Letter to the editor

Understanding the structure and function of *Bacillus thuringiensis* toxins.

Dear editor,

"If you want to understand function, study structure" (Francis Crick, 1988).

As biological control agents take an expanding share of the pesticides market and the production of insect-resistant crops increases, it is essential to understand the structure and function of the active agents, the invertebrate-active toxins that are the fundamental ingredients of these control systems. The potential for these agents in industry, agriculture and medicine necessitates a thorough investigation of their activity. The entomopathogenic bacterium *Bacillus thuringiensis* (Bt) is an important biological source of insecticidal proteins, with many strains bearing a wide variety of insecticidal genes. Bt delta-endotoxins (Cry and Cyt) (Figure 1) are synthesized during the stationary growth phase as crystalline parasporal inclusions, highly active against a wide range of insects (Schnepf, Crickmore et al. 1998). This bacterium also synthesizes other proteins during vegetative growth that are secreted into the culture medium. These have been designated as vegetative insecticidal proteins (Vips) (Estruch, Warren et al. 1996, Warren, Koziel et al. 1998) and secreted insecticidal protein (Sip) (Donovan, Engleman et al. 2006), and exhibit insecticidal activity against some coleopteran (the two-component Vip1/Vip2 toxin, and Sip) and lepidopteran pests (Vip3) (Estruch, Warren et al. 1996, Warren, Koziel et al. 1998). The insecticidal proteins of Bt are highly specific for their hosts and have gained worldwide importance as environmentally desirable alternatives to chemical insecticides. Bt products have the biggest market share of biological insecticides and are used successfully in crop protection and vector control programmes worldwide. Moreover, Bt strains are also the major source for insect resistance transgenes in transgenic plants. Despite the importance of a wide variety of toxins in the action of this entomopathogenic bacterium, structural information has only been published on a subset of toxin classes: (i) the 3-domain Cry toxins (eg (Li, Carroll et al. 1991)), (ii) the binary Cry34Ab/Cry35Ab toxin ((Kelker, Berry et al. 2014)), (iii) the Cyt toxins (eg (Li, Koni et al. 1996)); (iv) the Vip2Aa protein (an ADP-ribosyl transferase (Han, Craig et al. 1999)) and (v) aerolysin-like structures such as the Cry45 (anticancer parasporin protein), Cry46 (anticancer parasporin protein), and Cry51 insecticidal toxin (Akiba, Higuchi et al. 2006, Akiba, Abe et al. 2009, Xu, Chinte et al. 2015). The 3-domain Cry toxins (Figure 1) are the best-characterized group of insecticidal proteins and are toxic after crystal solubilisation and proteolytic activation by midgut proteases of susceptible insects (Schnepf, Crickmore et al. 1998). Even though different 3-domain Cry toxins display clear differences in their amino acid

sequences and biological activities, the activated toxins all share in common a remarkably similar and conserved 3-domain structure (de Maagd, Bravo et al. 2003, Bravo, Gill et al. 2007). The availability of structures for 3-domain Cry proteins (Figure 1) has opened the field for extensive mutagenesis to retarget toxins (Pigott and Ellar 2007) and to overcome resistance to the most used toxins to date (e.g. Cry1A) (Ferré and Van Rie 2002). The structures of the components for the binary Cry34/Cry35 toxin show similarities to the aegerolysin (Cry34) and aerolysin (Cry35) families of proteins, which are able to interact with cell membranes to form pores and kill coleopterans (Kelker, Berry et al. 2014). Although the roles of the two components in toxicity are not clear, Cry35 may be a beta-pore forming toxin and/or may interact with receptor via its lectin-like domain. The similarity of this protein with the better studied Bin toxins may also help in the elucidation of its activity. Cyt toxins directly interact with saturated membrane lipids and kill by causing cell lysis (Xu, Wang et al. 2014). Even though Cyt toxins are usually considered to be active against mosquitoes and black flies (de Maagd, Bravo et al. 2003), low activity has been reported against *Chironomus* larvae (Hughes, Stevens et al. 2005) and aphids (Porcar, Grenier et al. 2009) and the knowledge of Cyt toxin structure facilitated modification to enhance Cyt2Aa binding and toxicity against hemipteran pests (Chougule, Li et al. 2013). Hemipterans may show a general interaction in this class of toxins since related proteins from the bacterium *Dickeya dadantii* have been shown also to kill pea aphids (Loth, Costechareyre et al. 2015). However, despite the importance of increasing our knowledge of the structure of insecticidal toxins, a significant number of them do not share the 3-domain structure and for many of these, structural information still is not available. Consequently, our ability to carry out similar studies to exploit these toxins is severely limited, thereby inhibiting their development. Amongst the classes of other toxins lacking basic biochemical and structural characterisation are the following, important examples: (i) Vegetative insecticidal proteins Vip1 and Vip3 (Figure 1). Vip1 and Vip2 proteins together constitute a binary toxin and are commonly toxic against coleopteran and homopteran pests (Warren, Koziel et al. 1998). Vip2 exhibits homology with the enzymatic ADP-ribosyltransferase toxin and its structure has been already elucidated (Han, Craig et al. 1999). No structure-function studies have been developed for Vip1, the specificity-determining B component of the toxin. In addition, the mode of action of Vip3 toxins remains unclear and would be significantly enriched by studying the structure-activity relationships for this protein class with increasing interest in its development for use in transgenic plants. Variations in the insecticidal toxicity profiles of natural Vip3 sequences from different Bt strains will provide a background of sequence diversity with which to understand specificity and to map the variant amino acids with the structural data. (ii) Cry6 is a ~ 54-kDa protein exhibiting features of the Smc chromosome segregation protein family (Palma, Muñoz et al. 2014) showing activity against nematodes and coleopterans (van Frankenhuyzen 2013). (iii) Cry22 is active against

coleopteran pests and ants (Payne, Kennedy et al. 1997, Isaac, Krieger et al. 2003). It has regions of homology with cadherins and lectins but again, its structure has not been published. (iv) The small Cry37 protein (~14 kDa) that acts as a member of a two-component toxin that kills coleopterans (Donovan, Donovan et al. 2000). (v) Cry55 is active against coleopteran pests and nematodes (van Frankenhuyzen 2009) and, although some regional similarities to Toxin_10 family proteins are predicted, its overall fold and mechanism of action are unknown. Bringing new toxins to market involves numerous regulatory hurdles and structure function data greatly enhance our ability to address safety and target specificity issues. In addition, a deeper knowledge of structure and mechanism will be crucial in our efforts to avoid insect resistance (for example through understanding toxin-receptor interactions) and to be able to retarget toxins against new pests (as achieved previously with 3-domain toxins and dipteran active Cyt2Aa toxin). Therefore, a comprehensive understanding of the modes of action along with the understanding of structure will revolutionise our ability to exploit these proteins by providing new paradigms for the action of insect toxins and will assist the agri-business sector in their attempts to exploit new toxin types.

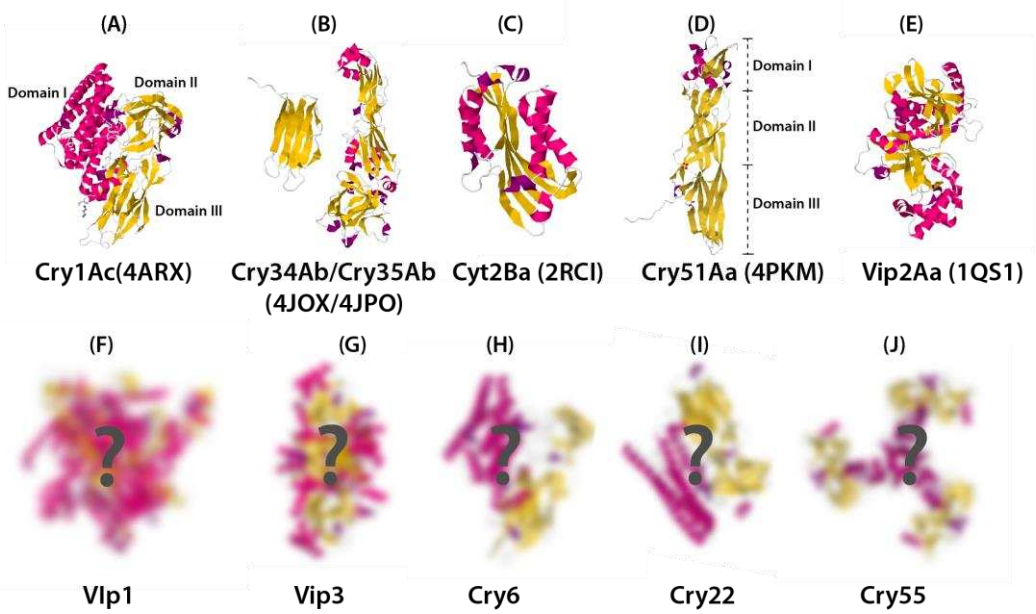

Figure 1. Bt toxin structures

Known three-dimensional structures of insecticidal toxins from Bt: (A) Three-domain Cry toxin Cry1Ac, Domain I (in pink) is the pore-forming domain whereas domains II and III (in yellow) have roles in toxin-receptor interactions. (B) Binary Cry34Ab/Cry35Ab toxin. (C) Cyt2Ba toxin (monomer). (D) Cry51 toxin (monomer) exhibits an aerolysin-like architecture that can be considered as 3-domains. (E) Vip2Aa protein from *Bacillus cereus*. Unknown toxin structures for insecticidal proteins of interest are represented by defocussed structural images: Vip1 (F) and Vip3 (G) and for insecticidal (crystal) toxins Cry6 (H), Cry22 (I) and Cry55 (J). Codes in parenthesis correspond to Protein Data Bank accession numbers.

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