

# Online Research @ Cardiff

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <http://orca.cf.ac.uk/81254/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Palma, Leopoldo and Berry, Colin 2016. Understanding the structure and function of Bacillus thuringiensis toxins [Letter]. *Toxicon* 109 , pp. 1-3. 10.1016/j.toxicon.2015.10.020 file

Publishers page: <http://dx.doi.org/10.1016/j.toxicon.2015.10.020>  
<<http://dx.doi.org/10.1016/j.toxicon.2015.10.020>>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



1 Letter to the editor

2  
3 **Understanding the structure and function of *Bacillus thuringiensis* toxins.**

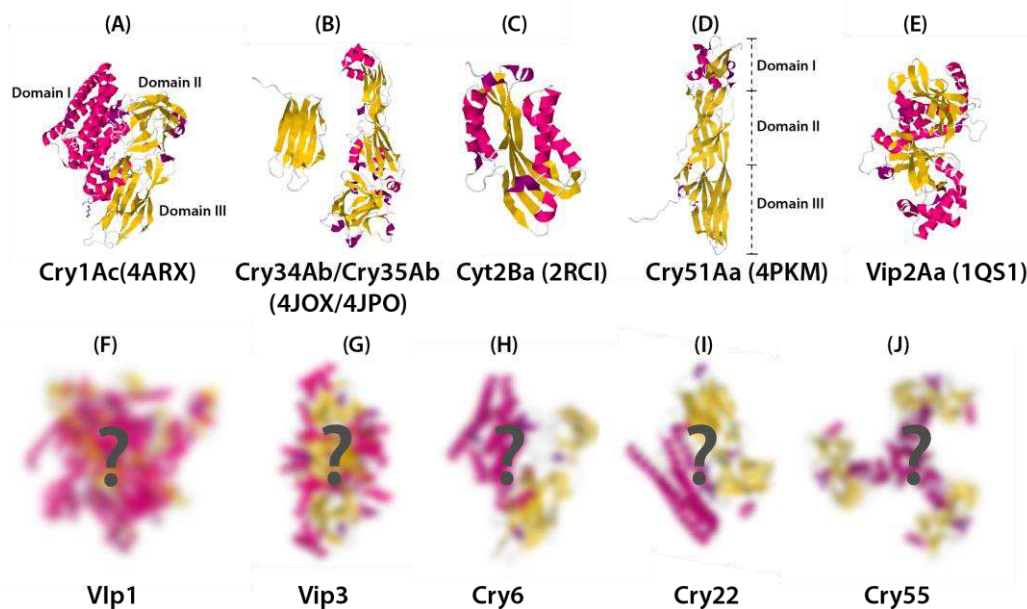
4  
5 Dear editor,

6  
7 “If you want to understand function, study structure” (Francis Crick, 1988).

8  
9 As biological control agents take an expanding share of the pesticides market and the  
10 production of insect-resistant crops increases, it is essential to understand the structure and  
11 function of the active agents, the invertebrate-active toxins that are the fundamental ingredients  
12 of these control systems. The potential for these agents in industry, agriculture and medicine  
13 necessitates a thorough investigation of their activity. The entomopathogenic bacterium *Bacillus*  
14 *thuringiensis* (Bt) is an important biological source of insecticidal proteins, with many strains  
15 bearing a wide variety of insecticidal genes. Bt delta-endotoxins (Cry and Cyt) (Figure 1) are  
16 synthesized during the stationary growth phase as crystalline parasporal inclusions, highly  
17 active against a wide range of insects ([Schnepf, Crickmore et al. 1998](#)). This bacterium also  
18 synthesizes other proteins during vegetative growth that are secreted into the culture medium.  
19 These have been designated as vegetative insecticidal proteins (Vips) ([Estruch, Warren et al.](#)  
20 [1996](#), [Warren, Koziel et al. 1998](#)) and secreted insecticidal protein (Sip) ([Donovan, Engleman et](#)  
21 [al. 2006](#)), and exhibit insecticidal activity against some coleopteran (the two-component  
22 Vip1/Vip2 toxin, and Sip) and lepidopteran pests (Vip3) ([Estruch, Warren et al. 1996](#), [Warren,](#)  
23 [Koziel et al. 1998](#)). The insecticidal proteins of Bt are highly specific for their hosts and have  
24 gained worldwide importance as environmentally desirable alternatives to chemical insecticides.  
25 Bt products have the biggest market share of biological insecticides and are used successfully in  
26 crop protection and vector control programmes worldwide. Moreover, Bt strains are also the  
27 major source for insect resistance transgenes in transgenic plants. Despite the importance of a  
28 wide variety of toxins in the action of this entomopathogenic bacterium, structural information  
29 has only been published on a subset of toxin classes: (i) the 3-domain Cry toxins (eg ([Li, Carroll](#)  
30 [et al. 1991](#))), (ii) the binary Cry34Ab/Cry35Ab toxin (([Kelker, Berry et al. 2014](#))), (iii) the Cyt  
31 toxins (eg ([Li, Koni et al. 1996](#))); (iv) the Vip2Aa protein (an ADP-ribosyl transferase ([Han,](#)  
32 [Craig et al. 1999](#))) and (v) aerolysin-like structures such as the Cry45 (anticancer parasporin  
33 protein), Cry46 (anticancer parasporin protein), and Cry51 insecticidal toxin ([Akiba, Higuchi et](#)  
34 [al. 2006](#), [Akiba, Abe et al. 2009](#), [Xu, Chinte et al. 2015](#)). The 3-domain Cry toxins (Figure 1)  
35 are the best-characterized group of insecticidal proteins and are toxic after crystal solubilisation  
36 and proteolytic activation by midgut proteases of susceptible insects ([Schnepf, Crickmore et al.](#)  
37 [1998](#)). Even though different 3-domain Cry toxins display clear differences in their amino acid

38 sequences and biological activities, the activated toxins all share in common a remarkably  
39 similar and conserved 3-domain structure ([de Maagd, Bravo et al. 2003](#), [Bravo, Gill et al. 2007](#)).  
40 The availability of structures for 3-domain Cry proteins (Figure 1) has opened the field for  
41 extensive mutagenesis to retarget toxins ([Pigott and Ellar 2007](#)) and to overcome resistance to  
42 the most used toxins to date (e.g. Cry1A) ([Ferré and Van Rie 2002](#)). The structures of the  
43 components for the binary Cry34/Cry35 toxin show similarities to the aegerolysin (Cry34) and  
44 aerolysin (Cry35) families of proteins, which are able to interact with cell membranes to form  
45 pores and kill coleopterans ([Kelker, Berry et al. 2014](#)). Although the roles of the two  
46 components in toxicity are not clear, Cry35 may be a beta-pore forming toxin and/or may  
47 interact with receptor via its lectin-like domain. The similarity of this protein with the better  
48 studied Bin toxins may also help in the elucidation of its activity. Cyt toxins directly interact  
49 with saturated membrane lipids and kill by causing cell lysis ([Xu, Wang et al. 2014](#)). Even  
50 though Cyt toxins are usually considered to be active against mosquitoes and black flies ([de](#)  
51 [Maagd, Bravo et al. 2003](#)), low activity has been reported against *Chironomus* larvae ([Hughes,](#)  
52 [Stevens et al. 2005](#)) and aphids ([Porcar, Grenier et al. 2009](#)) and the knowledge of Cyt toxin  
53 structure facilitated modification to enhance Cyt2Aa binding and toxicity against hemipteran  
54 pests ([Chougule, Li et al. 2013](#)). Hemipterans may show a general interaction in this class of  
55 toxins since related proteins from the bacterium *Dickeya dadantii* have been shown also to kill  
56 pea aphids ([Loth, Costechareyre et al. 2015](#)). However, despite the importance of increasing our  
57 knowledge of the structure of insecticidal toxins, a significant number of them do not share the  
58 3- domain structure and for many of these, structural information still is not available.  
59 Consequently, our ability to carry out similar studies to exploit these toxins is severely limited,  
60 thereby inhibiting their development. Amongst the classes of other toxins lacking basic  
61 biochemical and structural characterisation are the following, important examples: (i)  
62 Vegetative insecticidal proteins Vip1 and Vip3 (Figure 1). Vip1 and Vip2 proteins together  
63 constitute a binary toxin and are commonly toxic against coleopteran and homopteran pests  
64 ([Warren, Koziel et al. 1998](#)). Vip2 exhibits homology with the enzymatic ADP-  
65 ribosyltransferase toxin and its structure has been already elucidated ([Han, Craig et al. 1999](#)).  
66 No structure-function studies have been developed for Vip1, the specificity-determining B  
67 component of the toxin. In addition, the mode of action of Vip3 toxins remains unclear and  
68 would be significantly enriched by studying the structure-activity relationships for this protein  
69 class with increasing interest in its development for use in transgenic plants. Variations in the  
70 insecticidal toxicity profiles of natural Vip3 sequences from different Bt strains will provide a  
71 background of sequence diversity with which to understand specificity and to map the variant  
72 amino acids with the structural data. (ii) Cry6 is a ~ 54-kDa protein exhibiting features of the  
73 Smc chromosome segregation protein family ([Palma, Muñoz et al. 2014](#)) showing activity  
74 against nematodes and coleopterans ([van Frankenhuyzen 2013](#)). (iii) Cry22 is active against

75 coleopteran pests and ants ([Payne, Kennedy et al. 1997](#), [Isaac, Krieger et al. 2003](#)). It has  
 76 regions of homology with cadherins and lectins but again, its structure has not been published.  
 77 (iv) The small Cry37 protein (~14 kDa) that acts as a member of a two-component toxin that  
 78 kills coleopterans ([Donovan, Donovan et al. 2000](#)). (v) Cry55 is active against coleopteran pests  
 79 and nematodes ([van Frankenhuyzen 2009](#)) and, although some regional similarities to Toxin\_10  
 80 family proteins are predicted, its overall fold and mechanism of action are unknown. Bringing  
 81 new toxins to market involves numerous regulatory hurdles and structure function data greatly  
 82 enhance our ability to address safety and target specificity issues. In addition, a deeper  
 83 knowledge of structure and mechanism will be crucial in our efforts to avoid insect resistance  
 84 (for example through understanding toxin-receptor interactions) and to be able to retarget toxins  
 85 against new pests (as achieved previously with 3-domain toxins and dipteran active Cyt2Aa  
 86 toxin). Therefore, a comprehensive understanding of the modes of action along with the  
 87 understanding of structure will revolutionise our ability to exploit these proteins by providing  
 88 new paradigms for the action of insect toxins and will assist the agri-business sector in their  
 89 attempts to exploit new toxin types.



90

91 **Figure 1. Bt toxin structures**

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

100

101 **References**

- 102 Akiba, T., Y. Abe, S. Kitada, Y. Kusaka, A. Ito, T. Ichimatsu, H. Katayama, T. Akao, K.  
103 Higuchi, E. Mizuki, M. Ohba, R. Kanai and K. Harata (2009). "Crystal Structure of the  
104 Parasporin-2 *Bacillus thuringiensis* Toxin That Recognizes Cancer Cells." Journal of Molecular  
105 Biology **386**(1): 121-133.
- 106 Akiba, T., K. Higuchi, E. Mizuki, K. Ekino, T. Shin, M. Ohba, R. Kanai and K. Harata (2006).  
107 "Nontoxic crystal protein from *Bacillus thuringiensis* demonstrates a remarkable structural  
108 similarity to beta-pore-forming toxins." Proteins **63**(1): 243-248.
- 109 Bravo, A., S. S. Gill and M. Soberon (2007). "Mode of action of *Bacillus thuringiensis* Cry and  
110 Cyt toxins and their potential for insect control." Toxicon **49**(4): 423-435.
- 111 Chougule, N. P., H. Li, S. Liu, L. B. Linz, K. E. Narva, T. Meade and B. C. Bonning (2013).  
112 "Retargeting of the *Bacillus thuringiensis* toxin Cyt2Aa against hemipteran insect pests." Proc  
113 Natl Acad Sci U S A **110**(21): 8465-8470.
- 114 de Maagd, R. A., A. Bravo, C. Berry, N. Crickmore and H. E. Schnepf (2003). "Structure,  
115 diversity, and evolution of protein toxins from spore-forming entomopathogenic bacteria."  
116 Annu Rev Genet **37**: 409-433.
- 117 Donovan, W. P., J. C. Donovan and A. C. Slaney (2000). "*Bacillus thuringiensis* CryET33 and  
118 CryET34 compositions and uses thereof " US Patent 6063756.
- 119 Donovan, W. P., J. T. Engleman, J. C. Donovan, J. A. Baum, G. J. Bunkers, D. J. Chi, W. P.  
120 Clinton, L. English, G. R. Heck, O. M. Ilagan, K. C. Krasomil-Osterfeld, J. W. Pitkin, J. K.  
121 Roberts and M. R. Walters (2006). "Discovery and characterization of Sip1A: a novel secreted  
122 protein from *Bacillus thuringiensis* with activity against coleopteran larvae." Appl Microbiol  
123 Biotechnol **72**(4): 713-719.
- 124 Estruch, J. J., G. W. Warren, M. A. Mullins, G. J. Nye, J. A. Craig and M. G. Koziel (1996).  
125 "Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of  
126 activities against lepidopteran insects." Proc Natl Acad Sci USA **93**(11): 5389-5394.
- 127 Ferré, J. and J. Van Rie (2002). "Biochemistry and genetics of insect resistance to *Bacillus*  
128 *thuringiensis*." Annu Rev Entomol **47**: 501-533.
- 129 Han, S., J. A. Craig, C. D. Putnam, N. B. Carozzi and J. A. Tainer (1999). "Evolution and  
130 mechanism from structures of an ADP-ribosylating toxin and NAD complex." Nat Struct Biol  
131 **6**(10): 932-936.
- 132 Han, S., J. A. Craig, C. D. Putnam, N. B. Carozzi and J. A. Tainer (1999). "Evolution and  
133 mechanism from structures of an ADP-ribosylating toxin and NAD complex." Nature structural  
134 biology **6**(10): 932-936.

135 Hughes, P. A., M. M. Stevens, H.-W. Park, B. A. Federici, E. S. Dennis and R. Akhurst (2005).  
136 "Response of larval *Chironomus tepperi* (Diptera: Chironomidae) to individual *Bacillus*  
137 *thuringiensis* var. *israelensis* toxins and toxin mixtures." J. Invertebr. Pathol. **88**: 34-39.

138 Isaac, B., E. K. Krieger, L. Mettus and F. M. Sakuntala (2003). "Polypeptide compositions toxic  
139 to anthonomus insects, and methods of use." US Patent 6541448.

140 Kelker, M. S., C. Berry, S. L. Evans, R. Pai, D. G. McCaskill, N. X. Wang, J. C. Russell, M. D.  
141 Baker, C. Yang, J. W. Pflugrath, M. Wade, T. J. Wess and K. E. Narva (2014). "Structural and  
142 Biophysical Characterization of *Bacillus thuringiensis* Insecticidal Proteins Cry34Ab1 and  
143 Cry35Ab1." PLoS One **9**(11): e112555.

144 Li, J., P. A. Koni and D. J. Ellar (1996). "Structure of the mosquitocidal delta-endotoxin CytB  
145 from *Bacillus thuringiensis* sp. *kyushuensis* and implications for membrane pore formation." J.  
146 Mol. Biol. **257**(1): 129-152.

147 Li, J. D., J. Carroll and D. J. Ellar (1991). "Crystal structure of insecticidal delta-endotoxin from  
148 *Bacillus thuringiensis* at 2.5 A resolution." Nature **353**(6347): 815-821.

149 Loth, K., D. Costechareyre, G. Effantin, Y. Rahbe, G. Condemine, C. Landon and P. da Silva  
150 (2015). "New Cyt-like delta-endotoxins from *Dickeya dadantii*: structure and aphicidal  
151 activity." Sci Rep **5**: 8791.

152 Palma, L., D. Muñoz, C. Berry, J. Murillo and P. Caballero (2014). "*Bacillus thuringiensis*  
153 toxins: an overview of their biocidal activity." Toxins (Basel) **6**(12): 3296-3325.

154 Payne, J. M., K. Kennedy, J. B. Randall, H. Meier, H. J. Uick, L. Foncerrada, H. E. Schnepf, G.  
155 E. Schwab and J. Fu (1997). "*Bacillus thuringiensis* toxins active against hymenopteran pests."  
156 US Patent 5596071.

157 Pigott, C. R. and D. J. Ellar (2007). "Role of receptors in *Bacillus thuringiensis* crystal toxin  
158 activity." Microbiol Mol Biol Rev **1**(2): 255-281.

159 Porcar, M., A. M. Grenier, B. Federici and Y. Rahbe (2009). "Effects of *Bacillus thuringiensis*  
160 delta-endotoxins on the pea aphid (*Acyrtosiphon pisum*)." Appl Environ Microbiol **75**(14):  
161 4897-4900.

162 Schnepf, E., N. Crickmore, J. Van Rie, D. Lereclus, J. Baum, J. Feitelson, D. R. Zeigler and D.  
163 H. Dean (1998). "*Bacillus thuringiensis* and its pesticidal crystal proteins." Microbiol Mol Biol  
164 Rev **62**(3): 775-806.

165 van Frankenhuyzen, K. (2009). "Insecticidal activity of *Bacillus thuringiensis* crystal proteins."  
166 J Invertebr Pathol **101**(1): 1-16.

167 van Frankenhuyzen, K. (2013). "Cross-order and cross-phylum activity of *Bacillus thuringiensis*  
168 pesticidal proteins." J Invertebr Pathol **114**(1): 76-85.

169 Warren, G. W., M. G. Koziel, M. A. Mullins, G. J. Nye, B. Carr, N. M. Desai, K. Kostichka, N.  
170 B. Duck and J. J. Estruch (1998). "Auxiliary proteins for enhancing the insecticidal activity of  
171 pesticidal proteins." US patent 5770696.

172 Xu, C., U. Chinte, L. Chen, Q. Yao, Y. Meng, D. Zhou, L. J. Bi, J. Rose, M. J. Adang, B. C.  
173 Wang, Z. Yu and M. Sun (2015). "Crystal structure of Cry51Aa1: A potential novel insecticidal  
174 aerolysin-type beta-pore-forming toxin from *Bacillus thuringiensis*." Biochem Biophys Res  
175 Commun **462**(3): 184-189.

176 Xu, C., B. C. Wang, Z. Yu and M. Sun (2014). "Structural insights into *Bacillus thuringiensis*  
177 Cry, Cyt and parasporin toxins." Toxins (Basel) **6**(9): 2732-2770.

178

179 Leopoldo Palma\*

180 *Facultad de Ciencias Agrarias, Universidad Nacional del Litoral, Esperanza, Santa Fe,*  
181 *Argentina.*

182 *Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina.*

183 Colin Berry.

184 *Cardiff School of Biosciences, Cardiff University, Park Place, Cardiff CF10 3AT, UK.*

185

186 \*Corresponding author.

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

189