Supplementary information for

"Division of labour and terminal differentiation in a novel *Bacillus thuringiensis* strain"

by Deng et, al.

Text S1: DNA manipulation and transformation

Plasmid DNA was extracted from *E. coli* using QIAprep spin columns. Amplified DNA was purified using the QIAquick PCR purification Kit and digested DNA was purified using the QIAquick gel extraction Kit (QIAgen, France). Restriction enzymes, *Taq* DNA polymerase and T4 DNA ligase (New England Biolabs, USA) were used according to the manufacturer's instructions. Oligonucleotide primers (Table S2) were synthesized by Sigma-Proligo (Paris, France) and all constructs were confirmed by sequencing (Beckman Coulter Genomics, Takeley, UK). The transformation of *E. coli* was performed by a standard chemical competent method and *B. thuringiensis* was transformed by electroporation as described previously (Lereclus et al 1989).

Text S2: Microscopy

Strains were cultured in appropriate medium and were harvested at the indicated time points. The cells were observed with a Leica TCS SL Microsystem (Leica, Wetzlar, Germany) or a ZeissAxio Observer.Z1 system (Zeiss, Göttingen, Germany). Transmission electron microscopy (TEM) was performed with a New Bio-TEM H-7500 electron microscope (Hitachi Ltd., Japan) at an accelerating voltage of 80 kV as previously described (Yu et al 2006).TEM was performed using samples of T16 in SSM medium (the end of the exponential growth phase is defined as T_0, and T_n represents *n* hours after T_0).Laser confocal microscopy was performed with a Leica TCS SL laser confocal microscope. Cells were cultured in SSM medium, harvested at the time points indicated, washed with ddH_2O, and stained with a vital membrane stain, FM®-4-64 (Life Technologies, USA) as described previously(Yang et al 2012).

Text S3: Biochemical and microbiological phenotypic characterization

To test the hemolysis, ampicillin resistance, and motility of *B. thuringiensis* strains, cells were cultured in LB liquid medium until OD600 ≈ 1. One μl of each culture was spotted onto the following culture plates: a blood plate (Columbia agar + 5% sheep blood, bioMérieux, France), an ampicillin plate (LB with 1.3% agar + 100μg/ml ampicillin), or a semisolid plate (LB + 0.3% agar). Photos were taken after incubation at 37 ℃ for 24 h. The *B. thuringiensis* strain 407 Cry^-(Lereclus et al 1989) and its isogenic *plcR* mutant strain 407*ΔplcR* (Salamitou et al 2000) were used as controls.

Text S4: Genomic analysis

The 16s rRNA gene of LM1212 was identified by nucleotide blast (BioEdit) using the *B. thuringiensis* HD73 16s rRNA as input against the LM1212 genome (Hall 1999). The16s RNA gene was used to do a NCBI nucleotide BLAST to construct a phylogenetic tree. Twenty-five strains among the BLAST results (one *B. weihenstephanensis*, eight *B. thutingiensis*, ten *B. cereus*, five *B. anthracis*, and one *Bacillus sp.*) were selected for distance tree analysis with Neighbor Joining method. The Newick file of analysis result was displayed by MEGA 4.0 (Tamura et al 2007). Nucleotide CLUSTAL analysis with MEGA 4.0 was used for 16s rRNA typing, based on the typing method of Sacchi*et al.*(Sacchi et al 2002). For Multiple Locus Sequence Typing (MLST) analysis, the partial nucleotide sequences of seven LM1212 house keeping genes (http://pubmlst.org/bcereus/) (Priest et al 2004) were obtained. These sequences were linked as one query (2,838 bp in total) and used as input for an NCBI BLAST as 16s rRNA analysis. Delta-endotoxin genes in LM1212 were identified by protein sequence alignment against a local Bt toxins database.

Text S5: Crystal purification and peptide mass spectrometry identification

For crystal purification, LM1212 or HD73 Cry^- (HD73 acrystalliferous strain) harboring *Cry* gene expression plasmids were cultured on a HCT agar plate for 48h at 30 ℃. Cells were harvested from the plates with physiological saline and sonicated twice for 1 min. The crystal inclusions were purified

using a 72%-79% sucrose gradient and then analyzed by10% SDS-PAGE. The protein bands in SDS-PAGE were excised for analysis by the matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS) with an Ultraflex III TOF/TOF platform (BrukerDaltonik GmbH, Germany). MS data were screened against anin-houseLM1212 protein database using the Mascot software (Matrix Science)

Text S6: Bioassay of LM1212

For mammal infection assay, 7-8 week-old female mice were treated by hypodermic injection of 100 μL vegetative cells suspension (1.6×10^8 LM1212 cells or 3.5×10^7 A16R cells) or by intragastric administration of 100 μL spores suspension (2.5×10^8 spores). The *B. anthracis* vaccine strain A16R (Liu et al 2013) was used as a positive control. Each assay was performed on 10 mice.

Bt strains were grown in PB medium (0.5% peptone, 0.3% beef extract) for 40 h, and crystal-spore mixtures were prepared by centrifugation and lyophilized. The protein concentration was quantified with SDS-PAGE analysis and the Image Master 1D Elite software (Amersham Biosciences). Lyophilized spore-crystal mixtures were then diluted in 0.005% Triton X-100. The assays for *Plutella xylostella* (2^{nd} instar larvae) and *Colaphellus bowringi* (1^{st} instar larvae) were performed by a leaf dip method as previously described (Yan et al 2009). The assays for *Agrotis ypsilon* (1^{st} instar larvae) and *Spodoptera exigua* (2^{nd} instar larvae) were performed with an artificial diet method as previously described (Wang et al 2006). *B. thuringiensis* HD1 strain was used as a positive control for *Plutella xylostella* and *Agrotis ypsilon* assays. *B. thuringiensis* Bt22 strain was used as a positive control for *Colaphellus bowringi* assay. *B. thuringiensis* G03 strain (Wang et al 2006) was used as a positive control for *Spodoptera exigua* assay. All these larvae were tested with the spore-crystal mixture with a final protein concentration ~ 25 (LM1212)/ 10 (positive control) μg/ mL (for leaf dip method) or 25 (LM1212)/ 10 (positive control) μg/ g (for artificial diet method) and each assay was repeated three times. The assay for *Aedes aegypti* (4^{th} instar larvae) was performed using purified LM1212 crystals (at a final protein concentration of 0.025/ 0.25/ 5/ 10/ 25 μg/ mL) by the method previously described (Yuan et al 2001). A crystal preparation from *B. thuringiensis* Bti strain (Tilquin et al 2008) was used as a positive control at a final concentration of 0.25 μg/ mL. This assay was repeated twice.

The cytotoxicity of purified, protease-activated LM1212 crystals was tested in six cancer cell lines (Caco-2, HL60, Jurkat, HepG2, Hela and MOLT4) at a final concentration of 10 μg/ mL by the method previously described (Mizuki et al 1999). Crystals from HD73 Cry$^-$(p8E'*cry41Ca*) (containing Cry41Ca1) and HD73 Cry$^-$(p8E'*cry45Ba*) (containing Cry45Ba1, Cry74Aa, and NT32-like proteins) were purified and their cytotoxicity was tested in two cell lines (Caco-2 and HeLa) after protease-activation, at a final concentration of 30 μg/ mL. Two parasporins, PS1Aa1 (Cry31Aa1) (Mizuki et al 2000) and PS4Aa1 (Cry45Aa1) (Okumura et al 2011) were used as positive controls at a final concentration of 10 μg/ mL. Each assay was repeated three times.

Text S7: Plasmid constructions

The primer sets pcry32-83F/ pcry32-83R orpcry35-F/pcry35-R2 were used to amplify the promoter regions of *cry32Wa1* (p32) or *cry35-like* (p35) transcription units, respectively. LM1212 chromosomal DNA was used as template. The PCR product was digested with *Pst*I and *Bam*HI and the resulting fragments were ligated into a promoter-less *lacZ* reporter plasmid, pHT304-18Z (Agaisse and Lereclus 1994), cut with the same restriction enzymes, to generate p32'Z and p35'Z.

To construct *gfp* reporter plasmids, the promoter regions were amplified using pcry32-83-gfpF/pcry32-83-gfpR and pcry35-F/pcry35-R as primers. The PCR product was digested with *Kpn*I and *Bam*HI and then ligated into the promoterless *gfp* reporter plasmid, pHT-*gfp* (Yang et al 2012), cut with the same restriction enzymes, to generate p32'*gfp* and p35'*gfp*. The primer set pcry1Ac-F/pcry1Ac-R2 was used to clone the promoter region of *cry1Ac* gene.HD73 chromosomal DNA was used as a template. The primers pKan-F/pKan-R were used to amplify the promoter region of the *aphA3* from the pDG783 plasmid (Guerout-Fleury et al 1995). These two promoters were digested with *Kpn*I/*Xba*I and then cloned into the *Kpn*I/*Xba*I sites of pHT-*gfp* to create p1Ac'*gfp* and p*aphA*'*gfp*, respectively. The primers *gfp*-RBS/304R2 were used to clone the linker-*gfp* (l-*gfp*) fragment of pHT-*gfp* and to add a 16 bp ribosomal binding site (RBS) and start codon region (AGGAGGTTTACGC ATG) at the 5' end of l-*gfp*. This product was then digested with *Xba*I/*Hin*dIII

and inserted into pHT315 (Arantes and Lereclus 1991) cut with the same restriction enzymes, to generate the promoter-less plasmid with an RBS and a start codon, pHT-R-l-*gfp*.
The plasmid p1Ac'*cry35*, which harbors a transcriptional fusion between the promoter of the *cry1Ac* gene and the coding sequence of the *cry35-like* genes, was constructed by overlapping PCR: the *cry1Ac* promoter region (p_{cry1Ac}) was amplified by the primers pcry1AcBam-F/pcry1Ac-RL and the *cry35-like* operon (without its promoter region) was amplified with the primer pair cry35-FL/cry35opHind-R. These two PCR products were then mixed and amplified by the primers pcry1AcBam-F/cry35opHind-R. The products were digested with *Bam*HI/*Hin*dIII and then inserted into pHT315, digestedwith the same restriction enzymes, to generate p1Ac'*cry35*. The plasmids p8E'*cry32Wa*, p8E'*cry41Ca* and p8E'*cry45Ba*, in which the *cry* genes are under the control of *cry8Ea* promoter, were constructed by inserting the promoterless transcriptional units of *cry32Wa1* (primers: cry32F/cry32R), *cry41Ca1* (cry41CaF/cry41CaR), and *cry45Ba1* (cry45F/cry45R) into the *Bam*HI/*Sal*I sites of the expression plasmid pHT315-8E21b (laboratory stock).

Text S8: Competition experiment
Colonies of LM1212, HD73 and HD73 Cry$^-$ strains transformed with p35'Z were cultured in LB with antibiotics until an OD600 ≈ 3-5. These cultures were then diluted 100x in fresh LB medium with antibiotics. When the bacteria had reached exponential phase (OD600 ≈ 1-2), the cultures were adjusted to an OD600 = 1.0 using ddH_2O. HCT medium was inoculated with either LM(p35'Z)/ HD73(p35'Z) or LM(p35'Z)/ HD73 Cry$^-$(p35'Z) in various ratios (LM1212: HD73 or LM1212: HD73 Cry$^-$ =1:0, 9:1, 2:1, 1:1, 1:2, 1:9, 0:1). The total volume of the inoculum was 1% of the culture volume in all cases. The cells were cultured until more than 95% of the spores were released (as observed under the microscope, after approximately 48 h). A 1 mL sample of each culture was treated at 65 ℃ for 25 min, to kill any remaining vegetative cell. The heat-resistant spores were serially-diluted and plated on HCT agar containing antibiotics and 60 mg/mL X-gal and incubated at 30 ℃ for 2 days. The blue clones (corresponding to LM1212) and the white or very light blue clones (corresponding to HD73 and HD73 Cry$^-$) were counted and the spore yields were calculated and presented as spores/ mL culture.

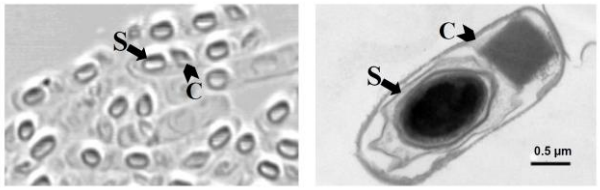

Fig. S1 Picture of *B. thuringiensis* HD73 by: A) optical micrograph and B) transmission electron microscopy (TEM). The spore and crystal are present in the same mother cell

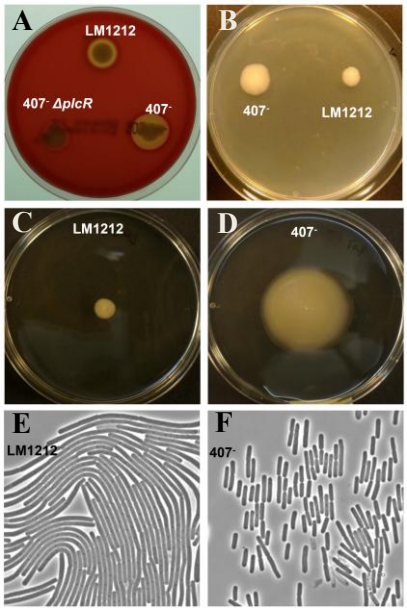

Fig. S2 Phenotypic analysis of LM1212. Cells were cultured in LB until OD600= 1 and examined under a microscope (E and F) or cultured on plates for 24 h at 37 °C (A – D). LM1212 is: A) hemolytic on sheep blood agar, and B) resistant to 100 µg /mL of ampicillin. C) LM1212 has no motility when cultured on 0.3% agar. D) 407$^-$ is mobile on 0.3% agar. E) LM1212 shows a more pronounced long-chain phenotype than F) 407$^-$. (407$^-$ = *B. thuringiensis* strain 407 Cry$^-$; 407$^-$*ΔplcR* = *plcR* mutant strain of 407$^-$)

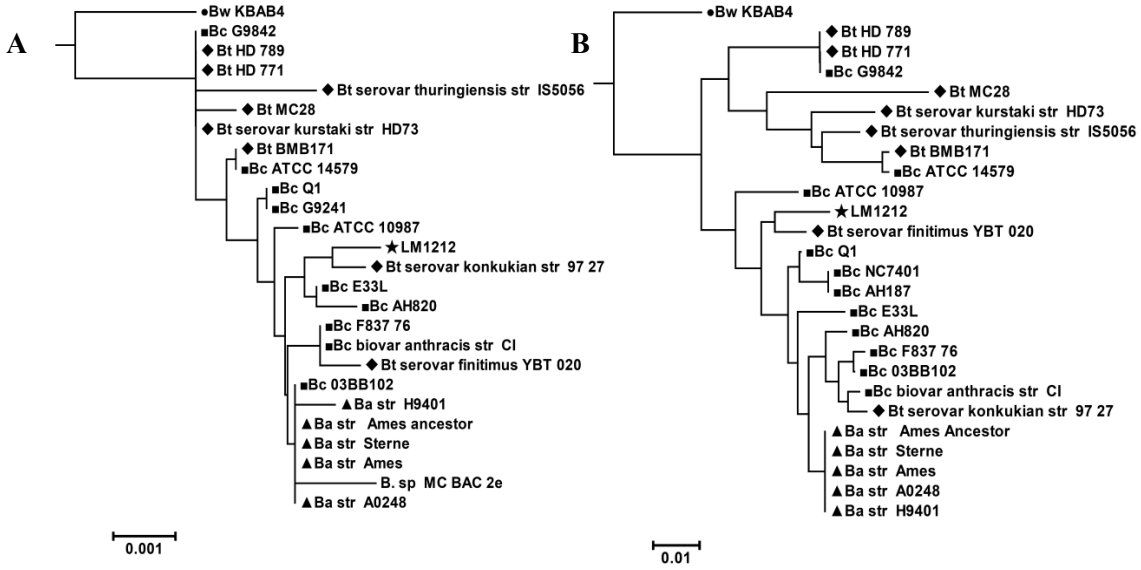

Fig. S3 Genetic typing analysis of LM1212. A) 16s rRNA analysis. LM1212 is most closely related to *B. thuringiensis* serovar konkukian strain 97-27. B) Multiple Locus Sequence Typing (MLST) analysis based on partial sequences of seven house-keeping genes. LM1212 is most closely related to *B. thuringiensis* serovar finitimus strain YBT-020. (▲ Ba = *B. anthracis*, ■ Bc = *B.cereus*, ◆ Bt = *B. thutingiensis*, ● Bw = *B. weihenstephanensis*,B. sp = *Bacillus sp.* ★ = LM1212. Distance is noted at lower-left of each graph.)

Fig. S4 Protein comparison analysis. A) Comparison of Cry32Wa1 and Cry41Ca1 with parasposin PS3Aa1. B) Comparison of Cry74Aa1 and Cry45Ba1 with parasposin PS4Aa1. C) Comparison of Cry35-like and Cry35-like2 with Cry35Ac1. D) Comparison of Cry35-like and Cry35-like2 with *Lysinibacillus sphaericus* BinA. E) Comparison of Cry35-like and Cry35-like2 with *Lysinibacillus sphaericus* BinB. F) Comparison of LM1212 Lef-like protein with Chain A of Lef protein of *B. anthracis* (Accession: PDB: 4DV8_A). Comparisons were done by ClustalW2 using default parameters.

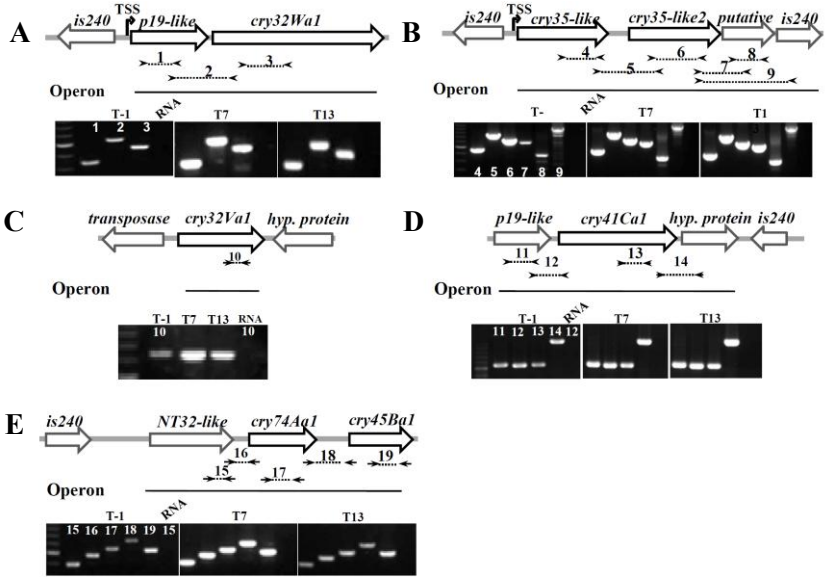

Fig. S5 Structures and RT-PCR analysis of *cry/cyt* genes in LM1212. A) *cry32Wa1* forms a transcription unit with an upstream *p19-like* ORF. B) *cry35-like* and *cry35-like2* are in the same transcription unit as the downstream putative ORF and *is240*. C) The *cry32Va1* gene is monocistronic. D) *cry41Ca1* co-transcribes with its upstream *p19-like* ORF and downstream hypothetical protein ORF. E) The *NT32-like* ORF, *cry74Aa1* and *cry45Ba1* are in the same transcriptional unit. Cells were cultured in SSM medium and RNAs were extracted at given time points (T0, T7 and T13,). The double arrow with dashed line indicates the location and length of RT-PCR amplicons. The numbers above the dashed lines (1 to 19) annotate the primers used and the corresponding RT-PCR products are indicated with the same number. Solid lines bellow ORFs indicate operons. Transcription start sites (TSS) are indicated by broken arrows. PCR of reverse transcriptase negative reactions was used as a control for DNA contamination. The *cry32Wa1* and *cry41Ca1* operons were highly similar in sequence and organization. Similar to the gene encoding parasporin PS2Ab which was co-transcribed with an upstream *p19* gene (Hayakawa et al 2007), *cry32Wa1* or *cry41Ca1* formed a transcription unit with an upstream *p19-like* ORF (A and D).

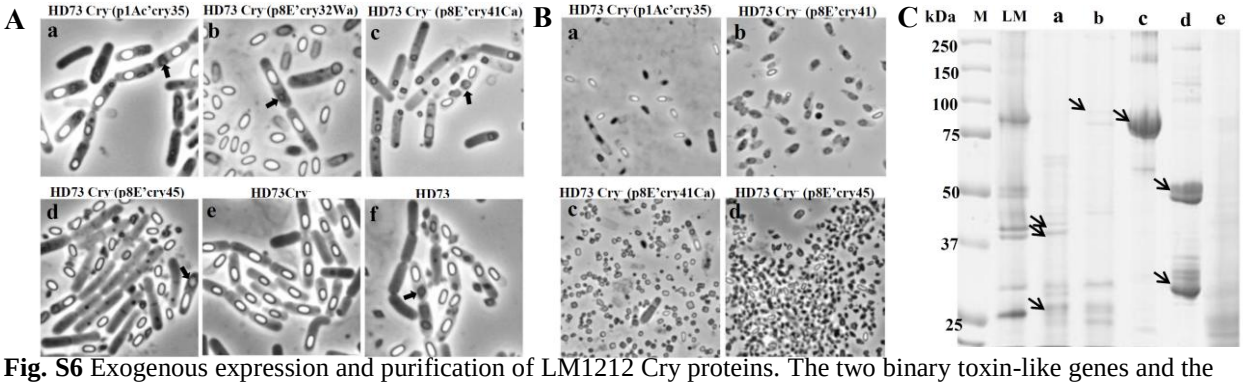

Fig. S6 Exogenous expression and purification of LM1212 Cry proteins. The two binary toxin-like genes and the four parasporin-like genes in four operons were expressed in the HD73Cry⁻ strain and purified. A) Expression of LM1212 *cry* genes in HD73 Cry⁻ strain. Strains harboring a) p1Ac'*cry35* and b) p8E'*cry32Wa* can form irregular inclusions. Strains harboring c) p8E'*cry41*Ca and d) P8E'*cry45Ba* can form crystals. No inclusion was detected in e) HD73 Cry⁻ strain. f) The wild type HD73 strain can form rhombic crystals. Inclusions/ crystals are indicated by arrows. Bacteria were cultured on HCT agar plates for 48h at 30 ℃. B) Purification of inclusions/ crystals by sucrose gradient centrifugation. a) and b): inclusions of HD73 Cry⁻(p1Ac'*cry35*) and HD73 Cry⁻ (p8E'*cry32Wa*) could not be efficiently purified. c) and d): Crystals were efficiently purified from HD73 Cry⁻ (p8E'*cry41Ca*) and HD73 Cry⁻(p8E'*cry45Ba*). C) SDS-PAGE analysis of purified inclusions/ crystals. All proteins expected to be present were detected (indicated by arrows). M: protein standard. LM: crystals of LM1212 (8 μg). a)- d): inclusions/ crystals showed in B) e): sample of crude HD73 Cry⁻. a), b) and e):50 μl samples, c) and d):5 μl samples.

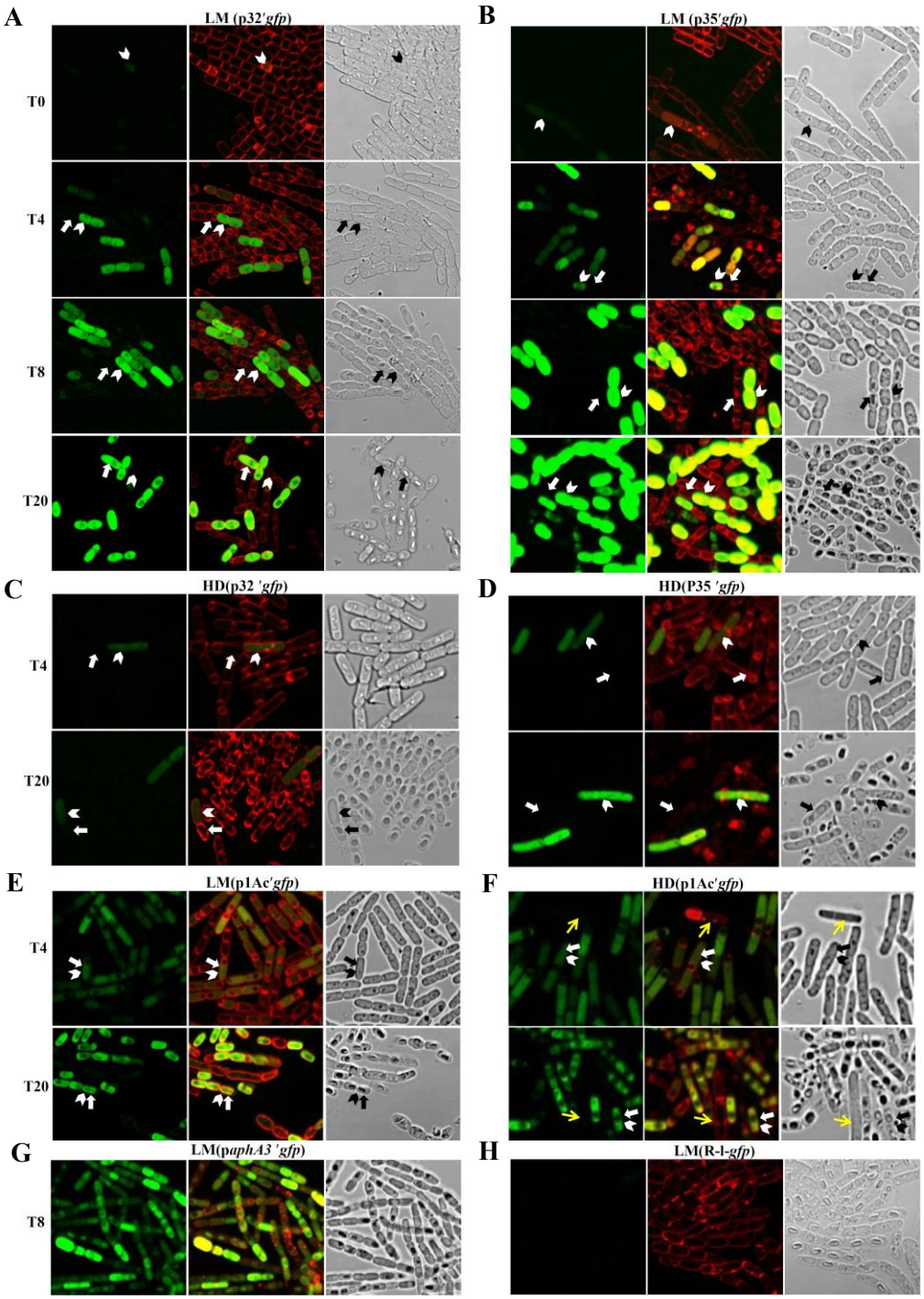

Fig. S7 The activity of LM1212 *cry* gene promoters as assessed using a *gfp* reporter gene. The activity of LM1212 *cry* promoters is restricted to non-sporulating cells in both LM1212 and HD73 strains. This contrasts to the sporulation-dependent *cry1Ac* promoter. A) and B) The activity of LM1212 *cry* gene promoters is restricted to the subpopulation of crystal-producers in LM1212. C) and D) The activity of LM1212 *cry* gene promoters is restricted to a small subpopulation of non-sporulating cells in HD73. E) The activity of the sporulation-dependent *cry* gene promoter P_{cry1Ac} is restricted to the subpopulation of spore-formers in LM1212. F) The activity of $P_{cry1Ac}$is restricted to sporulating cells in HD73. Non-sporulating cells are indicated by yellow arrows. G) The constitutive promoter P_{aphA3} is active in all cells of LM1212. H) The promoter-less plasmid pHT-R-l-*gfp* has very low background expression in LM1212. Left: GFP; Middle: GFP/ FM®-4-64 overlay; Right: bright field image. Sporulating cells (cell with asymmetric septum, prespore, or spore) are indicated by arrows and GFP-expressing cells are indicated by arrow heads. Cells were cultured in SSM medium with erythromycin. The cell walls were stained with FM®-4-64.

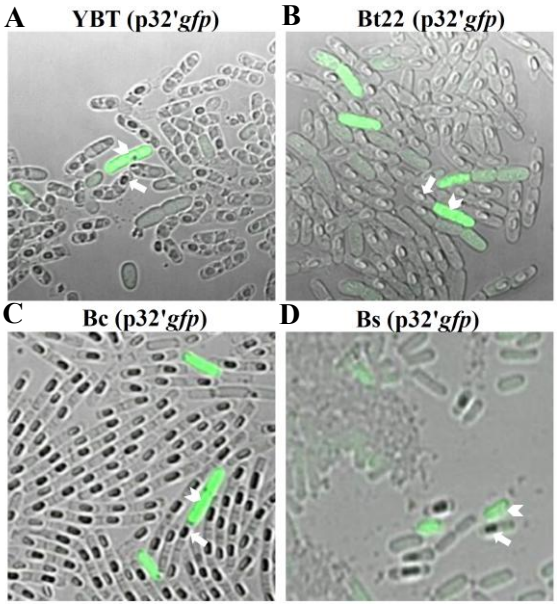

Fig. S8 Activity of LM1212 *cry* gene promoter in different strains and species. $P_{cry32Wa}$ is active in the small subpopulation of non-sporulating cells in A) *B. thuringiensis* strain YBT1520, B) *B. thuringiensis* strain BT22, C) *B. cereus* strain ATCC14579 and D) *B. subtilis* strain 168. Figures are overlay of GFP and bright field images. Spores are indicated by arrows and GFP-expressing cells are indicated by arrowheads. Cells were cultured in SSM medium for 24 h at 30 ℃.

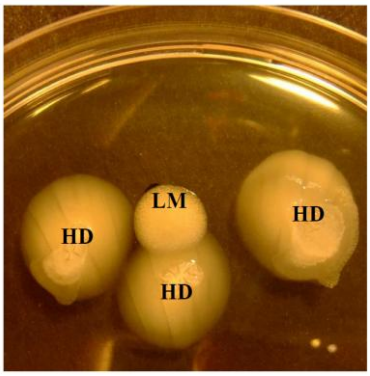

Fig. S9 There is no mutual antagonism between LM1212 and HD73 on agar plates. Cells were cultured on 0.3% agar plate for 24 h at 30 ℃. LM = LM1212, HD = HD73

Table S1 Strains and plasmids

Strains and plasmids	Description	Resource
***Bacillus thuringiensis* strains**		
LM1212	Wild-type strain	a
HD73	Wild-type strain carrying plasmid pHT73 containing *cry1Ac* gene	b
HD73 Cry$^-$	HD73 acrystalliferous strain by curing the pHT73 plasmid	b
LM (p35'Z)	LM1212 strain carrying p35'Z	a
HD73 (p35'Z)	HD73 strain carrying p35'Z	a
HD73$^-$(p35'Z)	HD73 Cry$^-$ strain carrying p35'Z	a
LM(p32'z)	LM1212 strain carrying p32'Z	a
HD73(p32'Z)	HD73 strain carrying p32'Z	a
HD73Cry$^-$(p32'Z)	HD73 Cry$^-$ strain carrying p32'Z	a
LM(p32'*gfp*)	LM1212 strain carrying p32'*gfp*	a
HD73(p32'*gfp*)	HD73 strain carrying p32'*gfp*	a
LM(p35'*gfp*)	LM1212 strain carrying p35'*gfp*	a
HD73(p35'*gfp*)	HD73 strain carrying p35'*gfp*	a
HD73 Cry$^-$(p1Ac'*cry35*)	HD73 Cry$^-$ strain carrying p1Ac'*cry35*	a
HD73 Cry$^-$(p8E'*cry32Wa*)	HD73 Cry$^-$ strain carrying p8E'*cry32Wa*	a
HD73 Cry$^-$(p8E'*cry41Ca*)	HD73 Cry$^-$strain carrying p8E'*cry41Ca*	a
HD73 Cry$^-$(p8E'*cry45Ba*)	HD73 Cry$^-$strain carrying p8E'*cry45Ba*	a
407 Cry$^-$	407 acrystalliferous strain	(Lereclus et al 1989)
407Δ*plcR*	407 Cry$^-$ strain deleted of *plcR*	(Salamitou et al 2000)
G03	Wild type strain	(Wang et al 2006)
HD1	Wild type strain	b
Bt22	Wild type strain	b
YBT1520	Wild type strain	b
Bt22(p32'*gfp*)	Bt22 strain carrying p32'*gfp*	a
YBT(p32'*gfp*)	YBT1520 strain carrying p32'*gfp*	a
LM(p*aphA*'*gfp*)	LM1212 strain carrying p*aphA*'*gfp*	a
LM(R-l-*gfp*)	LM1212 strain carrying pHT-R-l-*gfp*	a
LM(p1Ac'*gfp*)	LM1212 strain carrying p1Ac'*gfp*	a
HD(p1Ac'*gfp*)	HD73 strain carrying p1Ac'*gfp*	a
Other *Bacillus* strains		
A16R	*Bacillus anthracis* vaccine strain	(Liu et al 2013)
B. cereus ATCC14579	*Bacillus cereus* type strain	b
B. subtilis 168	*Bacillus subtilis* model strain	b
Bc(p32'*gfp*)	*B. cereus* ATCC14579 strain carrying p32'*gfp*	a
Bs(p32'*gfp*)	*B. subtilis* 168 strain carrying p32'*gfp*	a
Plasmids		
pHT304-18Z	*B. thuringiensis-E. coli* shuttle vector harboring the promoterless *lacZ* gene	(Agaisse and Lereclus 1994)
p32'Z	pHT304-18Z carring the *lacZ* gene under the control of the $P_{cry32Wa}$	a
p35'Z	pHT304-18Z carrying the *lacZ* gene under the control of the $P_{cry35\text{-}like}$	a
pHT-*gfp*	pHT315 containing promoterless *gfp* gene with linker	(Yang et al 2012)
pHT-R-l-*gfp*	pHT-*gfp* with an RBS and a start codon	a
p1Ac'*gfp*	pHT-*gfp* carrying the *gfp* gene under the control of the P_{cry1Ac}	a
p*aphA*'*gfp*	pHT-*gfp* carrying the *gfp* gene under the control of the P_{aphA3}	a
p32'*gfp*	pHT-*gfp* carrying the *gfp* gene under the control of the $P_{cry32Wa}$	a
p35'*gfp*	pHT-*gfp* carrying the *gfp* gene under the control of the $P_{cry35\text{-}like}$	a
pHT315	*B. thuringiensis-E. coli* shuttle vector	(Arantes and Lereclus 1991)
p1Ac'*cry35*	pHT315 carrying the *cry35* operon under the control of P*cry1Ac*	a
pHT315-8E21b	pHT315 carrying the *cry8Ea* promoter	b
p8E'*cry32Wa*	pHT315-8E21b carrying the *cry32Wa* operon under the control of the P_{cry8Ea}	a
p8E'*cry41Ca*	pHT315-8E21b carrying the *cry41Ca* operon under the control of the P_{cry8Ea}	a
p8E'*cry45*	pHT315-8E21b carrying the *cry45* operon under the control of the P_{cry8Ea}	a

a: this study. b: laboratory stock

Table S2 Oligonucleotide primers

Oligo	Sequence	Note
RT1-F	gaggagatacccatggaagag	
RT1-R	cgttcgcaatataaggcatac	
RT2-F	gtagaaggagaatgcgggaaa	
RT2-R	ggatagaaagcgctgtacgaa	
RT3-F	aggtttatgaaagcggtagag	
RT3-R	gattcggtgctaagttgtaag	
RT4-F	ctgttaccgtatccaatgct	
RT4-R	tcaagaaatgtccaagggtc	
RT5-F	gacccttggacatttcttga	
RT5-R	ggaaactgttcttggtcgtt	
RT6-F	aacgaccaagaacagtttcc	
RT6-R	caaggttctgcgactaatga	
RT7-F	tcattagtcgcagaaccttg	
RT7-R	tccaaattgaatgagacgag	
RT8-F	gagagcatatggacagcaac	
RT9-R	cttttgcatttcgtctctga	
RT10-F	caactgcttacgtcgtgtgg	
RT10-R	gtaactgctgggacgcttct	
RT11-F	ggaggatatgcacatggaagag	
RT11-R	accatgatcgtacacgggttt	
RT12-F	gtagaaggagaatgcgggaaa	
RT12-R	ggagggctgttcttaaatcgt	
RT13-F	ttgagccaagatggttcgagt	
RT13-R	tagctccatcatcggcaattc	
RT14-F	caaccgctaccaataccaaa	
RT14-R	tggttggcattctatcctct	
RT15-F	taacggtatgttaacggcttc	
RT15-R	catattgtcactttgcgtagg	
RT16-F	ccaacgaaacagcaatgacaa	
RT16-R	cgtcgaaacgagatagtctga	
RT17-F	gaggcgttgagaatatcgagt	
RT17-R	cacagtacctgaacccgtaaa	
RT18-F	gtcaactggggcttcaaacct	
RT18-R	aattctccgcaaccgctctaa	
RT19-F	acagtctaccacaacggaaca	
RT19-R	ccggaatttgatatcttcgag	
Pcry41-83F	aactgcaggtaccaacgtatcgttaacca	*Pst*I
Pcry41-83R	cgcggatccgggtatctcctcctttctact	*Bam*HI
Pcry35-F	ggggtacctgcagaccagtatggccaaatttga	*Kpn*I/*Pst*I[a]
Pcry35-R	cgggatcctaccacatttcgatttgcc	*Bam*HI
Pcry32-83-gfp F	ggggtaccgtaccaacgtatcgttaacca	*Kpn*I
Pcry32-83-gfp R	cgcggatccatgggtatctcctcctttc	*Bam*HI
pcry1Ac-F	ggggtaccgcaggtaaatggttctaaca	*Kpn*I
pcry1Ac-R	gctctagaagttacctccatctcttttatt	*Xba*I
pKan-F	ggggtaccgataaacccagcgaacca	*Kpn*I
pKan-R	gctctagacaattccggtgatattctca	*Xba*I
gfp-RBS	gctctagaaggaggtttacgcatggtcgactcaggtggaggcgg	*Xba*I
304R2	atgaccatgattacgccaagct	*Hin*dIII
pcry1AcBam-F	cgggatcctgcaggtaaatggttctaaca	*Bam*HI
pcry1Ac-RL	catcaaacagttccccttttctccataagttacctccatctc	*Bam*HI
cry35-FL	gagatggaggtaacttatggagaaaggggaactgtttgatg	
cry35opHind-R	cccaagctttccaaattgaatgagacgag	*Hin*dIII
cry32Ca-F	cgggatccgtaaggtactacctatttcag	*Bam*HI
cry32Ca-R	acgcgtcgacttattcgcaattcatccg	*Sal*I
cry41-F	cgggatccgtagaaaggaggagatacccatg	*Bam*HI
cry41-R	acgcgtcgacttataacttattcagtttgaatttttg	*Sal*I
cry45-F	cgggatcccggcacattccgtaggcatg	*Bam*HI
cry45-R	acgcgtcgacctattgacgagataggactgggtcc	*Sal*I
P35-PE	accacatttcgatttgcccttaaagc	

P32-PE	tcttcaggtagaaattcgagtgggt

Restriction sites are underlined. [a] two overlapping restriction sites

Table S3 16S rRNA typing analysis of LM1212

			Positions[a]												
16S type	**Species**	**No. of strains**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
			73	**77**	**90**	**92**	**182**	**189**	**192**	**200**	**208**	**1015**	**1036**	**1045**	**1146**	**1462**
2	**Bc**	**1**	G	R[b]	Y	W	C	A	T	T	G	A	T	A	A	A
3	**Bc**	**1**	G	G	C	A	Y	A	T	T	G	A	T	A	A	A
6	**Ba**	**86**	G	A	T	T	C	A	C	T	G	C	T	A	W	T
7	**Bc**	**3**	G	A	T	T	C	A	C	T	G	C	T	A	A	T
9	**Bc**	**2**	G	A	T	T	C	A	C	T	G	A	T	A	A	T
10	**Bt**	**11**	G	A	T	T	C	A	Y	T	G	A	T	A	A	T
12	**Bc**	**2**	G	A	T	T	Y	A	T	T	G	A	T	A	A	T
13	**Bc**	**1**	G	A	T	T	C	A	C	T	G	C	T	A	T	T
1	**Bm**	**2**	G	A	T	T	C	C	C	G	C	C	C	G	A	-[c]
4	**Bt**	**3**	G	G	C	A	C	A	T	T	G	A	T	A	A	-
5	**Bc**	**8**	G	G	C	A	C	A	C	T	G	A	T	A	A	-
7	**Ba**	**3**	G	A	T	T	C	A	C	T	G	C	T	A	A	T
	LM	**1**	A[d]	A	T	T	C	A	T	T	G	A	T	A	T	T

[a]Numbers in the top row refer to the number of positions where mismatches are found. Numbers in the bottom row refers to positions in the 16S rRNA gene. [b]R refers to a purine (A or G) at that position; Y refers to a pyrimidine (C or T) at that position; and W refers to an A or T at that position. [c]The last position (position 14) on 16S types 1, 4, and 5 is missing because their GenBank sequences are shorter. [d]This specific A was only found in Bt 97-27 and Bc-AH820 among 100 16s rRNA sequences from the NCBI searching result with LM1212 16S rRNA as query. Bc = *Bacillus cereus*, Bt = *Bacillus thuringiensis*, Ba =*Bacillus anthracis*, Bm =*Bacillus mycoides*, LM = LM1212

Table S4 LM1212 pathogenicity assay in mouse models of infection

		Mouse									
		1	2	3	4	5	6	7	8	9	10
Assay	**Strains**	**Survival time post-infection[a]**									
Hypodermic injection	A16R	23	37	37	44	47	59	62	87	-[b]	-
	LM1212	-	-	-	-	-	-	-	-	-	-
Intragastric administration	A16R	6	7	7	8	--[c]	--	--	--	--	--
	LM1212	--	--	--	--	--	--	--	--	--	--

[a]hours for hypodermic injection treatment and days for intragastric administration treatment; [b] animal did not die after 168 h; [c] animal did not die after 14 d.

Table S5 List of *cry* and *cyt-like* genes in LM1212

Gene	Translation (aa)	Calculated MW (kDa)	Similar with	Coverage (%)	Identity (%)	Positive (%)	Locus Tag	Note
cry32Va1	1258	142	Cry32Aa1	99	59	71	LM_02513	
cry74Aa1	289	31.3	Cry45Aa1	96	36	56	LM_04540	PS4-like
cry45Ba1	273	30.3	Cry45Aa1	100	52	69	LM_04541	PS4-like
cry41Ca1	851	96.6	Cry41Aa1	99	44	61	LM_04405	PS3-like
cry32Wa1	799	89.2	Cry41Aa1	99	40	55	LM_02757	PS3-like
cry35-like	345	38	Cry35Ac1	82	25	49	LM_02499	Bin-like
cry35-like2	354	38.9	Cry35Ac1	66	25	46	LM_02500	Bin-like
cyt1Aa-like	274	30.5	Cyt1Aa1	91	71	86	LM_05513	Not in crystal
cyt1Ca-like	520	58.9	Cyt1Ca1	99	48	67	LM_02750	Not in crystal
cry32-t1	517	58.1	Cry32Da1	96	60	73	LM_05541	Truncated
cry41-t2	305	35.5	Cry41Aa1	93	46	62	LM_05540	Truncated

Table S6 Cytotoxicity tests of LM1212 Cry proteins

Cell lines	NC	PS1	PS4	LM T	LM C	LM P	41 T	41 C	45 T	45 P
Molt-4	-	-	-	-	-	-	NT	NT	NT	NT
Jurkat	-	-	+	-	-	-	NT	NT	NT	NT
HL-60	-	-	-	-	-	-	NT	NT	NT	NT
HeLa	-	+	-	-	-	-	-	-	-	-
Caco-2	-	-	-	-	-	-	-	-	-	-
HepG2	-	+	+	-	-	-	NT	NT	NT	NT

PS1: PS1Aa1 activated with trypsin; PS4: PS4Aa1 activated with trypsin; LM T/C/P: crystals purified from LM1212 and activated with trypsin/ chymotrypsin/ proteinase K; 41 T/ C: crystals purified from HD73 Cry^-(p8E'*cry41Ca*) and activated with trypsin/ proteinase K; 45 T/ C: crystals purified from HD73 Cry^-(p8E'*cry45Ba*) and activated with trypsin/ proteinase K; NC: negative control, buffer with trypsin ; +: more than 90% cell lysis after 24h; -: no significant cell lysis after 24h; NT: not tested

Table S7 Insect larvea tests of LM1212

Treatments	*Plutella xylostella* 2nd instars	*Agrotis ypsilon* 1st instars	*Spodoptera exigua* 2nd instars	*Colaphellus bowringi* 1st instars	*Aedes aegypti* 4th instars
	Toxicity				
Positive	+	+	+	+	+
Negative	-	-	-	-	-
LM1212	-	-	-	-	-

Positive: positive control (see supplementary Text S9 for details on the strain used for each insect);
Negative: negative control dd H_2O; +: mortality > 85% after 48 h; -: mortality < 10% after 72 h

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