Specific binding between *Bacillus thuringiensis* Cry9Aa and Vip3Aa toxins synergizes their toxicity against Asiatic rice borer (*Chilo suppressalis*)

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List of the material included: two supporting figures, four supporting tables and one supporting method.

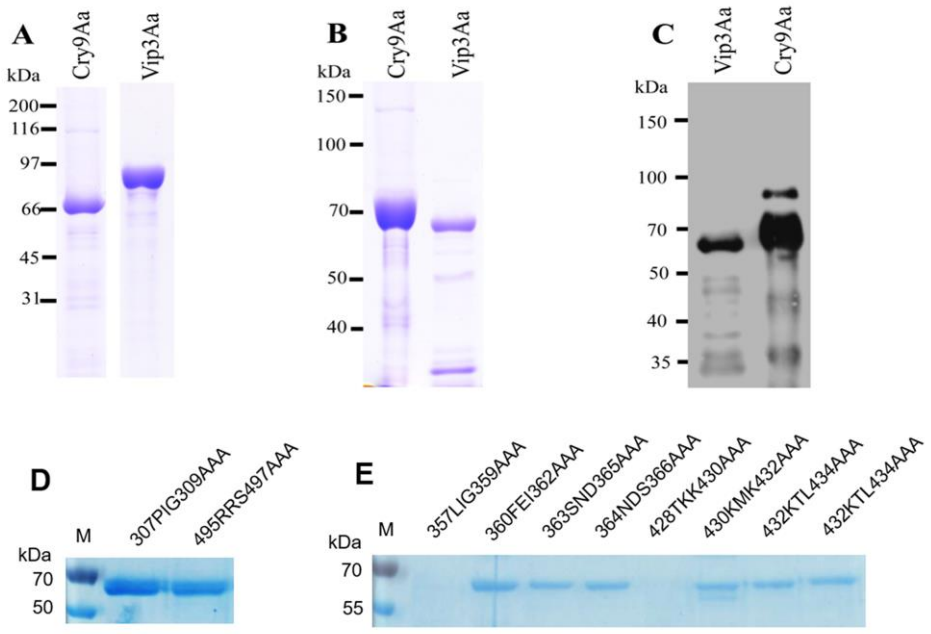

Figure S1. Analysis of Cry9Aa and Vip3Aa proteins. (Panel A) shows SDS-PAGE of the soluble Vip3Aa protoxin and Cry9Aa toxin fragment (655 amino acids) purified by Ni-affinity purification. (Panel B) shows SDS-PAGE of trypsin activated Cry9Aa and Vip3Aa toxins. (Panel C) shows Western-blot of the biotinylated Cry9Aa and Vip3Aa toxins detected with Streptavidin. (Panel D) shows trypsin activation of Cry9Aa-R495A/R496A/S497A mutant and another representative mutant (Cry9Aa-P307A/I308A/G309A mutant). (Panel E) shows trypsin activation of different Vip3A mutants including Vip3Aa-K432A/T433A/L434A.

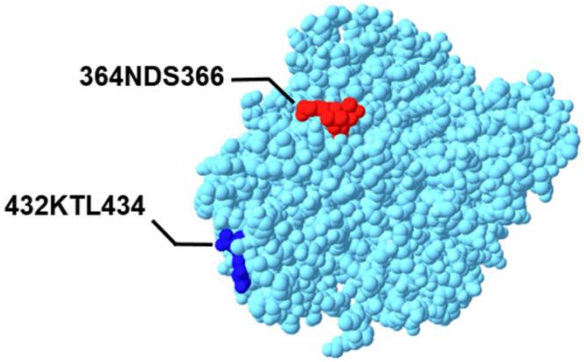

Figure S2. Model of the three dimensional structure of Vip3Aa constructed by using QUARK protein structure prediction since this is suitable for proteins which are considered without homologous templates (https://zhanglab.ccmb.med.umiC.edu/QUARK/).

Table S1. Bioassays of insecticidal activity of Cry9Aa and Vip3Aa toxins against *Ostrinia furnacalis* larvae.

Toxins	Observed LC_{50} in μg/g diet (95% confidence limits)	Expected LC_{50} in μg/g diet	Synergy factor (SF)
Cry9Aa	5.89 (4.39-7.88)		
Vip3Aa	>150		
Cry9Aa+ Vip3Aa	2.51 (1.65-3.81)	11.3	4.5
Cry9Aa-R495A/R496A/S497A	21.7 (14.5-34.9)		
Vip3Aa-K432A/T433A/L434A	>150		
Cry9Aa- R495A/R496A/S497A + Vip3Aa	50.9[a]	37.9	0.76
Cry9Aa+ Vip3Aa-4K432A/T433A/L434A	1.99 (0.359-10.2)	11.3	5.7
Cry9Aa-R495A/R496A/S497A+ Vip3Aa- K432A/T433A/L434A	60.5 (42.3-112)	37.9	0.63

[a], confidence limits not statistical significant

Table S2. Primers for amplification of the different Cry9Aa and Vip3Aa fragments

Primer name	DNA Sequence (5'→3')	Restriction site
Cry9Aa D1-F	CGCGGATCCGATGAATCAAAATAAACACGG	*Bam*HⅠ
Cry9Aa D1-R	CCGCTCGAGCGGCCAACTTTCTCCCCTAAGACTAC	*Xho*Ⅰ
Cry9Aa D2-F	CGCGGATCCGTACCCAATAGAAACAGATTTTCAGTTGAGTAGGG	*Bam*HⅠ
Cry9Aa D2-R	CCGCTCGAGCGGACGAGCCAGACTTTTATGTGTCCAACCATAC	*Xho*Ⅰ
Cry9Aa D3-F	CGCGGATCCGAACAATACCATTAATCCAGATAGAATTAC	*Bam*HⅠ
Cry9Aa D3-R	CCGCTCGAGCGGAACTGGAATGAACTCAATTCTATCTAC	*Xho*Ⅰ
Vip3Aa F1-F	CGGATCCGATGAACAAGAATAATACTAAATTAAG	*Bam*HⅠ
Vip3Aa F1-R	CCGCTCGAGCGGGTAAACAATTGCAAGAGATTTCT	*Xho*Ⅰ
Vip3Aa F2-F	CGGATCCGGATGTTAATAACAAACTCGATG	*Bam*HⅠ
Vip3Aa F2-R	CCGCTCGAGCGGCCCGAATAAATTATTTCCTAC	*Xho*Ⅰ
Vip3Aa F3-F	CGGATCCGGATAAGTTGGATATTATTAATG	*Bam*HⅠ
Vip3Aa F3-R	CCGCTCGAGCGGATCTGCTAAGCCTAATAATTTTC	*Xho*Ⅰ
Vip3Aa F4-F	CGGATCCGCGTTCAGCTTTAAAAACTGC	*Bam*HⅠ
Vip3Aa F4-R	CCGCTCGAGCGGTAATTTATCCATATCACCATAAATAAC	*Xho*Ⅰ
Vip3Aa F5-F	CGGATCCGATTGATTATACTTCTATTATG	*Bam*HⅠ
Vip3Aa F5-R	CCGCTCGAGCGGGTCAATTTCTCCTGTAGAAG	*Xho*Ⅰ
Vip3Aa F6-F	CGGATCCGAAACCAGGACATGCATTGATTG	*Bam*HⅠ
Vip3Aa F6-R	CCGCTCGAGCGGTGAATTTTCATCAGCTTG	*Xho*Ⅰ
Vip3Aa F7-F	CGGATCCGTGCCCAGATCAATCTGAAC	*Bam*HⅠ
Vip3Aa F7-R	CCGCTCGAGCGGCTCTAAATTGTCCTCTTCTATG	*Xho*Ⅰ
Vip3Aa F8-F	CGGATCCGTTAATTACTTTAACATGTAAATC	*Bam*HⅠ
Vip3Aa F8-R	CCGCTCGAGCGGACTTTTTAAAATTAAATACAC	*Xho*Ⅰ
Vip3Aa F9-F	CGGATCCGAAAGGAAAACCTTCTATTC	*Bam*HⅠ
Vip3Aa F9-R	CCGCTCGAGCGGAGCACCGCTCATATATCTTTTTTC	*Xho*Ⅰ
Vip3Aa F10-F	CGGATCCGCAAAATGGAGATGAAGCTTG	*Bam*HⅠ
Vip3Aa F10-R	CCGCTCGAGCGGCTTAATAGAGACATCGTAAAAATG	*Xho*Ⅰ

Restriction sites were underlined

Table S3. Primers used for mutagenesis of Cry9Aa and Vip3Aa proteins

Primer name	DNA Sequence (5'→3')
Cry9Aa 307PIG309AAA	GGGTCATTTATACAGATGCAGCTGCTTTTGTACATCGTAGTAGTCTTAGGGG
Cry9Aa 316LRH318AAA	GGTTTTGTACATCGTAGTAGTGCTGCGGCAGAAAGTTGGTTTAGCTTTG
Cry9Aa 325VNR327AAA	GAAAGTTGGTTTAGCTTTGCTGCTGCAGCTAATTTCTCAGATTTAG
Cry9Aa 359PVS361AAA	CTACTGGTTCACTTACATTGGCGGCTGCCCCAAGTACTGATAGAGCGAGGG
Cry9Aa 364TDR366AAA	CATTGCCGGTTAGCCCAAGTGCTGCTGCAGCGAGGGTATGGTATGGAAGTCG
Cry9Aa 393HTT395AAA	CTGAACTAATCTCTGGACAAGCTGCGGCTGCTACACAAACTATT
Cry9Aa 416NDT418AAA	GATTCTCAAGCTTGTAATTTAGCTGCTGCCACATATGGAGTGAATAGGGCGG
Cry9Aa 422VNR424AAA	GTAATTTAAATGATACCACATATGGAGCGGCTGCGGCGGTATTTTATCATGATGCG
Cry9Aa 488LRG490AAA	CAACAATAAATTTAACAGGAGGAGCTGCAGCAGTAGCATCTAATCGCCGTTC
Cry9Aa 495RRS497AAA	GGACTTAGACAAGTAGCATCTAATGCCGCTGCATCTTTAGTAATGTATGGTTGG
Vip3Aa 357LIG359AAA	GAAGCTAAACCAGGACATGCAGCGGCTGCGTTTGAAATTAGTAATGATTC
Vip3Aa 360FEI362AAA	CCAGGACATGCATTGATTGGGGCTGCAGCTAGTAATGATTCAATTACAG
Vip3Aa 363SND365AAA	CATTGATTGGGTTTGAAATTGCTGCTGCTTCAATTACAGTATTAAAAG
Vip3Aa 364NDS366AAA	CATTGATTGGGTTTGAAATTGCTGCTGCTTCAATTACAGTATTAAAAG
Vip3Aa 428TKK430AAA	GTAATTACTAAAATTGATTTCGCTGCAGCAATGAAAACTTTAAGATATG
Vip3Aa 430KMK432AAA	CTAAAATTGATTTCACTAAAGCAGCGGCAACTTTAAGATATGAGGTAAC
Vip3Aa 432KTL435AAA	GATTTCACTAAAAAAATGGCAGCTGCAAGATATGAGGTAACAGCG

Table S4. Determination of APN enzymatic activity in *Chilo suppressalis* midgut membrane

Extraction from midgut	APN enzymatic activity (U/mg)	The ratio of BBMV/ Midgut homogenate
BBMV	38.04	4.25 folds
Midgut homogenate	8.95	

The ratio of BBMV/ midgut homogenate suggests that the BBMV sample is well prepared.

Supporting methods

The Preparation of Cry9Aa and Vip3Aa Antibodies. The anti-Cry9Aa or anti-Vip3Aa polyclonal antibodies were raised in New Zealand white rabbits (from facilities of IBT-UNAM) after subcutaneous immunization with purified Vip3Aa or Cry9Aa toxins. The rabbits were boosted three times with 1 mg of the toxins mixed with incomplete Freund´s adjuvant, at 15-day intervals. Blood serum was obtained and the specificity and sensitivity of the polyclonal antisera was determined in a dot blot assay using different concentrations of Vip3Aa or Cry9Aa toxins spotted on nitrocellulose strips and analyzed with different concentrations of the polyclonal anti-Vip3Aa or anti-Cry9Aa antibodies (from 1: 10,000 to 1: 50,000 dilutions) and the secondary goat anti-rabbit antibody coupled to HRP (diluted 1: 10,000).