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MOLECULAR EVIDENCE ON SYMBIOTIC RELATIONSHIPS BETWEEN *BRACOVIRUS* AND *SPODOPTERA LITTORALIS* (LEPIDOPTERA: NOCTUIDAE)

SUMMARY

Bracoviruses (BVs) from the Polydnaviridae family are symbionts of parasitic braconid wasps. BVs are used by parasitoid wasps to manipulate their lepidopteran host physiology. The virus is transmitted into the haemocoel of the host during oviposition, together with the parasitoid egg and other maternal protein secretions. Viral products encode proteins that lower host immunity; allowing the development of parasitoid wasp larvae in the host they ensure wasp survival in the lepidopteran larvae. The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.), is an important pest that causes extensive damages in many vegetable, fodder, and fiber crops. Although resistance has been developed to different types of insecticides, chemical-based control methods are still used as a management strategy for *S. littoralis*.

In the present study, two transcripts that are members of the C-type lectin family encoding *BV* proteins were annotated from a cDNA library generated from the hemolymph of the fifth instar larvae of *S. littoralis*. Characterizations of the nucleotide and deduced amino acid sequences of these genes approved the bracoviral deriving genes, and also showed sequence similarity to lectin proteins of a parasitoid wasp, *Cotesia* sp. (*Hymenoptera*: Braconidae). A phylogenetic analysis showed a relationship between the C-type lectins from Lepidoptera and Hymenoptera suggesting that there is *bracovirus-mediated* gene flux *between* two orders. We suggest that the *symbiotic* relationship between *bracovirus* and S. littoralis might have an important role in the benefit of wasps, resulting in a suppressed host immune system and also for the evolution process of the interacted genomes.

Keywords: *Bracovirus, Spodopteralittoralis, Lectins.*

INTRODUCTION

Insects have unique adaptation strategies in order to survive. They have developed several defense mechanisms, including the immune system. The immune system of insects involves physical barriers, cellular responses and humoral responses that allow them to interact with microorganisms recognize and remove pathogens, and repair wound and tissue damage (Gillespie *et al.*,

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1997). Sclerotized cuticle, the integument, the peritrophic matrix of the midgut epithelium and the chitin lining of the tracheal system serve as initial barriers against wounds and invading microorganisms (Lavine and Strand, 2002; Wigglesworth, 1972). The hallmark of humoral defense reaction is the production of antimicrobial peptides (AMPs), reactive intermediates of oxygen and nitrogen species (ROS, RNS), and the complex enzymatic cascades leading to melanisation and hemolymph coagulation (Meister *et al.*, 2000 and Lowenberger, 2001; Bogdan *et al.*, 2000 and Vass and Nappi, 2001; Castro *et al.*, 2009; Lavine and Strand, 2002). In contrast, cellular defense refers to hemocyte-mediated immune responses like phagocytosis, nodulation and encapsulation (Strand and Pech, 1995; Schmidt *et al.*, 2001).

A key step before the initiation of an immune response is the detection and recognition of microorganisms. Insects produce some elicitors in order to recognize characteristic molecular patterns of microbial walls known as pathogen-associated molecular patterns (PAMPs) (Gillespie *et al.*, 1997; Jiravanichpaisal *et al.*, 2006). The recognition of these PAMPs is achieved predominantly by peptidoglycan recognition proteins (PRPs) (Elftherianos *et al.*, 2007). The best characterized PRPs in insects are the C-type lectins (CTLs). CTLs have a key role in the recognition of different kinds of pathogens during the early phase of microbial infection (Watanabe *et al.*, 2006). Invertebrate CTLs are involved in immune responses including PPO (*prophenoloxidase*) activation (Yu and Kanost, 2000), hemocyte nodule formation (Koizumi *et al.*, 1999), opsonization and microbial clearance (Jomori and Natori, 1992; Yu and Kanost, 2003). Lepidopteran C-type lectins can also recognize the insects' hemocytes and enhance hemocyte-related responses (Chai *et al.*, 2008).

On the other hand CTLs associated with the bracoviruses have been identified from Hymenopteran parasitoid viral genomes during parasitization which are involved in host immune and developmental suppression (Glatz *et al.*, 2003; Teramato and Tanaka, 2003). Polydnaviruses, comprise two genera Ichnoviruses (IVs) and Bracoviruses (BVs), are a group of insect viruses that have a symbiotic relationship with parasitic wasps.

The primary function of most polydnavirus genes expressed in lepidopteran larvae appears to be suppression of the immunity of its*host.* The endoparasitoid, *Cotesia* (Hymenoptera: Braconidae) maintain its development by attacking larval stages of Lepidopteran insects. During parasitoid oviposition the bracoviral particles are transmitted into the hemocoel of the host, and finally polydnaviruses express their own genes to manipulate the host physiology (Stoltz, 1993; Webb and Strand, 2005).

Here, we report two bracoviral proteins encoded by different transcripts of CTL family found in the transcriptome of the lepidopteran *S. littoralis*. Phylogenetic reconstruction and structural bioinformatics of each gene revealed two major clades that represent the two different CTLs. We further discussed our findings in the context of bracoviruses-host-parasite associations.

MATERIALS AND METHODS

Insect cultures were maintained on an artificial diet at 25 ± 1°C with 60% relative humidity and 16:8 h light-dark photoperiod (Sorour *et al.* 2011). Total RNA from last instar larvae was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA was treated with DNase-free (Ambion, Austin, TX, USA) using 1.5 units/µg of total RNA. Quantification and integrity was assessed by using ethidium bromide-stained 1% agarose gel and Nanodrop ND-1000 spectrophotometry (Thermo Scientific, Waltham, MA, USA), with a cut-off value of 1.8 for the A260: 280 ratio.

RNA was fragmented with a zinc chloride solution. Fragmented RNA was quantified using the Agilent 2100 Bioanalyzer system using PicoGreen dsDNA Assay Kit (Invitrogen). cDNA was synthesized using the cDNA Synthesis System Kit with random hexamer primers (Roche Applied Science, Indianapolis, IN, USA). The cDNA fragments were subjected to ligation to the sequencing adaptors provided with the GS FLX Titanium Rapid Library Preparation Kit (Roche Applied Science), and small fragments were removed with AMPure XP (Beckman Coulter, Fullerton, CA, USA). Sequencing was performed on a GS FLX platform with Titanium chemistry (Roche/454) using a Small region of a Pico Titer Plate (PTP) per library, following the manufacturer's instructions.

Annotations of assembled sequences were carried out by BLASTx against NCBI (National Center for Biotechnology Information) non-redundant protein sequence databases using the software Blast2GO (Conesa *et al.*, 2005). The partial cDNA and deduced amino acid sequences were compared using the BLASTx tool and EXPASY. Sequences were aligned using the MUSCLE software. Phylogenetic trees were inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Evolutionary analyses were performed with MEGA6 (Tamura *et al.*, 2013).

RESULTS AND DISCUSSION

We have constructed cDNA library from the hemolymph of the fifth instar larvae of *S. littoralis* and identified two different contigs encoding bracovirus-like lectin proteins. Contig 00225 corresponded to a transcript identified as *S. littoralis bracovirus-like lectin 1* (*Spli-BLL1*) while Contig 00253 corresponded to *S. littoralis bracovirus-like lectin 2* (*Spli-BLL2*). The *Spli-BLL1* was 1053 bp in length comprising of putative open reading frame (ORF) encoding 131 aa, while Spli-*BLL2* was 653 bp in length containing of putative ORF encoding 147 aa. BLAST analysis revealed that the *Spli-BLL1* showed highest similarity to *Spodoptera exigua BLL2* with 69% identity and *Spli-BLL2* shows highest similarity to *Spodoptera frugiperda CTL* with 87% identity.

Figure 1. Phylogenetic analysis of Spli-BLLs. The name of the used species and accession numbers of the GenBank of the aminoacid sequences are as follows:*Spodopterafrugiperda* proteins obtained from Spodobase (http://bioweb.ensam.inra.fr/spodobase) *S.frugiperda* lectin 3 (Sf1H08856-3-1) and *S. frugiperda* lectin 5 (Sf2H07501-5-1); *Spodopteraexigua*bracovirus lectin like 1-6 KP406769-74; *S. exigua* lectin like AKP99429.1; C-type-lectins from bracoviruses of *Cotesia* species CCQ71085.1; AEE09562.1; AEE09593.1; AGO14401.1; AAS10157.1; AAO74641.1; *B. mori* C-type lectin 19; NP_001165396.1; *B. mori* C-type lectin 21 NP_001037056.1; *Helicoverpaarmigera* lectin ABF83203.1; *H. armigera* C-type lectin 8 AFI47453.1; *H. armigera* C-type lectin 6 AFI47451.1; *H. armigera* C-type lectin 2 ACI32834.1; *Antheraeapernyi* C-type lectin AGN70857.1; *Mamestraconfigurata* C-type lectin AEA76325.1; *Anopheles stephensi* C-type lectin ACP43727.1; *Aedesaegypti* C-type lectin ABF18196.1; *Drosophila melanogaster* C-type lectin NP_001260046.1; *Musca domestica* C type lectin XP_005189940.1

The molecular phylogeny of *S. littoralis* BLLs were examined using amino acid sequences from different *insects and bracovirus genes* (Figure 1). Phylogenetic analysis result showed that Spli-BLL1 and Spli-BLL2 were clustered closely with the BLLs from other *Spodoptera* species and also Spli-BLL2 was clustered together with the Polydnavirus Lectins from *S. frugiperda.* Furthermore, both Spli-BLLs were situated on the same branch with the Bracovirus C-Type Lectin from Hymenoptera. It might be considered that Spli-BLLs have a mutual evolutionary background with BLLs, Polydnavirus Lectins from Lepidoptera and CTLs from Hymenopteran species. Previous bioinformatic analysis listing a series of bracovirus insertions suggested that there is a bracovirus-mediated gene flux between Hymenopteran and Lepidopteran orders (Gasmi *et al.*, 2015). Furthermore it was previously shown that bracoviral DNA integration into hosts and integration back into the wasp genome in a same manner involving a conserved viral site named Host Integration Motif (Beck et al., 2011; Herniou *et al.*, 2013). Altogether, we can suggest that there might be an integration of the BLLs to the ancestral *S. littoralis*, due to the transmission of the bracoviral DNA into the germlines of the Lepidopters.

Humoral or cell-associated lectins are the key component of innate immune responses of animals with the ability to recognize the exposed glycans on the cell surface of potential pathogens (Sun *et al.* 2008). Lectins from hemolymph of cockroaches have been reported to specifically bind bacterial LPS (Jomori and Natori, 1991) and to stimulate hemolymph phenol oxidase activation (Chen *et al.*, 1995). Immulectin synthesis is expressed in response to bacterial challenge in the hemolymph of *Manducasexta* and appears to interact with bacterial LPS to activate the prophenol oxidase system in plasma (Yu *et al.*, 1999). Similarly, bracovirus C-lectin sequences were highly expressed in the *S. exigua* hemocytes (Gasmi *et al.* 2015). We have also detected *Spli-BLL1* and *Spli-BLL2* genes in the hemolymph of larvae using transcriptomic analysis suggesting that these genes might be involved in the immune response of *S. littoralis*.

Several bracovirus sequences expressed by Lepidoptera have been reported that could result in adaptive advantages for the host (Gasmi *et al.*, 2015). Therefore further functional analysis will highlight the association of host parasitoid and bracoviral products in terms of host immune responses and development. Also full length sequences of the Spodopteran *BLL* genes will give more information about the mechanism involved in the integration of the viral gene into Lepidopteran *S. littoralis*.

CONCLUSIONS

Bracoviruses are symbiotic viruses of parasitic wasps which are used to manipulate host physiology. On the other hand CTLs are one of the important molecules involved in pathogen recognition. Here we report novel *Spli-BLL1* and *Spli-BLL2* genes from *S. littoralis* transcriptome analysis. Expression of both genes in the hemocytes of *S. littoralis* suggests that they could be implicated in

the immune response of the cotton leaf worm. We also hypothesize a possible integration of the bracoviral DNA into the germlines of the *S. littoralis* may lead to have a common phylogenetic history among different orders. However further investigation needs to be conducted about the acquisition and integration mechanisms of the bracoviral genes and their role in insect immune system.

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