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In-silico Determination of Insecticidal Potential in Lepidopteron Specific Crystal Toxins with Midgut Alkaline Phosphatase Using Molecular Docking

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***Abstract*:** Alkaline phosphatase (ALP) enzyme plays an important role in binding of lepidopteran specific insecticidal crystal toxins Cry1Ac and Cry1Ab on GPI-anchored membrane receptor. In most of the cases crystal toxin interaction with ALP or Aminopeptidase N (APN) mediated by the terminal N-acetyl galactosamine (GalNAc) moiety. Among toxins Cry1Ac (PDB Id-4ARY) and Cry1Ab (PDB Id - n642) expresses less binding affinity about (-5.79 and -5.62) to GalNAc in ALP or APN region, respectively. The diptera specific crystal toxins Cry4Ba structure resembles to the lepidopteran specific toxins as it mimics like Cry1Aa for binding to ALP receptor by showing least binding affinity about (-6.99). Cry4Ba may be repurposed along with the novel *Bt* crystal toxins for lepidopteron insects as toxicity over Cry1Ac or Cry1Ab which will effective to bind with ALP or APN for toxin receptor interaction and help to minimize the rate of resistance. The Cry4Ba pyramided in combination with other Cry genes in different crops like cotton and maize would be an efficient strategy to increase crop protection by delaying the lepidopteran insect resistance.

Keywords: Lepidoptera, Alkaline phosphatase, Receptor crystal toxin, Resistance

Bacillus thuringiensis (*Bt*) a gram positive bacterium member of the *Bacillaceae* family was firstly identified and isolated in Japan by Ishiwata in 1902 who considered this microbe responsible for *Bombyx mori* infection in silkworm, followed by the study of Berliner on moth *Ephestia kuehniella* larvae evaluated as cry δ-endotoxins capable of killing insects produced by *Bt* (Melo et al 2016, Zhang et al 2017). There are several strains of *Bacillus thuringiensis (Bt)* which known as biological control agent to produce crystalline proteins by sporulation during their stationary phase of growth, which are demonstrated as lethal to lepidopeterous, coleopeterous and dipterous insects due to their specificity and toxicity toward certain insect orders (Jisha et al 2013, Lucena et al 2014). Cry toxins can cause death by two mechanism by inactive protoxin complex of (Cry alone or Cry and Cyt toxins together) with high molecular mass, which is cleaved upon ingestion into the active component proteins known as inactive protoxin crystals which were solubilized by the high alkaline environment in the digestive tract in the insect midgut with their ability to induce both pore formation (sequential binding model) and ion channel activation (signalling pathway model) lead to insect death (Bravo et al 2007, Zhang et al 2017). The another mechanism of crystal toxin like Adenylyl cyclase belongs to signalling pathway in which cadherin receptors used to report toxicity in insects by stimulation of protein kinase A by involving "G protein" activation via upregulation of cAMP levels leads to cytological changes such as cell swelling and lysis in insects (Zhang et al 2006, Bravo et al 2007, Fernandez-Chapa et al 2019). For different crystal toxins there were major receptors such as Alkaline phosphatase (ALP), Midgut membrane bound cadherin-like protein, Aminopeptidase N (APN) as binding sites in different lepidopteran insects (Du et al 1994, Zhao et al 2017, Wang et al 2019). After binding of crystal structures on such membrane receptors, oligomer formation and pre-pore complex takes place which is more flexible than its monomeric form and will be stabilized by the alkaline pH in the midgut of lepidopteran and dipteran larva (Rausell et al 2004, Parker and Feil 2005). Alteration or mutation in any one of the mechanisms such as solubilisation of crystal toxin in protoxin to active form, binding of crystal toxins to ALP, APN or Cadherin (CAD) receptors, pre-pore oligomeric structure leads to intoxication and development of resistance (Herrero et al 2001, Bravo et al 2007). To reverse the intoxication mechanism and resistance potential in insects, second generation transgenic cotton Cry2Ab alone or in combination with Cry1Ac has been developed (Tabashnik et al 2002). *B.*

thuringiensis endotoxins that are currently utilized in commercial transgenic insect resistant crops also pyramiding of multiple *B. thuringiensis* genes that encode different insecticidal proteins with several modes of action has greatly increased to control of major pest species. These toxin oligomers binds with high affinity to APN and ALP which are GPI anchored receptor located in specific membrane micro domain called lipid-rafts, leading to the membrane insertion by forming ion leakage pores that causes osmotic lysis which results in extensive damage to the midgut epithelial cells and eventual larval death (Ning et al 2010, Sengupta et al 2013, Song et al 2015, Tay et al 2015, Wei et al 2019). The first generation of transgenic *Bt* cotton, Cry1Ac shown to be highly effective on susceptible strain of pink bollworm (PBW) (Ojha et al 2014). Reduced Cry1Ac binding activity of ALP receptor on PBW midgut membrane seen to be resistant to ligand Cry1Ac (Ojha et al 2014). The development of insect resistance known as complex phenomenon involving several mechanisms which operates simultaneously within lepidopteron insect strain (Welling et al 1976, Jurat-Fuentes et al 2011).

The crystal toxin receptors characterized as a glycosylated protein implying that carbohydrate residue GalNAc in active catalytic site of ALP which plays an important role in toxin receptor interaction and subsequent cry toxin specificity (Boonserm et al 2005, Bravo et al 2007, Sengupta et al 2013). Multiple structure alignment of Cry4Ba belongs to Lepidoptera and Diptera selective classes structurally similar to Cry1Aa and Cry2Aa than the Coleoptera specific Cry3Aa (Boonserm et al 2005). Cry4Ba which was isolated from *Bacillus thuringiensis* subspecies *israelensis* have toxic action against the larvae of *Aedes* and *Anopheles* mosquitoes. The *Aedes aegypti* mosquito belongs to diptera order have membrane bound ALP functions as a Cry4Ba receptor, responsible for mediating Cry4Ba toxicity while GPI-APN isoforms showed a dramatic increase of resistance in *A. aegypti* mosquito larvae (Dechklar et al 2011, Saengwiman et al 2011). So, the main objective of our study is to investigate the insect resistance molecular mechanism of midgut enzyme ALP and its interactions with various insecticidal crystal toxins via *In-silico* Molecular Docking analysis. It is a computational method of studying binding interactions in terms of binding energies is immensely used in the process of drug discovery which shows position, orientation and conformation of ligand in the active site of protein. This study is performed to analyse the most suitable crystal toxins such as Cry1Ac, Cry2Ab and Cry4Ba which shows binding affinity towards GalNAc present in ALP binding pocket in a lepidopteran larval midgut and mediate the toxin-receptor interaction.

MATERIAL AND METHODS

To perform molecular docking studies computer generated representation of the ligand GalNAc retrieved from PubChem were used to study their binding affinity with different lepidopteran specific *Bt* Crystal toxins (Cry1Ac, Cry1Ab and Cry4Ba). The main objective of molecular docking study is to finding out conformation with their least binding energies.

Protein Structure: The lepidopteran specific biological macromolecular protein structures Cry1Ac (PDB Id- 4ARX), Cry1Ac (PDB Id-4ARY), Cry1Ac (PDB Id-4W8J), Cry4Ba (PDB Id-1w99) were retrieved from RCSB databank which contains experimentally-determined structures of proteins. Ligplots were obtained from PDBSum-Generate database which utilizes 4 characters, to get the ligands with their ligplots. Crystal toxins were taken for study having resolution above 2.0 Å.

Homology modelling: Since Cry2Ab structure was not available in RCSB-PDB; FASTA sequence was retrieved from UniProtKB (P21254) (CR2AB_BACTK) and it has been utilised for homology modelling. Further due to unavailability of ligands in its homologous 3D model, Cry1Ab similar to Cry1Ac was considered for molecular docking analysis (Lee et al 1995, Karim et al 2000, Iva´n Arenas et al 2009). Homology modelling of insecticidal and pesticidal crystal protein Cry1Ab for *Bacillus thuringenesis* sub species *kurstaki* (Lepidopteran specific) (UniprotKB-POA370) (CR1AB_BACTK) was envisaged. Then we got the Swiss model of Cry1Ab (PDB Id: n642) (sequence identity-88.1 %) for further molecular docking procedures (Fig. 1). The ligplots were used for studying interacting sites of the protein of interest and ALP present in the midgut of PBW with accordance to GalNAc (ligand) receptor for sugar binding moiety of crystal toxins in the active site of the enzyme.

Molecular docking: The AutoDock 4 suite (*ver.* 1.5 6rC2) was used to perform the docking of GalNAc into the carbohydrate binding site of various crystal toxins like Cry1Ac, Cry2Ab and Cry4Ba. The results were further analysed by a statistical scoring function which converts interacting energies into numerical values called docking score (Moon et al 2018). Auto Dock 4 consists of two main programs: Auto Dock which perform docking of the ligand to a set of grids that will describes rigid target protein and Auto Grid. Graphical user interface of Auto Dock, Auto Dock Tools (ADT) built on Python Molecular Viewer was used for docking studies. Proteins and ligand were cleaned in Discovery Studio 3.1 Visualizer and Marwin view. Hydrogen atoms were added to the modelled structure and converted into PDBQT format by AutoDock. A 3D grid box of size (60 x 60 x 62) was defined, with a grid space that covered the above-mentioned

residue. The standard docking protocol was performed using Lamarckian genetic algorithm by keeping receptor as rigid and ligand as flexible.

RESULTS AND DISCUSSION

Structural and functional analyses of midgut ALP and APN have major role which binds to Cry1Ac and Cry2Ab receptor with high affinity in susceptible insects of *Plutella xylostella*, *Pectinophora Gossypiella*, *Helicoverpa Armigera* and *Helicoverpa Zea* in monomeric form and then bound to cadherin in midgut converts it into oligomeric form. *In-silico* molecular docking analysis of various lepidopteron specific crystal toxins with target receptor ALP in which GalNAc existing on the active site shows various interacting sites with their docking score (Fig. 2, Fig. 3). In case of Cry2Ab and Cry1Ac resistant insects toxin binding activities were altered by their binding receptors (Tabashnik et al 2002, Iva´n Arenas et al 2009, Caccia et al 2012, Malthankar and Gujar 2014, Chen et al 2015, Wei et al 2019, Xiao and Wu 2019). *In vitro* interaction studies of GalNAc states that it is binding determinant in the oligomeric structure of Cry1Ac affiliated to APN (Aminopeptidase N) which induced a conformational change in the toxin and enhanced its insertion into lipid membranes (Pardo et al 2006). Mutation in ABCC genes or

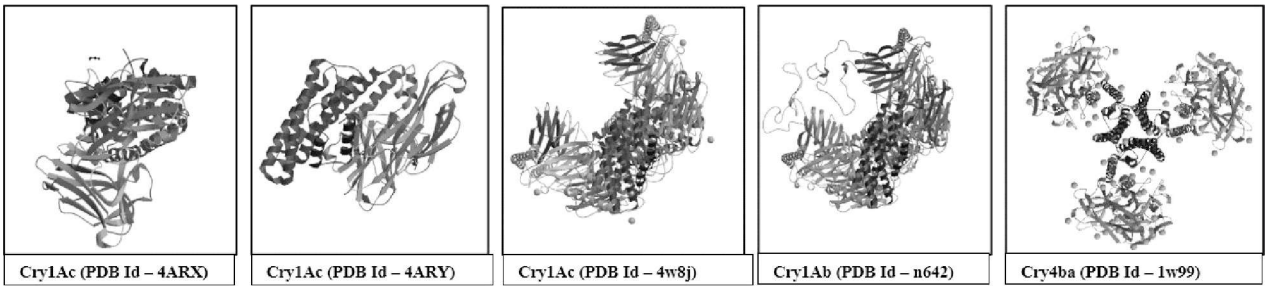

Fig. 1. PDB structure of different lepidopteran specific crystal toxins

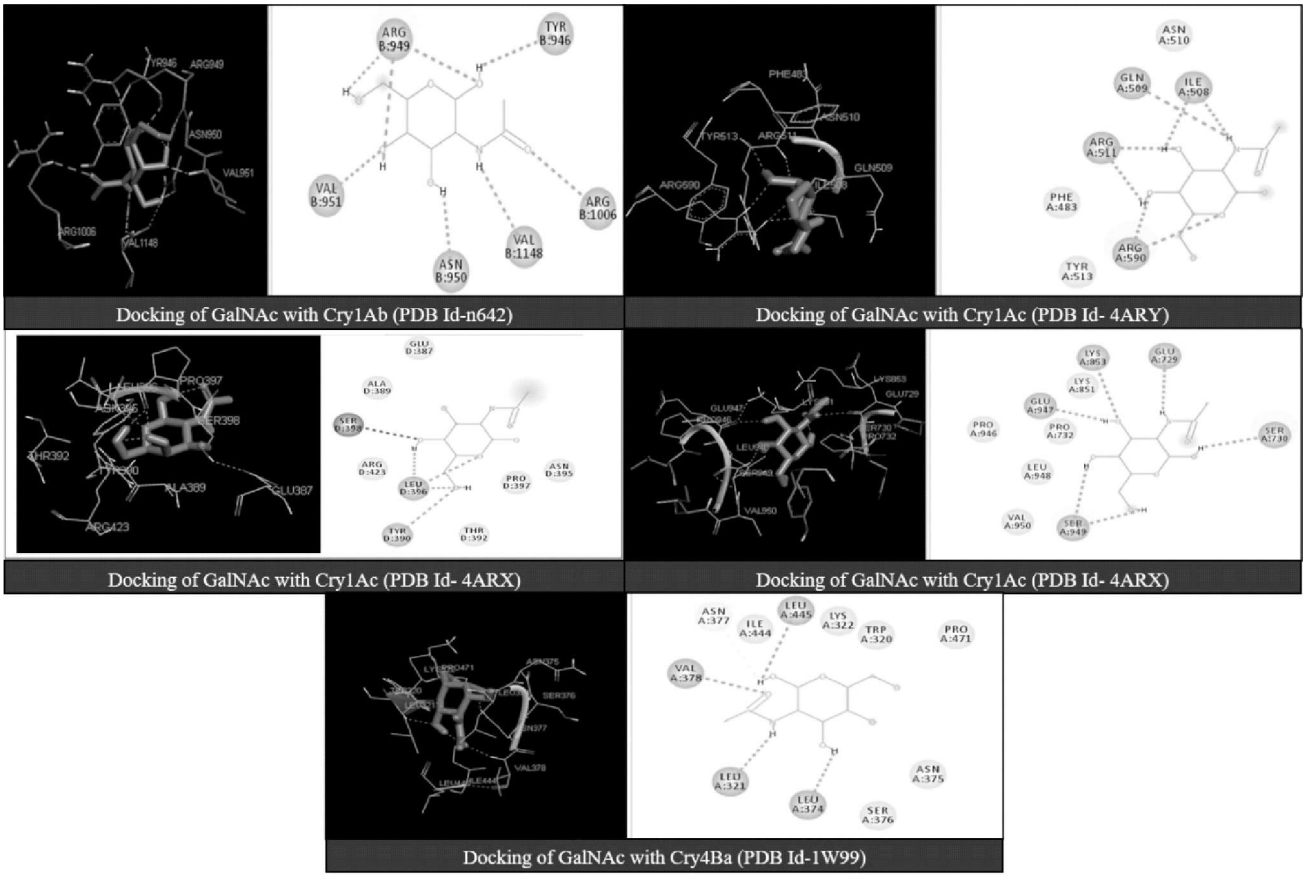

Fig. 2. 3D and 2D images of molecular docking of GalNAc with various crystal toxins

alteration in MAPK signalling pathway also resists the crystal toxin binding to midgut ALP leads for resistance for Cry1Ac toxin in Diamond back Moth (Guo et al 2015).

In-silico data analysis, diptera specific crystal toxin Cry4Ba holds a good docking score with GalNAc in ALP as it shows structural similarity with lepidopteran specific crystal toxins, than performed assay with crystal toxin Cry1Ac and Cry1Ab (Boonserm et al 2005). Cry4Ba holds a lowest docking score (-6.99) which states that it possesses higher affinity towards ALP GalNAc receptor which shows 5 interacting sites i.e. LEU A 374, VAL A 378, LEU A 445, LEU A 321, ASN A 377. While Cry1Ac (4ARY) and Cry1Ab (n642) also shows good toxin receptor interaction. Cry1Ac (PDB Id-4ARY) and Cry1Ab (PDB Id - n642) shows lowest binding docking score -5.79 and -5.62 towards GalNAc receptor respectively so both can show a good toxin receptor interaction with ALP in the midgut of lepidopteran insects. In response to all crystal toxins, Cry1Ab shows maximum 6 interactive sites i.e. ARG B 1006, VAL B 1148, ASN B 950, VAL B 951, TRYP B 946 and ARG B 949 with different amino acids (Table 1). None of the similar interaction was found in crystal toxins when compared to their ligplots so it may be concluded that, toxin receptor interaction not occurred on ligplot interacting sites but occurred via distinct amino acids. The interpretation drawn from the *In-silico* data analyses, that GalNAc moiety on the receptor ALP is recognised by the domain III of crystal toxins at its monomeric form. Then the oligomer formation of crystal toxins takes place when it gets interacted to other midgut receptors like CAD (Cadherins) and facilitates the oligomer formation and pore formation will

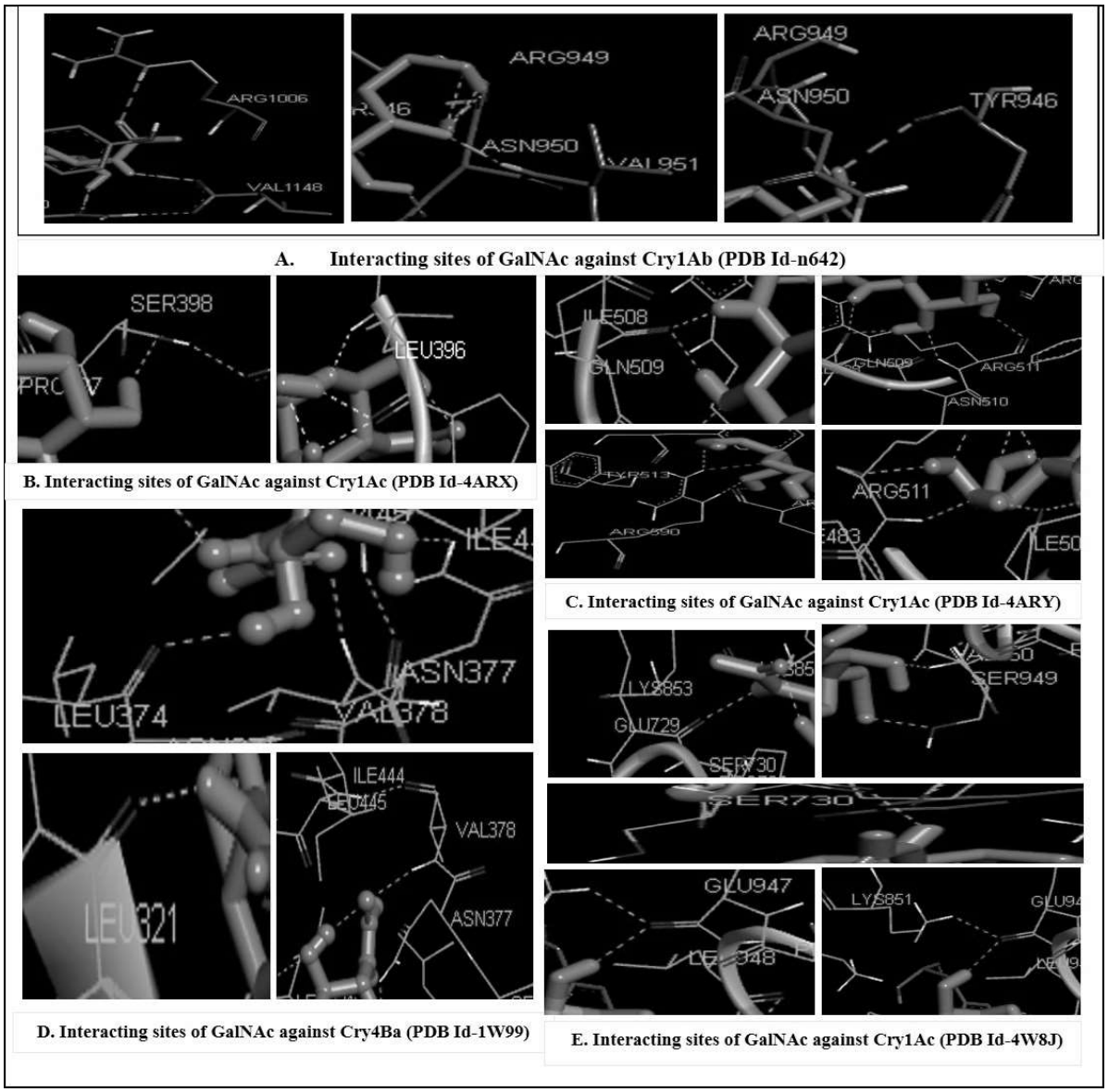

Fig. 3. Interacting sites of ligand GalNAc with various crystal toxins

Table 1. Docking score of crystal toxins for ALP receptor expressing GalNAc in their active site

Crystal toxins	N – Acetylgalactosamine (GalNAc)	
	Docking score	Interacting sites
Cry1Ac (PDB Id-4ARX)	-4.36	LEU A 396, TYR A 390, SER D 398
Cry1Ac (PDB Id-4ARY)	-5.79	ILE A 508, ARG A 511, ARG A 590, GLN A 509
Cry1Ac (PDB Id-4W8J)	-5.65	LYS A 851, GLU A 947, GLU A 729, SER A 730, SER A 949
Cry1Ab (PDB Id-n642)	-5.62	ARG B 1006, VAL B 1148, ASN B 950, VAL B 951, TRYP B 946, ARG B 949
Cry4Ba (PDB Id-1w99)	-6.99	LEU A 374, VAL A 378, LEU A 445, LEU A 321, ASN A 377

lead to larval death. But with a continuous exposure to crystal toxins, lepidopteran insects get resistant to them by altering mutations in their receptor binding site in the midgut ultimately leads to low cross affinity for binding of ALP, APN or cadherin receptors due to absence of midgut site for toxin receptor interaction which will reduce oligomer formation and insertion.

Insects exhibit low genetic diversity having uniform populations with rear mutations due to which alteration in receptor genes mediates resistance to diverse control strategies. Introducing novel *Bt* crystal toxins is a need for increasing susceptibility of insects towards toxins killing mechanisms as lepidopteran insects are now becoming resistant to previous *Bt* crystal toxins (Ahmed et al 2015, Xiao and Wu 2019). Cry toxin mutants are innovative and efficient tools that can be applied to transformed plants for insect control (Lucena et al 2014). CRISPR /Cas9-mediated knockout and host mediated RNAi has a potential avenue for increasing crop resistance by inhibiting egg production of target pests (Xiao and Wu 2019). Fusion of two insecticidal crystal proteins or fusion of crystal proteins with vegetative insecticidal protein like Vip3Aa with Cry1Ac which will improve the insecticidal activity against various midgut receptors targets (Ahmed et al 2015, Javaid et al 2018). Genetically modified Crystal toxins Cry1AbMod and Cry1AcMod were also effective against a laboratory-selected strain of pink bollworm resistant to Cry2Ab as well as to Cry1Ab and Cry1Ac (Tabashnik et al 2013). According to *In-silico* findings, Cry4Ba may be repurposed for lepidopteran insect toxicity over Cry1Ac or Cry2Ab, along with the novel *Bt* crystal toxins is a need for increasing susceptibility of insects towards toxins killing mechanisms as lepidopteran insects are now becoming resistant to previous *Bt* crystal toxins. It will be effective in binding of toxin receptor interaction and help to minimize the rate of resistance.

CONCLUSION

In-silico molecular docking analysis of various lepidopteran specific crystal toxins with GalNAc in their active site of midgut receptor ALP addresses its important role in toxin receptor interaction. Dipteran specific crystal toxin Cry4Ba having similarity with lepidoptera specific crystal toxins holds a good docking score of -6.99 with GalNAc ligand. As Lepidoptera already acquires resistance to various known crystal toxins like Cry1Ac and Cry2Ab via altering their receptors so pyramiding of transgenic crops with Cry4Ba might prolong resistivity. This study states that, along with Cry1Ac and Cry1Ab, Cry4Ba may be repurposed for lepidopteran which will facilitate for more toxin receptor interaction between crystal toxins and midgut receptors like ALP or APN on binding determinant GalNAc. It will lower the resistivity of lepidopteran insects to crystal toxins which will lead to larval death Replace with The resistivity of lepidopteran larvae towards thr cry toxinn will be lower leading to the larval mortality.

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REFERENCES

Ahmad A, Javed MR, Rao AQ, Khan MAU, Ahad A, Din SU, Shahid AA and Husnain T 2015. In-Silico determination of insecticidal potential of Vip3Aa-Cry1Ac fusion protein against lepidopteran targets using molecular docking. *Frontiers of Plant Science* **6**: 1081.

Boonserm P, Davis P, Ellar DJ and Li J 2005. Crystal structure of the mosquito-larvicidal toxin Cry4Ba and its biological implications. *Journal of Molecular Biology* **348**: 363-382.

Bravo A, Gill SS and Soberón M 2007. Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. *Toxicon* **49**: 423-433.

Caccia S, Moar WJ, Chandrashekhar J, Oppert C, Anil Kumar KJ, Jurat-Fuentes JL and Ferré J 2012. Association of Cry1Ac toxin resistance in *Helicoverpa zea* (Boddie) with increased alkaline phosphatase levels in the midgut lumen. *Applied and Environmental Microbiology* **78**: 5690-5698.

Chen W, Liu C, Xiao Y, Zhang D, Zhang Y, Li X, Tabashnik BE and Wu K 2015. A toxin-binding alkaline phosphatase fragment synergizes *Bt* toxin Cry1Ac against susceptible and resistant *Helicoverpa armigera*. *PLoS One* **10**: 4-8.

Dechklar M, Tiewsiri K, Angsuthanasombat C and Pootanakit K 2011. Functional expression in insect cells of glycosyl phosphatidylinositol-linked alkaline phosphatase from *Aedes aegypti* larval midgut: A *Bacillus thuringiensis* Cry4Ba toxin receptor. *Insect Biochemistry and Insect Molecular Biology* **41**: 159-166.

Du C, Martin PA and Nickerson W 1994. Comparison of disulfide contents and solubility at alkaline PH of insecticidal and non-insecticidal *Bacillus thuringiensis* protein crystals. *Applied and Environmental Microbiology* **60**: 3847-3853.

Fernández-Chapa D, Ramírez-Villalobos J and Galán-Wong L 2019. Toxic potential of *Bacillus thuringiensis*: An overview. In: *Protecting Rice Grains in the Post-Genomic Era*. IntechOpen.

Guo Z, Kang S, Chen D, Wu Q, Wang S, Xie W, Zhu X, Baxter SW, Zhou X, Jurat-Fuentes JL and Zhang Y 2015. MAPK signalling pathway alters expression of midgut ALP and ABCC genes and causes resistance to *Bacillus thuringiensis* Cry1Ac toxin in diamond back moth. *PLoS Genetics* **11**: 4-8.

Herrero S, Oppert B and Ferré J 2001. Different Mechanisms of resistance to *Bacillus thuringiensis* toxins in the Indian meal moth. *Applied and Environmental Microbiology* **67:** 1085-1089.

Iva´n Arenas, Alejandra Bravo, Mario Sobero N and Isabel Go´Mez1 2009. Role of alkaline phosphatase from *Manduca sexta* in the mechanism of action of *Bacillus thuringiensis* Cry1Ab toxin. *Journal of Biological Chemistry* **285**: 12497-12503.

Javaid S, Naz S, Amin I, Jander G, Ul-Haq Z and Mansoor S 2018. Computational and biological characterization of fusion proteins of two insecticidal proteins for control of insect pests. *Scientific Reports* **8**: 4835-4837.

Jisha VN, Smitha RB and Benjamin S 2013. An overview on the crystal toxins from *Bacillus thuringiensis*. *Advances in Applied Microbiology* **3**: 462.

Jurat-Fuente JL, Lohitash K, Siva Rama Krishna Jakka, Changming N, Chenxi Liu, Kongming Wu and Jerreme J 2011. Reduced levels of membrane-bound alkaline phosphatase are common to lepidopteran strains resistant to Cry toxins from *Bacillus thuringiensis*. *PLoS One* **6**: E-17606e17606.

Karim S, Riazuddin S, Gould F and Dean DH 2000. Determination of receptor binding properties of *Bacillus thuringiensis* deltaendotoxins to cotton bollworm (*Helicoverpa zea*) and pink bollworm (*Pectinophora gossypiella*) midgut brush border membrane vesicles. *Pesticide Biochemistry and Physiology* **67**: 198-216.

Lee MK, Rajamohan F, Gould F and Dean DH 1995. Resistance to *Bacillus thuringiensis* CrylA delta-endotoxins in a laboratory-selected *Heliothis virescens* strain is related to receptor alteration. *Applied and Environmental Microbiology* **61**: 3836-3842.

Lucena WA, Pelegrini PB, Martins-de-Sa D, Fonseca FC, Gomes JE, De Macedo LL and Grossi-de-Sa MF 2014. Molecular approaches to improve the insecticidal activity of *Bacillus thuringiensis* Cry toxins. *Toxins* **6:** 2393-2423.

Malthankar PA and Gujar GT 2015. Association of Cry2Ab resistance with alkaline phosphatase in pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae). *Biopesticides International* **10**: 143-151.

Melo A, Soccol VT and Soccol CR 2016. *Bacillus thuringiensis*: mechanism of action, resistance, and new applications: a review. *Critical Reviews in Biotechnology* **36**: 317-326.

Moon A, Khan D, Gajbhiye P, Jariya M and Taksande A 2018. Molecular and in silico studies on dhfr of uropathogenic *Klebsiella Pneumoniae*. *World Journal of Pharmacy and Pharmaceutical Sciences* **1**: 900-913.

Ning C, Wu K, Liu C, Gao Y, Jurat-Fuentes JL and Gao X 2010. Characterization of a Cry1Ac toxin-binding alkaline phosphatase in the midgut from *Helicoverpa armigera* (Hübner) larvae. *Journal of Insect Physiology* **56**: 666-672.

Ojha A, Sree KS, Sachdev B, Rashmi MA, Ravi KC, Suresh PJ and Bhatnagar RK 2014. Analysis of resistance to Cry1Ac in field-collected pink bollworm, *Pectinophora gossypiella* (Lepidoptera: Gelechiidae), populations. *Genetically modified Crops and Food* **5**: 280-286.

Oppenoorth FJ and Welling W 1976. Biochemistry and physiology of resistance, in "*Insecticide Biochemistry and Physiology*" (CF Wilkinson, Ed.).

Pardo-López L, Gómez I, Rausell C, Sánchez J, Soberón M and Bravo A 2006. A structural change of the Cry1Ac oligomeric pre-pore from *Bacillus thuringiensis* induced by N-acetyl galactosamine facilitates toxin membrane insertion. *Biochemistry* **45**: 10329-10336.

Parker MW and Feil SC 2005. Pore-forming protein toxins: from structure to function. *Progress in Biophysics and Molecular Biology* **88**: 91-142.

Rausell C, Pardo-López L, Sánchez J, Muñoz-Garay C, Morera C, Soberón M and Bravo A 2004. Unfolding events in the water-soluble monomeric Cry1Ab toxin during transition to oligomeric pre-pore and membrane-inserted pore channel. *Journal of Biological Chemistry* **279**: 55168-55175.

Saengwiman S, Aroonkesorn A and Dedvisitsakul P et al 2011. In vivo identification of *Bacillus thuringiensis* Cry4Ba toxin receptors by RNA interference knockdown of glycosyl phosphatidylinositol-linked aminopeptidase N transcripts in *Aedes aegypti* larvae. *Biochemical and Biophysical Research Communications* **407:** 708-713.

Sengupta A, Sarkar A, Priya P, Dastidar SG and Das S 2013. New insight to structure-function relationship of GalNAc mediated primary interaction between insecticidal Cry1Ac toxin and HaALP receptor of *Helicoverpa armigera*. *PloS One* **8**: e78249.

Song X, Kain W, Cassidy D and Wang P 2015. Resistance to *Bacillus thuringiensis* toxin Cry2Ab in *Trichoplusia ni* is conferred by a novel genetic mechanism. *Applied and Environmental Microbiology* **81:** 5184-5195.

Tabashnik BE, Dennehy TJ, Sims MA, Larkin K, Head GP, Moar WJ and Carriere Y 2002. Control of resistant pink bollworm (*Pectinophora gossypiella*) by transgenic cotton that produces *Bacillus thuringiensis* toxin Cry2Ab. *Applied and Environmental Microbiology* **68**: 3790-3794.

Tabashnik BE, Fabrick JA, Unnithan GC, Yelich AJ, Masson L, Zhang J and Soberón M 2013. Efficacy of genetically modified Bt toxins alone and in combinations against pink bollworm resistant to Cry1Ac and Cry2Ab. *PloS One* **8**: 80496.

Tay WT, Mahon RJ, Heckel DG, Walsh TK, Downes S, James WJ and Gordon KH 2015. Insect resistance to *Bacillus thuringiensis* toxin Cry2Ab is conferred by mutations in an ABC transporter subfamily A protein. *PLoS Genetics* **11**: e1005534.

Wang L, Ma Y, Guo X, Wan P, Liu K, Cong S and Tabashnik BE 2019. Pink bollworm resistance to Bt toxin Cry1Ac associated with an insertion in cadherin exon 20. *Toxins* **11**: 186.

Wei J, Zhang M, Liang G and Li X 2019. Alkaline phosphatase 2 is a functional receptor of Cry1Ac but not Cry2Ab in *Helicoverpa zea*. *Insect Molecular Biology* **28**: 372-379.

Xiao Y and Wu K 2019. Recent progress on the interaction between insects and *Bacillus thuringiensis* crops. *Philosophical Transactions of the Royal Society B: Biological Sciences* **374**: 1767-1769.

Zhang Q, Hua G and Adang MJ 2017. Effects and mechanisms of *Bacillus thuringiensis* crystal toxins for mosquito larvae. *Insect Science* **24**: 714-729.

Zhang X, Candas M, Griko NB, Taussig R and Bulla LA 2006. A mechanism of cell death involving an adenylyl cyclase/PKA signalling pathway is induced by the Cry1Ab toxin of *Bacillus thuringiensis*. *Proceedings of the National Academy of Sciences* **103**: 9897-9902.

Zhao M, Yuan X, Wei J, Zhang W, Wang B, Khaing MM and Liang G 2017. Functional roles of cadherin, aminopeptidase-N and alkaline phosphatase from *Helicoverpa armigera* (Hübner) in the action mechanism of *Bacillus thuringiensis* Cry2Aa. *Scientific Reports* **7**: 4655.

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