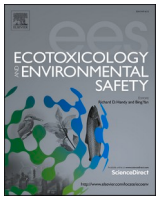
Ecotoxicology and Environmental Safety 228 (2021) 112975

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv



Bacillus thuringiensis strain QBT220 pBtoxis plasmid structural instability enhances δ-endotoxins synthesis and bioinsecticidal activity

Kavita Nair, Roda Al-Thani, Samir Jaoua *

Environmental Science Program, Department of Biological & Environmental Sciences, College of Arts & Sciences, Qatar University, 2713, Doha, Qatar

ARTICLE INFO

Edited by Dr Yong Liang

Keywords:
Bacillus thuringiensis
PBtoxis plasmid instability
Bioinsecticide production

ABSTRACT

Bacillus thuringiensis subsp. *israelensis* (*Bti*) spherical parasporal crystal contains several insecticidal proteins used as environmentally safe alternative to toxic chemical pesticides. The exploration of a *Bti* strain isolated from Qatar QBT220 genes encoding the δ-endotoxins responsible of the insecticidal activities revealed the alteration of a 14-kb DNA region including the δ-endotoxins *cry10A* and *cyt1C* genes of pBtoxis plasmid. The presence of all the insecticidal genes except *cry10A* and *cyt1C* was explained by a structural instability of the plasmid pBtoxis. However, when compared with the *Bti* reference strains H14 and QBT217 that carry all δ-endotoxins coding genes, it was found that QBT220, has a significantly higher insecticidal activity against the dipteran insect *Aedes aegypti* larvae despite of the plasmid pBtoxis structural instability due to the alteration of *cry10A* and *cyt1C* genes. In addition, QBT220 showed the highest δ-endotoxin synthesis per spore, compared with that of the wildtype strains. These findings confirm that the altered genes *cry10A* and *cyt1C* are not mandatory for *Bti* insecticidal activities and on the other hand show a possible inhibitory effect played by the 2 proteins Cry10A and Cyt1C on the insecticidal activities of the other insecticidal proteins. In addition, the QBT220 increased δ-endotoxins synthesis per cell, makes this strain a good candidate for possible applications in the industrial production of bioinsecticides.

1. Introduction

Bacillus thuringiensis israelensis (*Bti*) is a Gram-positive, spore forming, aerobic, soil microbe that has larvicidal properties against dipteran insects owing to their parasporal crystal proteins (Lacey, 2007; Mittal, 2003). They are known widely for their ability to kill mosquito larvae when ingested by the latter (Jacups et al., 2013). These insecticidal proteins are highly target specific with next to none harmful effect on other species including humans (Lacey, 2007; Merritt et al., 1989; Roh et al., 2007). Compared to the chemical insecticides, used widely today, *Bti* proteins have very low potential to develop any resistance among the target insects and do not lose their efficiency in the process (Lee and Scott, 1989). Due to the advantages, various *Bacillus thuringiensis* species have been studied and adopted for commercial production of insecticidal products against various insects. These insecticides occupy 10% of all insecticidal markets and about 97% of all biological insecticides available in the market today (Brar et al., 2005).

The toxicity of the *Bti* towards Dipteran insects is due to the insecticidal spherical protein crystals produced by *Bti* during its sporulation stage (Poopathi and Archana, 2012; Mittal, 2003). The efficacy and the low potential for development of resistance among the target species are attributed to the different δ-endotoxins that form the spherical crystal (Palma et al., 2014). These δ-endotoxins consist of several Cry (crystal) proteins and Cyt (cytolytic) proteins. The protein crystals, when ingested by the larvae, reach its midgut and gets solubilized into different δ-endotoxins. The solubilized endotoxins (pro-toxins) are then activated (to toxins) by specific midgut enzymes. The activated Cry toxins bind to specific cell receptors to initiate cell lysis and consequent larval death (Zhang et al., 2018). The activated Cyt toxins, on the other hand, have a non-specific detergent like action on the cells (Ben-Dov E, 2014; Palma et al., 2014; Cohen et al., 2011). The combined dual mode of action not only increases the efficiency of the *Bti*, but also, delays the onset of any resistance among the insects (Ben-Dov, 2014; Wirth et al., 1997, 2005). In *Bti*, the insecticidal δ-endotoxin proteins are encoded by genes carried by a large 128 kb plasmid called pBtoxis (Berry et al., 2002). The *cry* genes present on this plasmid include *cry4A*, *cry4B*, *cry10A* and *cry11A*. The plasmid also carries three main *cyt* genes *cyt1A*, *cyt1C* and *cyt2B*. These genes are controlled by sporulation dependent promoters and are

* Corresponding author.
E-mail address: samirjaoua@qu.edu.qa (S. Jaoua).

https://doi.org/10.1016/j.ecoenv.2021.112975
Received 12 August 2021; Received in revised form 20 October 2021; Accepted 5 November 2021
Available online 10 November 2021
0147-6513/© 2021 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

hence expressed only during the sporulation stage (Wang et al., 2013). During sporulation along with the spores, parasporal protein crystals are formed, consisting of four main insecticidal proteins; Cry4A, Cry4B, Cry11A and Cyt1A (Berry et al., 2002). In addition, minor traces of Cry10A and Cyt2B can also be detected (Lee et al., 1985; Garduno et al., 1988). On the other hand, Cyt1C protein has been undetectable because of either the low quantity or the instability of the transcript (Manasherob et al., 2006). Among these minor insecticidal proteins, Cry10A and Cyt2B have been reported to enhance the insecticidal activity of Cry4A and Cry4B when present in high quantities; provided by cloning individual *cry10A* and *cyt2B* genes into acrystalliferous *Bti* strain with strong promoters (Valtierra-De-Luis et al., 2020). In case of Cyt1C, insecticidal activity and the synergistic function were reported to be absent (Itsko et al., 2005).

Previous studies have shown that pBtoxis plasmid might sometimes be subjected to structural instability leading to DNA rearrangements in pBtoxis, as reported in a *Bti* strain BUPM97 (Zghal and Jaoua, 2006; Schnepf et al., 1998) that showed the absence of *cry4B* and *cry10* gene and that significantly reduced the insecticidal activity of BUPM97.

In this article, another case of pBtoxis plasmid structural instability is evidenced in another *Bti* strain isolated in Qatar, revealing an alteration of an important region including *cry10A* and *cyt1C* genes and a significantly high delta-endotoxin synthesis per cell and high insecticidal activity against the dipteran insect *Aedes aegypti* larvae in spite of the plasmid pBtoxis structural instability and consequent loss of Cry10A and Cyt1C toxins.

2. Materials and methods

2.1. Bt strains and culture conditions

The study focuses on the *Bacillus thuringiensis israelensis* strain QBT220 previously isolated in the lab from Qatari soil. This *Bti* strain produces smooth spherical parasporal crystals during sporulation stage along with its spores. For the δ-endotoxin production, two reference strains were used as positive controls, the *Bti* strain H14 (Schnepf et al., 1998) and another local *Bti* strain QBT217 (Nair et al., 2020). The negative control used in the study was the strain *Bti* 4Q7 (Schnepf et al., 1998), which is *Bti* H14 that has lost all its plasmids and hence does not produce any parasporal crystals during sporulation. All the strains were grown in Luria Bertini medium (Sambrook et al., 1989) and incubated at 30 °C overnight. A single isolated colony was transferred every time from an overnight LB agar plate of each strain to the sporulation T3 broth (Travers et al., 1987; Zouari et al., 1998). The cultures were then incubated at 30 °C for 96 h until complete sporulation was achieved.

2.2. Dipteran insects and raring conditions

The dipteran insect *Aedes aegypti Bora Bora* was used for the insecticidal bioassay. The eggs of *A.aegypti* were obtained from *Laboratoire de Lutte contre les Insectes Nuisibles* (*LIN*), *Montpellier*, *France* on filter papers. These eggs were then transferred to water and allowed to develop into 3rd instar larvae by incubation at 26 (± 2) °C with a 12:12 h light & dark photoperiod.

2.3. Quantitative insecticidal bioassay and statistical analysis

The quantitative bioassays were performed by the standard protocols of WHO (WHO, 2005). The 3rd instar larvae were added to 100 ml water in plastic cups. Each concentration was triplicated, and the larvae were exposed to the spore-crystal mixture at 27 (± 2) °C with 12:12 light and dark photoperiod. The number of dead and live larvae were counted after 24 h. Each concentration was tested in triplicate and % of killed larvae are presented as mean + /- standard deviation error values. Probit analysis software (WHO, 2005) was used to determine the LC_{50}.

2.4. Detection of Bti toxins genes by polymerase chain reaction (PCR)

The *Bti* δ-endotoxin genes content was explored by PCR, using control strains *Bti* H14 and *Bti* 4Q7. The plasmid DNA was isolated from these strains by alkaline lysis and alcohol precipitation method (Sambrook et al., 1989). The primers used in this study and the list of genes studied are mentioned in Supplementary Table 1. The amplifications were obtained using the Applied Biosystems 96 wells Veriti Thermal Cycler (USA) using the protocol from Jaoua et al. (1996) with necessary modifications. The amplified products were loaded and run on a 1.2% agarose gel with Ethidium bromide to stain the DNA and observed under UV.

2.5. Plasmid regional mapping

Primers were designed in order to amplify about 750–1000 kb fragments in pBtoxis plasmid of the region between *cry4B* gene and *cyt1C* gene. The details of the primer are given in Supplementary Table 2. The PCR were run using the same protocol as above. The reference strain H14 was used as positive control and strain 4Q7 was used as negative controls. The amplified PCR products were run on 1.2% agarose gel with Ethidium bromide as the staining solution.

2.6. Isolation and purification of spore-crystal mixture

Bt strains were cultured for 96 h in Soya meal starch based medium (Starch, 15 g/l; Soyameal, 25 g/l; Calcium carbonate, 20 g/l; Monopotassium phosphate, 1 g/l; Dipotassium phosphate, 1 g/l; Manganese sulphate, 0.01 g/; Magnesium sulphate, 3 g/l; Ferrous sulphate, 0.01 g/l; pH7 (Zouari et al., 1998). Then the mixtures spores/crystals were collected by centrifugation at 4,000 rpm. The pellets were washed with NaCl 1 M, then with cold water thrice. Then, the spore-crystal mixtures were resuspended in NaOH 50 mM for 1 h to solubilize the crystals, then centrifuged at 14000 rpm for 5 min. The solubilized δ-endotoxins were transferred to a fresh tube and stored at 4 °C.

2.7. Determination of the δ-endotoxins concentration in Soya meal starch based medium

The determination of the δ-endotoxin concentration was estimated by the Bradford's method (Bradford, 1976). 20 µl of the solubilized δ-endotoxins was added to 1 ml of Bradford reagent and incubated for 15 min. Then the optic density (OD) was measured at 595 nm. A standard graph of OD versus protein concentration of Bovine serum albumin (BSA) allowed the determination of the δ-endotoxins concentrations. The non-crystal forming strain 4Q7 was used as a negative control. The CFU (colony forming unit) was estimated by plating serial dilutions on LB medium. The δ-endotoxins yield per spore is calculated by dividing the concentration of δ-endotoxins synthesised per ml by the CFU per ml.

3. Results and discussion

3.1. Evaluation of the presence of Bti endotoxin genes by PCR

Eight *Bti* important genes (*cry4A/cry4B, cry11A, cyt1A, cyt2B*, *cry10, cyt1C, p19, p20)* carried by the plasmid pBtoxis (Supplementary Fig. 1) and responsible for the *Bti* insecticidal activities, were explored by PCR using specific the pairs of primers listed in Supplementary Tables 1 and 2. The results of the PCR amplifications (Supplementary Table 3) showed that the Qatari QBT220 had all the expected *Bti* genes *cry4A*, *cry4B*, *cry11A, cyt1A*, *cyt2B*, *p19* and *p20*. It was noticed that the *Bti* QBT220 showed the presence of all the important endotoxin and accessory protein coding genes except *cry10* and *cyt1C*.

Analyzing the positions of the genes on pBtoxis plasmid of reference strains *Bti* H14, it was realized that the two missing genes are placed next to each other on this mega plasmid. Also, the gene *cry10* and *cyt1C* are

downstream of another important gene *cry4B*. As the *cry4B* gene is present in QBT220, it was necessary to check the entire region downstream from *cry4B* to understand the possible structural instability that is responsible for the absence of *cry10* and *cyt1C* genes.

3.2. Investigation of plasmid structural instability by regional mapping

Knowing the sequence of the pBtoxis plasmid DNA, walking technique was applied where primers were designed to amplify overlapping specific regions of about 1 kb each. Primers were designed for the region between the positions 30 kb and 50 kb in pBtoxis. This region of pBtoxis contains the two genes under investigation, *cry10A* and *cyt1C*. This region also includes the gene *cry4B*, which was shown by PCR to be present in the reference H14 as well as in QBT220. The primer sets F1-R1, F1-Dip1B, Dip1A-Dip1B and Dip1A-R1 gave the expected PCR products for the reference as well as QBT220; while all other primer sets gave expected PCR products for the reference strain H14 only, but not for QBT220 (Fig. 1). As mentioned in this figure, all the horizontal red lines indicate the regions that could not be amplified in the pBtoxis plasmid of QBT220 but were amplified correctly when using the pBtoxis of the reference strain H14. These data demonstrate that in QBT220, a very big region of about 14-kb was altered in QBT220. This region is comprised between the position of the primer Dip1B and the primer Cy1C-2.

This is considered as pBtoxis, plasmid structural instability occurring in QBT220 but not in all explored strains. Such structural instability was reported for other *Bti* strains isolated in north Africa, but also accompanied with the reduction of insecticidal activities (Zghal and Jaoua, 2006).

Therefore, it was necessary to explore QBT220 δ-endotoxins production and insecticidal activities and compare with those of the reference strains to understand the effect of plasmid structural instability.

3.3. Investigation of the insecticidal activity of QBT220 against Aedes aegypti larvae

The quantitative bioassay for *Bti* H14 against 3rd instar *Aedes aegypti* larvae was performed using different concentrations to estimate its LC50 value (Fig. 2). Among the concentrations tested, all the tests for concentrations of 0.01 μg/l and below could not kill any larvae. On the other hand, all tests for concentrations of 0.25 μg/l and above could kill 100% of larvae. The concentration (estimated LC50) that killed about 50% of the tested larvae was 0.1 μg/l. This estimated LC50 was used to check the efficiency of the local *Bti* strain QBT220. Spore-crystal mixture

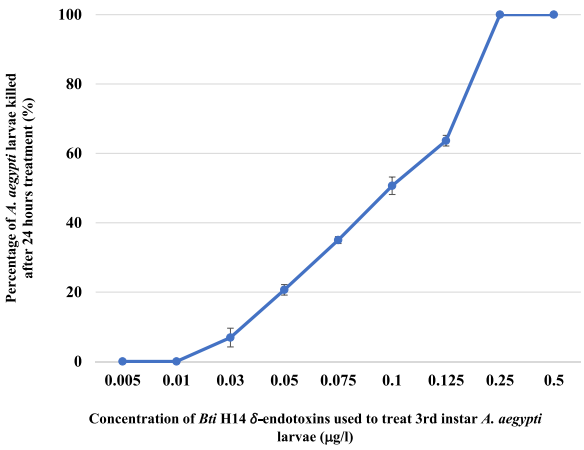


Fig. 2. Estimation of lethal concentration (50%) of the reference strain Bti H14. Red dot shows the LC_{50} value of Bti H14 that killed about 50% of the tested larvae. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of QBT220 was diluted to get a 100 ml solution of concentration 0.1 μg/l (estimated LC50 of H14). QBT220 at this tested concentration could kill more than 70% of the tested larvae. The *Bti* strain QBT220 was further studied to estimate its LC50. The larvae were treated with five different concentrations of spore-crystal mixture of QBT220. After 24 h of treatment, the number of live and dead larvae were counted, it was found that QBT220 could kill more than 50% of larvae even at 0.075 μg/l (Table 1).

Probit analysis software showed that the calculated LC_{50} for the reference strain H14 was 0.095 μg/l. On the other hand, the calculated LC_{50} for QBT220 was 0.06 μg/l (Table 2). It was concluded by statistical analysis that the local Bti strain QBT220 was the most efficient when compared to the commercially used reference strain H14.

3.4. Estimation of the δ-endotoxin production by the highly insecticidal Bt strain QBT220

The investigation of the δ-endotoxin synthesis per spore by the candidate highly insecticidal *Bt* strain QBT220, using the reference strains H14 and QBT217 as positive controls, was carried out for 72 h at 30 °C to allow complete sporulation and synthesis of crystals. Then, the

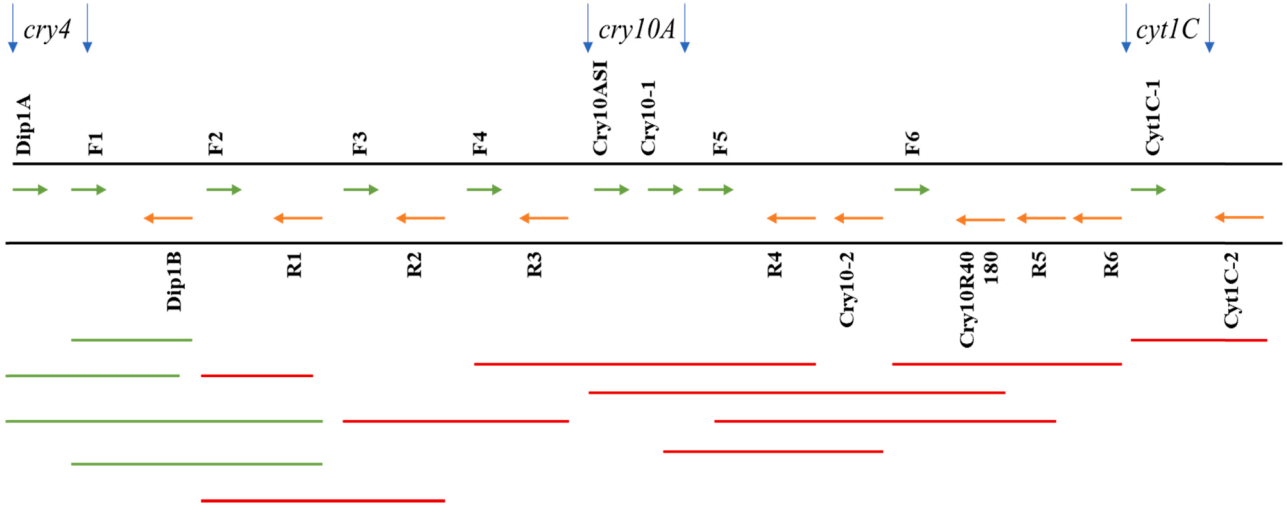


Fig. 1. QBT220 pBtoxis plasmid 50 kb region PCR mapping. The green arrows indicate the forward primers and the orange arrows indicate the reverse primers (listed in Supplementary Table 2); the green lines depict the PCR products obtained as expected and red lines depict the region where no amplifications were obtained with corresponding primers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Quantitative bioassay of Bt QBT220 strain δ-endotoxins against 3rd instar larvae of *Aedes aegypti*.

Concentrations (μg/l)	Average of killed larvae (%)[a]
0.005	0
0.03	20.7 ± 2.9
0.05	41.3 ± 2.9
0.075	60.3 ± 5.8.
0.1	71.3 ± 9.5
0.125	89.0 ± 3.0
0.25	100

[a] Different concentrations of QBT220 δ-endotoxins were used in Bioassays against 25 larvae of 3rd instar larvae of *Aedes aegypti*. Each concentration was tested in triplicate and % of killed larvae are presented as mean + /- standard deviation error values. Probit analysis software showed that the calculated LC_{50} for the reference strain H14 was 0.095 μg/l and the calculated LC_{50} for QBT220 was 0.06 μg/l (Table 2).

Table 2
Probit analysis of quantitative bioassay of Bti QBT220 and H14 against 3rd instar larvae of *Aedes aegypti*.

Strains			Probit analysis output	
H14	LC50 (%)	LC (μg /l)	95% Fiducial CI	
			Lower	Upper
		0.094797	0.0675826	0.1329701
QBT220	LC50 (%)	LC (μg /l)	95% Fiducial CI	
			Lower	Upper
		0.060121	0.0452774	0.0798318

95% fiducial limit indicates the range of LC50 for each strain tested.

δ-endotoxin concentration and the CFU per ml were determined.

It was noticed that QBT220 had the lowest cell growth with a CFU of 1013.33 (+/- 75.35) x 10^5 CFU/ml when compared with the two control strains H14 (1883.33 (+/- 93.86) x 10^5 CFU/ml) and QBT217 (1370.00 (+/- 95.39) x 10^5 CFU/ml) (Table 3, Fig. 3). The control strain H14 showed the highest δ-endotoxin production of 147.251 (+/- 1.73) μg/ml, followed by QBT217, 111.895 (+/- 8.73) μg/ml and QBT220 with 101.858 (+/- 4.57) μg/ml (Table 3, Fig. 3). These data demonstrate that QBT220 had the highest δ-endotoxin synthesis per spore of 100.94 (+/- 2.95) x 10^{-5} ng/cell, followed by QBT217 with 81.71 (+/- 3.02) 10^{-5} ng/spore and H14 with 78.48 (+/- 2.99) x 10^{-5} ng/spore (Table 3, Fig. 3). QBT220 showed the highest production capacity. This could be attributed to the loss of plasmid DNA. Several studies have been conducted where the plasmid DNA or the genomic DNA are mutated to lose huge sections of "unwanted genes" to enhance the expression of proteins of interest (Manabe et al., 2011; Li et al., 2016). This could be due to the loss of genes expressing inhibitory proteins and proteolytic proteins or simply due to lesser number of genes to be transcribed and translated (Suárez et al., 2019).

Table 3
Determination and statistical analysis of δ-endotoxin yield for Qatari *Bti* strains with references in soya meal starch based.

Strains	δ-endotoxin (μg/ml)	Cell biomass (10^5) cfu/ml	δ endotoxin content (ng/10^5 cfu)
H14	147.251 + /- 1.73	1883.33 + /- 93.86	78.48 + /- 2.99
***QBT*217**	111.895 + /- 8.73	1370.00 + /- 95.39	81.71 + /- 3.02
***QBT*220**	101.858 + /- 4.57	1013.33 + /- 75.35	100.94 + /- 2.96

Positive control is *Bti* strain H14; Negative control is *Bti* strain 4Q7; + /- standard deviation error values

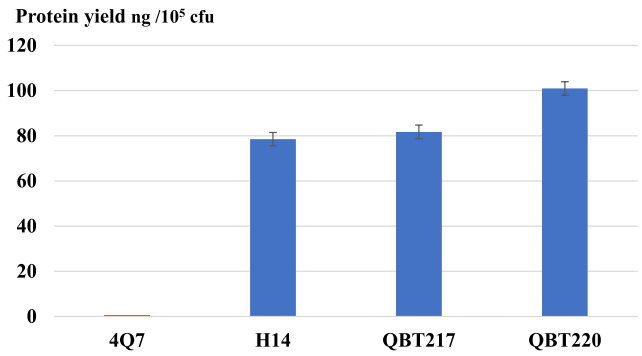


Fig. 3. Comparing the protein yield per spore (ng/10^5 cfu) of Qatari *Bti* strains with references in soya meal starch based medium. *Bti* H14 is the positive control; *Bti* 4Q7 is the negative control; Qatari *Bti* strains: QBT217, QBT220; error bars are standard error values plotted for positive and negative values.

4. Conclusion

In conclusion, the *Bti* strain QBT220, isolated from Qatar, showed a pBtoxis plasmid structural instability evidenced by the alteration of a region of 14-kb carrying the genes *cry10* and *cyt1C* encoding two of its insecticidal proteins. These findings confirm the existence of another case of pBtoxis plasmid DNA structural instability in a Qatari *Bti* strain, which is consistent with other such cases reported, like the one in north African region (Zghal and Jaoua, 2006).

From these reports, it was expected that the plasmid structural instability in Qatari *Bti* strain would also have some effect on its δ-endotoxin production capacity and insecticidal activity as the *Bti* toxins have synergistic effect on insect larvae (Schnepf et al., 1998; Monnerat et al., 2014). As reported by Carvalho et al. (2018), even after intensive selection pressure, *Aedes aegypti* larvae remain more susceptible when exposed to all *Bti* crystal proteins rather than to individual δ-endotoxin, due to well-known crystal proteins synergistic effects.

But, despite of the observed plasmid structural instability and the absence of two insecticidal δ-endotoxin genes *cry10* and *cyt1C*, QBT220 was found to be even more insecticidal than the reference H14. These findings not only confirm the phenomenon of pBtoxis structural instability observed rarely in *Bti* strains, but also show that the deleted genes *cry10A* and *cyt1C* are not crucial in *Bti* toxicity.

In case of insecticidal activity, Cry10A protein has a synergistic function but as it is expressed in such low quantity in *Bti*, its absence may not show a significant loss of insecticidal activity. On the other hand, absence of Cyt1C can enhance the insecticidal activity. Cyt1C protein consists of an N terminal that resembles other Cyt proteins in its family, but the C terminal is unique to Cyt1C. Unlike other Cyt proteins, with non-specific and detergent like cytolytic action, Cyt1C possess a membrane receptor binding C terminal similar to that of Mosquito-larvicidal Mtx-1 toxin (Berry et al., 2002). From these reports, it is clear that Cyt1C can bind to larval midgut but has no insecticidal property. In other words, Cyt1C can compete with larval midgut membrane binding Cry4A/4B, which is completely lost in case of QBT220 and hence shows higher insecticidal activity against *Aedes aegypti* larvae. This is the first report of the possible inhibitory effect of Cyt1C reported in a *Bti* strain. The absence of certain genes and hence their associated proteins has ensured that Qatari *Bti* strains QBT220 has a significantly lower LC50 value than the reference strains H14. Further studies need to be done to quantitatively evaluate this competition between the Cyt1C protein and the Cry4A/Cry4B proteins. Also, there is a speculation of how the genes encoding insecticidal Cyt1A has evolved from that of the Cyt1C by losing the specific membrane binding domain and hence contributing highly to the insecticidal activity (Manasherob et al., 2006) of *Bti*. Does that mean that Cyt1C is now "redundant" and hence lost in QBT220?

In case of δ-endotoxin production per spore, QBT220 showed the highest production capacity. This could be attributed to the loss of

plasmid genome. Several studies have been conducted where the plasmid DNA or the genomic DNA are mutated to lose huge sections of "unwanted genes" to enhance the expression of proteins of interest (Manabe et al., 2011; Li et al., 2016). This could be due to the loss of genes expressing inhibitory proteins and proteolytic proteins or simply due to lesser number of genes to be transcribed and translated (Suárez et al., 2019).

In conclusion, it is clear that QBT220 would be an excellent candidate for industrial production of bioinsecticides due to both its high insecticidal activities and δ-endotoxin production. As reported by Nair et al. (2018), a mutated Cyt1A protein from another Qatari *Bti* strain had enhanced its anti-cancer activity as compared to the reference strain H14. Similarly, a naturally occurring QBT220 needs to be explored further to confirm the hypotheses made here as it could help understand many unanswered questions regarding *Bti* toxicity and the evolution of *Bti* δ-endotoxins.

CRediT authorship contribution statement

K.N. and S.J.: Conceptualization; **K.N. and S.J.**: Methodology; **K.N, R.A. and S.J.**: Validation and analysis of results; **S.J**: Resources provided; **K.N. and S.J.**: Writing – review & editing; **S.J.**: Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We are very grateful to Prof. Fabrice Chandre and Mrs. Carole Ginibre, from the "*Laboratoire de Lutte contre les Insectes Nuisibles* (*LIN*), *Montpellier*, *France*" for providing us with the eggs of *A.aegypti* and all methodologies related to the insecticidal activity determination, bioassays and data analysis. This publication was made possible by GSRA (Graduate Student Research Award) grant no. GSRA2-1-0604-14015 from the Qatar National Research Fund (a member of Qatar foundation), Qatar. The findings achieved herein are solely the responsibility of the authors. The publication of this article was supported by the Qatar National Library, member of the Qatar foundation.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.112975.

References

Ben-Dov, E., 2014. Bacillus thuringiensis subsp. israelensis and Its dipteran-specific toxins. Toxins 6, 1222–1243. https://doi.org/10.3390/toxins6041222.

Berry, C., O'Neil, S., Ben-Dov, E., Jones, A.F., Murphy, L., Quail, M.A., Parkhill, J., 2002. Complete sequence and organization of pBtoxis, the toxin-coding plasmid of Bacillus thuringiensis subsp. israelensis. Appl. Environ. Microbiol 68, 5082–5095. https://doi.org/10.1128/aem.68.10.5082-5095.2002.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Brar, S.K., Verma, M., Tyagi, R.D., Valéro, J.R., Surampalli, R.Y., 2005. Starch industry wastewater-based stable Bacillus thuringiensis liquid formulations. J. Econ. Entomol. 98, 1890–1898. https://doi.org/10.1093/jee/98.6.1890.

Carvalho, K.S., Crespo, M.M., Araújo, A.P., Silva, R.S., de Melo-Santos, M.A.V., de Oliveira, C.M.F., Silva-Filha, M.H.N.L., 2018. Long-term exposure of Aedes aegypti to Bacillus thuringiensis svar. israelensis did not involve altered susceptibility to this microbial larvicide or to other control agents. Parasites Vectors 11, 673–683. https://doi.org/10.1186/s13071-018-3246-1.

Cohen, S., Albeck, S., Ben-Dov, E., Cahan, R., Firer, M., Zaritsky, A., Dym, O., 2011. Cyt1Aa toxin: crystal structure reveals implications for its membrane-perforating function. J. Mol. Biol. 413, 804–814. https://doi.org/10.1016/j.jmb.2011.09.021.

Garduno, F., Thorne, L., Walfield, A., Pollock, T., 1988. Structural relatedness between mosquitocidal endotoxins of Bacillus thuringiensis subsp. israelensis. Appl. Environ. Microbiol. 54, 277–279. https://doi.org/10.1128/AEM.54.1.277-279.1988.

Itsko, M., Manasherob, R., Zaritsky, A., 2005. Partial restoration of antibacterial activity of the protein encoded by a cryptic open reading frame (cyt1Ca) from Bacillus thuringiensis subsp. israelensis by site-directed mutagenesis. J. Bacteriol. 2005 (187), 6379–6385. https://doi.org/10.1128/JB.187.18.6379-6385.2005.

Jacups, S.P., Rapley, L.P., Johnson, P.H., Benjamin, S., Ritchie, S.A., 2013. Bacillus thuringiensis var. israelensis misting for control of Aedes in cryptic ground containers in North Queensland, Australia. Am. J. Trop. Med. Hyg. 88, 490–496. https://doi.org/10.4269/ajtmh.12-0385.

Jaoua, S., Zouari, N., Tounsi, S., Ellouz, R., 1996. Study of the δ-endotoxins produced by three recently isolated strains of Bacillus thuringiensis. FEMS Microbiol. Lett. 145, 349–354. https://doi.org/10.1111/j.1574-6968.1996.tb08599.

Lacey, L.A., 2007. Bacillus thuringiensis serovariety israelensis and Bacillus sphaericus for mosquito control. J. Am. Mosq. Contr. Assoc. 23, 133–163. ⟨https://pubmed.ncbi.nlm.nih.gov/17853604/⟩.

Lee, S., Eckblad, W., Bulla, L., 1985. Diversity of protein inclusion bodies and identification of mosquitocidal protein in Bacillus thuringiensis subsp. israelensis. Biochem. Biophys. Res. Commun. 126, 953–960. https://doi.org/10.1016/0006-291X(85)90278-5.

Lee, B.M., Scott, G.I., 1989. Acute toxicity of temephos, fenoxycarb, diflubenzuron, and methoprene and Bacillus thuringiensis var. israelensis to the Mumrnichog (Fundulus heteroclitus). Bull. Environ. Contam. Toxicol. 43, 827–832. https://doi.org/10.1007/BF01702051.

Li, Y., Zhu, X., Zhang, X., Fu, J., Wang, Z., Chen, T., Zhao, X., 2016. Characterization of genome - reduced Bacillus subtilis strains and their application for the production of guanosine and thymidine. Microb. Cell Fact. 15, 1–15. https://doi.org/10.1186/s12934-016-0494-7.

Manabe, K., Kageyama, Y., Morimoto, T., Ozawa, T., Sawada, K., Endo, K., Tohata, M., Ara, K., Ozaki, K., Ogasawara, N., 2011. Combined effect of improved cell yield and increased specific productivity enhances recombinant enzyme production in genome-reduced Bacillus subtilis strain MGB874. Appl. Environ. Microbiol. 77, 8370–8381. https://doi.org/10.1128/AEM.06136-11.

Manasherob, R., Itsko, M., Sela-Baranes, N., Ben-Dov, E., Berry, C., Cohen, S., Zaritsky, A., 2006. Cyt1Ca from Bacillus thuringiensis subsp. israelensis: production in Escherichia coli and comparison of its biological activities with those of other Cyt-like proteins. Microbiology 152, 2651–2659. https://doi.org/10.1099/MIC.0.28981-0.

Merritt, R.W., Walker, E.D., Wilzbach, M.A., Cummins, K.W., Morgan, W.T., 1989. A broad evaluation of Bti. for black fly (Diptera: Simuliidae) control in a Michigan river: efficacy, carry and nontarget effects on invertebrates and fish. J. Am. Mosq. Contr. Assoc. 5, 397–415. ⟨https://pubmed.ncbi.nlm.nih.gov/2584974/⟩.

Mittal, P.K., 2003. Biolarvicides in vector control: challenges and prospects. J. Vect. Borne Dis. 40, 20–32. ⟨https://pubmed.ncbi.nlm.nih.gov/15119068/⟩.

Monnerat, R., Pereira, E., Teles, B., Martins, E., Praça, L., Queiroz, P., Soares, C.M., 2014. Synergistic activity of Bacillus thuringiensis toxins against Simulium spp. larvae. J. Invertebr. Pathol. 121, 70–73. https://doi.org/10.1016/J.JIP.2014.07.003.

Nair, K., Iskandarani, A., Al-Thani, R., Mohammad, R., Jaoua, S., 2018. The replacement of five consecutive amino acids in the Cyt1A Protein of Bacillus thuringiensis enhances its cytotoxic activity against lung epithelial cancer cells. Toxins 10, 125. https://doi.org/10.3390/toxins10030125.

Nair, K., Al-Thani, R., Ginibre, C., Chandre, F., Alsafran, M., Jaoua, S., 2020. Bacillus thuringiensis strains isolated from Qatari soil, synthesizing δ-endotoxins highly active against the disease vector insect Aedes aegypti Bora Bora. Heliyon 6, e05003. ⟨https://authors.elsevier.com/sd/article/S2405-8440(20)31846-6⟩.

Palma, L., Muñoz, D., Berry, C., Murillo, J., Caballero, P., 2014. Bacillus thuringiensis toxins: an overview of their biocidal activity. Toxins 6, 3296–3325. https://doi.org/10.3390/toxins6123296.

Poopathi, S., Archana, B., 2012. A novel cost-effective medium for the production of Bacillus thuringiensis subsp. israelensis for mosquito control. Trop. Biomed. 29, 81–91. ⟨https://pubmed.ncbi.nlm.nih.gov/22543607/⟩.

Roh, J.Y., Choi, J.Y., Li, M.S., Jin, B.R., Je, Y.H., 2007. Bacillus thuringiensis as a specific, safe, and effective tool for insect pest control. J. Microbiol. Biotechnol. 17, 547–559. ⟨https://pubmed.ncbi.nlm.nih.gov/18051264/⟩.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning. A Laboratory Manual, 2nd ed. Cold Spring Harbor,NY, USA: Cold Spring Harbor Laboratory. https://doi.org/10.1002/jobm.3620300824.

Schnepf, E., Crickmore, N., Van Rie, J., Lereclus, D., Baum, J., Feitelson, J., Zeigler, D.R., Dean, D.H., 1998. Bacillus thuringiensis and its pesticidal crystal proteins. Microbiol. Mol. Biol. Rev. 62, 775–806. ⟨https://pubmed.ncbi.nlm.nih.gov/9729609/⟩.

Suárez, R.A., Stülke, J., van Dijl, J.M., 2019. Less is more: toward a genome-reduced Bacillus cell factory for "difficult proteins". ACS Synth. Biol. 8, 99–108. https://doi.org/10.1021/acssynbio.8b00342.

Travers, R.S., Martin, P.A., Reichelderfer, C.F., 1987. Selective process for efficient isolation of soil Bacillus spp. Appl. Environ. Microbiol. 53, 1263–1266. ⟨https://www.ncbi.nlm.nih.gov/pmc/articles/PMC203852/pdf/aem00123-0067.pdf⟩.

Valtierra-De-Luis, D., Villanueva, M., Lai, L., Williams, T., Caballero, P., 2020. Potential of Cry10Aa and Cyt2Ba, two minority δ-endotoxins produced by Bacillus thuringiensis ser. israelensis, for the control of Aedes aegypti larvae. Toxins 12 (6), 355. https://doi.org/10.3390/TOXINS12060355.

Wang, J., Mei, H., Qian, H., Tang, Q., Liu, X., Yu, Z., He, J., 2013. Expression profile and regulation of spore and parasporal crystal formation-associated genes in Bacillus thuringiensis. J. Proteome Res. 12, 5487–5501. https://doi.org/10.1021/pr4003728.

WHO , 2005. Guidelines for laboratory and field testing of mosquito larvicides. ⟨https://www.who.int/whopes/resources/who_cds_whopes_gcdpp_2005.13/en/⟩.

Wirth, M.C., Georghiou, G.P., Federici, B.A., 1997. CytA enables CryIV endotoxins of Bacillus thuringiensis to overcome high levels of CryIV resistance in the mosquito, Culex quinquefasciatus. Proc. Natl. Acad. Sci. USA 94, 10536–10540. https://doi.org/10.1073/pnas.94.20.10536.

Wirth, M.C., Jiannino, J.A., Federici, B.A., Walton, W.E., 2005. Evolution of resistance toward Bacillus sphaericus or a mixture of B. sphaericus Cyt1A from Bacillus thuringiensis, in the mosquito, Culex quinquefasciatus (Diptera: Culicidae). J. Invertebr. Pathol. 88, 154–162. https://doi.org/10.1016/j.jip.2005.01.003.

Zghal, R.Z., Jaoua, S., 2006. Evidence of DNA rearrangements in the 128-kilobase pBtoxis plasmid of Bacillus thuringiensis israelensis. Mol. Biotechnol. 33, 191–198. https://doi.org/10.1385/MB:33:3:191.

Zhang, J., Pan, Z.Z., Xu, L., Liu, B., Chen, Z., Li, J., Niu, L.Y., Zhu, Y.J., Chen, Q.X., 2018. Proteolytic activation of Bacillus thuringiensis Vip3Aa protein by Spodoptera exigua midgut protease. I. J. Biol. Macromol. 107, 1220–1226. https://doi.org/10.1016/J.IJBIOMAC.2017.09.101.

Zouari, N., Dhouib, A., Ellouz, R., Jaoua, S., 1998. Nutritional requirements of a Bacillus thuringiensis subsp. kurstaki strain and use of gruel hydrolysate for the formulation of a new medium for δ-endotoxin production. Appl. Biochem. Biotechnol. 69, 41–52. https://doi.org/10.1007/BF02786020.