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Research Article

# Putative Receptors for *Bacillus Thuringiensis Var. Israelensis* Cry11Aa in *Haemonchus Contortus* Larvae

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### Abstract

**Objectives:** Through bioinformatics analysis, predict *Haemonchus contortus* larvae putative receptors for Cry11Aa.

**Results:** Using a PyDOCK predicted high affinity interaction, as demonstrated by the highly negative binding energies of -57.2, -72.8, and -68.4 respectively for HC23, H-GAL-GP, H11, and all receptors docked to the domain II of Cry11Aa.

**Conclusion:** These results suggest that Bti Cry11Aa toxin may use the proteins HC23, H-GAL-GP, and H11 as a receptor in *Haemonchus contortus* larvae and by doing so, carry out its nematocidal effect.

### Introduction

The *Bacillus thuringiensis* crystal toxin proteins are widely used as biological control agents [1]. At sporulation, the bacterium produces one or more parasporal crystalline inclusions ( $\delta$ -endotoxins), which are toxic to a wide range of insects (Orders Hymenoptera, Homoptera, Orthoptera, Mallophaga, Coleoptera). Once ingested by target insects, these crystal proteins (Cry proteins) are proteolytically activated in the larvae midgut and bind to membrane gut receptors, leading to cell pore formation and death [1]. One of the main characteristics of Cry toxins is its specificity, which suggests that it is mainly mediated by the specific binding to a surface receptor localized in the host midgut cells. The main receptors for the Cry toxin are cadherin-like proteins, Glycosylphosphatidylinositol (GPI)-anchored Aminopeptidase-N (APN), GPI-anchored Alkaline Phosphatase (ALP) and an  $\alpha$ -amilase [2]. Studies using *Bacillus* sp. evidenced a significant toxic activity against larvae of the important livestock parasite, the nematode *Haemonchus contortus* [3,4]. *Bacillus thuringiensis var. israelensis* (Bti) possesses more than one toxin within the range of 10-120 kDa (i.e., Cry4Aa, Cry4Ba, Cry11Aa and Cyt1Aa), however, the main toxicity effect for *Haemonchus contortus* larvae is mediated by Cry11Aa. We were able to confirm by cloning and expressed Cry11Aa in *E. coli* and demonstrated its major role in the Bti toxicity for *Haemonchus contortus* larvae [3]. We hypothesize that the integral membrane proteins HC23, H-GAL-GP and H11 present in the microvilli of *Haemonchus* intestinal cells might act as a putative receptor for Cry11Aa toxin. These membrane proteins consist of a family of microsomal aminopeptidases, a complex containing aspartyl and metallo proteases; and it is presumed that is involved in the digestion of the parasite blood meal [5]. Thus, this study aims, through bioinformatics, to predict a putative receptor for Cry11Aa Bti toxin in *Haemonchus contortus* larvae.

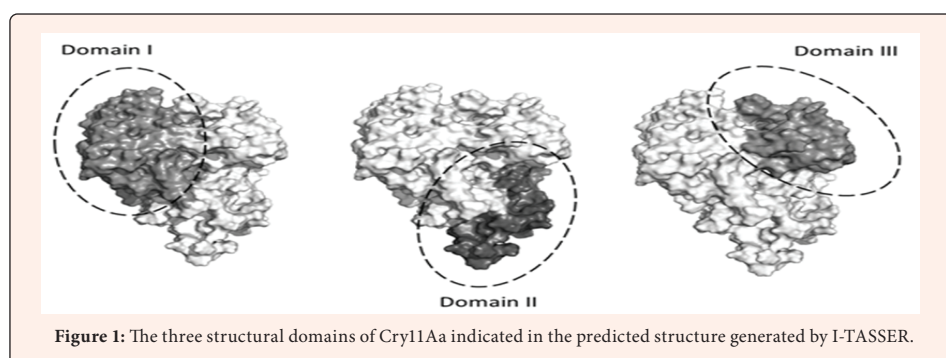
### Materials and Methods

#### Bioinformatics analysis

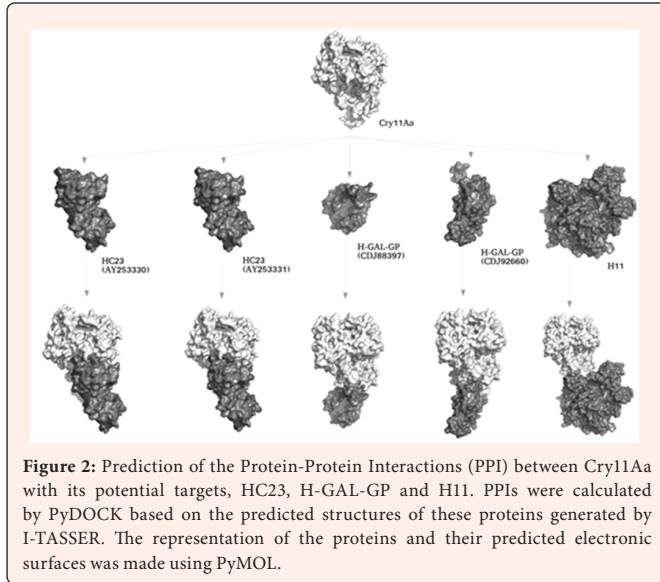
To identify which receptor would be involved in the recognition of the toxin Cry11Aa in *Haemonchus contortus*, the amino acid sequence of Cry11Aa (Uniprot: P21256), H11 (Uniprot: Q10737), HC23 (GenBank: CDJ92660.1, CDJ88397.1) and H-GAL-GP (GenBank: AY253330.1, AY253331.1) were processed by I-TASSER [6] to predict their respective tertiary structures, which were evaluated using the Ramachandran Plot implemented in the Rampage server (<http://mordred.bioc.cam.ac.uk/~rapper/rampage.php>). Based on these structures, the prediction of the Protein-Protein Interaction (PPIs) was performed using PyDock [7,8]. The results were visualized using PyMOL (<https://www.pymol.org/>).

### Results

#### Bioinformatics analysis



The result from the structure prediction made with I-TASSER for Cry11Aa is indicated in Figure 1, with indications of its 3 domains (I, II and III). The modes of binding predicted by PyDOCK between Cry11Aa and its potential receptors, are indicated in Figure 2, and binding energies are summarized in Table 1. For all potential targets (receptors) PyDOCK predicted a high affinity interaction, as demonstrated by the highly negative binding energies, and all receptors docked to the domain II of Cry11Aa.



**Figure 2:** Prediction of the Protein-Protein Interactions (PPI) between Cry11Aa with its potential targets, HC23, H-GAL-GP and H11. PPIs were calculated by PyDOCK based on the predicted structures of these proteins generated by I-TASSER. The representation of the proteins and their predicted electronic surfaces was made using PyMOL.

**Table 1:** Summary of the PyDock analysis results for the interaction of Cry11Aa and its potential targets: HC23, H-GAL-GP and H11. The degree of affinity is measured as values of predicted free energies for the best binding mode, which are illustrated by the electrostatic free energy, desolvation free energy, Van der Waals free energy and an overall score. Energies are measured in kilocalories per mol (kcal/mol).

Target	Electrostatic	Desolvation	Van der Waals	Total
HC23 (GenBank: AY253330)	-22.816	-34.009	-3.920	-57.217
HC23 (GenBank: AY253331)	-12.577	-49.680	90.662	-53.191
H-GAL-GP (GenBank: CDJ88397)	-5.954	-62.083	18.530	-66.184
H-GAL-GP (GenBank: CDJ92660)	-9.097	-72.478	87.580	-72.817
H11 (Uniprot: Q10737)	-37.764	-39.266	86.627	-68.367

## Discussion

In this study we have demonstrated, using the Cry11Aa predicted structure, that the possible putative receptors for it are: the aminopeptidase H11, an intestinal membrane glycoprotein; the native somatic protein HC23; and H-GAL-GP containing glycoprotein complex (Figure 1 and 2). The protein structure prediction is still a hard task and is always limited by the availability of experimental data in public databases and proteins that share sequence similarity/homology (homology modeling) or structural similarity (threading modeling) with already-solved structures, which may be used as templates by the modeling algorithms [9]. However, the protein-protein interaction interface between Cry11Aa and HC23, H-GAL-GP and H11, predicted by PyDOCK, attributed a total energy (score) of -57.217, -72.817 and -68.367 Kcal/mol respectively, for the interaction of Cry11Aa with these three proteins, thus suggesting that this interaction might occur in the larvae midgut cells (Table 1). The best characterized gut membrane proteins, or protein complexes, are known as H11 and H-gal-GP. The protein known as H11 is an integral membrane glycoprotein derived from the intestinal microvilli of the parasite. Based on its amino acid sequence and enzyme assays, H11 has been shown to be a microsomal aminopeptidase [10]. The fraction H-gal-GP has

been termed *Haemonchus* galactose-containing Glycoprotein (H-gal-GP) complex since it binds selectively to lectins with a specificity for N-acetylgalactosamine. The proteins, respectively, consist of a microsomal aminopeptidase's family, and a complex containing protective aspartyl and metallo proteases, and it is presumed that all three protease families are involved in the digestion of the blood meal [5]. The HC23 protein is a galectin present in adult worms of *Haemonchus contortus*, and its recombinant form showed that hemagglutinated human A, B, O type, dog, rabbit, chicken and mouse erythrocytes, but did not hemagglutinate erythrocytes of the natural host (sheep) of *Haemonchus contortus*. For the Cry11Aa protoxin, proteolytic activation involves amino-terminal processing and intramolecular cleavage, leading to two fragments of 36 and 32 kDa that remain associated and retain insect toxicity [11]. One of the main characteristics of Cry toxins is its specificity, which suggests it is mainly mediated by the specific binding to a surface receptor localized in the host midgut cells. The main receptors for the Cry toxin are cadherin-like proteins, Glycosylphosphatidylinositol (GPI)-anchored Aminopeptidase-N (APN), GPI-anchored Alkaline Phosphatase (ALP) and an  $\alpha$ -amilase [2]. In addition, glycolipids were proposed to act as Cry toxin receptors, as was demonstrated for the nematode *Caenorhabditis elegans* [12]. The cadherin proteins have been identified as receptor for Cry11Aa in *Aedes aegypti* and *Anopheles gambiae* [13], and the GPI anchored proteins (aminopeptidase-N and alkaline phosphatase) have been reported to act as a Cry11Aa receptors [14].

Another interesting attribute of Cry11Aa toxins activity is the synergistic effect with Cyt1Aa toxin. Cry11Aa can binds to Cyt1Aa through domain II loop regions, which are involved in receptor interaction [15], and this binding facilitates the formation of an oligomeric structure, which plays a role in cell pore formation, suggesting that Cyt1Aa can have a role similar of the cadherin regarding oligomer formation [16]. The Cry11Aa toxin is a homolog of other Cry proteins toxic to different orders of insects, and probably requires at least one specific membrane receptor to bind to microvilli and cause toxicity through formation of transmembrane cationic pores [1]. For the Cry11Aa toxic activity to occur, an interaction with a receptor needs to happen, so, the three putative protein receptors HC23, H-GAL-GP and H11 here identified suggesting that this interaction might occur in the *Haemonchus contortus* larvae midgut cells [17-25].

## Conclusion

Briefly, our findings demonstrate the promising potential of HC23, H-GAL-GP and H11 proteins as putative receptors for Cry11Aa in *Haemonchus contortus* larvae. This finding may pave the way to develop new tools for the *Haemonchus contortus* control.

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## Author Contributions

A.P.S.S.L. and F.P.L.L. conceived and designed the experiments; F.S.K. and L.S.P. bioinformatic analysis; N.L.C and F.P.L.L. wrote the paper.

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