was followed using EconoTac DNA polymerase. PCR conditions were as follows 94°C for 2 min, 13 cycles of 94°C for 15 s, 74°C for 15 s (-1°C/ Cycle Incr.) and 72°C for 30 s, 16 cycles of 94°C for 15 s, 62°C for 15 s and 72°C for 30 s before final elongation of 72°C for 2 min. The RT-PCR products were run at 1% agarose gel at 90 V for 90 min. After getting the 5' end sequences the primers were designed again to isolate the full length genes through RT-PCR of the *B. hebetor* purified RNA samples. The PCR conditions used were 94°C for 3 min, 30 cycles of 94°C for 30 s, 62°C for 30 s and 72°C for 1 min before final elongation of 72°C for 6 min. PCR products were visualized on 1% agarose gel.

Cloning and Sequencing of Target Genes

The gel bands were excised under UV lamp and purified using Qiagen Gel Extraction Kit. The purified genes were ligated through PGEM-T Easy Vector System I and transformed into DH5 alpha competent cells. IPTG and X gal added plates containing Ampicillin as antibiotic were used for Blue white screening of the colonies. Colony PCR for selected white colonies was performed and through gel electrophoresis, successful cloning of the desired genes was confirmed. Plasmid extraction of the selected colony was performed using E.Z.N.A plasmid DNA mini kit II to purify the gene of interest with plasmid vector and sequenced.

Bioinformatic Analysis

ExPASy-Translate tool (https://web.expasy.org/translate/) was used to translate gene sequences using standard amino acids category. The deduced amino acid sequences for desired genes were aligned with previously characterized protein sequences present on NCBI database for different species using online multiple sequence alignment tool, Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) which also provides the cladograms showing phylogenetic relationships of different species with our tested insect species. Lastly, SignalP4.1 server



A Description	Max score	Total score	Query cover	E value	Ident	Accession
PREDICTED: venom acid phosphatase Acph-1-like isoform X2 [Diachasma alloeum]	486	486	97%	3e-170	65%	XP_015115861.1
PREDICTED: venom acid phosphatase Acph-1-like isoform X1 [Diachasma alloeum]	486	486	97%	7e-169	65%	XP_015115859.1
PREDICTED: venom acid phosphatase Acph-1-like isoform X2 [Fopius arisanus]	479	479	95%	2e-167	65%	XP_011301485.1
PREDICTED: venom acid phosphatase Acph-1-like isoform X1 [Fopius arisanus]	480	480	95%	1e-166	65%	XP_011301483.1
histidine acid phosphatase [Glyptapanteles flavicoxis]	414	414	99%	5e-141	54%	ACE75179.1

B Description	Max score	Total score	Query cover	E value	Ident	Accession
PREDICTED: tryptase-2 [Diachasma alloeum]	303	303	81%	2e-99	87%	XP_015112900.1
PREDICTED: serine protease 52 [Fopius arisanus]	293	293	81%	1e-95	82%	XP_011299038.1
Serine proteinase stubble [Camponotus floridanus]	271	271	80%	3e-89	78%	EFN72618.1
serine protease homolog 21 precursor [Nasonia vitripennis]	275	275	80%	2e-88	79%	NP_001155060.1
hypothetical protein TSAR_005224 [Trichomalopsis sarcophagae]	275	275	80%	4e-88	78%	OXU21553.1

C Description	Max score	Total score	Query cover	E value	Ident	Accession
PREDICTED: CTD nuclear envelope phosphatase 1 homolog isoform X2 [Diachasma alloeum]	418	418	67%	1e-145	100%	XP_015111989.1
PREDICTED: CTD nuclear envelope phosphatase 1 homolog isoform X2 [Fopius arisanus]	416	416	67%	2e-144	99%	XP_011314789.1
PREDICTED: CTD nuclear envelope phosphatase 1 homolog isoform X2 [Microplitis demolitor]	413	413	67%	2e-143	99%	XP_014299864.1
PREDICTED: CTD nuclear envelope phosphatase 1 homolog isoform X1 [Diachasma alloeum]	413	413	67%	2e-143	99%	XP_015111988.1
PREDICTED: CTD nuclear envelope phosphatase 1 homolog isoform X2 [Orussus abietinus]	412	412	66%	7e-143	99%	XP_012282831.1

Fig. 1: Blast X homology for A. Venom acid phosphatase contig B. Tryptase 2 contig and C. CTD nuclear envelope phosphatase contig

Fig. 2: Blast 2 Go screenshot for *B. hebetor* contigs annotation

(http://www.cbs.dtu.dk/services/SignalP/) was used for signal peptide prediction of deduced protein sequences.

Results

Analysis of Transcriptome Assembly

Three genes (contigs) were selected from Transcriptomic data of *B. hebetor* on the basis of their predicted immunosuppressive activity. Blast X analysis and functional annotation results are shown in Fig. 1 and 2, respectively. First contig showed 65% similarity with venom acid phosphatase Acph-1 like protein and the other two contigs showed 87 and 100% similarities with Tryptase-2 and CTD nuclear envelope phosphatase proteins, respectively. Their function as immunosuppressive proteins were observed under recent annotation results and already published research work.

RT-PCR

RT-PCR gels for 5'end genes and full length genes are shown in Fig. 3 and 4, respectively. Three 5' end fragments of 490, 1006 and 712 bp were amplified for Venom acid

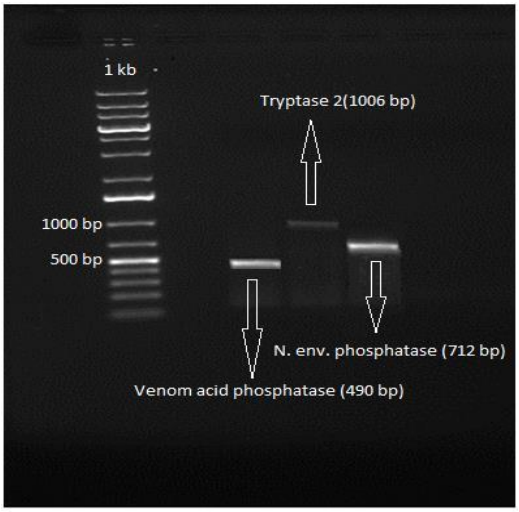


Fig. 3: RT-PCR gel for 5' end genes (Racer products) of *B. hebetor*

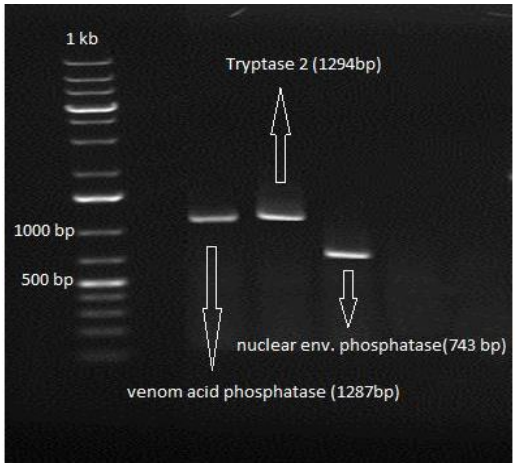


Fig. 4: RT-PCR gel for full length genes of *B. hebetor*

phosphatase, Tryptase-2 and CTD Nuclear envelope phosphatase, respectively, while for the amplified full length fragments were of 1287, 1294 and 743 bp length for all three genes, respectively. Nucleotide sequence of all three genes and their deduced amino acid sequences are shown in Fig. 5–7.

Multiple Sequence Alignments

B. hebetor deduced amino acid sequences for all three genes showed significant similarities with other Braconid species according to sequence alignment results (Fig. 8–11). Maximum similarity was observed toward the *Diachasma alloeum* in case of all three genes *i.e*., 71.50, 91.96 and 100% similarity for venom acid phosphatase, Tryptase-2 and CTD nuclear envelope phosphatase genes, respectively which were decreased gradually in case of *Fopiusarisanus*and*Microplitis demolitor* genes, respectively.

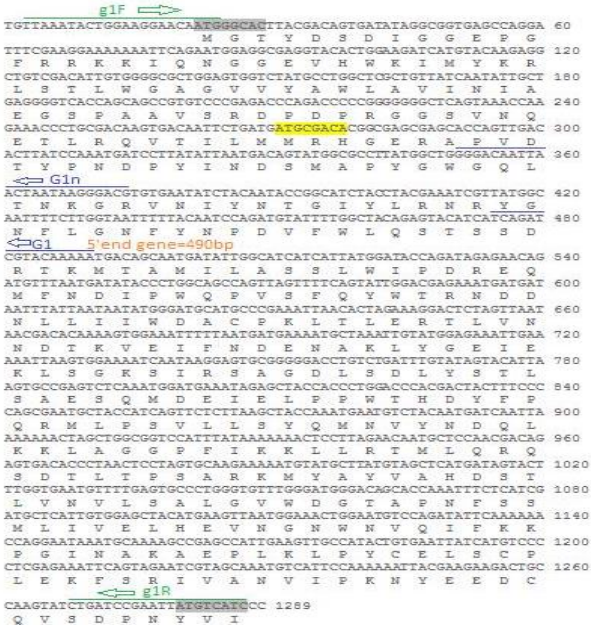


Fig. 5: Nucleotide and deduced amino acid sequence for Venom acid phosphatase-1 like gene, isoform X1, from *B. hebetor*. Primers locations are shown with above lined regions containing arrowheads for direction. The coding sequence start and end sites are highlighted with grey colour. The yellow colour highlighted area indicates the selected contig start site or the overlapping start site for 5' end gene sequence and selected contig sequence

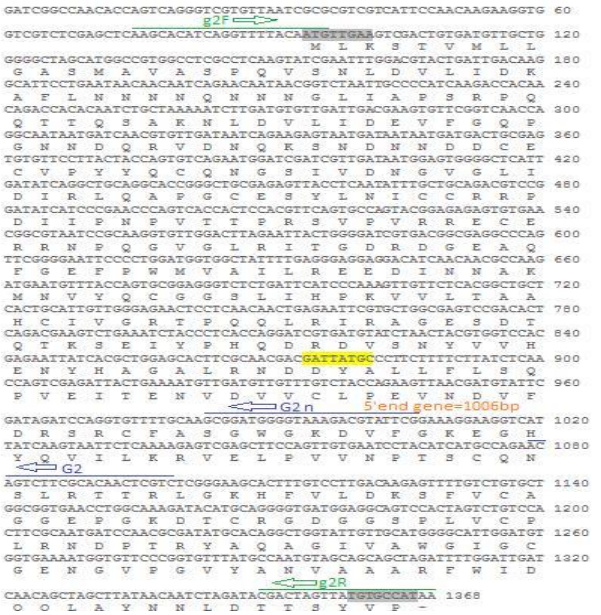


Fig. 6: Nucleotide and deduced amino acid sequence for Tryptase-2 gene from *B. hebetor*. Primers locations are shown with above lined regions containing arrowheads for direction. The coding sequence start and end sites are highlighted with grey color. The yellow color highlighted area indicates the selected contig start site or the overlapping start site for 5' end gene sequence and selected contig sequence

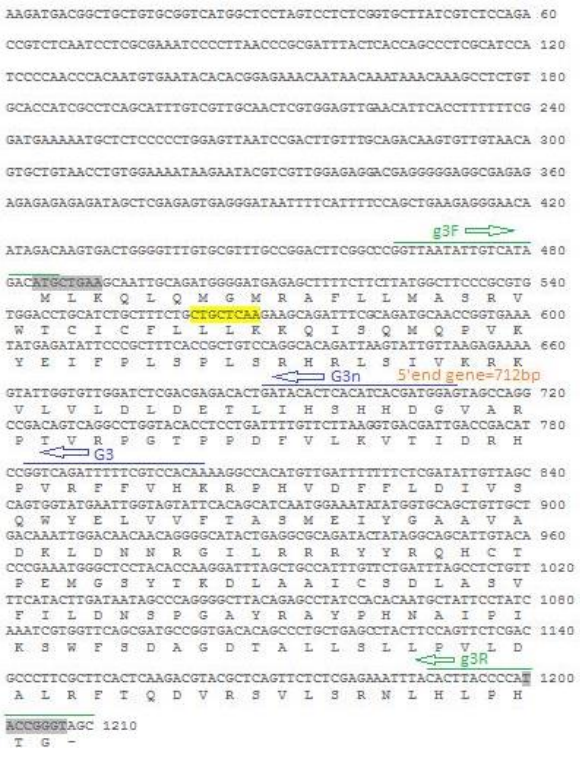


Fig. 7: Nucleotide and deduced amino acid sequence for CTD nuclear envelope phosphatase1 homolog isoform X2 gene from *B. hebetor*. Primers locations are shown with above lined regions containing arrowheads for direction. The coding sequence start and end sites are highlighted with grey colour. The yellow colour highlighted area indicates the selected contig start site or the overlapping start site for 5' end gene sequence and selected contig sequence

Fig. 8: Multiple amino acid sequence alignment of *B. hebetor* venom protein, Venom acid phosphatase deduced amino acid sequence with related species protein sequences: *Diachasma alloeum* (XP 015115859.1, identity 71.50%), *Fopius arisanus* (XP 011301483.1, identity 62.72%) and *Microplitis demolitor* (XP 008550915.1, identity 52.09%). Sequences were aligned using Clustal Omega (online software). Consensus sequences are shown with symbols "***" and highlighted with different colors

Signal Peptides Prediction

Signal P results depicted that tryptase 2 and nuclear envelope phosphatase had good secretory role whereas venom acid phosphatase showed poor secretory nature (Fig. 12–14).

Discussion

Parasitic wasp's venom, either singly or in mixture containing additional maternal toxic factors are particularly recognized to have various functions, including obstruction of the host defense, in numerous conditions delaying or reducing hemocyte-intervened reactions (Beckage and Gelman, 2004; Moreau and Guillot, 2005; Asgari, 2006, 2007). The parasitic wasp species lacking PDVs or other associated viruses such as *Nasonia vitripenns* (Rivers *et al*., 2002), *P. turionellae* (Osman, 1978) and *Pimpla hypochondriaca* (Parkinson and Weaver, 1999; Richards and Parkinson, 2000), venoms would perform immune suppressive activity in the absence of other immune inhibitive factors. Wu *et al*. (2008) initially characterized Vn.11 protein from *Pteromalus puparum* and recognized it as an immune suppressive component linked with host cellular immunity in parasitic wasps lacking PDVs or VLPs. SPIs (Serine protease inhibitors) have been recognized in parasitic wasp venoms *i.e*., *Cotesia inanitus* (Vincent *et al.,* 2010), *P. hypochondriaca* (Parkinson *et al.,* 2004), *P. puparum* (Zhu *et al.,* 2010), *Cotesia rubecula* (Asgari *et al*., 2003), *Nasonia vitripennis* (Graaf *et al*., 2010) and *Anisopteromalus calandrae* (Perkin *et al.,* 2015) involved in suppressing melanization and encapsulation by taking advantage of interfering phenol oxidase activating mechanism in host insect. In contrast, CrCRT, a calreticulin-like protein isolated from the similar venom has been proved to inhibit encapsulation and spreading of the hemocytes (Zhang *et al*., 2006).

Likewise, N. *vitripennis* venom diminished host cellular defense responses (Rivers *et al.,* 2002) *i.e.,* calreticulin and C1q-like venom protein from this category were supposed to play a role in immune suppression (Graaf *et al.,* 2009). The immunoglobulin-like protein, a third member, was found to have two similar domains in common with immune or defense related proteins of higher animals, but might have an extremely diverse biological function in insects fauna (Graaf *et al.,* 2010).

Fig. 9: Multiple amino acid sequence alignment of *B. hebetor* venom protein, Tryptase 2 deduced amino acid sequence with related species protein sequences: *Diachasma alloeum* (XP 015112900.1, identity 91.96%), *Fopius arisanus* (XP 011299038.1, identity 74.05%) and *Microplitis demolitor* (XP 008545410.1, identity 57.31%). Sequences were aligned using Clustal Omega (online software). Consensus sequences are shown with symbols "***" and highlighted with different colours

Fig. 10: Multiple amino acid sequence alignment of *B. hebetor* venom protein, CTD nuclear envelope phosphatase deduced amino acid sequence with related species protein sequences: *Diachasma alloeum* (XP 015111989.1, identity 100%), *Fopius arisanus* (XP 011314789.1, identity 99.58%) and *Microplitis demolitor* (XP 014299864.1, identity 98.33%). Sequences were aligned using Clustal Omega (online software). Consensus sequences are shown with symbols "***" and highlighted with different colors

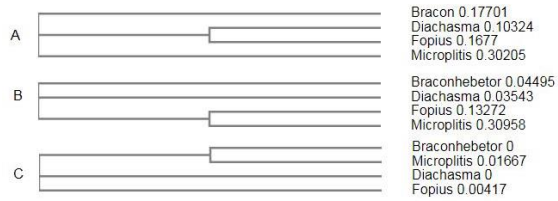


Fig. 11: Cladograms showing phylogenetic relationships of *B. hebetor* with other Braconid species based on amino acid sequence alignments for A. Venom Acid Phosphatase B. Tryptase 2 C. CTD nuclear envelope phosphatase

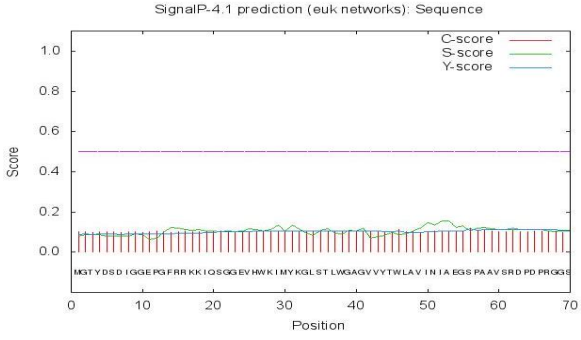


Fig. 12: Signal peptide prediction for Venom acid phosphatase

Our Blast X analysis results indicated that the protein conserved domain for venom acid phosphatase gene (1st gene) belongs to HP-HAP Superfamily (Histidine phosphatases) which have functions like metabolism, signaling and regulation. Moreover, in its predicted functions, this gene acts as a venom allergen and is also involved in dephosphorylation of immune proteins.

Perkin *et al.* (2015) has provided evidence about the function of venom acid phosphatase gene in which 64 different types of transcripts in the venom gland, ovipositor, venom reservoir and Dufour gland of the agriculturally vital wasp *Anisopteromalus calandrae* were recognized. They have categorized the venom acid phosphatase in the group of immunesuppressive genes/proteins. Moreover, acid phosphatases from a disease causing fungus were proposed to target the defense system of a desert locust, *Schistocerca gregaria* by dephosphorylation of immune proteins and inhibiting the host immune responses (Xia *et al.*, 2000).

The 2nd gene, tryptase 2 which we isolated in the current research, its protein conserved domain belong to the Tryp-SPc Superfamily (trypsin like serine proteases). It is also considered as homolog of venom 50 (venom protein) identified in different parasitoids *i.e.*, *C. rubicula*, inhibit encapsulation response by host hemocytes, *i.e.*, A Vn50 (50 kDa protein) from *C. rubecula* was reported to hinder the stimulation of prophenoloxidase (proPO) to phenoloxidase (PO), an important enzyme in the melanization pathway (Asgari *et al.*, 2003). This is because

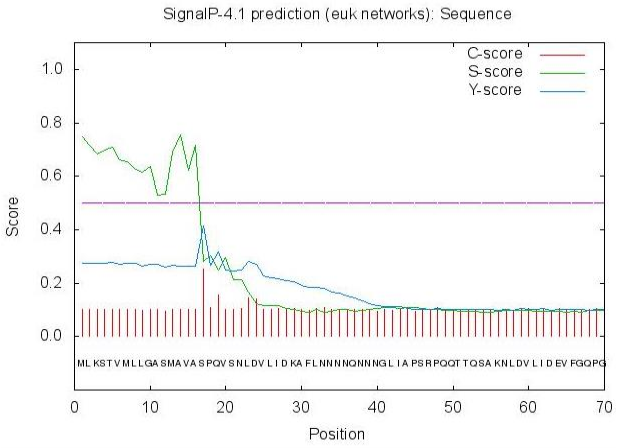


Fig. 13: Signal peptide prediction for Tryptase-2

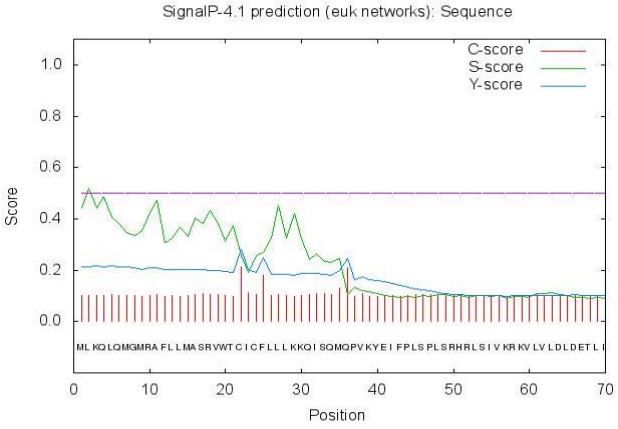


Fig. 14: Signal peptide prediction for CTD nuclear envelope phosphatase

of the structural similarity of Vn50 to serine protease homologs (SPHs) (Thomas and Asgari, 2011) which usually assist activation of the enzyme by proPO activating protein (PAP) (Jiang and Kanost, 2000) and aggressive binding to proPO and PAP (Zhang *et al.*, 2004). Likewise, in *Leptopilina boulardi*, venom was found to be an important factor to stifle the encapsulation response (Labrosse *et al.*, 2005). The main protein is RhoGAP (Rac GTPase Activating protein) that affects the spreading and aggregation of lamellocytes making them unable to form a capsule (Labrosse *et al.*, 2005).

The 3rd gene was CTD nuclear envelope phosphatase for which protein conserved domain belong to HAD like superfamily of proteins having catalytic action. The predicted function of the nuclear envelope phosphatase from annotation results were found as a cytokins inhibitor, involving in dephosphorylation of immune proteins and interfering with cadherin gene expression, indicating that this gene has a broad function. Cytokine is a neural protein which is related to *G. mellonella* immunity. Vogel *et al.* (2011) reported that one immune pathway includes proteolytic activation of a cytokine called Spaetzle, which plays role in dorsal-ventral patterning in before time embryonic development and during the antimicrobial defense in host larvae and adults. It was manifested that introduction of Spaetzle into *M. sexta* larvae activated the expression of many immune-related peptides and proteins, together with cecropin, moricin, attacin and lysozyme (An *et al.*, 2010).

The deduced proteins sequence alignment indicated that *B. hebetor* venom proteins have a lot of conserved regions or similarities with other parasitoids belonging to the same family *i.e*., Braconidae. Maximum similarity (nearly 100%) was found for CTD nuclear envelope phosphatase protein (Fig. 10). Moreover, on the basis of alignments and phylogenetic relationship results we can foretell that our test insect showed more homology with *D. alloeum* as compared to other braconid wasps. Likewise, signal peptide prediction results indicate that tryptase-2 protein showed an excellent secretory role (role in secretion) and CTD nuclear envelope phosphatase is also a good secreted protein but venom acid phosphatase showed no or poor secretory role. These results concede the venomous nature of these proteins.

Conclusion

Immune Suppressive Genes naturally occurring in the venom blend of the wasp *B. hebetor* have insecticidal potential for the control of insect pests of Pyralidae. Venom gland Transcriptome of *B. hebetor* was analyzed bioinformatically, revealing a number of bioactive genes with potential insecticidal activity for the development of sustainable insect control programs.

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