



Published in final edited form as:

*Invert Neurosci.* 2012 June ; 12(1): 37–41. doi:10.1007/s10158-012-0135-8.

## Bacterial pore-forming proteins as anthelmintics

Yan Hu and

Section of Cell and Developmental Biology, Division of Biological Sciences, University of California, San Diego, 9500 Gilman Drive, Mail Code 0322, La Jolla, CA 92093-0322, USA

Raffi V. Aroian

Section of Cell and Developmental Biology, Division of Biological Sciences, University of California, San Diego, 9500 Gilman Drive, Mail Code 0322, La Jolla, CA 92093-0322, USA

Yan Hu: yahu@ucsd.edu; Raffi V. Aroian: raroian@ucsd.edu

### Abstract

Crystal (Cry) proteins are made by the Gram-positive bacterium *Bacillus thuringiensis* (Bt). Cry proteins are pore-forming proteins and are the most widely used biological insecticides in the world. Our laboratory found some Cry proteins are highly effective against a broad range of nematodes (roundworms). Here, we discuss our results of Cry protein activity against intestinal roundworms. Both Cry5B and Cry21A have therapeutic activities against infections of the roundworm *Heligmosomoides polygyrus bakeri* in mice. Cry5B also shows highly therapeutic activity against *Ancylostoma ceylanicum* infection in hamsters. *A. ceylanicum* is a minor hookworm parasite of humans, and it is closely related to the more prevalent *Ancylostoma duodenale*. In addition, Cry proteins show excellent combinatorial therapeutic properties with nicotinic acetylcholine receptor (nAChR) agonists, one of the two classes of compounds approved by the World Health Organization for the treatment for intestinal roundworms in humans. Given their non-toxicity to humans and their broad spectrum of nematocidal action, Cry proteins show great potential as next-generation anthelmintics.

### Keywords

*Bacillus thuringiensis*; Crystal proteins; Anthelmintic; Cry5B; Soil-transmitted helminths; Pore-forming proteins

---

Hundreds of millions of children and pregnant women who live in impoverished conditions are infected with the soil-transmitted helminths known more commonly as hookworm (*Ancylostoma duodenale* and *Necator americanus*), whipworm (*Trichuris trichiura*), and giant roundworm (*Ascaris lumbricoides*) (Bethony et al. 2006; Hall et al. 2008; Hotez et al. 2007). In children, these roundworm (nematode) parasites are significant sources of malnutrition, anorexia, growth stunting, cognitive retardation, lethargy, high rates of school absenteeism, and immune defects; children who grow up with these roundworm infections make significantly less money as adults than children who do not have roundworms (Bethony et al. 2006; Hall et al. 2008; Harhay et al. 2010; Hotez et al. 2007; Lim et al. 2009; Tchuem Tchuente 2011). Pregnant women with roundworm infections, especially hookworm infections, are more likely to die, have their baby die, or give birth to low

birthweight babies than uninfected women (Haider et al. 2009; Hotez et al. 2004). As such, soil-transmitted helminths are the leading contributors to poverty worldwide.

Because of the paucity of resources in most places where soil-transmitted helminths are endemic, mass drug administration treatment programs require that drugs be cheap, easy to dose, work as a single dose, and affect as many parasites as possible (Keiser and Utzinger 2008). For these reasons, the drugs of choice are the benzimidazoles—mebendazole and albendazole. Pyrantel and levamisole, both L-subtype nicotinic acetylcholine receptor (nAChR) agonists, are less commonly used alternatives. A new drug in a similar class as pyrantel and levamisole, tribendimidine, is now in use in China and may be superior to these two (Xiao et al. 2005). Because the benzimidazoles are variably effective against the parasites and because resistance to these drugs appears to be emerging (Adugna et al. 2007; Albonico et al. 2003; Flohr et al. 2007; Gunawardena et al. 2008; Humphries et al. 2011; Smits 2009; Soukhathammavong et al. 2012; Stepek et al. 2006; Stothard et al. 2009), new and more efficacious anthelmintics (antiroundworm drugs) are urgently needed.

*Bacillus thuringiensis* (Bt) is a Gram-positive soil bacterium with a long history as the premier biologically produced insect control agent (Sanahuja et al. 2011). Bt was discovered in 1901 and first commercialized as an insecticide in the 1930s. During sporulation, the mother cell produces copious amounts of proteins called Cry proteins (Bulla et al. 1980; Ibrahim et al. 2010; Soberon et al. 2010). Following sporulation, a Bt culture will contain spores, crystals, and mother cell lysate (hence, the end product of Bt sporulation is called spore-crystal lysates). Cry proteins are ingestible pore-forming proteins that bind to the insect midgut, forming pores in these cells and leading to death of the insect (Ibrahim et al. 2010; Soberon et al. 2010). Because Bt and its Cry proteins are non-toxic to vertebrates, Bt spore-crystal lysates are ideal insecticides and in fact are used by organic farmers (Sanahuja et al. 2011; Zehnder et al. 2007). Moreover, transgenic plants that express Cry proteins are now widespread in agriculture around the world in order to improve crop yields and decrease reliance on toxic chemical pesticides (Sanahuja et al. 2011).

Over 200 Cry proteins are known, some of which kill Lepidoptera (caterpillars), some of which kill Coleoptera (beetles), and some of which kill Diptera (mosquitoes) (Sanahuja et al. 2011; Soberon et al. 2010). A small number are also demonstrated to kill nematodes or roundworms (Wei et al. 2003).

Feeding of Cry proteins, most notably Cry5B, to free-living roundworms like *Caenorhabditis elegans*, results in lethargy, anorexia, pale coloration, brood size reduction, developmental arrest, and/or death of the roundworm (Bischof et al. 2008; Griffiths et al. 2001; Marroquin et al. 2000; Wei et al. 2003). Cry5B is predicted to have significant structural similarity to insecticidal Cry proteins, like Cry1 family proteins used in transgenic crops (Xia et al. 2008). Cry5B also has pore-forming activity similar to insecticidal Cry proteins (Kao et al. 2011). Genetic, molecular, cell biological, and biochemical analyses reveal that the receptors for Cry5B in *C. elegans* are carbohydrate structures present on lipids on the intestinal surface of the roundworm (Barrows et al. 2006, 2007; Griffiths and Aroian 2005; Griffiths et al. 2001, 2003, 2005). Similar lipids appear to bind Cry5B in the hookworm *Ancylostoma ceylanicum* (Cappello et al. 2006). These glycolipids that bind Cry5B are known as arthroseries glycolipids and are specific to insects and roundworms (nematodes); they are lacking in mammals and vertebrates (Griffiths and Aroian 2005; Griffiths et al. 2005). Thus, at least part of the reason that Cry proteins like Cry5B are non-toxic to vertebrates is the lack of the Cry5B arthroseries glycolipid receptors in vertebrates.

Because they are safe to vertebrates and have significant intoxicating effects against free-living nematodes, it was investigated whether nematicidal Cry proteins can cure parasitic

roundworm infections in vivo. To date, two different rodent–parasite systems have been tested in this regard, with promising results (Cappello et al. 2006; Hu et al. 2010a, b; Table 1). Hamsters infected with the human hookworm *A. ceylanicum* (Fig. 1a) are cleared of 89 % of their parasites when treated with a triple dose (~14 mg/kg; 100 nM/kg) of Cry5B (Cappello et al. 2006). Production of parasite eggs is also profoundly affected with this treatment, showing about 80 % reduction (Cappello et al. 2006). Mice infected with the mouse parasite *Heligmosomoides polygyrus bakeri* (aka *H. polygyrus*, aka *H. bakeri* Fig. 1b) are cleared of about 70 % of their parasites when treated with single-dose Cry5B spore-crystal lysates (90–100 mg/kg; ~715 nM/kg; Hu et al. 2010a). Parasite egg production is also profoundly impacted by this treatment, showing a 98 % reduction (Hu et al. 2010a). Relative to the treatments with other anthelmintics against the same parasite, *H. polygyrus bakeri*, Cry5B efficacy is excellent when compared on a molar basis (Table 2). Furthermore, greater than 99 % of the protein is digested in simulated gastric fluids (Hu et al. 2010a), conditions analogous to those in the mammalian stomach. Based on the fact that Cry5B compares very favorably to current anthelmintics without even taking into account this degradation, then these results suggest that Cry proteins have the potential to be anthelmintics superior to those currently in use. Although less studied, Cry21A also has therapeutic activity in vivo against *H. polygyrus bakeri* (Hu et al. 2010b).

An important consideration in the establishment of any new anthelmintic is its mechanism of action relative to known anthelmintics such that roundworms/nematodes resistant to one anthelmintic are still susceptible to the other class. The benzimidazoles are thought to act via their effects on tubulin, whereas the nAChR agonists act at the neuromuscular junction (Holden-Dye and Walker 2007). Both mechanisms are different than plasma membrane-acting pore-forming Cry proteins. The independence of resistance pathways was formally tested by (1) taking *C. elegans* mutants resistant to nAChR agonists and benzimidazoles and treating them with Cry5B; and (2) taking *C. elegans* mutants resistant to Cry5B and treating them with nAChR agonists (Hu et al. 2010b). The results of these experiments confirmed independence of resistance pathways between Cry5B and nAChR agonists and benzimidazoles. Moreover, a very striking result was seen. *C. elegans* resistant to nAChR agonists were hypersusceptible to Cry5B, and *C. elegans* resistant to Cry5B were hypersusceptible to nAChR agonists (Hu et al. 2010b; Table 3). Hypersusceptibility is a phenomenon whereby as organisms targeted by Drug A become resistant to Drug A and they conversely become more susceptible than wild-type organisms to a Drug B. This phenomenon has been well characterized in human immunodeficiency virus (HIV) chemotherapy, and HIV drug treatment combinations that take advantage of hypersusceptibility have good clinical efficacy (Demeter et al. 2008; Katzenstein et al. 2003; Zaccarelli et al. 2004). The source of mutual hypersusceptibility between a pore-forming protein that attacks the roundworm intestine and acetylcholine receptor agonists that target the neuromuscular junction is as yet unknown and an interesting question for future research.

These results suggested that Cry5B and nAChR agonists might be usefully combined. In the follow-up, Cry5B and nAChR agonists were then tested for synergistic interactions (Hu et al. 2010b). Synergy is defined as a combination of drugs that shows efficacy greater than predicted from the additive effects of each drug individually. Using the experimental design and algorithm for synergy of Chou and Talalay (Chou 2006), we found that Cry5B and nAChR agonists show strong synergy (Table 4). Taken together with the hypersusceptibility experiments, these results indicate that Cry5B and nAChR agonists represent a potentially powerful combination therapy against roundworm parasites.

## Acknowledgments

This work was supported by National Institutes of Health grant to RVA (NIAID R01 AI056189).

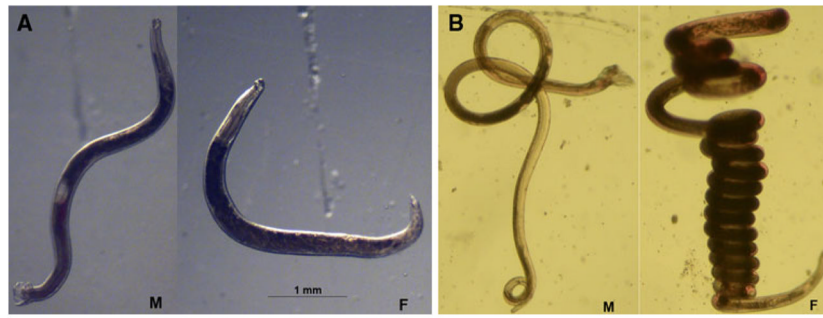
## References

- Adugna S, Kebede Y, Moges F, Tiruneh M. Efficacy of mebendazole and albendazole for *Ascaris lumbricoides* and hookworm infections in an area with long time exposure for anthelmintics, Northwest Ethiopia. *Ethiop Med J.* 2007; 45:301–306. [PubMed: 18330331]
- Albonico M, Bickle Q, Ramsan M, Montresor A, Savioli L, Taylor M. Efficacy of mebendazole and levamisole alone or in combination against intestinal nematode infections after repeated targeted mebendazole treatment in Zanzibar. *Bull World Health Organ.* 2003; 81:343–352. [PubMed: 12856052]
- Barrows BD, Griffiths JS, Aroian RV. *Caenorhabditis elegans* carbohydrates in bacterial toxin resistance. *Methods Enzymol.* 2006; 417:340–358. [PubMed: 17132513]
- Barrows BD, Haslam SM, Bischof LJ, Morris HR, Dell A, Aroian RV. Resistance to *Bacillus thuringiensis* toxin in *Caenorhabditis elegans* from loss of fucose. *J Biol Chem.* 2007; 282:3302–3311. [PubMed: 17135259]
- Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diemert D, Hotez PJ. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet.* 2006; 367:1521–1532. [PubMed: 16679166]
- Bischof LJ, Kao CY, Los FC, Gonzalez MR, Shen Z, Briggs SP, van der Goot FG, Aroian RV. Activation of the unfolded protein response is required for defenses against bacterial pore-forming toxin in vivo. *PLoS Pathog.* 2008; 4:e1000176. [PubMed: 18846208]
- Bulla LA Jr, Bechtel DB, Kramer KJ, Shethna YI, Aronson AI, Fitz-James PC. Ultrastructure, physiology, and biochemistry of *Bacillus thuringiensis*. *Crit Rev Microbiol.* 1980; 8:147–204. [PubMed: 7000441]
- Cappello M, Bungiro RD, Harrison LM, Bischof LJ, Griffiths JS, Barrows BD, Aroian RV. A purified *Bacillus thuringiensis* crystal protein with therapeutic activity against the hookworm parasite *Ancylostoma ceylanicum*. *Proc Natl Acad Sci USA.* 2006; 103:15154–15159. [PubMed: 17005719]
- Chou TC. Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. *Pharmacol Rev.* 2006; 58:621–681. [PubMed: 16968952]
- Demeter LM, DeGruttola V, Lustgarten S, Bettendorf D, Fischl M, Eshleman S, Spreen W, Nguyen BY, Koval CE, Eron JJ, Hammer S, Squires K. Association of efavirenz hypersusceptibility with virologic response in ACTG 368, a randomized trial of abacavir (ABC) in combination with efavirenz (EFV) and indinavir (IDV) in HIV-infected subjects with prior nucleoside analog experience. *HIV Clin Trials.* 2008; 9:11–25. [PubMed: 18215978]
- Flohr C, Tuyen LN, Lewis S, Minh TT, Campbell J, Britton J, Williams H, Hien TT, Farrar J, Quinnell RJ. Low efficacy of mebendazole against hookworm in Vietnam: two randomized controlled trials. *Am J Trop Med Hyg.* 2007; 76:732–736. [PubMed: 17426180]
- Fonseca-Salamanca F, Martinez-Grueiro MM, Martinez-Fernandez AR. Nematocidal activity of nitazoxanide in laboratory models. *Parasitol Res.* 2003; 91:321–324. [PubMed: 14574563]
- Githiori JB, Høglund J, Waller PJ, Baker RL. The anthelmintic efficacy of the plant, *Albizia anthelmintica*, against the nematode parasites *Haemonchus contortus* of sheep and *Heligmosomoides polygyrus* of mice. *Vet Parasitol.* 2003a; 116:23–34. [PubMed: 14519324]
- Githiori JB, Høglund J, Waller PJ, Leyden Baker R. Evaluation of anthelmintic properties of extracts from some plants used as livestock dewormers by pastoralist and smallholder farmers in Kenya against *Heligmosomoides polygyrus* infections in mice. *Vet Parasitol.* 2003b; 118:215–226. [PubMed: 14729169]
- Griffiths JS, Aroian RV. Many roads to resistance: how invertebrates adapt to Bt toxins. *BioEssays.* 2005; 27:614–624. [PubMed: 15892110]
- Griffiths JS, Whitacre JL, Stevens DE, Aroian RV. Bt toxin resistance from loss of a putative carbohydrate-modifying enzyme. *Science.* 2001; 293:860–864. [PubMed: 11486087]

- Griffitts JS, Huffman DL, Whitacre JL, Barrows BD, Marroquin LD, Muller R, Brown JR, Hennet T, Esko JD, Aroian RV. Resistance to a bacterial toxin is mediated by removal of a conserved glycosylation pathway required for toxin-host interactions. *J Biol Chem*. 2003; 278:45594–45602. [PubMed: 12944392]
- Griffitts JS, Haslam SM, Yang T, Garczynski SF, Mulloy B, Morris H, Cremer PS, Dell A, Adang MJ, Aroian RV. Glycolipids as receptors for *Bacillus thuringiensis* crystal toxin. *Science*. 2005; 307:922–925. [PubMed: 15705852]
- Gunawardena NK, Amarasekera ND, Pathmeswaran A, de Silva NR. Effect of repeated mass chemotherapy for filariasis control on soil-transmitted helminth infections in Sri Lanka. *Ceylon Med J*. 2008; 53:13–16. [PubMed: 18590264]
- Haider BA, Humayun Q, Bhutta ZA. Effect of administration of anthelmintics for soil transmitted helminths during pregnancy. *Cochrane Database Syst Rev*. 2009; 10:1002/14651858.CD005547.pub2
- Hall A, Hewitt G, Tuffrey V, de Silva N. A review and meta-analysis of the impact of intestinal worms on child growth and nutrition. *Matern Child Nutr*. 2008; 4(Suppl 1):118–236. [PubMed: 18289159]
- Harhay MO, Horton J, Olliaro PL. Epidemiology and control of human gastrointestinal parasites in children. *Expert Rev Anti Infect Ther*. 2010; 8:219–234. [PubMed: 20109051]
- Holden-Dye, L.; Walker, RJ. Anthelmintic drugs. *WormBook*; 2007. p. 1-13.
- Hotez PJ, Brooker S, Bethony JM, Bottazzi ME, Loukas A, Xiao S. Hookworm infection. *N Engl J Med*. 2004; 351:799–807. [PubMed: 15317893]
- Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Sachs SE, Sachs JD, Savioli L. Control of neglected tropical diseases. *N Engl J Med*. 2007; 357:1018–1027. [PubMed: 17804846]
- Hu, Y.; Aroian, RV. Promise of *Bacillus thuringiensis* crystal proteins as Anthelmintics. In: Selzer, P., editor. *Drug discovery in infectious diseases*. Vol. 4. Wiley; New York: 2012.
- Hu Y, Georghiou SB, Kelleher AJ, Aroian RV. *Bacillus thuringiensis* Cry5B protein is highly efficacious as a single-dose therapy against an intestinal roundworm infection in mice. *PLoS Negl Trop Dis*. 2010a; 4:e614. [PubMed: 20209154]
- Hu Y, Platzer EG, Bellier A, Aroian RV. Discovery of a highly synergistic anthelmintic combination that shows mutual hypersusceptibility. *Proc Natl Acad Sci USA*. 2010b; 107:5955–5960. [PubMed: 20231450]
- Humphries D, Mosites E, Otchere J, Twum WA, Woo L, Jones-Sanpei H, Harrison LM, Bungiro RD, Benham-Pyle B, Bimi L, Edoh D, Bosompem K, Wilson M, Cappello M. Epidemiology of hookworm infection in Kintampo North Municipality, Ghana: patterns of malaria coinfection, anemia, and albendazole treatment failure. *Am J Trop Med Hyg*. 2011; 84:792–800. [PubMed: 21540391]
- Ibrahim MA, Griko N, Junker M, Bulla LA. *Bacillus thuringiensis*: a genomics and proteomics perspective. *Bioeng Bugs*. 2010; 1:31–50. [PubMed: 21327125]
- Kao CY, Los FC, Huffman DL, Wachi S, Kloft N, Husmann M, Karabrahimi V, Schwartz JL, Bellier A, Ha C, Sagong Y, Fan H, Ghosh P, Hsieh M, Hsu CS, Chen L, Aroian RV. Global functional analyses of cellular responses to pore-forming toxins. *PLoS Pathog*. 2011; 7:e1001314. [PubMed: 21408619]
- Katzenstein DA, Bosch RJ, Hellmann N, Wang N, Bacheler L, Albrecht MA. Phenotypic susceptibility and virological outcome in nucleoside-experienced patients receiving three or four antiretroviral drugs. *AIDS*. 2003; 17:821–830. [PubMed: 12660529]
- Keiser J, Utzinger J. Efficacy of current drugs against soil-transmitted helminth infections: systematic review and meta-analysis. *JAMA*. 2008; 299:1937–1948. [PubMed: 18430913]
- Lim YA, Romano N, Colin N, Chow SC, Smith HV. Intestinal parasitic infections amongst Orang Asli (indigenous) in Malaysia: has socioeconomic development alleviated the problem? *Trop Biomed*. 2009; 26:110–122. [PubMed: 19901897]
- Marroquin LD, Elyassnia D, Griffitts JS, Feitelson JS, Aroian RV. *Bacillus thuringiensis* (Bt) toxin susceptibility and isolation of resistance mutants in the nematode *Caenorhabditis elegans*. *Genetics*. 2000; 155:1693–1699. [PubMed: 10924467]



- Sanahuja G, Banakar R, Twyman RM, Capell T, Christou P. *Bacillus thuringiensis*: a century of research, development and commercial applications. *Plant Biotechnol J*. 2011; 9:283–300. [PubMed: 21375687]
- Smits HL. Prospects for the control of neglected tropical diseases by mass drug administration. *Expert Rev Anti Infect Ther*. 2009; 7:37–56. [PubMed: 19622056]
- Soberon M, Pardo L, Munoz-Garay C, Sanchez J, Gomez I, Porta H, Bravo A. Pore formation by cry toxins. *Adv Exp Med Biol*. 2010; 677:127–142. [PubMed: 20687486]
- Soukhathammavong PA, Sayasone S, Phongluxa K, Xayaseng V, Utzinger J, Vounatsou P, Hatz C, Akkhavong K, Keiser J, Odermatt P. Low efficacy of single-dose albendazole and mebendazole against hookworm and effect on concomitant helminth infection in Lao PDR. *PLoS Negl Trop Dis*. 2012; 6:e1417. [PubMed: 22235353]
- Stepek G, Buttle DJ, Duce IR, Behnke JM. Human gastrointestinal nematode infections: are new control methods required? *Int J Exp Pathol*. 2006; 87:325–341. [PubMed: 16965561]
- Stothard JR, Rollinson D, Imison E, Khamis IS. A spot-check of the efficacies of albendazole or levamisole, against soil-transmitted helminthiasis in young Ungujan children, reveals low frequencies of cure. *Ann Trop Med Parasitol*. 2009; 103:357–360. [PubMed: 19508754]
- Tchuem Tchuente LA. Control of soil-transmitted helminths in sub-Saharan Africa: diagnosis, drug efficacy concerns and challenges. *Acta Trop*. 2011; 120(Suppl 1):S4–S11. [PubMed: 20654570]
- Wabo Pone J, Mbida M, Bilong Bilong CF. In vivo evaluation of potential nematicidal properties of ethanol extract of *Canthium mannii* (Rubiaceae) on *Heligmosomoides polygyrus* parasite of rodents. *Vet Parasitol*. 2009; 166:103–107. [PubMed: 19744792]
- Wei JZ, Hale K, Carta L, Platzer E, Wong C, Fang SC, Aroian RV. *Bacillus thuringiensis* crystal proteins that target nematodes. *Proc Natl Acad Sci USA*. 2003; 100:2760–2765. [PubMed: 12598644]
- Xia LQ, Zhao XM, Ding XZ, Wang FX, Sun YJ. The theoretical 3D structure of *Bacillus thuringiensis* Cry5Ba. *J Mol Model*. 2008; 14:843–848. [PubMed: 18504623]
- Xiao SH, Hui-Ming W, Tanner M, Utzinger J, Chong W. Tribendimidine: a promising, safe and broad-spectrum anthelmintic agent from China. *Acta Trop*. 2005; 94:1–14. [PubMed: 15777691]
- Zaccarelli M, Tozzi V, Perno CF, Antinori A. The challenge of antiretroviral-drug-resistant HIV: is there any possible clinical advantage? *Curr HIV Res*. 2004; 2:283–292. [PubMed: 15279592]
- Zehnder G, Gurr GM, Kuhne S, Wade MR, Wratten SD, Wyss E. Arthropod pest management in organic crops. *Annu Rev Entomol*. 2007; 52:57–80. [PubMed: 16846384]



**Fig. 1.** Parasitic roundworms covered in these studies. **a** *A. ceylanicum*, **b** *Heligmosomoides polygyrus bakeri*. *M* male; *F* female

**Table 1**

Summary of the efficacy of Cry proteins against roundworms infection in vivo

<b>Cry protein</b>	<b>Dose</b>	<b>Roundworm</b>	<b>% Intestinal worm reduction</b>	<b>References</b>
Cry5B	~100 nM/kg (14 mg/kg), triple dose	<i>A. ceylanicum</i>	89	Cappello et al. (2006)
Cry5B	~715 nM/kg (100 mg/kg), single dose	<i>H. polygyrus bakeri</i>	70	Hu et al. (2010a)
Cry21A	99 nM/kg (13 mg/kg), triple dose	<i>H. polygyrus bakeri</i>	40	Hu et al. (2010b)



**Table 2**

Efficacy of different anthelmintics against *H. bakeri* as compared to Cry5B in vivo (Modified from Hu and Aroian 2012)

Anthelmintic	Dose given ( $\mu$ M/kg)	Dose relative to Cry5B	Day(s) treatment was given (PI = post infection)	% Reduction in intestinal worm burden	Publication references
Cry5B	0.7	1x	15 days PI	70	Hu et al. (2010a)
Levamisole	49	70x	12 days PI	90	Fonseca-Salamanca et al. (2003)
Ivermectin	5.7	8x	18 days PI	87	Githiori et al. (2003a)
Pyrantel	84	120x	18 days PI	99	Githiori et al. (2003b)
Mebendazole	75	107x	9–15 days PI	84	Wabo Pone et al. (2009)
Tribendimidine	2.2	3x	15 days PI	70	Hu et al. (2010a)

Table 3

Sensitivity of various *C. elegans* mutants to anthelmintics

Genotype	Drug	LC <sub>50</sub> (μg/mL)	LC <sub>50</sub> (μM)	95 % Confidence limits	Fold change <sup>c</sup>	Result <sup>d</sup>
N2 (wild type)	Cry5B	7.16	0.05	6.56–7.82		
<i>lev-8(ye493)</i> <sup>a</sup>		5.02	0.04	4.62–5.46	0.70	H
<i>unc-50(ye494)</i> <sup>a</sup>		5.61	0.04	5.01–6.26	0.78	H
N2 (wild type)	Tribendimidine	51.76	114.4	45.70–58.97		
<i>bre-5(ye17)</i> <sup>b</sup>		9.39	20.7	8.28–10.64	0.18	H
N2 (wild type)	Levamisole	18.07	75.1	15.10–21.32		
<i>bre-5(ye17)</i> <sup>b</sup>		9.19	38.2	7.57–10.81	0.50	H

The LC<sub>50</sub> values along with 95 % confidence limits are shown (modified from Hu et al. 2010b)<sup>a</sup>These mutants are resistant to nAChR agonists like tribendimidine and levamisole<sup>b</sup>This mutant is resistant to Cry5B<sup>c</sup>The ratio of the LC<sub>50</sub> of the mutant to the LC<sub>50</sub> of wild-type. Fold change values <1 indicate the mutant is more sensitive to the drug than wild type (i.e., hypersusceptible)<sup>d</sup>H hypersusceptible

**Table 4**

Combination index values of Cry5B and nAChR agonists at different effect dose (ED) levels (e.g., at the combination dose at which 50 % of the *C. elegans* roundworms are killed, Cry5B and levamisole show a combination index value of 0.38)

Combinations	Combination index value <sup>a</sup>		
	ED <sub>50</sub>	ED <sub>75</sub>	ED <sub>95</sub>
Cry5B + tribendimidine	0.52	0.30	0.12
Cry5B + levamisole	0.38	0.26	0.15
Cry5B + pyrantel	0.64	0.54	0.40

From (Hu et al. 2010b)

<sup>a</sup>Combination index values <1 are indicative of synergy. The combination index values shown here are generally indicative of strong synergy