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Letter to the Editor

Understanding the structure and function of *Bacillus thuringiensis* toxins

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A B S T R A C T

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.

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) (Fig. 1) are synthesized during the stationary growth phase as crystalline parasporal inclusions, highly active against a wide range of insects (Schnepf 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 et al., 1996; Warren et al., 1998) and secreted insecticidal protein (Sip) (Donovan et al., 2006), and exhibit insecticidal activity against some coleopteran (the two-component Vip1/Vip2 toxin, and Sip) and lepidopteran pests (Vip3) (Estruch et al., 1996; Warren 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 (Li et al., 1991), (ii) the binary Cry34Ab/Cry35Ab toxin (Kelker et al., 2014), (iii) the Cyt toxins (Li et al., 1996); (iv) the Vip2Aa protein (an ADP-ribosyltransferase) (Han et al., 1999b) and (v) aerolysin-like structures such as the Cry45 (anticancer parasporin protein), Cry46 (anticancer parasporin protein), and Cry51 insecticidal toxin (Akiba et al., 2009, 2006; Xu et al., 2015). The 3-domain Cry toxins are the best-characterized group of insecticidal proteins and are toxic after crystal solubilisation and proteolytic activation by midgut proteases of susceptible insects (Schnepf 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 (Bravo et al., 2007; de Maagd et al., 2003). The availability of structures for 3-domain Cry proteins (Fig. 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 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 et al., 2014). Even though Cyt toxins are usually considered to be active against mosquitoes and black flies (de Maagd et al., 2003), low activity has been reported against *Chironomus* larvae (Hughes et al., 2005) and aphids (Porcar et al., 2009) and the knowledge of Cyt toxin structure facilitated modification to enhance Cyt2Aa binding and toxicity against hemipteran pests (Chougule 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 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,

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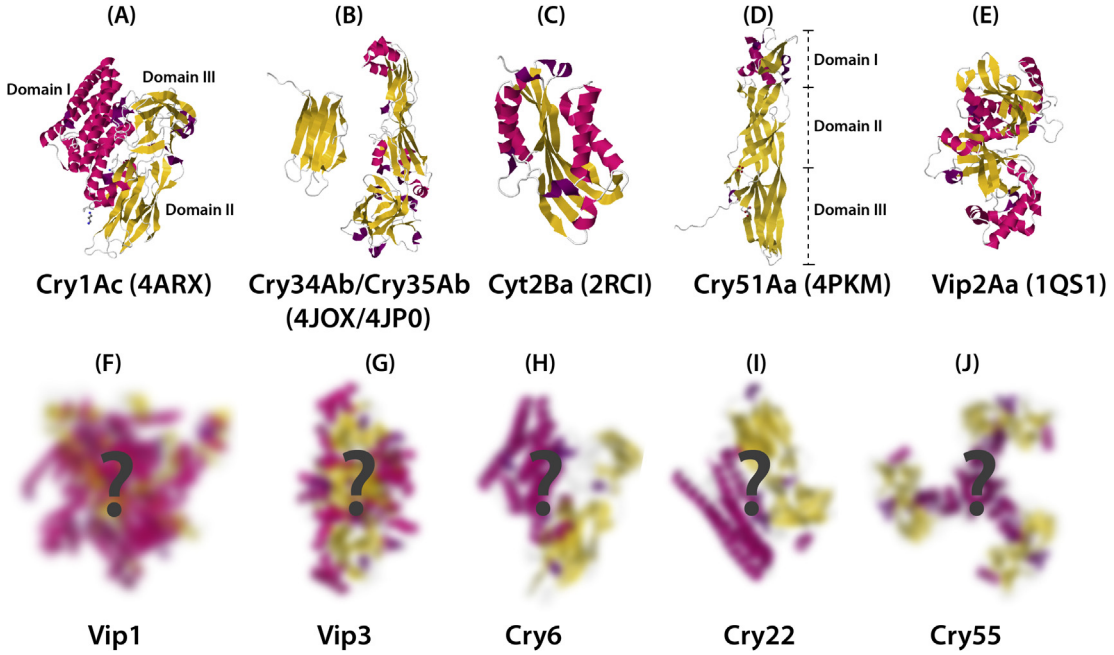

Fig. 1. Bt toxin structures. Known three-dimensional structures of insecticidal toxins from Bt: (A) Three-domain Cry toxin Cry1Ac, Domain I (in pink) is the pore-forming domain whereas domains II and III (in yellow) have roles in toxin-receptor interactions. (B) Binary Cry34Ab/Cry35Ab toxin. (C) Cyt2Ba 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 defocused 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. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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. Vip1 and Vip2 proteins (Fig. 1) together constitute a binary toxin and are commonly toxic against coleopteran and homopteran pests (Warren et al., 1998). Vip2 exhibits homology with the enzymatic ADP-ribosyltransferase toxin and its structure has been already elucidated (Han et al., 1999a). 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 et al., 2014) showing activity against nematodes and coleopterans (van Frankenhuyzen, 2013). (iii) Cry22 is active against coleopteran pests and ants (Isaac et al., 2003; Payne et al., 1997). 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, with non-insecticidal Cry23 protein, and exhibiting activity against coleopterans (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.

Transparency document

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Leopoldo Palma*
Facultad de Ciencias Agrarias, Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina

Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina

Colin Berry
Cardiff School of Biosciences, Cardiff University, Park Place, Cardiff, CF10 3AT, UK

* Corresponding author. Facultad de Ciencias Agrarias, Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina.
E-mail address: lpalma@fca.unl.edu.ar (L. Palma).

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