Fig S1 Light microscopy images (100X) of *B. thuringiensis* DB27 cultures used in *C. elegans* killing assays. A. Culture which kills nematodes in 16 hours and marked "BT DB27 veg cells/spores" in Fig 1A. Mixture of vegetative cells (often represented by chains of cells) and spores is shown. B. Culture which kills worms in 10 hours and marked "BT DB27 spores" in Fig 1A. Pure spores are shown. C. Culture which is not virulent to nematodes and marked "BT DB27 veg cells" in Fig 1A. Pure vegetative cells that form long chains are shown. Scale bar is 20 μm.

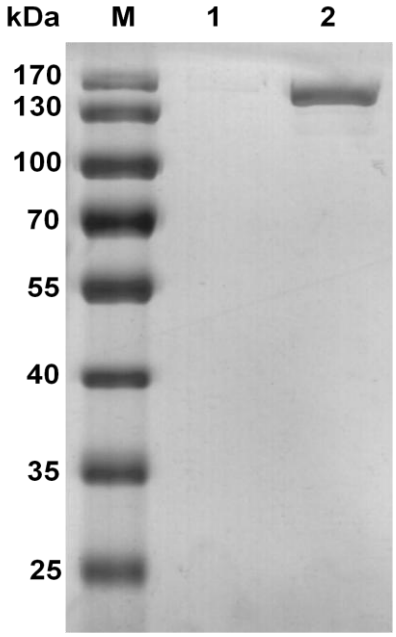

Fig S2 SDS-PAGE confirmation of Cry toxin production. Spore-crystal mixtures of *B. thuringiensis* DB27 (lane 2) and of plasmidless strain (lane 1) grown in BT sporulation medium were treated with alkaline solubilization buffer. Obtained proteins were resolved using SDS-PAGE, stained with Coomassie Briliant Blue.

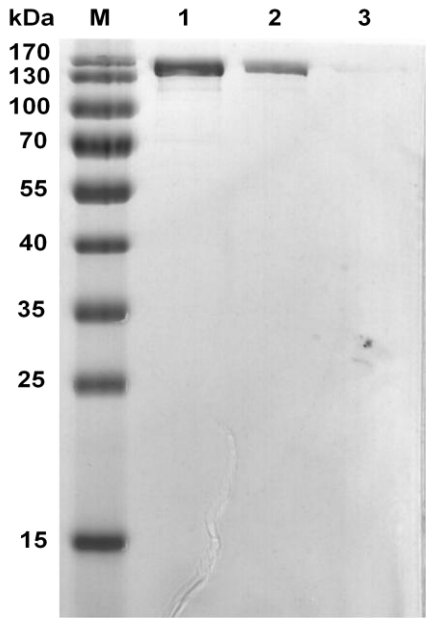

Fig S3 SDS-PAGE image of Cry proteins isolated from *B. thuringiensis* DB27 pure spores (lane 1), mixed culture of spores and vegetative cells (lane 2), pure vegetative cells (lane 3). Analyzed samples were normalized to total protein content. Vegetative cells do not produce Cry proteins, spores showed the highest production.

Fig S4 Multiple sequence alignment (Clustal Omega) of three novel Cry21 protoxins discovered in this study in comparison with Cry21Ba1 toxin, which shows the highest similarity to all three proteins

*Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega*Sievers F, Wilm A, Dineen DG, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins DMolecular Systems Biology 7 Article number: 539doi:10.1038/msb.2011.75

An * (asterisk) indicates positions which have a single, fully conserved residue.
A : (colon) indicates conservation between groups of strongly similar properties.
A . (period) indicates conservation between groups of weakly similar properties.

Colours mark the residues according to their physicochemical properties:

RED – small and hydrophobic
BLUE – acidic
MAGENTA – basic
GREEN - Hydroxyl + sulfhydryl + amine

```
Cry21Ga1      MADLSNIYPIPYNTVSSQYFYQNQLDVDPNGENNPLTKNEQLIEDFKKTLKEKPGNLLTA      60
Cry21Ha1      MVVLNNIYKGPYNVLATPTF----LDTQEGSFDDLITDLQSAWDNFNKT-----GAFS--      49
Cry21Ba1      MADLTELYPSYHNVLARPIR----LDSIFDPFIDIFNALKGGWEEFAKT-----GYKD-P      50
Cry21Fa1      MVILNDIYKRPYNVLANPPIIVE-EGPTPGSFMDIFEDIKKAFEEFQKT-----GNLQ--      52
              *. *.::*   :*.::                 : :   :   ::* **     *

Cry21Ga1      GADIFKDIYNAI-DKQEVDYLSLTTSILGLVSIFVPEIGFVAPLLGLFYRAMG---TGNT      116
Cry21Ha1      -TEVLNQAYKMYENGGSFDYLALFKAGITVVGSVFPEIAPAVPFITMIANFIFPHLFGGT      108
Cry21Ba1      LEQHLKIAWNAS-QNGTIDYLALTKASISFIGL-IPDADAVVPFINMFVDFIFPKLFGEG      108
Cry21Fa1      -TTALQQAWNAY-QGGTIDYLALLKSSLSLVGLLIPGGEAAVPFIGMFLDFVFPKLFGAS      110
                  ::  ::   :   .***:* .: : .:.  .*    ..*:: ::   :     *

Cry21Ga1      TSDPNMNDIFEALKPKIEEMIDSKLTQEQEDFLNKTVEGLQDNLSNYRNAVRTFTIAKQS      176
Cry21Ha1      SDN--KQTIINIIDDEVNRLLNERLEQDKKDELQGYLNGMGNNIKDFGQKIVDTLFNS--      164
Cry21Ba1      SQQNSQAQFFELIIEKVKEIVDQEFRNFTLNTLLNDLDGMQTTLEHFQNDVQIAICQGEQ      168
Cry21Fa1      GSN--SDNVFEIIIKEVKQWTNQQFENFTLNSLNNTLIGIQSNISSFNEMIQIAICQEET      168
               .:     .:: :  :::.  :..: :   : *   : *:  .:. : : :

Cry21Ga1      KNTDKI--------------------KAAKTFLQRTIDIIDQIFTNQLAHLLSSVHVKL      215
Cry21Ha1      --------NKKPLIPNSHSLHDVYQSYSGFIGNVNTVIDQFR-----------LKSYEKM      205
Cry21Ba1      PGLILD-EKHPPCTPTKNHLVSVKESFKNARTSIETVLPHFKNPMTNNKTPDFNSDTVLL      227
Cry21Fa1      PGDDKSSTPSPLCTPTAEHLKNVWTQFQIARTQIEASLPYFKNPMQLDASADFQSNYIML      228
                                        .     ::  :  :             .    :

Cry21Ga1      SLPYYAMMGTLYLALLKDTITNGVEWGYEDVVL----------NTKKYELREKIKTNTER      265
Cry21Ha1      SLPYYCLAVTLVLNVYRDFIRYGKKWIYTITDETDYTTYENYINTAIKNMNQLTSKATKY      265
Cry21Ba1      TLPMYTTAATLNLILHQGYIQFVERWKSVDYDEAFI-------NQTKADLQHRIQEYSTT      280
Cry21Fa1      TLPLYTMAATLNLTLYQSFIQFADKHKDVYYDLGTM-------EQTKANHRKNIKSYTAT      281
              :** *    ** * : :  *    .                   :    : ..  .  :
```

```
Cry21Ga1      ALAVFKSQLQTKLTPLETSDYNKQVDYITGATVNCLDIIRLWPTFDPLDYPIEID--LEL      323
Cry21Ha1      VLNTFKTYL-PTYNYNSKSQLNTYNSYIRSMTISCLDYVAIWPTLNPIDYPIPTQ--LDK      322
Cry21Ba1      VSTTFEKFK-PTLSN-KKSSINTYNKYVRSMTLNCLDIAATWPTLDNVNYPSNVEIQLDQ      338
Cry21Fa1      VRSTVINFQ-PTGDRSNKDKANLFNQYMRVMTVQCLDFVAIWPTLDPFDYPIQTE--LNP      338
              .  .. .    .    .... *   .*:   *:.***    ***:: .:**   :  *:

Cry21Ga1      TRVLRNGIYGDQNITPPQQPFPTDT----WKADSTVDINDLQTGGIGVPIYGNKNLSGIT      379
Cry21Ha1      TRIIFQDIVGPTGKDIHFDLFDIYGK--KLP-------N----NDVFRYSYGGFQLSSAR      369
Cry21Ba1      TRLVFSNLVGPFEGNDDISTYTRRSIMNYSKGDTPGDVN----SAIQSLRYPRLELSKVQ      394
Cry21Fa1      TRIILGDIVGPVEGVGDLQFSYIDADKNPLKNNDPLN----------YFYNGYQLSFAS      387
              **::   : *        .                              *   :**

Cry21Ga1      IHQYSDSSNWFSGPDGITQQYTDGSIISYGKTDQQ----------ASL-----TPIAID      423
Cry21Ha1      F---LTSSY---HPSDIKHCIFSGFTAENINYKSEVTQQNEYDLQNGSLYADSSWSNTIT      423
Cry21Ba1      F---Y---THDQRSNGVRHCYTSGFNLTFNDNSSM-----SAKQ-DESATA---DSPPLT      439
Cry21Fa1      M---NYENIKDNGGRGREHIFPKSLHTSYSNHDGTT---LTFDS-DNSSYN---GSFSFP      437
              :                 :   ..      . .              *         :

Cry21Ga1      NPLFGINFRYP------WPKAFIDLIFFKNQDN----------YYYGNFRYLANHKIHAV      467
Cry21Ha1      SPMYLFNMASQNTMYLDYSALYNSDKYFTIAGCYPLNDNSTGKGYHSNVTSTANHKIQAI      483
Cry21Ba1      APIKNMNANSQNSQYYDYSSINIDN---QGGGGCS---AFP--SYQSNNPILPNQKINVF      491
Cry21Fa1      MPLTAINFASMYNSNEDV--VAVYA---NPGGRMLEGNYYLSYTDKSGNQVIGNQKVQAI      492
               *:  :*                                      .      *:*:...

Cry21Ga1      YPAFLPGDPIPTH----------AVGASFIPLELFPQNILGMPDVNGNIN---AKGFPFE      514
Cry21Ha1      YAMHNNPDV-------EQDGRKRGILASLVPFEVTPTNIIGQTDQNENQK---IQAFPIE      533
Cry21Ba1      YPYGSSAHPIDPHTTDPDTWFKLGYVSSHIPYDLTPQNVIGEIDQDTKQPSLILKGFPAE      551
Cry21Fa1      YPVTSYQNQVWGNG------TKYGLIYTLIPATTSPTIIIKKD-QI--------TTFSAE      537
              *      .                .   : :*    *  ::                 *  *

Cry21Ga1      KSSKN-----SSVRLEWINGSNPVYMKIYDSNLIMNITNITTTNYKIRFRIATNTDNETL      569
Cry21Ha1      KINHL---DYNKIKVERSNGANSISMETG-DVYSIYFTNVLHQNYQIRVRAATSSNNIQI      589
Cry21Ba1      KG--YG--GSIEYVSEPLNGANAAKLTLN-QILYMQVTNLTTQKYQIRLRYATKNDTTAS      606
Cry21Fa1      NTSSFKNSSTNNHKYELINGASAIQLNTK-EEVTYKLTIQDPGRYQIRVRASTNTDSQTL      596
              :          .   *  **:.   :    .     .*     .*:**.* :*..:.

Cry21Ga1      TISIPNLLSPTTIPLPNTSTMTNEF------NDPDTAHAVGPKGEVNSQYVLVDGP----      619
Cry21Ha1      KGSIYNEDLNGTY----VASFQPSLSNTSILNTENPELNNLIEGINGDIYKLFSSSEETL      645
Cry21Ba1      VW-FHIIGPNNQDIINHSPDIPPRSNNKMFVQGEN------------GKYV-LDTLVDSI      652
Cry21Fa1      SFQIKELGFTNSLTLPNTSKYN--NDSQKGLSGEQTTYILIPESKHDGNFI-YQTPTENI      653
                 :                          .  :              :   .

Cry21Ga1      AITIPSGSIELNIE--VNSDDPIYLDRIEFIPIPNQIKFKRIN-----IKGDQVYEIWKD      672
Cry21Ha1      NLTLSPGNYRLEF-SLQTGDTPVILDRVEFIPIPSQIKPQNLN-----IQDNKSYEIWRD      699
Cry21Ba1      E--LPSGQLTILIQNINP-DQDLFLDRIEFVPIPTLPTNPNISIPKTDTSPKDSKVLWEA      709
Cry21Fa1      VNFNRSGTFTLTLQNTNSQPSPIVLDRIEFLSLPPTEFE----IPILSGVENTIYEIWKN      709
                    *   : :          : ***:**: :*                  .    :*.

Cry21Ga1      NDYY----SSYLSGTVIS--IINPAYDNPTVTFEYFDGETSLGTTSKTFNQPEFY-----      721
Cry21Ha1      NNNN---YASYLSGTVVP--IFNPTTDNPIVTFEYFDGEESLGTIPKKYSNVGFD-----      749
Cry21Ba1      SPDIPIANTITLTGSVY---------DFADITFELYKNGNMVTSYPIKGPGPIPHRSHGN      760
Cry21Fa1      DIYY----SKYLSGTIDLFQPWDTENSKINLTFEYFDGNTSLGTITQKYSGPDANKVHD-      764
              .       :  *:*::          .   :*** :.    : :   .

Cry21Ga1      ------GIPKWN------GKKFNRVTVTTSN--FTYSATSTLVFDSLTINDITSKPSQFT      767
Cry21Ha1      ------DIPKWL------GKKFNRVTVTVSN--FTFPPGSGITLANLIIQDIKNKYSQFT      795
Cry21Ba1      YVSCSQGILSYNYENKPVLDGFDQLRININSDPSFYDSNSGCDTKNQYSAEI-KINPNLS      819
Cry21Fa1      -------IEKWT------GKNFNRVTVKISD----LDDSGTIYLENLIVNELTNQPSQFT      807
                     * .:        . *::: :. ..        .     .    :: .   :::

Cry21Ga1      APEDLEKITNQANQLFTSSSQTELANTVTDYRIDQVVLKVNALSDDKFGVEKKALRKLVN      827
Cry21Ha1      TPEDLEKITNQVNQLFTSSSQTELTPTVTDYGIDQMVLKVDALSDDVFGMEKKTLRKLVN      855
Cry21Ba1      ATTDLEKITNQVNQLFTSSSQTELANTITDYRIDQIVMKVDALSNNVFGVEKKALRKLVN      879
Cry21Fa1      APEDLEKITNQVNQLFSSSSQTELAHTVSDYKIDQVVLKVNALSDDVFGVEKKALRKLVN      867
              :  ********.****:*******: *::** ***:*:**:***:: **:***:******
```

```
Cry21Ga1    QAKQLSKARNVLVGGNFEKGHKWVLGRKATMVANHDLFKGDHLLLPPPTLYPSYAYQKID   887
Cry21Ha1    QAKQLSKARNVLVGGNFEKGHEWVLGRKATMVANHDLFKGDHLLLPPPTLYPSYAYQKID   915
Cry21Ba1    QAKQLSKARNVLAGGNFEKGHEWVLGREATMIANHELFKGDHLLLPPPTLYPSYAYQKID   939
Cry21Fa1    QAKQLSKTRNVLVGGNFEKGHEWALGREATMVANHELFKGDHLLLPPPSLYPSYAYQKID   927
            *******:****.********:*.***:***:***:***********:***********

Cry21Ga1    ESKLQSNTRYTVSGFIAQSEHVEVVVSRYGKEVHDMLDVPYEEALPISSDESPNCCKPAA   947
Cry21Ha1    ESKLQPNTRYTVSGFVAQSEHLEVVVSRYGKEVHDMLDVPYEEALPISSDESPNCCKPAA   975
Cry21Ba1    ESKLKSNTRYTVSGFIAQSEHLEVIVSRYGKEVHDMLDVPYEEALPISSDESPNCCKPAT   999
Cry21Fa1    ESKLKSNTRYTVSGFIAQSEHVEVIVSRYGKEVHDMLDVPYEEALPISSDESPNCCKPAA   987
            ****: *********:*****:**:************************************:

Cry21Ga1    CQCQSCDGSKRDSHFFSYSIDVGSVQSDVNLGIELGLRITKPNGFAKISNLEIKEDRPLT   1007
Cry21Ha1    CQCSSCDGSQPDSHFFSYSIDVGSLQSDVNLGIEFGLRIAKPNGFAKISNLEIKEDRPLT   1035
Cry21Ba1    CQCPSCDGSQPDSHFFSYSIDVGSVQSDVNLGIEFGLRIAKPNGFAKISNLEIKEDRPLT   1059
Cry21Fa1    CQCSSCDGSQPDSHFFSYNIDVGSVQSDVNLGIEFGLRIAKPNGFAKISNLEIKEDRPLT   1047
            *** *****: *******.*****:*********:****:*******************

Cry21Ga1    EKEIKKVQRKEQKWKKAFDQEQTEVTARLQPTLDQINALYQNEDWNGSLHPHVTYQDLSA   1067
Cry21Ha1    EKEIKKVQRKEQKWKKAFDQEQAEVAATLQPTLDQINALYQNEDWNGSLHPHVTYQHLSA   1095
Cry21Ba1    DQEIKKIQRKEQKWKKAFDQEQAEVAATFQPTLDQINALYQNEDWNGSLHPHVTYQHLSA   1119
Cry21Fa1    EKEIKKVQRKEQKWKKAFDQEQAELATTLQPTLDQINALYQNEDWNSSLHSHVTYQHLSA   1107
            ::****:****************:::: :*****************.*** *****.***

Cry21Ga1    VVVPTLPKQRHWFMEDRKGEHSGLTQQFQQALDRAFQQIEEQNLIHNGSFTNGLTDWTVT   1127
Cry21Ha1    VVLPTLPKQRHWFMEDREGEHYSVTQQFQQALDRAFQQIEEQNLIHNGSFANGLTDWTVT   1155
Cry21Ba1    VVLPTLPKQRHWFMEDREGEHYGVTQQFQQALDRGFQQIEEQNLIHNGSFANGLTDWTVT   1179
Cry21Fa1    VVLPALPKQRHWFMEDREGEHYGVTQQFQQALDRAFQQIEEQNLIHNGSFANGLTDWTVT   1167
            **:*:************:*** .:**********.***************:*********

Cry21Ga1    GDAQLTIFDEDPVLELAHWDASVSQTIEIMDFEEETEYKLRVRGKGKGTVTVQHGEEELE   1187
Cry21Ha1    GDAQLTIFDEDPVLELAHWDASVSQTIEIMDFEEDTEYKLRVRGKGKGTVTVQHGEEELE   1215
Cry21Ba1    GDAQLTIFDEDPVLELAHWDASVSQTIEIMDFEEETEYKLRVRGKGKGTVTVQHGEEELE   1239
Cry21Fa1    GDAQLTIFDEDPVLELAHWDASVSQTIEIMDFEEDTEYKLRVRGKGKGTVTVQHGEEELE   1227
            ************************************:************************

Cry21Ga1    TMTFNTTSFTTQEQTFYFEGKTVDVHVQSENNTFLVDSVELIEVVEE-  1234
Cry21Ha1    TMTFNTTSFTTQEQTFYFEGDTVDVHVQSENNTFLVDSVELIEVVEEE  1263
Cry21Ba1    TMTFNTTSFTTQEQTFYFEGDTVDVHVQSENNTFLVDSVELIEVVEE-  1286
Cry21Fa1    TMTFNTTSFTTQEQTFYFEGNTVDVHVQSENNTFLVDSAELIEVVEEE  1275
            ********************.*****************.********
```

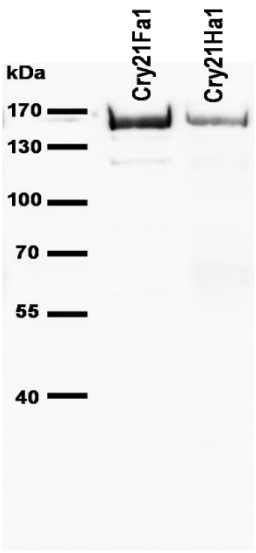

Fig S5 Representative western blot image of purified Cry21Fa1 and Cry21Ha1 proteins. Western blot was used to confirm that size-selected proteins in chromatography-separated fractions are proteins of interest. Anti-His primary antibodies were used in 1:1000 dilution. Horseradish peroxidase-conjugated secondary antibody (1:5000) and enhanced chemiluminescence were used to visualize the signal.

Table S1 Log-rank statistical analysis of survival curves

Figures	Treatment[a]	Mean survival±SD[b]	Comparison[c]	P value[d]
1A	DB27 veg. cells	20.0±0.0[3]	Veg. cells vs mix cells/spores	<0.00001
	DB27 mix cells/spores	10.7±0.6[3]	Veg. cells vs spores	<0.00001
	DB27 spores	5.4±0.48[3]	Mix cells/spores vs spores	<0.0001
1G	*C. elegans* N2	16.0±0.0[3]	*C. elegans* N2 vs *P. strongyloides*	<0.0001
	P. strongyloides	35.8±3.79[3]		
	O. carolinensis	50.0±4.1[3]	*C. elegans* N2 vs *O. carolinensis*	<0.0001
1H	*C. elegans* N2	10.7±0.6[3]	*C. elegans* N2 vs *P. redivivus*	<0.001
	P. redivivus	7.8±0.43[3]		
3A	Vector	n. a.[*]	Vector vs Fa1	<0.00001[***]
	Cry21Fa1	42.0±2.23[2]	Vector vs Ga1	0.82[**]
	Cry21Ga1	n. a.[*]	Vector vs Ha1	<0.00001[**]
	Cry21Ha1	84.4±6.18[2]	Fa1 vs Ha1	<0.0001
3D	Cry21Fa1+vector	83.3±5.19[2]	Fa1+v vs Fa1+Ha1	<0.0001
	Cry21Ha1+vector	109.2±4.07[2]	Ha1+v vs Fa1+Ha1	<0.0001
	Cry21Fa1+Cry21Ha1	55.0±3.37[2]	Fa1+v vs Ha1+v	<0.001
	Cry21Ha1+Cry21Ga1	104.4±4.69[2]	Fa1+v vs Fa1+Ga1	0.612
	Cry21Ga1+Cry21Fa1	80.2±4.63[2]	Fa1+v vs Fa1+Ha1+Ga1	0.014
	Cry21Ha1+Cry21Fa1+ Cry21Ga1	63.9±4.84[2]	Ha1+v vs Ha1+Ga1	0.525
			Ha1+v vs Fa1+Ha1+Ga1	<0.0001
3E	N2 Cry21Fa1	47.05±1.23[2]	N2 vs *nasp-1* Cry21Fa1	<0.0001
	N2 Cry21Ha1	83.3±6.26[2]	N2 vs *nasp-1* Cry21Ha1	<0.001
	nasp-1 cry21Fa1	67.25±4.66[2]		
	nasp-1 cry21Ha1	114.0±4.88[2]		
4A	N2 Cry21Fa1	42.8±1.95[2]	N2 vs *bre-2* Fa1	0.804
	N2 Cry21Ha1	84.4±6.18[2]	N2 vs *bre-2* Ha1	0.532
	bre-2 Cry21Fa1	44.0±2.43[2]	N2 vs *bre-3* Fa1	0.552
	bre-2 Cry21Ha1	89.6±6.4[2]	N2 vs *bre-3* Ha1	0.303
	bre-3 Cry21Fa1	44.8±2.35[2]		
	bre-3 Cry21Ha1	93.6±6,25[2]		
4B	N2 wt	41.6±1.89[2]	N2 vs *xbp-1*	0.006
	xbp-1	34.4±1.75[2]	N2 vs *jun-1*	0.012
	jun-1	34.4±1.93[2]	N2 vs *pmk-1*	<0.0001
	pmk-1	27.6±0.91[2]	N2 vs *kgb-1*	0.223
	kgb-1	38.8±1.77[2]		

Mean survival and statistical significance were calculated for each experiment as detailed in Materials and Methods.

[a] Treatment indicates which nematode species, mutant genotype and/or toxins were analysed.

[b] Mean survival and standard error for indicated treatment. Each treatment was tested in at least three replicates and repeated at least two times. Superscript indicates the number of repetitions of that experiment. The reported mean is based on the average survival curve of the replicates of the representative experiment.

[c] Comparison shows which treatments are statistically compared.

[d] P value for a given comparison.

n. a. * survival curves for these treatments are not completed, therefore it is not possible to calculate mean survival time

** P values for time point 120 hours

*** P value for time point 48 hours

Table S2 Potential virulence factors of *B. thuringiensis* DB27 detected by whole genome sequencing

Potential virulence factor	Function	Quantity	Location
Microbial collagenase	Peptidase M9	1	plasmid
Hemolysin BL	enterotoxin	3	plasmid
NheA, NheB, NheC	non-hemolytic enterotoxin	1	chromosome
Phospholipase (pipls, cerA, cerB)	lipase	3	chromosome
Collagenase	Peptidase M9	2	chromosome
Immune inhibitor A	InhA peptidase M6 superfamily	3	chromosome
Chitinase	chitinase	2	chromosome
Lipase	lipase	9	chromosome
Bacillolysin	Neutral protease	4	chromosome
Proteases	protease	>40	chromosome
Enhancin	mettaloprotease	1	chromosome
Hemolysin(CytK, Hly3)	cytotoxins	3	chromosome

Table S3 Features of *B. thuringiensis* DB27 plasmids and Cry toxins detected by whole genome sequencing

Plasmid name	Plasmid size, bp	Detected Cry toxins	% of protein similarity to known toxins	Designated new names	GenBank accession number
pDB27210	201029	Cry21Ba1-like	54	Cry21Fa1	KF701307
pDB27104	104550	-			
pDB2743	43904	-			
pDB278	8003	Cry21Ba1-like	49	Cry21Ga1	KF771885
pDB276	6525	Cry21Ba1-like	50	Cry21Ha1	KF771886
pDB275	5336	-			
pDB274	4121	-			