RESEARCH ARTICLE
ScienceAsia 49 (2023): 754–759
doi: 10.2306/scienceasia1513-1874.2023.077

Characterization of a novel ETX/MTX2 domain-presenting parasporal crystal protein from *Bacillus toyonensis* HSY140 strain

Yinghong Jin[a], **Yiming Huang**[a], **Jiali He**[a], **Yangyang Wang**[a], **Yuquan Xu**[b], **Ruiping Wang**[a], **Wenfei Zhang**[a,*]

[a] Ministry of Education Key Laboratory for Ecology of Tropical Islands, Key Laboratory of Tropical Animal and Plant Ecology of Hainan Province, College of Life Sciences, Hainan Normal University, Haikou 571158 China
[b] Biotechnology Research Institute, The Chinese Academy of Agricultural Sciences, Beijing 100081 China

*Corresponding author, e-mail: wenfei2007@163.com, wfzhang@hainnu.edu.cn

Received 14 Sep 2022, Accepted 4 Jul 2023
Available online 12 Oct 2023

ABSTRACT: The soil-derived *Bacillus toyonensis* HSY140 strain was found to produce parasporal crystalline inclusions. Genome sequencing and annotation did not find the three-domain Cry (3d-Cry) and Cyt toxins, but a novel *mpp23-like* gene (918 bp) containing an ETX/MTX2 domain was detected. Alignment and phylogenetic analysis revealed that the translated Mpp23-like protein had the highest homology to Cry23Aa1 (now named Mpp23Aa1, AAA22333.1). Cloning and expression of the *mpp23-like* gene into the acrystalliferous *Bacillus thuringiensis* BMB171 (*cry*$^{-}$) generated a recombinant strain able to produce elliptical parasporal crystals with the same shape as those of the HSY140 strain. Finally, bioassay experiments indicated that the expressed Mpp23-like protein did not exhibit any activity to the lepidopteran insects of *Plutella xylostella*, *Helicoverpa armigera*, and *Spodoptera exigua* and the dipteran insect of *Aedes aegypti*. Altogether, we have characterized for the first time a new toxin, Mpp23-like protein, responsible for the formation of crystalline inclusions in *B. toyonensis* HSY140 strain.

KEYWORDS: *Bacillus toyonensis*, parasporal crystal, genome, Mpp23-like protein, ETX/MTX2

INTRODUCTION

Bacillus thuringiensis (Bt), a Gram-positive, spore-forming, and parasporal crystal-secreting bacterium, has been successfully applied worldwide as a microbial insecticide or bio-pesticide to reduce the amount of chemical pesticides [1, 2]. Within the genus *Bacillus*, Bt presents similarities to other species such as *Bacillus cereus*, *Bacillus anthracis*, *Bacillus toyonensis*, and *Bacillus wiedmannii* [3, 4]. Bt is a ubiquitous gram-positive, spore-forming bacterium, generally known to produce parasporal crystals through the transcription of *cry* or *cyt* genes during the sporulation phase of its life cycle.

Cry proteins can be divided into different groups according to their homology and structure. The largest group of Cry toxins comprises the well-known three-domain "classical" model whereas other Cry toxins pertaining to the "non-three-domain" category belong to distinct groups such as binary (Bin) toxin, ETX/MTX2 toxin, and aerolysin [5–7]. Cry toxins (e.g., Cry15, Cry23, Cry45, and Cry64) show features of the ETX/MTX2 family which includes the Mtx2 protein from *Lysinibacillus sphaericus* and the *Clostridium* epsilon toxin. At present, there are only a few studies addressing the role and function of these Cry proteins. Baum et al [8] reported the Cry51Aa2 toxin (ETX/MTX2 type) showed a median lethal concentration (LC_{50}) of 72.9 µg/ml against *Lygus hesperus*. Liu et al [9] discovered that 2 ETX/MTX2 type Cry toxins had high insecticidal activity against Hemipteran pests. Nevertheless, the characterization of Cry toxins belonging to the ETX/MTX2 group remains largely unexplored.

In this study, the HSY140 strain was isolated, and the formation of parasporal crystals was further observed by scanning electron microscope (SEM). To gain insight into parasporal crystal inclusions of the HSY140 strain, its draft genome was further sequenced. Remarkably, genome annotation and gene expression analysis indicated that the parasporal crystals are encoded by a novel *mpp23-like* gene (918 bp) containing an ETX/MTX2 domain. Further, bioassay experiments helped us to explore the properties of this toxin protein.

MATERIALS AND METHODS

Strains and plasmids

The HSY140 strain was isolated from a soil sample of Limushan National Forest Park in Hainan province, China, and preserved at College of Life Sciences, Hainan Normal University. This strain was incubated in BP medium (0.3% beef extract, 0.5% peptone, 0.5% NaCl, 1.5% agar, pH 7.0–7.2) at 28 °C for parasporal protein preparation [10, 11]. The parasporal inclusions were observed under the oil lens optical microscope and scanning electron microscope (SEM) as previously described [12].

Escherichia coli DH5α and JM110 were used for common transformation and nonmethylated recombinant plasmids, respectively. All *E. coli* strains were grown at 37 °C in Luria-Bertani (LB) medium (1.0% tryptone, 0.5% yeast extract, 1.0% NaCl, pH 7.2–7.4)

supplemented with 100 µg/ml ampicillin for plasmid selection. The plasmid pSTAB (15 µg/ml), containing ampicillin and erythromycin resistance genes, was used as *E. coli*/Bt shuttle vector [13–15]. An acrystalliferous Bt mutant strain BMB171 [16] was used as the host strain for spore-crystal protein expression.

Genome sequencing and annotation

The strain HSY140 was cultivated in LB medium for 6 h at 28 °C, and then its genomic DNA was extracted and purified by using a commercial genomic DNA isolation kit (Tiangen Corporation Ltd., Beijing, China). The draft genome was sequenced at Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China) with paired-end sequencing technology (HiSeq 4000, Illumina, Inc., USA) and assembled with SOAP de novo v2.04 [17]. The Rapid Annotation using Subsystem Technology (RAST) server online was performed for the genome annotation [18]. The 16S rRNA was identified by using EzBioCloud server and compared with 16S rDNA sequences (Bacteria and Archaea) deposited in the GenBank database by BLASTN [19]. The translated genes were aligned with the COG database [20, 21] using National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) [22] to elaborate their putative function. The phylogenetic analysis with the genome sequences of the HSY140 strain and several nearby neighbor species downloaded from NCBI was performed using an orthologous average nucleotide identity tool (OAT) [23].

Bioinformatic analysis of the putative toxin sequence

A stand-alone BLAST was applied to identify Bt toxins of the HSY140 strain by searching against the comprehensive Bt toxin protein database, whose amino acid sequences were downloaded from Bacterial Pesticidal Protein Resource Center (https://www.bpprc.org) [24]. The phylogenetic tree was constructed by the neighbor-joining method using MEGA 11 with 1000 boot-strapping replications [25]. Signal peptides and conserved domains were predicted using the SignalP 5.0 tool [26] and InterPro database [27], respectively. Based on the bioinformatic analysis, the putative toxin encoded by *orf_4894* gene was named Mpp23-like, and the amino acid sequence of the toxin was submitted online to SWISS-MODEL workspace to model the three-dimensional space structure [28].

Molecular cloning and expression of the *mpp23-like* gene

The *mpp23-like* gene was amplified by Polymerase Chain Reaction (PCR) from the HSY140 strain genomic DNA using primers with a *Pst I* site added to the forward primer (5′-aagcgtcgacGAT AAA GGA GGA TTT ACA ATG ACAG-3′) and an *EcoR I* site added to the reverse primer (5′-gacgggatccTTA ACT ATA AAT AGG GAT AGG TGA AC-3′). The fragment was cloned into the shuttle vector pSTAB under the control of *cyt1A* gene promoters and the STAB-SD stabilizer sequence [29]. The recombinant plasmids were demethylated by transformation into *E. coli* JM110. Purified plasmids from this strain were then electroporated into the acrystalliferous *B. thuringiensis* BMB171 strain (*cry*$^-$) for protein expression. Positive clones were confirmed by PCR and sequenced. The method for Mpp23-like protein expression, extraction, and quantitative analysis from the recombinant strains was performed as described previously [30].

Table 1 Genome properties and statistics of the HSY140 strain.

Feature	Value
Genome size (bp)	6,603,054
DNA scaffold (bp)	753
DNA G + C content (%)	35.18
DNA coding (bp)	5,502,193
Scaffold N50 length (bp)	23,269
Total gene	7,539
Protein coding gene	7,383
RNA gene	156
Gene with function prediction	5406
Gene assigned to COG	5101
Gene with Pfam domain	4121
Gene with signal peptide	698
Gene with transmembrane helix	1918
CRISPR repeat	2

Bioassay

The spore-crystal pellets were harvested and washed 3 times with ice-cold 0.5 M NaCl containing 1 mM phenylmethylsulfonyl fluoride (PMSF) [10, 31]. Then the proteins were separated by Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The insecticidal activity of the Mpp23-like protein was tested against second instar lepidopteran larvae of *Plutella xylostella* [12], *Helicoverpa armigera* [32, 33], and *Spodoptera exigua* [34, 35] and fourth instar larvae of *Aedes aegypti* [30, 36] following the procedure described previously [12].

Nucleotide sequence accession number

The Whole Genome Shotgun project has been deposited at GenBank under the accession with BioProject ID PRJNA856049.

RESULTS AND DISCUSSION

General features and draft genome sequence of the HSY140 strain

Bacillus sp. HSY140 strain was isolated from soil samples based on parasporal crystals production observed under the oil lens optical microscope (Fig. 1a). The

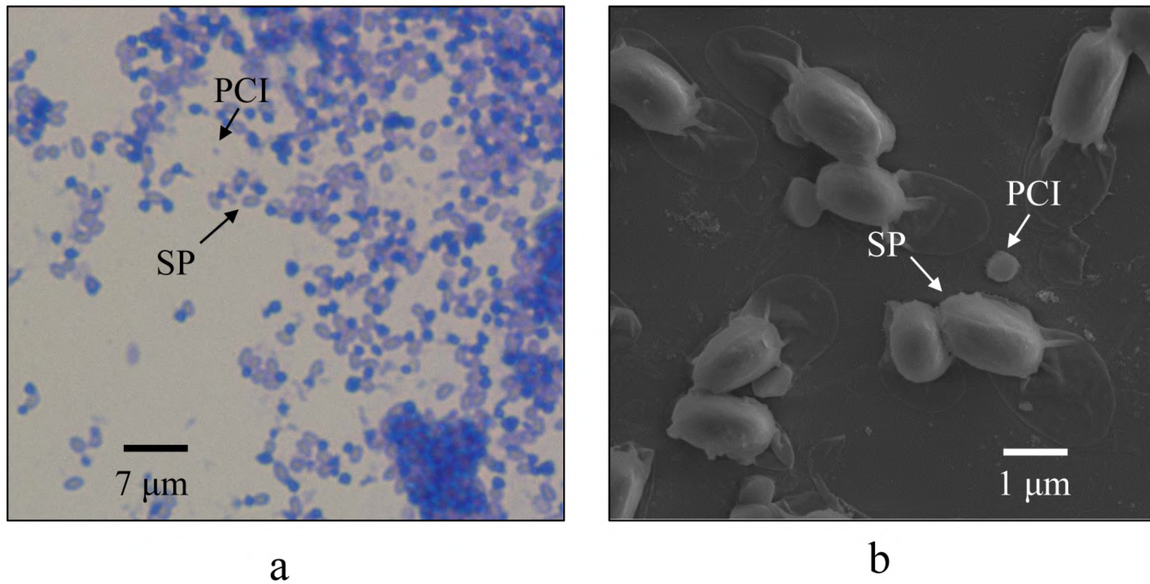

Fig. 1 Spores (SP) and parasporal crystals (PCI) of HSY140 strain observed under (a) the ×100 oil lens optical microscope and (b) the scanning electron microscope.

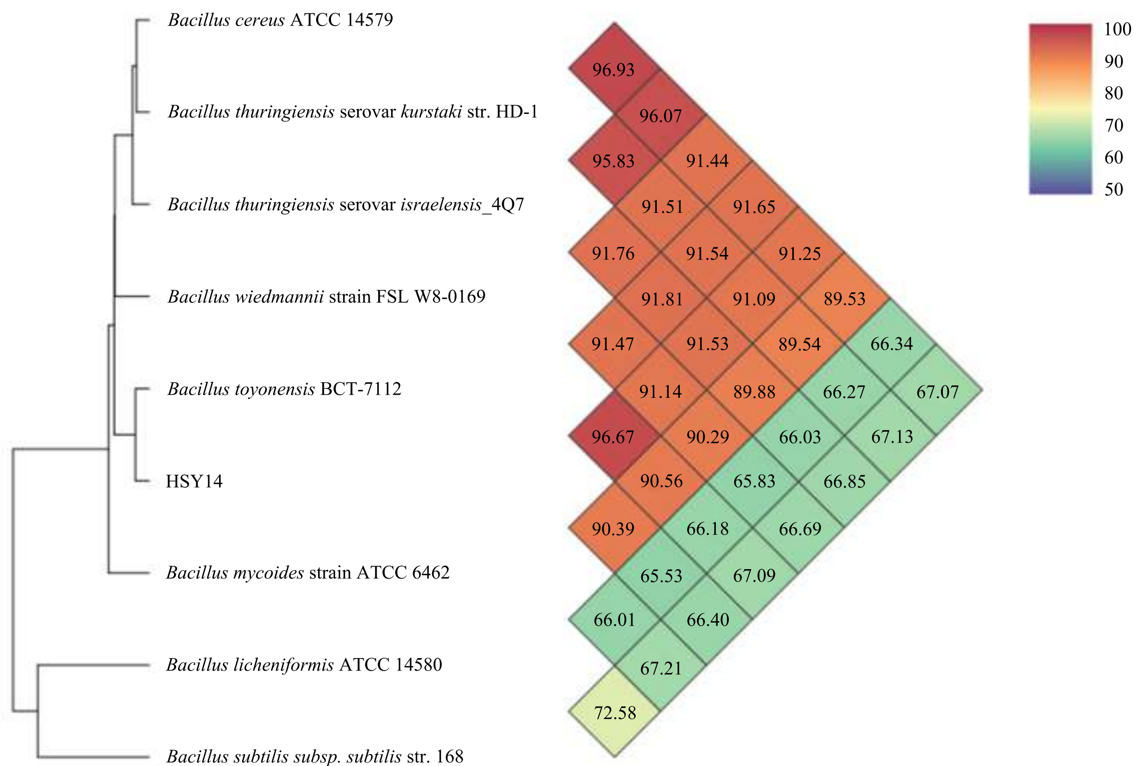

Fig. 2 Phylogenetic analyses of nine genomes of *Bacillus* sp. HSY140 strain and representative related *Bacillus* species by OAT. The genomic sequences of *B. cereus* ATCC 14579 (NC004722), *B. thuringiensis* serovar *kurstaki* strain HD-1 (CP004870), *B. thuringiensis* serovar *israelensis* strain 4Q7 (JEOC01000001), *B. wiedmannii* strain FSL W8-0169 (LOBC01000001), *B. toyonensis* BCT-7112 (NC022781), *B. mycoides* strain ATCC 6462 (CP009692), *B. licheniformis* ATCC 14580 (CP000002), and *B. subtilis* strain 168 (NC000964) were downloaded from NCBI for phylogenetic analysis.

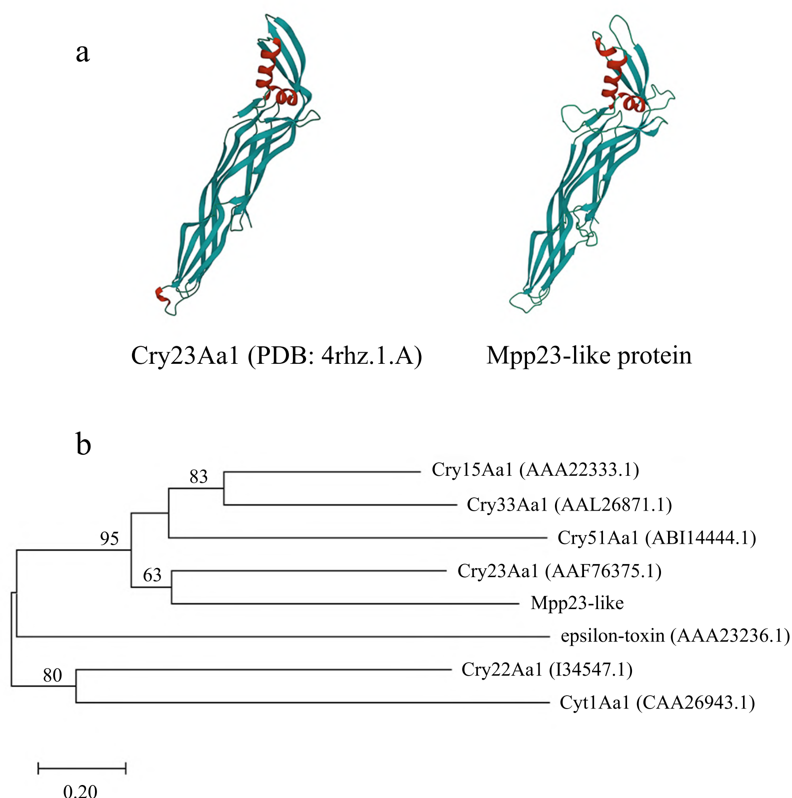

Fig. 3 The simulated spatial 3D structure and phylogenetic tree of Mpp23-like toxin. (a) Cry23Aa1 (PDB4rhz.1.A) selected as a model for homology modeling in SWISS-MODEL. α-helices are depicted in red, and β-sheets are in green. (b) The phylogenetic tree constructed by MEGA 11 using a neighbor-joining method with a bootstrap of 1,000 replications.

elliptical parasporal crystals and adherent spores were further examined by SEM (Fig. 1b).

In order to identify the gene responsible for synthesis of parasporal crystalline inclusions, genomic DNA of the HSY140 strain was isolated and sequenced by a high-throughput sequencing technology. The draft genome revealed a total length of 6,603,054 base pairs (bp) with an average coverage depth exceeding 200× and an average GC content of 35.18% (see Table 1 for full list of HSY140 strain features).

Blast search against the GenBank database showed that the 16S rRNA gene sequence of the HSY140 strain was 99% identical to more than 20 sequences of members of the *Bacillus* genus such as *B. toyonensis* BCT-7112, *B. thuringiensis* ATCC 10792, and *B. cereus* ATCC 14579. The phylogenetic tree was constructed with the whole genome sequences of HSY140 strain neighbor species using the OAT tool. Strikingly, results revealed that the HSY140 strain shared high similarity (96.67%) with *B. toyonensis* BCT-7112 (Fig. 2). According to current bacterial taxonomy, the HSY140 strain may belong to the species of *B. toyonensis* given their average nucleotide identity (ANI) > 95% [23].

Identification of paraporal crystal gene with ETX/MTX2 domain

To identify the gene responsible for the formation of parasporal inclusions, gene prediction and annotation were performed using BLAST searches against the comprehensive Bt toxin protein database. BLAST results revealed the protein encoded by the *orf_4894* gene (918 bp) was identical to one putative *cry* toxin, which was most similar to the known Cry23Aa1 toxin (now named Mpp23Aa1; Percent identity: 26.2%; Query Cover: 77%) [24]. Additionally, it also contained an ETX/MTX2 domain (PF03318) located at residue positions 46-280. Considering all these characteristics, we named this new toxin Mpp23-like, a protein consisting of 305 amino acid residues with a molecular mass of 31.91 kDa without the signal peptide.

With a Top score of 0.60 at GMQE (Global Model Quality Estimate), Cry23Aa1 (Protein Data Base: 4rhz.1.A) was used as the template for modeling and building a homology model of Mpp23-like online. Data regarding the three-dimensional (3D) structure of both proteins showed some differences in the α-helix

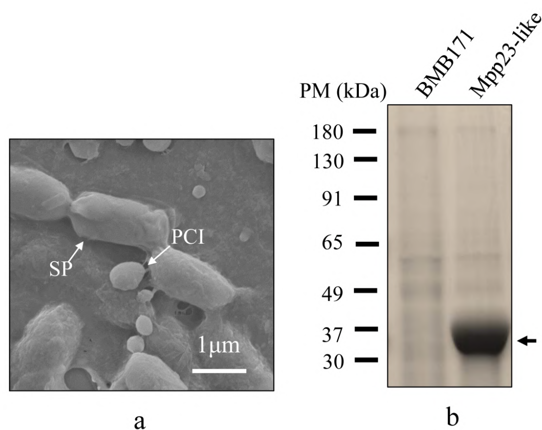

Fig. 4 Expression of Mpp23-like toxin gene. (a) Scanning electron micrograph of recombinant *B. thuringiensis* BMB171 strains producing Mpp23-like (SP, Spores; PCI, parasporal crystals). (b) SDS-PAGE analysis of Mpp23-like gene expressed in recombinant *B. thuringiensis* BMB171 strains. Lane PM, the protein molecular weight marker (Sangon Biotech, Shanghai, China); lane BMB171, the acrystalliferous strain; lane Mpp23-like, the recombinant strain. Mpp23-like proteins are indicated by arrows at 32 kDa.

between Cry23Aa1 and Mpp23-like proteins (Fig. 3a). Results obtained from the constructed phylogenetic tree indicated that this Mpp23-like protein was most closely related to Cry23Aa1 (Fig. 3b).

Mpp23-like protein expression and bioassay

The *mpp23-like* gene was cloned into the vector of pSTAB, and the purified plasmids were further electroporated into the acrystalliferous *B. thuringiensis* BMB171 (*cry*$^{-}$) to allow protein expression. Recombinant strains were found to produce visible parasporal crystals with the same shape as those of the HSY140 strain (Fig. 4a). The expected protein band of about 32 kDa was demonstrated by SDS-PAGE electrophoresis analysis (Fig. 4b). Finally, the expressed Mpp23-like protein was conducted to bioassay to test its insecticidal activity against lepidopteran insects of *P. xylostella*, *H. armigera*, and *S. exigua* and a dipteran insect of *A. aegypti*. However, Mpp23-like protein did not exhibit any activity to the tested insects (data not shown).

CONCLUSION

In this study, we conducted a whole-genome sequencing of *B. toyonensis* HSY140 strain. Gene prediction and annotation, performed to find a *cry* or *cyt* gene responsible for the synthesis of the crystalline inclusions, allowed the identification of a novel Mpp23-like toxin containing an ETX/MTX2 domain. Cloning and expression of the *mpp23-like* gene into the acrystalliferous *B. thuringiensis* BMB171 generated a recombinant strain that was able to sporulate normally in BP medium. Moreover, this strain produced clear crystalline inclusions that were visible under scanning electron microscopy. Furthermore, the bioassay results showed that the Mpp23-like protein did not exhibit any insecticidal activity against the tested insects, including *P. xylostella*, *H. armigera*, *S. exigua*, and *A. aegypti*. In conclusion, *B. toyonensis* HSY140 is able to produce parasporal inclusions during sporulation just like Bt. We found that the novel Mpp23-like protein belonging to ETX/MTX2-type toxin is responsible for the production of parasporal inclusions.

Acknowledgements: This study was supported by the National Natural Science Foundation of China (project number: 31960012), the Education Department of Hainan Province (project number: Hnky2020-23), and the Hainan Province Science and Technology Special Fund (ZDKJ2021035).

REFERENCES

1. Sanahuja G, Banakar R, Twyman RM, Capell T, Christou P (2011) *Bacillus thuringiensis*: a century of research, development and commercial applications. *Plant Biotechnol J* **9**, 283–300.
2. Schnepf E, Crickmore N, Van Rie J, Lereclus D, Baum J, Feitelson J, Zeigler DR, Dean DH (1998) *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol Mol Biol Rev* **62**, 775–806.
3. Read TD, Peterson SN, Tourasse N, Baillie LW, Paulsen IT, Nelson KE, Tettelin H, Fouts DE, et al (2003) The genome sequence of *Bacillus anthracis* Ames and comparison to closely related bacteria. *Nature* **423**, 81–86.
4. Helgason E, Okstad OA, Caugant DA, Johansen HA, Fouet A, Mock M, Hegna I, Kolsto AB (2000) *Bacillus anthracis*, *Bacillus cereus*, and *Bacillus thuringiensis* – one species on the basis of genetic evidence. *Appl Environ Microbiol* **66**, 2627–2630.
5. Palma L, Munoz D, Berry C, Murillo J, Caballero P (2014) *Bacillus thuringiensis* toxins: an overview of their biocidal activity. *Toxins (Basel)* **6**, 3296–3325.
6. Alves GG, Machado De Avila RA, Chavez-Olortegui CD, Lobato FC (2014) *Clostridium perfringens* epsilon toxin: the third most potent bacterial toxin known. *Anaerobe* **30**, 102–107.
7. Pardo-Lopez L, Soberon M, Bravo A (2013) *Bacillus thuringiensis* insecticidal three-domain Cry toxins: mode of action, insect resistance and consequences for crop protection. *FEMS Microbiol Rev* **37**, 3–22.
8. Baum JA, Sukuru UR, Penn SR, Meyer SE, Subbarao S, Shi X, Flasinski S, Heck GR, et al (2012) Cotton plants expressing a hemipteran-active *Bacillus thuringiensis* crystal protein impact the development and survival of *Lygus hesperus* (Hemiptera: Miridae) nymphs. *J Econ Entomol* **105**, 616–624.
9. Liu Y, Wang Y, Shu C, Lin K, Zhang J (2017) Cry64Ba and Cry64Ca, two ETX/MTX2 *Bacillus thuringiensis* insecticidal proteins against hemipteran pests. *Appl Environ Microbiol* **84**, e01996–01917.
10. Jin Y, Wang J, Liu Q, Qiu H, Wang Y, Zhou J, Sun J, Li P, et al (2020) Genome of *Bacillus* sp. strain QHF158

provides insights into its parasporal inclusions encoded by the S-layer gene. *Braz J Microbiol* **51**, 1145–1150.

11. Zhang W, Zhang J, Crickmore N, Wu Z, Yang Y, Qian J, Wu H, Wang R, et al (2014) Identification of a mosquitocidal toxin from *Bacillus thuringiensis* using mass spectrometry. *World J Microbiol Biotechnol* **30**, 3273–3277.
12. Zhang W, Crickmore N, George Z, Xie L, He YQ, Li Y, Tang JL, Tian L, et al (2012) Characterization of a new highly mosquitocidal isolate of *Bacillus thuringiensis* – an alternative to Bti? *J Invertebr Pathol* **109**, 217–222.
13. Park HW, Ge B, Bauer LS, Federici BA (1998) Optimization of Cry3A yields in *Bacillus thuringiensis* by use of sporulation-dependent promoters in combination with the STAB-SD mRNA sequence. *Appl Environ Microbiol* **64**, 3932–3938.
14. Park H (1999) Differential enhancement of Cry2A versus Cry11A yields in *Bacillus thuringiensis* by use of the cry3A STAB mRNA sequence. *FEMS Microbiol Lett* **181**, 319–327.
15. Arantes O, Lereclus D (1991) Construction of cloning vectors for *Bacillus thuringiensis*. *Gene* **108**, 115–119.
16. He J, Shao X, Zheng H, Li M, Wang J, Zhang Q, Li L, Liu Z, et al (2010) Complete genome sequence of *Bacillus thuringiensis* mutant strain BMB171. *J Bacteriol* **192**, 4074–4075.
17. Li R, Zhu H, Ruan J, Qian W, Fang X, Shi Z, Li Y, Li S, et al (2010) *De novo* assembly of human genomes with massively parallel short read sequencing. *Genome Res* **20**, 265–272.
18. Aziz RK, Bartels D, Best AA, Dejongh M, Disz T, Edwards RA, Formsma K, Gerdes S, et al (2008) The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* **9**, 75.
19. Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* **67**, 1613–1617.
20. Tatusov RL, Natale DA, Garkavtsev IV, Tatusova TA, Shankavaram UT, Rao BS, Kiryutin B, Galperin MY, et al (2001) The COG database: new developments in phylogenetic classification of proteins from complete genomes. *Nucleic Acids Res* **29**, 22–28.
21. Tatusov RL, Galperin MY, Natale DA, Koonin EV (2000) The COG database: a tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Res* **28**, 33–36.
22. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL (2009) BLAST+: architecture and applications. *BMC Bioinformatics* **10**, 421.
23. Lee I, Ouk Kim Y, Park SC, Chun J (2016) OrthoANI: An improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Microbiol* **66**, 1100–1103.
24. Crickmore N, Berry C, Panneerselvam S, Mishra R, Connor TR, Bonning BC (2021) A structure-based nomenclature for *Bacillus thuringiensis* and other bacteria-derived pesticidal proteins. *J Invertebr Pathol* **186**, 107438.
25. Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular evolutionary genetics analysis version 11. *Mol Biol Evol* **38**, 3022–3027.
26. Almagro Armenteros JJ, Tsirigos KD, Sonderby CK, Petersen TN, Winther O, Brunak S, Von Heijne G, Nielsen H (2019) SignalP 5.0 improves signal peptide predictions using deep neural networks. *Nat Biotechnol* **37**, 420–423.
27. Blum M, Chang HY, Chuguransky S, Grego T, Kandasaamy S, Mitchell A, Nuka G, Paysan-Lafosse T, et al (2021) The InterPro protein families and domains database: 20 years on. *Nucleic Acids Res* **49**, D344–D354.
28. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, Heer FT, De Beer TaP, et al (2018) SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res* **46**, W296–W303.
29. Sun Y, Zhao Q, Xia L, Ding X, Hu Q, Federici BA, Park HW (2013) Identification and characterization of three previously undescribed crystal proteins from *Bacillus thuringiensis* subsp. jegathesan. *Appl Environ Microbiol* **79**, 3364–3370.
30. Wang J, Wang J, He J, Wu J, Sun J, Wang R, Zhang W (2020) Vip3Aa from *Bacillus thuringiensis* subsp. kurstaki HD1 is toxic to *Aedes aegypti* (Diptera: Culicidae). *J Invertebr Pathol* **171**, 107342.
31. Guo G, Zhang L, Zhou Z, Ma Q, Liu J, Zhu C, Zhu L, Yu Z, et al (2008) A new group of parasporal inclusions encoded by the S-layer gene of *Bacillus thuringiensis*. *FEMS Microbiol Lett* **282**, 1–7.
32. Navon A, Klein M, Braun S (1990) *Bacillus thuringiensis* potency bioassays against *Heliothis armigera*, *Earias insulana*, and *Spodoptera littoralis* larvae based on standardized diets. *J Invertebr Pathol* **55**, 387–393.
33. Wu K, Guo Y, Lv N (1999) Geographic variation in susceptibility of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to *Bacillus thuringiensis* insecticidal protein in China. *J Econ Entomol* **92**, 273–278.
34. Visser B, Munsterman E, Stoker A, Dirkse WG (1991) A novel *Bacillus thuringiensis* gene encoding a *Spodoptera exigua*-specific crystal protein. *J Bacteriol* **172**, 6783–6788.
35. Ouying P, Promdonkoy B, Kubera A (2022) Biological activities of the vegetative insecticidal protein Vip3Aa against beet armyworm (*Spodoptera exigua*). *ScienceAsia* **48**, 165–170.
36. Poncet S, Delécluse A, Klier A, Rapoport G (1995) Evaluation of synergistic interactions among the CryIVA, CryIVB, and CryIVD toxic components of *B. thuringiensis* subsp. *israelensis* crystals. *J Invertebr Pathol* **66**, 131–135.