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Analysis of Antigenic Behavior of Bt (*Bacillus thuringiensis*) based CRY Proteins upon its Parenteral Adsorption or Administration by using JambW

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Abstract

Today in the world of plant biotechnology, Bt (*Bacillus thuringiensis*) has become buzz word. Everyone working in this field, wish to develop some biotic resistances in the crops without studying the need of such crops in the society. Except few companies like Monsanto- Mahyco which are involved in the development of Bt based crops, most have focused their research on development of Bt based recombinants without much concern on study of toxicity and antigenecity of Bt products on animal and human health. Reports suggest that not more than 90 days of immunological studies were reported on all Bt based crops developed till date. There are no reports on horizontal gene transfers and their permanent accumulation in mammalian genome. In our study we have focused our efforts on studying the immunological consequences that might arise upon administration or absorption of complete / partially digested or horizontally transferred synthesis of Bt based CRY protein in mammalian circulatory system by using bioinformatics based tools.

In the present study we have selected four major classes of Bt based CRY proteins namely CRY 1 , CRY 3, CRY 4 and CRY 8 which are extensively used in development of Bt based recombinant varieties of various crops like Cotton, Maize, Potato and Brinjal. In the present study through the simple bioinformatics based approach we wish to suggest the cost effective and rapid method for studying immunological aspects of Bt based CRY proteins upon parenteral absorption or administration.

Key words: CRY antigenecity, Bt CRY, GM crops, antigenic index and JambW.

Introduction

Bacillus thuringiensis is an entomopathogen found naturally in the soil, and it is characterized by the production of paraspores inclusion bodies during sporulation, formed by proteins toxic to different insect groups (Hofte and Witheley, 1989). The protein product of *cry* gene is called as CRY (Crystal) protein.

These crystal proteins from the Gram-positive spore forming bacterium *Bacillus thuringiensis* are toxic to a wide variety of insects that are economically important as pests. Over 100 *cry* gene sequences encoding the *B. thuringiensis* endotoxin have been isolated, characterized and classified in 22 groups and different subgroups with regard to their amino acid similarity (Crickmore et al., 1998). The proteins toxic for lepidopteran insects belong to the CRY1, CRY9, and CRY2 groups; toxins active against coleopteran insects are the CRY3, CRY7, and CRY8 proteins as well as the CRY1B and CRY1I proteins, which have dual activity. The CRY5, CRY12, CRY13, and CRY14 proteins are nematocidal, and the CRY2, CRY4, CRY10, CRY11,

CRY16, CRY17, CRY19, and CYT proteins are toxic for diptera insects. The characterizations for most of the *B. thuringiensis* collections were based on bioassays against different insect larvae without identification of the *cry* genes present in the *B. thuringiensis* strains (Nariman, 2007).

Though the Bt based crops show the potential insecticidal activity and now a days are commonly cloned and successfully expressed into various crops like cotton, brinjal, maize etc. Various studies reported till date suggests that there are uncertainties about the effects of CRY toxins on mammals and humans. Very few have been tested for their effects on humans (Tayabali and Seligy, 2000). Some CRY proteins are cytotoxic to human or mouse cells, but surprisingly not to insects (Vazquez et al., 2000 and Ito et al., 2004). Moreover, the toxicity was cell-type specific, meaning that if the wrong kind of cellular tissue culture is used in the assay, toxicity may be underestimated. Some CRY proteins are even being considered for use as new chemotherapy agents due to their ability to kill certain kinds of human cells (Kim et al., 2000 and Akiba et al., 2004). CRY proteins may also stimulate an immune response leading to the need to test them as allergens.

Assessment of the immunotoxicological effects of GMOs (Genetically Modified Organisms) has mainly focused on the allergic potential of genetically modified proteins whereas general immunotoxicological investigations of whole GMOs are not described in those literatures. The given study is focused on study of immunological aspects of parenteral injection or absorption of Bt CRY protein using bioinformatics based approach. Furthermore the same study will also be carried out and proved its relatedness with present study through wet lab experiments.

Material and Methods

The CRY proteins selected in the present study are CRY 1, CRY 3, CRY 4, and CRY 8 with their subgroups, except CRY 4 which is Nematocidal rest all has Insecticidal activity and are commonly used in development of Bt CRY protein based varieties of Cotton, Maize, Brinjal, Potato etc. since long time.

The given work was initiated with the collection of Bt CRY proteins sequences from various databanks like National Centre for Biotechnology Information (www.ncbi.nlm.nih.gov), Protein Data Bank (www.rcsb.org), Protein Information Resource (www.PIR.georgetown.com) etc. in FASTA (Fast Alignment) format. The collected sequences were primarily screened for sequential identity among them by using ClustalW (www.ebi.ac.uk) alignment tool. After confirming that the selected CRY proteins shares no significant sequence homology / similarity all the CRY proteins were screened for their antigenic index individually using JambW (www.bioinformatics.org) software. The software is used for calculation of antigenic index of a short peptide and proteins. Upon giving a sequence of amino acids, this program computes and plots the antigenecity along the polypeptide chain, as predicted by the algorithm of Hopp (Hopp and Woods, 1981). The basic principal used in calculation of antigenic index is the percent amount of aromatic amino acids with ionic R side chains. Higher the amount of these amino acids in the input sequence higher will be the antigenic index. Upon calculating the antigenic index of all the selected CRY proteins as per the instructions given in software the final discussion is made by comparing obtained results with the experimental proof based suggestions given by various workers working on Bt based *cry* genes and CRY protein.

Observations

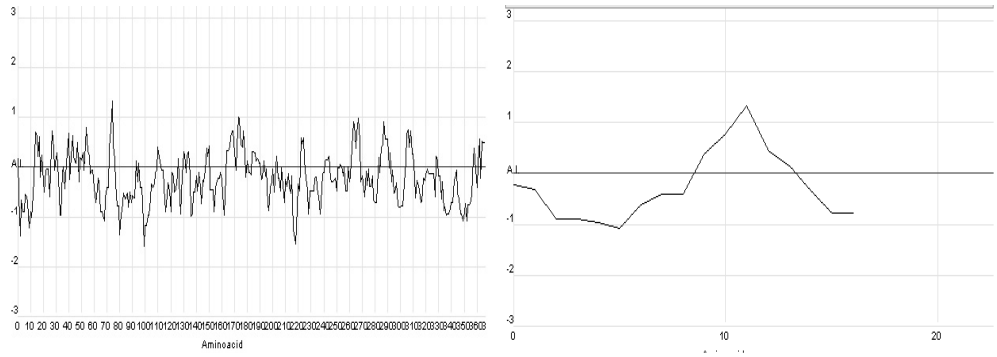

Figure 1: Showing antigenic index of whole CRY 1 Ab protein and its region having significant antigenic index.

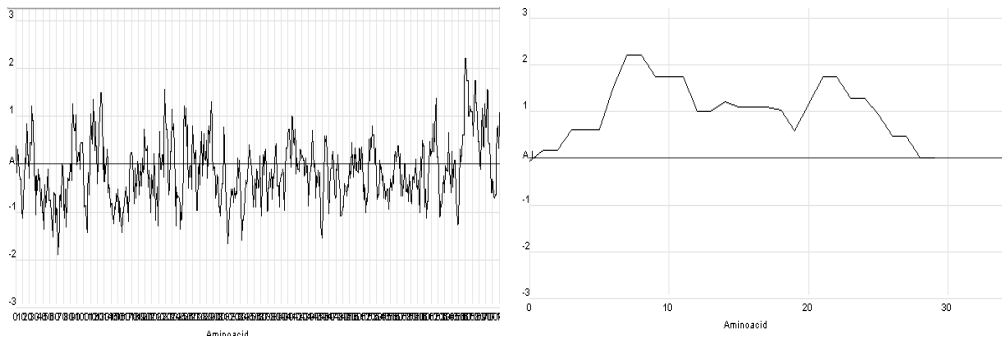

Figure 2: Showing antigenic index of whole CRY 1 Ac protein and its region having significant antigenic index.

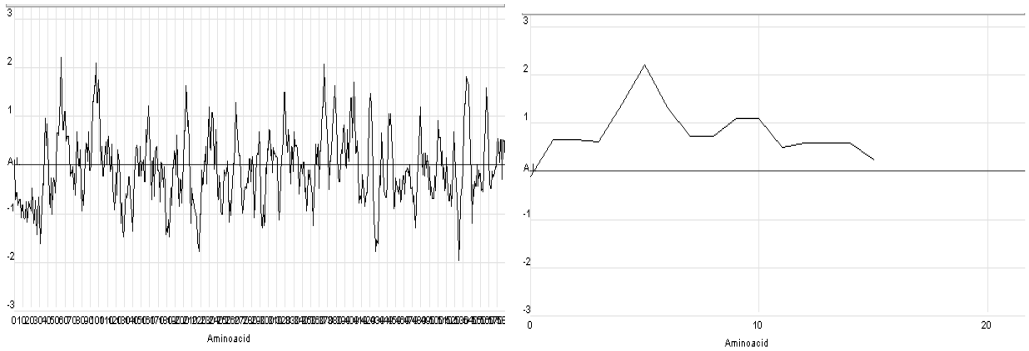

Figure 3: Showing antigenic index of whole CRY 3BB protein and its region having significant antigenic index.

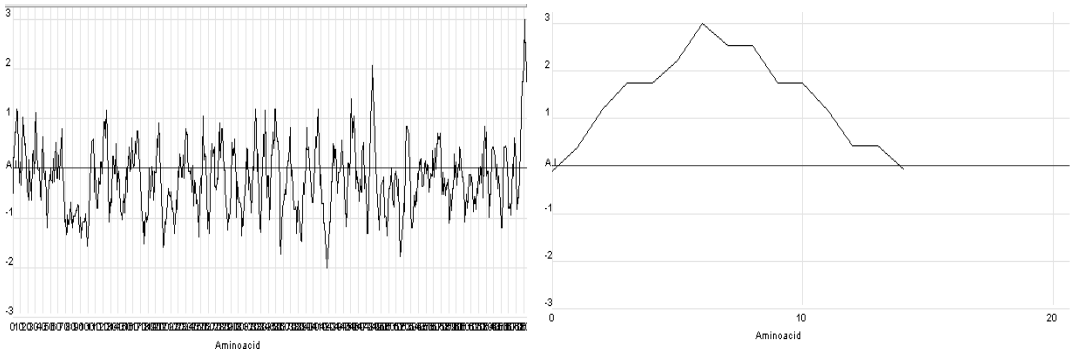

Figure 4: Showing antigenic index of whole CRY 4A protein and its region having significant antigenic index.

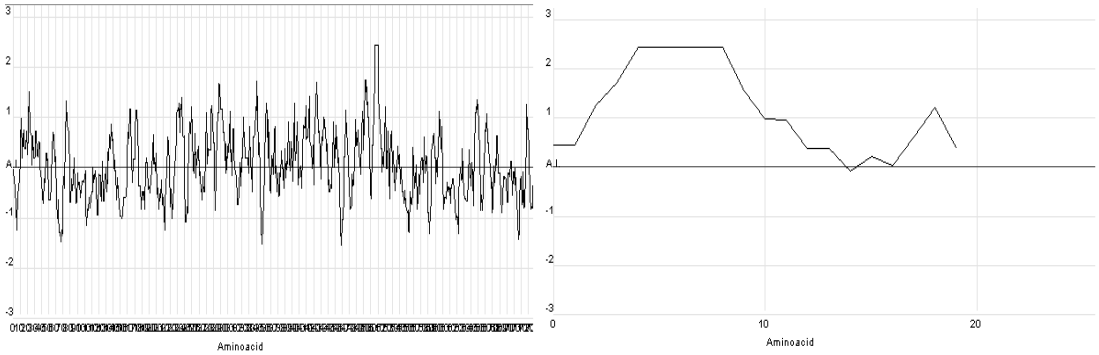

Figure 5: Showing antigenic index of whole CRY 8AAW protein and its region having significant antigenic index.

Results

Out of the various CRY proteins undertaken in given study for calculation of their antigenic index and correlating it with its behavior upon parenteral entry in animal body, four classes of CRY have shown significant antigenic index as shown in observations (fig 1-5). CRY 1 Ab and 1 Ac are commonly used in development of CRY based Bt varieties of cotton and maize, of which CRY 1 Ab shows its antigenic index >1 in comparison with its counterpart CRY 1 Ac with 2 as its higher antigenic

index. The other CRY proteins are CRY 3, CRY 4, CRY 8 with antigenic index 2, 3 and >2 respectively. These CRY proteins are used in development of Nematocidal and Insecticidal resistant Bt crop varieties.

Implementation

If used for studying the antigenic behavior of known foreign proteins, the software gives comparatively accurate information. Further the same protocol can be used in immunodiagnostics and development of monoclonal antibodies for passive immunity.

Discussion

When GM (Genetically Modified) crops and foods were first introduced in the 1990s, scientists raised concerns that genetic modification was imprecise and unpredictable. According to them, GM could create foods that are toxic, allergic and less nutritious than their Non-GM counterparts; they could damage vulnerable wild plant and animal populations and may harm biodiversity**.** Especially Bt transgenes not only risk killing more species of insects than intended, but may also contain previously unknown toxicities for other animals and human beings.

Besides these concerns some group of peoples are continuing their work in development of more and more new classes of GM varieties day after another. One of the genes extensively used for development of GM crop is *cry*. Various reports suggest the *cry* gene is not only harmful to grazing animals but also to humans and has great intension to environmental pollution.

In one of the survey in India it was reported that at least 1820 sheep were reported dead after grazing on post-harvest Bt cotton crops. This was uncovered in a preliminary investigation conducted by civil society organizations in just four villages in the Warangal district of Andhra Pradesh in India (Mae Wan Ho, 2006).

In present study we have demonstrated the effects of CRY protein when absorbed partially or completely into animal circulatory system using simpler, reliable and cost effective bioinformatics based approach by using JambW software.

The genes namely *cry* 1 Ab and 1 Ac are one of those *cry* genes which are widely used in development of Bt based cotton, maize and more recently brinjal **(**Ho. M.W., 2006**)**. Though the companies developing these varieties suggests that these products are environmentally safe and fit for human and animal consumption, studies by Sebesta and Vankova exhibited data that show the thuringiensin is more toxic if given intraperitoneally than orally (Diouneia Lisiane Berlitz, 2006). It was observed that GM DNA can survive processing and is detectable in the digestive tract of sheep. This raises the possibility that antibiotic resistance and Bt insecticide genes can move into gut bacteria, a process known as horizontal gene transfer (J. A. Thomson, 2001). We through our experiment wish to show the same that as CRY 1 Ab and 1 Ac used in brinjal and maize because of their significant antigenic index (Fig 1 and fig 2) upon its penetration / absorption into animal circulatory system either completely or partially digested or fragmented form it will act as a antigen and will induce immunological consequences in animals. Also if these proteins are absorbed into liver there also it may exhibit its toxic effects. The reports resembling this statement was

published in May 2007, by French researchers in their reanalysis of Monsanto data and concluded that there were indications of liver/kidney toxicity in rats fed Bt corn MON863, saying that "with the present data it cannot be concluded that GM corn MON863 is a safe product" (Seralini et al., 2007). Also in November 2008, Italian researchers concluded that "the consumption of Bt MON810 maize induced alterations in intestinal and peripheral immune response of weaning and old mice (Finamore et al., 2008).

Apart from CRY 1 Ab and 1 Ac the new classes of Bt based genes currently used in development of Bt varieties are CRY 3, 4 and 8. In our study we have also analysed the protein product of these genes and found that in comparison to CRY 1 Ab and 1 Ac the CRY products of these genes shows higher antigenic index (Fig 3, 4, 5). Suggesting, to prevent the use of these genes in future development of *cry* gene based Bt varieties.

Few reports also suggests that Bt based GM crops also affecting the environment and its severity is much more as it has been spread worldwide, corn and cotton engineered with the *cry* genes covered 114 million ha in 2007. 1300 metric tons of *Bacillus thuringiensis* were annually produced in fermenters (Anonymous, 1999 and Youngsteadt et al., 2008) and it is contaminating the drinking water. Studies of *Bacillus thuringiensis* loads in drinking water in Japan found on average 0.45 colony forming unit/ml. (Ichimatsu et al., 2000).

Through our simple experiment we wish to make an appeal to the scientific society to lower down the development of newer Bt based crops and society to minimize use of such crops in consumption because they are neither beneficial nor safe for consumption wherever possible.

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