Supplementary Material

Fragoso P. *et al*., Molecular Characterization of the cry Gene profile of Bacillus thuringiensis Isolated from a Caribbean Region of Colombia,
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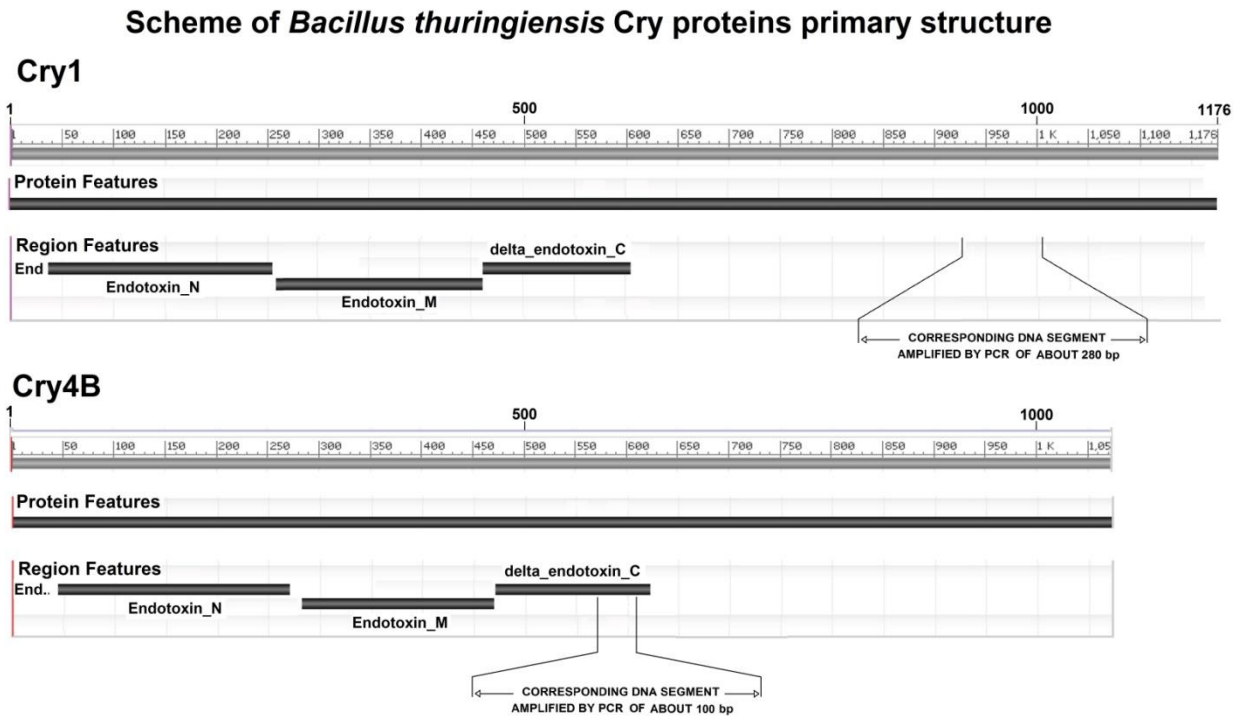

Fig. S1. Scheme of *Bacillus thuringiensis* **Cry1 and Cry4B protein primary structures, indicating the corresponding locations of the PCR products.**
The first amino acid sequence corresponds to the open reading frame of the *B.thuringiensis cry1A* gene (GenBank accession code: D17518.1) and the second to the open reading frame of the full length *cry4B* gene corresponding to 1135 amino acids of *B. thuringiensis* serovar *israelensis* (ID code:BAAA00178.1).

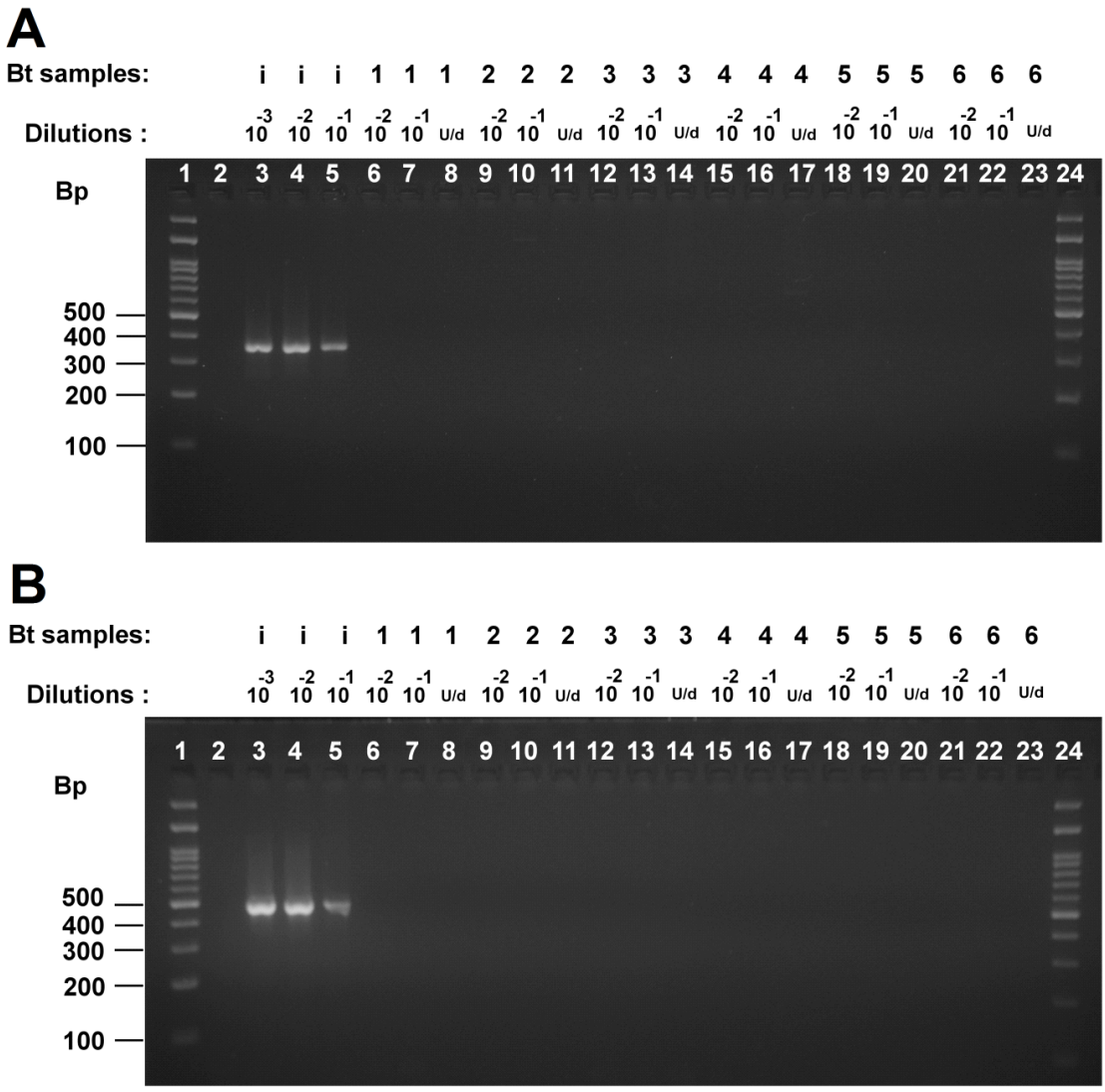

Fig.S2. Detection of *cry10* and *cyt1* in different dilutions of *Bacillus thuringiensis* (Bt) DNA samples.

The electrophoresis was performed in 2.5% agarose gels, showing the PCR amplification products for the *cry10* gene segment of 348 base pairs (panel A) and for the *cyt1* gene segment of 480 bp (panel B) using the primers and protocols described by Santos *et al.* (2012). In both panels the Bt samples were the same as indicated on top of each figure. The samples were the following: 100 bp DNA ladder marker (lanes 1 and 24), negative control with no sample (lane 2), different concentrations of *B. thuringiensis* var. *israelensis* (Bti) DNA (lanes 3 - 5) corresponding to 0.17; 1.7; and 17 ng/µl, respectively (lane 3); DNA samples from the Colombian Caribbean Bt-UPC-1 to Bt-UPC-6 isolates (Lanes 6 – 23). A volume of 1 µl in an assay volume of 20 µl was tested for all the samples with the exception of undiluted samples (U/d) in which were only assayed with 0.5 µl (see Materials and Methods for more details).

Table S1

Sampling locations of the *B. thuringiensis* isolates utilized in toxicity bioassays against third and fourth instar larvae of *A. aegypti*.

Isolates	Type of sample	Sampling site	Geographic coordinates (latitude - longitude)	Sample date
Bt – UPC – 1	Treatment plant mud	[a]Valledupar	10° 31' 04" - 073°18' 07"	5/2012
Bt – UPC – 2	Urban soil	El Carmen	10°28' 24.7"- 073°14' 27.2"	3/2011
Bt – UPC – 3	Urban soil	Villa Mirian	10°27'08.8"- 073° 16' 08.3"	4/2011
Bt – UPC – 4	Urban soil	La Popa	10° 27' 44.4"-073°16' 16.8"	6/2011
Bt – UPC – 5	Urban soil	Villa taxi	10°27' 07.5"- 073°16' 41.2"	6/2011
Bt – UPC – 6	Urban soil	El progreso	10°27' 07.4"-073° 16' 48.4"	4/2011
Bt – UPC – 7	Urban soil	Mayales	10°27' 23.8"-073° 14' 20.9"	8/2011
Bt – UPC – 8	Treatment plant mud	[a]Valledupar	10° 31' 04"- 073° 18' 07"	5/2012
Bt – UPC – 9	Treatment plant mud	[a]Valledupar	10° 31' 04"- 073° 18' 07"	4/2012
Bt – UPC – 10	Treatment plant mud	[a]Valledupar	10° 31' 04"- 073° 18' 07"	6/2012
Bt – UPC – 11	Urban soil	5 de Noviembre	10°27'36.1"- 073° 14' 31.2"	8/2011
Bt – UPC – 12	Urban soil	Mareigua	10°26'06.4"- 073° 15' 15.7"	10/2011
Bt – UPC – 13	Urban soil	12 de Octubre	10°27'26.7"- 073° 14' 44.5"	7/2011
Bt – UPC – 14	Urban soil	Novalito	10°28'58.7"- 073° 15' 22.0"	4/2011
Bt – UPC – 15	Urban soil	Villa Clara	10°27'49.0"- 073° 14' 15.9"	9/2011
Bt – UPC – 16	Urban soil	Francisco de Paula	10°26'58.5"- 073° 15' 48.2"	9/2011
Bt – UPC – 17	Urban soil	Villa Jaidi	10°26'04.8"- 073° 15' 22.2"	11/2011
Bt – UPC – 18	Urban soil	7 de Agosto	10°27' 04.8"-073° 15' 22.8"	11/2011
Bt – UPC – 19	Urban soil	Pescaito	10°28' 44.3"-073° 14' 29.1"	3/2011
Bt – UPC – 20	Urban soil	San Joaquin	10°28'37,7"- 073° 15' 20.7"	3/2011
Bt – UPC – 21	Urban soil	Nuevo Milenio	10°26' 18.7"-073° 15' 02,0"	5/2011

Bt – UPC – 22	Urban soil	Los Cocos	10°27' 13.9"-073° 13' 53.1"	5/2011
Bt – UPC – 23	Urban soil	25 de Diciembre	10°26' 18.7"-073° 14' 41.7"	6/2011
Bt – UPC – 24	Urban soil	Don Alberto	10°27'57.4"- 073° 16' 53.5"	8/2011
Bt – UPC – 25	Urban soil	Alamos III	10°26'51.8"- 073° 15' 55.7"	2/2011
Bt – UPC – 26	Urban soil	Barrio la Nevada	10°28'43.7"- 073° 16' 54.9"	9/2011
Bt – UPC – 27	Urban soil	Alamos II	10°26'51.0" - 073°16' 00.4"	2/2011
Bt – UPC – 28	Urban soil	Milagros	10°27'28.5" - 073°13' 56.4"	10/2011
B. thuringiensis var. *Kurstaki*	Commercial	_		_
B. thuringiensis var. *israeliensis*	Commercial	_		_

[a]Valledupar public service company.

Table SII

Mortality percentages of *A. aegypti* larvae after co-incubation during 48 hours with bacterial suspension of *B. thuringiensis* isolates and the presence of *cry* genes.

Isolates[a]	**Maximal percentage of larval mortality**	**Concentration of bacterial suspension (mg/l)**	**Presence of *cry* genes:**			
			cry1[b]	***cry2***[b]	***cry4B***[c] **fragment (bp)**	***cry10***[c]
Bt – UPC – 1	0	0	+	-	-	-
Bt – UPC – 2	0	0	-	-	-	-
Bt – UPC – 3	0	0	+	-	-	-
Bt – UPC – 4	0	0	-	-	-	-
Bt – UPC – 5	**17**	**10**	-	-	-	-
Bt – UPC – 6	**23**	**10**	-	-	-	-
Bt – UPC – 7	0	0	-	-	**100 + 120**	-
Bt – UPC – 8	0	0	+	+	-	-
Bt – UPC – 9	0	0	-	-	-	-
Bt – UPC – 10	0	0	-	-	-	-
Bt – UPC – 11	0	0	-	-	-	-
Bt – UPC – 12	0	0	-	-	**150**	-
Bt – UPC – 13	0	0	-	-	-	-
Bt – UPC – 14	0	0	-	-	**100**	-
Bt – UPC – 15	**27**	**0.1**	-	-	-	-
Bt – UPC – 16	0	0	+	+	-	-
Bt – UPC – 17	0	0	+	+	-	-
Bt – UPC – 18	0	0	+	+	-	-
Bt – UPC – 19	0	0	+	-	**100**	-
Bt – UPC – 20	**30**	**0.1**	-	-	-	-
Bt – UPC – 21	0	0	+	-	-	-
Bt – UPC – 22	0	0	-	+	**150**	-
Bt – UPC – 23	0	0	-	+	**100**	-
Bt – UPC – 24	0	0	-	-	-	-

Bt – UPC – 25	**27**	**0.1**	**-**	**-**	**-**	**-**
Bt – UPC – 26	0	0	**+**	**+**	**150**	**-**
Bt – UPC – 27	0	0	**-**	**+**	**100 + 150**	**-**
Bt – UPC – 28	0	0	**+**	**+**	**-**	**-**
Total number (%)			**10 (35.7)**	**9 (32.1)**	**8 (28.6)**	**0**
Bt. var. *Kurstaki*	**80**	**10**	**+**	**+**	**-**	**ND[d]**
Bt. var. *israelensis*	**93**	**10**	277	-	321	348

[a]Isolated from the mud of water treatment plants and urban soils of the city of Valledupar (Materials and Methods).
[b] The *cry1* and *cry2* genes were detected by the specific primers and protocols described by Ben-Dov *et al.* (1997).
[c]The *cry4B, cry10 and cyt1* genes were assayed using the specific primers and protocols described by Santos *et al.* (2012).
[d]ND = not determined.