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Original article

Biotoxicity assessment of cloned cry 11 protein gene from *Bacillus thuringiensis* 9NF

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ABSTRACT

The current investigation describes the isolation and characterization of toxic *Bt*. local isolates harboring 99% homology with *Bti.* prototoxin *Bacillus thuringiensis* (AXJ97553.1 and novel OUB27301.1) which contains full length *cry11* gene (1.9 kb). Initially, it was cloned in pTZ57R/T and then sub-cloned in pET30a(+) for expression. The optimized conditions for good expression were found 1 mM IPTG, 3.5–4 h incubation time, and 37 °C. Toxicological assays were determined against 3rd instar larvae of *Aedes aegypti* with expressed partially purified and crude recombinant protein using recombinant *E. coli* BL21, DE3 transformed with *cry11* gene. It was found that partially purified *Bt.* protein is highly toxic against *A. aegypti* larvae with LC_{50} value of 42.883 ± 6 μg/ml. *B. thuringiensis* strains producing Cry 11 toxic protein can be used as biopesticide to control resistance in insects.

1. Introduction

The World Health Organization (WHO) suggested the exercise of biopesticides built on microorganisms to control vector-borne diseases. The tons of insecticides are being used to control pest, a short cut approach to increase agriculture production. The WHO recommended the use of insecticides founded in microorganism to keep in check with mosquito borne diseases (WHO, 2009).

Fernandez et al. (2009) described the Cry11 δ- endotoxins protein tertiary structure which was not resolute by X-rays crystallography. Alvaro et al. (2018) reported three discrete structural domains of the Cry11 family which goes to a large group of δ-endotoxins. The Cry11Aa and Cry2A ICP from *Bti, Btj*, *Btk*, have been analytically considered which specify dipteran-active toxins. The mysterious potentiality of Cry11 toxin remains incomplete because of novelty and mutation in *cry11* gene which is a successful approach to control resistance in dipteran insects. *Bti* (*Bacillus thuringiensis* subsp. *israelensis*), *Btj*, *Btko* and *Btk* are current biotic remedies for this biological problem. Five Cry11 deviations found by DNA shuffling exhibited lethal activity against *Culex quinquefasciatus* and *Aedes aegypti*. Three of these variants were categorized on the basis of protein docking and 3D modeling (Wang et al., 2019).

Mutation resulted due to point mutations, deletions, and insertions cause a change in structural domain, toxin-receptor interactions and toxic activities. The toxicity increased many fold due to variability in domain III which causes high sequence erraticism as compared to deletion of the N-terminal segment in domain I. The specific domains in *cry11* genes family expose new visions into the solicitation of engaged evolution policies to homework on the genetic variability. The strain produced endotoxin, assessed by bioassay against dipteran and lepidopteran insects, which showed a higher toxicity index than control HD500. The prominent molecular masses are 130, 65–72 kDa of Cry1, Cry2, Cry10, Cry11 protein, respectively. The outcomes of this study may approve the constant *Bt.* screening programmed from different ecological regions of the world (Baig et al., 2010; Crickmore et al., 2020).

The Cry toxin structures help to explore toxin specificity which is based on amino acid sequence differences and host specificity. All types of Cry toxins have 3 domains (I, II, and III). Domain I has an α-helical roll in which 6 helices frame around the core helix and its amphipathic, aromatic, hydrophobic nature involve in membrane insertion (Li et al., 2001). Domain II consists of 3 antiparallel sheets which are stick together like a prism with

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pseudo 3 fold symmetry. Its specificity is apex loops and its variability is due to variable length, conformation and sequence. Domain III is less variable and the difference is due to length, conformation, orientation of loop binding pocket associated in receptor joining (Pigott et al., 2008; Wang et al., 2019). Domain III of different toxin proteins was compared and significant similarity index to Carbohydrate binding module (CBMs) was observed in microbial lyases, esterases, glycoside hydrolases (Wang et al., 2019).

In the present study the full length *cry11* gene from highly toxic *Bt*. local isolate was amplified, purified and was cloned and sequenced. The gene showed 99% homology of the cry11gene encoding Bti. prototoxin of *B. thuringiensis* (AXJ97553.1 and novel OUB27301.1), and it was cloned into the expression vector pET 30 a (+) and transformed in *E. coli* BL21 DE3. The transformed organism was used for bioassays which showed extraordinary toxicity against dipteran insects.

2. Materials and methods

2.1. Screening and characterization of local Bt. isolates

The screening of *Bt.* isolates was done as proposed by Martin and Travers (1989). Briefly, soil samples (0.5 g) were suspended LB medium (10 ml) containing 0.2 M sodium acetate, shaken well, and incubated for 4 h at 30 °C. The incubated samples were filtered using filter paper (0.25 nm) and heated at 80 °C for 15 min to isolate spore formers. The above treated samples were diluted 1:2 and then spread on LB agar plates and incubated overnight at 30 °C. Colonies with B.t. like morphology were picked, streaked on LB agar plates, and incubated 24 h at 30 °C (Bukhari and Shakoori, 2010). Genomic DNA from screened *Bt*. was isolated using phenol chloroform extraction method according to Sambrook and Russel (2001) and confirmed by agarose gel (1%) electrophoresis, 2–3 µl of RNAase solution (1 mg/0.1 ml), was mixed by inverting 2–4 times and reared at 37 °C for 25 min. DNA was stored at –20 °C. PCR amplification for the conserved and full length 16S rRNA gene was done (0.5 Kb and 1.6 Kb) using *Bt*. specific primers (Bukhari and Shakoori (2009).

2.2. Amplification of conserve region of cry11gene

To confirm the presence of *cry 11* gene in *Bt.* isolates, shorter fragment of *cry11* gene (0.65 Kb) was amplified having an annealing temperature 51 °C from local *Bt*. isolates using reported primer by Bravo et al. (2011).

2.3. Biotoxicity assays with Bt. spores and total cell protein

Bt. spore and total cell protein diet from *Bt.* isolates were prepared according to the method described by Makino et al. (1994) and Bukhari and Shakoori (2010), respectively. Protein content was estimated by Lowry method (1951) and studied by SDS-PAGE. The local population of *Aedes aegypti* was used to assess the toxicity of spore and total cell protein of *Bt*. isolates (Bukhari and Shakoori, 2009,2010).

2.4. Amplification and purification of cry11 gene

The polymerase chain reaction was performed to screen *Bt.* isolates positive for *cry11* gene. The amplification of 1.9-kb full length *cry11* gene was done by using following reported primers (Bukhari and Shakoori, 2009).

Forward: 5′ ATGGAAGATAGTTCTTTAGAT 3′.

Reverse: 5′ CTACTTTAGTAACGGATT 3′.

The optimized PCR conditions for amplification of full length *cry11* gene was denaturation at 94 °C for two min, annealing temperature 48 °C for 1.5 min and extension at 72.5 °C for 2.5 min with final extension of 10 min with 35 cycles. The full length *cry11* gene was purified according to procedure described by Sambrook et al. (1998) through Fermentas gene clean kit (#K0153).

2.5. Cloning of full length cry11 gene

2.5.1. Ligation of cry11gene in pTZ57R/T

Amplified *cry11* gene (1.9 Kb) was cloned in pTZ57R/T Fermentas Ins TAclone ™ PCR cloning Kit (# K1214). For ligation, 30 µl reaction mixture was prepared using 3 µl vector, 5X ligation buffer 6 µl, purified PCR product 4 µl, T4 ligase (5U) 1 µl, and nuclease free water 16 µl. The mixture was incubated overnight at 16 °C and stored at −20 °C.

2.5.2. Competent cells preparation

Competent cells of *E. coli* (DH5α) were prepared according to protocol described by Sambrook and Russel (2001). Single colony of 18 h old culture was inoculated in 5 ml LB broth and was incubated at 37 °C overnight. Initially 1% inoculum of DH5α was transferred in 50 ml LB broth and incubated at 37 °C in a shaking incubator for 2–3 h until its OD value reached to.0.2–0.3. Then, it was centrifuged at 5400 xg at 4 °C for 10 min in a sterile falcon tube (50 ml). Supernatant discarded and the pellet was resuspended in ice cold 20 ml $CaCl_2$ (50 mM) and was left for 40 min on ice. After 40 min, centrifugation was done at 5400 xg at 4 °C (10–15 min). The resultant supernatant was discarded and the pellet was again resuspended in 3–4 ml ice cold $CaCl_2$ (50 mM). The cells were stored on ice until needed.

2.5.3. Transformation of E. coli DH5α with cry11gene

For transformation, 15 µl of ligated mixture (pTZ-*cry11*) was added in 200 µl competent cells. It was mixed gently and kept on ice for 45 min. After this, cells were quickly transferred to 42 °C for 90 min and again placed on ice for 6 min for heat shock purpose. Later, 1 ml LB broth was added, mixed, and was kept at 37 °C for 1–2 h without quaking. For screening, LB agar plates containing ampicillin, IPTG and X-gal were prepared. Then, 200 µl transformed cells were grown on LB agar plates containing 2% X-gal (20 mg dissolved in 1 ml of N, N-dimethylformamide DMF) 150 µl, IPTG 150 µl (100 Mm = 23.8 mg/ml) plates, and ampicillin 100 µl (100 mg/ml). For blue and white colonies selection, the plates were placed in an incubator at 37 °C for 24 h. Positive transformed white colonies were selected and re-streaked on an ampicillin plate. Colony PCR, mini-prep, restriction analysis of ligated gene, and sequencing was done to confirm the presence of insert (full length 1.9 Kb *cry11* gene) as described by Sambrook and Russel (2001).

2.5.4. Expression of full length cry11 gene from most toxic Bt. isolates in E. coli

For expression the *cry11* gene of most toxic *Bt.* isolate was then cloned in expression vector pET30a(+) and was transformed in *E. coli* BL21(DE3) competent cells as a host for plasmid propagation. To screen positive transformants LB agar kanamycin (50 µg/ml) plates were made and 200 µl of transformed cells [(ligated with pET30a(+)] were then spreaded. For confirmation restriction digestion of *cry11* full length gene previously cloned in expression vector pET30a(+) was done by using *EcoR1* and *Hind111* (Fermentas # ER027). The colony PCR was done for further confirmation of the presence of 1.9 kb ligated *cry11* gene (Bukhari and Shakoori, 2009).

For expression of Cry11 protein 1% inoculum from of overnight culture of recombinant organisms (*E. coli* BL21) transformed with ligated *cry11* gene in pET30a(+) was inoculated in 15 ml LB med-

ium containing kanamycin (50ug/ml). For negative control organism without insert in the expression vector was incubated at 37 °C till O.D reached to 0.2–0.6. The conditions were optimized for good expression of *cry11* protein gene regarding IPTG concentration ranging (0.5, 1.0, and 1.5 mM), incubation temperature (25, 30, 37, 40, and 45 °C, and incubation time (3, 5, 7, and 10 h).

2.6. Isolation and purification of expressed recombinant Cry11 protein

Single colony of positive transformant (BL21 DE3 cells transformed with pET30a (+) ligated with *cry11* gene) was inoculated in 5 ml LB kanamycin broth, placed in incubator at 37 °C for 24 h, and centrifuged at 13000 rpm for 5 min. The resultant pellet was washed with double deionized water and vortexed with 200 µl of lysis buffer (0.01% mercaptoethanol, 1% SDS). The centrifugation was done at 13000 rpm (10 min) and the resultant supernatant was discarded carefully in the new tube without disturbing the pellet. The expressed protein was visualized by 12% SDS-PAGE (12% resolving, 5% stacking) with negative control (Laemmli, 1970). Protein was partially purified by using two methods viz. heat shock method and high alkaline pH stress (alkaline buffer 20 mM $NaHCO_3$, 30 mM Na_2CO_3, pH 11.0–11.5). Optimization of conditions regarding good expression of recombinant Cry11 protein was previously done.

2.7. Bioassays with BL21(DE3) transformed with cry11 gene

Biotoxicity of cloned expressed recombinants Cry11 protein was performed with the eggs of 3rd instar larvae of *A. aegypti* received by insectary GCU Lahore and reared time to time with optimized conditions. The positive control HD500 (Culture collection number DSM 6087, NRRL, HD-500) was provided kindly by *Bacillus* Genetic Stock Centre (BGSC), Columbus, Ohio State, United States. Single transformed colony (BL21 (DE3) cells transformed with pET30a(+) ligated with *cry11* gene) was inoculated in 200 ml LB medium with kanamycin (50 µg/ml), IPTG (1 Mm), and was placed in shaking incubator at 37 °C for 7 h. Cells were harvested at 10,000 rpm. Pellet was then washed twice with distilled water. Different concentration of transformed organism ranging 0, 50, to 500 µg/ml in a wide mouthed cup having 20 ml autoclaved distilled water. Twenty 3rd instar larvae of mosquitoes per cup was added and incubated at 24 °C for 24 h to monitor mortality of the organism (Bukhari and Shakoori, 2010).

2.7.1. Biotoxicity assay with total expressed recombinant Cry11 protein

The toxicity of expressed recombinant Cry11 protein was determined. The transformed organism was cultured in 200 ml LB broth with kanamycin (50 µg/ml) and IPTG (1 mM) and was placed in shaking incubator at 37 °C for 3.5–4 h. Biotoxicity assays were done with total cell protein of *E. coli* transformed with *cry11* gene against third instar larvae of *A. aegypti*. The collected pellet was washed with Tris-HCl (pH 7.2), lysis buffer was added to burst the cells to release total cell protein of transformed organisms, and was estimated by Lowry method (1951). Different concentrations of crude expressed recombinant Cry 11 protein extract ranging from 0, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 µg/ml were added in each wide mouth cup containing 20 ml of distilled water with 20 larvae (*Aedes aegypti*) incubated at 20 °C for 24 h in triplicate. After 24 h, knocked down larvae were numbered in each cup and percentage mortality was determined through Log-probit analysis (Finney, 1952).

2.7.2. Bioassay with partially purified expressed recombinant Cry11 proteins

The toxicity of partially purified expressed Cry11 protein is checked against 3rd instar larvae of *A. aegypti* as described previously (Bukhari and Shakoori, 2010). Quantification of expressed recombinants partially purified Cry11 proteins was measured by method described by Lowry et al. (1951) and Bradford (1976). Different concentrations (0, 20, 40, 60, 80, 100, 120, 140, 160, 180 200 µg/ml) were used in triplicate. The larvae knocked down, counted, and their toxicity was calculated by Log probit analysis (Finney, 1952).

3. Results

On the basis of colony morphology (erect margin, boiled egg, off white colony and rich vegetative growth), staining and biochemical tests 30 *Bt.* isolates were shortlisted from different ecological environments of Pakistan. Microscopic examination revealed that these *Bt.* isolates were rod shape, gram positive and purple in color (Fig. S1a). The spores and crystal (ICPs) are released in medium after 48 to 72 h and stained green with malachite green (Fig. S1b). Ribotyping confirms that *Bt.* isolates having accession numbers, KT216626-27, MN218726-29 showed maximum homology with already reported sequence1 JQ435683.1.

3.1. Biotoxicity assays with Bt. spore and total Bt. cell protein

Bioassays performed with *Bt.* spore and total cell protein revealed that eleven *Bt.* isolates harboring *cry 11* gene were found toxic against 3rd instar larvae of *Aedes aegypti.* Three *Bt.* isolates (9NF, 6NF, and 3NF) were found toxic from 11 *Bt.* isolates. Among them 9NF was found the most toxic with LC_{50} 327.8 ± 0.17 µg/ml of spore diet against 3rd instar larvae. The mortality was 100% at 1 mg of spore/ml as compared to the positive control HD-500 (94% mortality). It was found that LC_{50} values (442.7, 460.8 µg/ml) of 6 and 4NF were comparatively less than 9NF isolate. Total cell protein LC_{50} of 9, 6, and 4NF was 69.130 ± 5, 84.1 ± 5, and 95.1 ± 407 µg/ml against 3rd instar larvae (Table.1).

Total *Bt.* cell protein profile of selected *Bt.*isolates (1NF- 9NF) was analyzed by SDS-PAGE (Fig. 1a,b). The diversity of different protein sizes were observed in SDS-PAGE having different molecular weights ranging from130 to 65 kDa. Low molecular weights protein bands of 28 and 17 kDa were also visualized in the total protein profile of *Bt.* isolates and positive control HD500.

3.2. Amplification of full length cry 11 gene

Full length *cry11*gene 1900 bp (MT840204) of most toxic *Bt.* isolate was amplified using PCR showing 99% similarity with *Bti.* prototoxin *Bacillus thuringiensis* (AXJ97553.1 & novel OUB27301.1) (Fig. 2a). The amplified *cry11* gene (1.9 Kb: MT840204) from highly toxic *Bt.* strain (9NF: accession number MT840204) was ligated in TA cloning vector pTZ57R/T and transformed in DH5α (*E.coli*). The positive transformants were screened by the selection of white colonies containing recombinant plasmid and gene cloning was confirmed by restriction analysis and colony PCR. Cry11 protein gene of most toxic with accession no MT840204 has 99% similarity with *Bti* prototoxin *Bacillus thuringiensis* (AXJ97553.1) already reported ICP gene in GENbank DNA data base (Fig. 2b).

3.3. Cry11 gene expression in BL21D3

For expression of *cry11* gene of toxic *Bt.* isolate, cloned in pET30a(+), was expressed in *E. coli* BL21(DE3) as a host for plasmid propagation whereas pET30a(+) without insert was used as a neg-

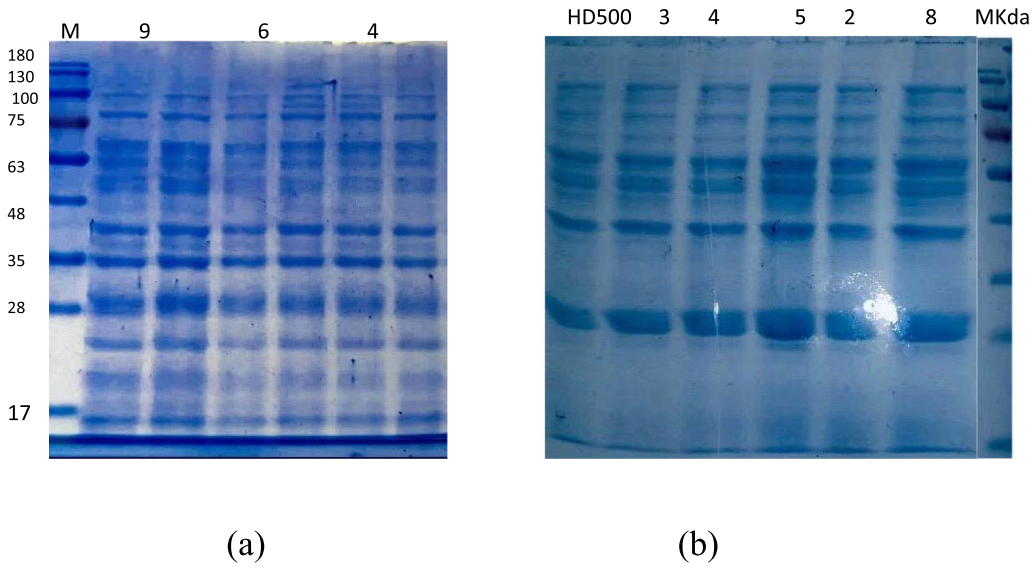

Fig. 1. a,b: Polyacrylamide gel electrophoretic profile of total protein of 9, 6, 4, 3, 2, 5, 8NF and HD500 *Cry11* gene.

Table 1
Comparison of LC_{50} of different preparations of Cry11 protein against 3rd instar larvae of *A. aegypti*.

Sr. No	Bt. isolates	Sampling sites	Soil texture and source	Spore (µg/ml)	Total Cell protein (µg/ml)	Organism transformed with *cry11* gene (µg/ml)	Crude recombinent expressed protein ng/ml (ng/ml)	Partially purified expressed RecombinantCry11 protein (ng/ml)
1.	9NF	Kashmir KSM Neelam valley	Animal dung dry waste	327.842 ± 846	69.130 ± 5	150.360 ± 5	237.098 ± 2	42.883 ± 6
2	HD500	BGSC	Reference strain	683.077 ± 2	124.9.9 ± 7	314.292 ± 7	394	108.54 ± 2
Neg. control (*transformed E. coli* BL21D3 having plasmid without *cry11* gene with pET 30				0	0	0	0	0
Zero dose				0	0	0	0	0

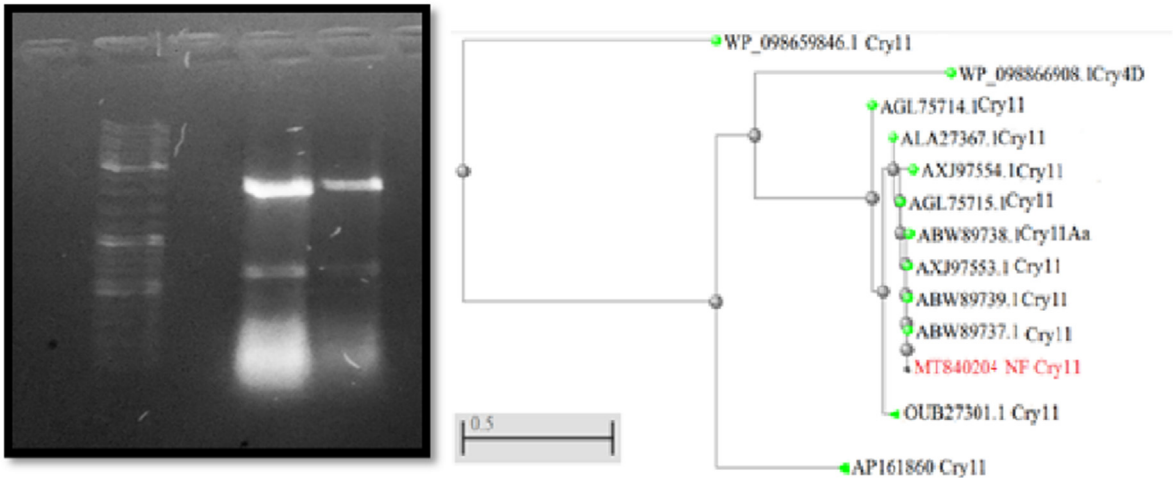

Fig. 2. (**a**) PCR product of *cry11* gene and (**b**) *cry11* gene dendrogram has shown the relatedness of Cry11 protein gene of 9NF with accession no MT840204 with the already reported ICP gene in the GENbank data base.

ative control. The recombinant plasmid DNA was isolated and digested with *EcoR1* and *Hind111* showings the presence of insert i.e. 1.9 Kb. Positive transformants were later confirmed by colony PCR showing 1.9 Kb product of *cry11* gene.

3.4. Optimum conditions and purification for the expression of Cry11 protein

Optimized conditions for expression of Cry11 protein gene i.e. temperature (16, 28, 30, 35, 37 °C) incubation time (3, 4, 5, 7 h)

and IPTG (0.5, 1.5 mM) concentration were optimized to get good expression results. It was found that IPTG concentration of 1 mM, incubation time period of 4 h and temperature of 37 °C were found optimum for the expression of *cry11* gene (Fig. 3a,b).

For purification expressed recombinant Cry 11 protein was solubilized in high alkaline pH carbonate buffer (30 mM Na2CO3, 20 mM NaHCO3, pH 11.0–11.5) and then was used in bioassays to confirm toxicity of partially purified protein (Fig. 3c).

3.5. Biotoxicity assays with crude recombinant and partially purified expressed Cry11 protein

Toxicological assays with crude recombinant expressed Cry11 protein were performed and showed 100% mortality at 140 µg/ml as compared to the crude recombinant protein with LC_{50} 237.09 ± 22 and showed 100% mortality at 450ug/ml. The expressed partially purified recombinant Cry11 protein is highly toxic against 3rd instar larvae of *A. aegypti* having LC_{50} 42.883 ± 6. Further screening from different ecological habitats must be necessary to search novel *cry* genes to form biopesticide to overcome resistance in insects.

4. Discussion

Bacillus thuringiensis is an entopathogenic, producing different forms of crystalline inclusion bodies which was highly toxic to dipteran insects. Currently various crystal and vegetative insecticidal proteins (cry/vip) are used to develop transgenic plants resistant to insects. Cry/vip proteins have expedient pesticidal properties and may be abused to switch off pests in agriculture (Crickmore et al., 2014; Gupta et al., 2021). Ahmad and Shakoori (2013) and Ashraf et al. (2017) reported that spores are chief providers to the toxicity of *Bt.* δ-endotoxin.

The present study was intended to screen native environments for *cry11* positive *Bt.* isolates which could later urbanize into biopesticide against mosquito larvae. Out of thirty strains, fifteen strains harboring *cry11* gene were selected according to their toxicity. First six stains were selected on the basis of toxicity in descending order. Finally, 9NF highly toxic strain out of three most toxic *Bt.* isolates was selected for cloning and characterization of full length *cry11* gene.

Various reports cover the distribution of the organism in various environments (Martin and Travers, 1989) considering temper-

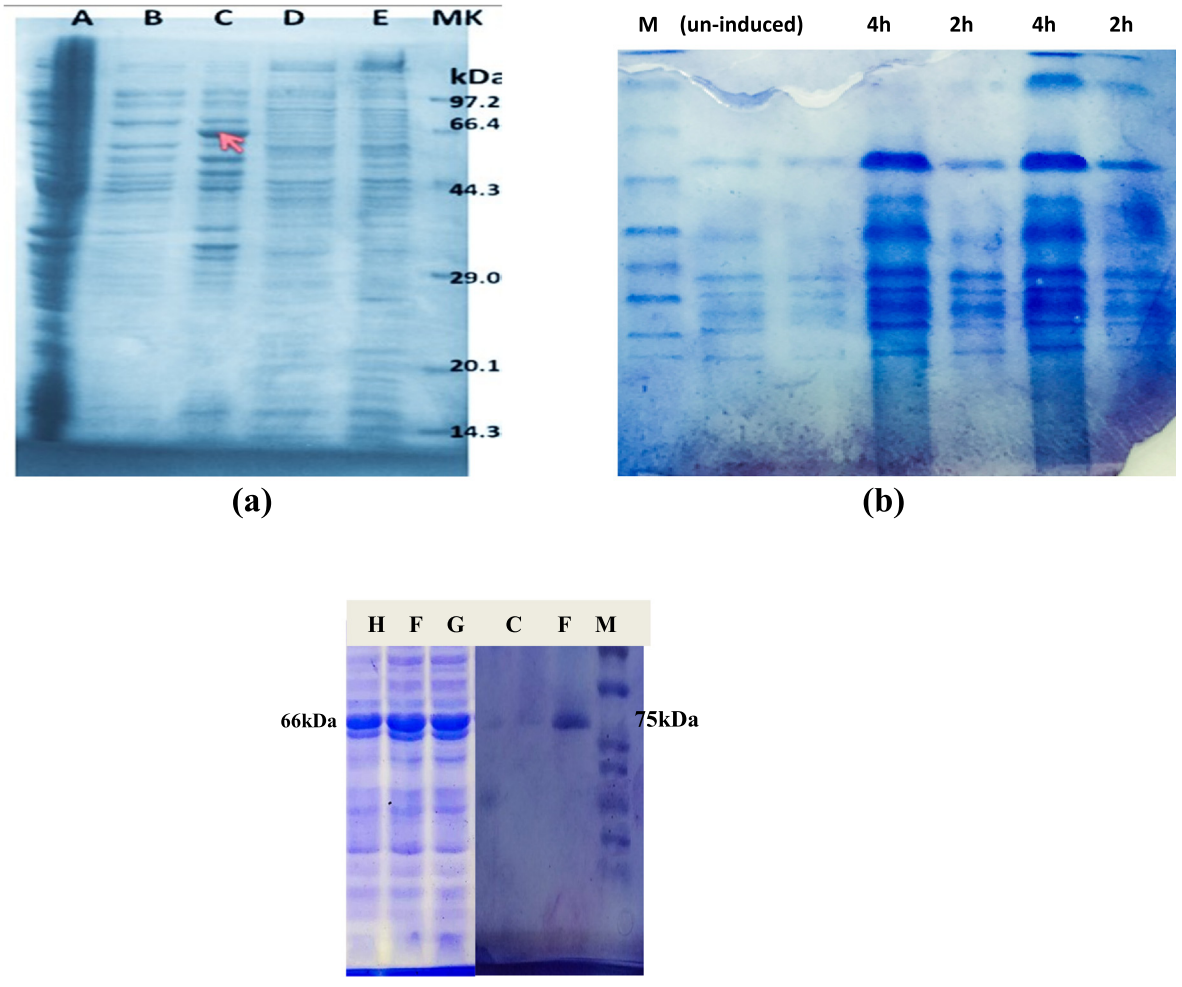

Fig. 3. (a) Lane A: Negative control (without IPTG); Lane B: Lysate supernatant (37 °C); Lane C: Lysate precipitate [with IPTG (1 mM) for 4 h at 37 °C] Lane D: Lysate supernatant (without IPTG) Lane E: Lysate precipitate (without IPTG). **(b)** Lanes 2–3, without IPTG; Lanes 4–5, induced with 1 mM IPTG for 4 and 2 h incubation (9NF accession number—); Lane 6–7,positive control (HD500) induced with 1 mM IPTG for 4 and 2 h incubation. **(c)** Lanes H, F, and G optimized expressed recombinant Cry11 protein. Lane C partially purified expressed Cry 11 protein by heat shock method and lane F partially purified expressed Cry 11 protein by high alkaline pH stress. M is a protein marker.

ate and tropical regions. There are many strains of *Bt*. which are active against different host ranges depending on the type of toxins they have like *Btk.is* active against caterpillars, *Bti*. is active against mosquitoes and black flies. *Bt*. var aizawai is toxic to several species of moths and *Bt.* var tenebrionis against larvae of leaf beetles (Schnepf et al., 1998; deMaagd et al., 2001; Bari et al., 2021).

In the current investigation, different areas of the Pakistan ecosystem were surveyed for samples collection and noticed that 36%, 22%, 20%, 12%, and 10% *Bt.* were quarantined from dry soil, moist soil, soil containing cattle waste, garden soil, and sandy soil, respectively. The prevalence of *cry11* gene is maximum 50% in dung containing soil which shows maximum toxicity against *A. aegypti* in the current research work.

Fernández-Chapa et al. (2019) declared that *Bt*-based products have become the best-selling biological insecticides and are potentially effective against Lepidoptera, Diptera, Coleoptera. Devendra et al. (2017) valued the PCR technology for *cry11* gene detection and its cloning strategy will be proved very beneficial in the area of integrated pest management for sustainable agriculture (Carozzi et al., 1991). In the present study, molecular analysis reveals that 9NF harboring *cry11* gene (1.9 kb) has a 99% similarity index with reported *Bacillus thuringiensis* strain JW, crystal protein (*cry11)* gene which is highly toxic to *A. aegypti.*

Lone et al. (2017) reported that the toxic isolates have a remarkable use in the pest control. So, commercially abused to control insect pest and revealed the confirmed significance of innovative *Bt*. strain from diverse ecological region of world. The prominent molecular masses are (130, 72, 65 kDa) observed correspond to the Cry1, Cry2 and Cry11 protein respectively. The results of present research work recommend the importance of constant search for new *Bt.* stains from the diverse ecological regions of the world.

Present study explores the production of crystalline inclusion bodies highly susceptible to different factors like UV light, temperature, humidity, and pH. The *E. coli* BL21(DE3) expression system is an efficient system for protein expression work. This system contains a prophage DE3 derived from a bacteriophage λ, carries the T7 RNA polymerase gene which works in the control of the lacUV5 promoter. In this study, optimized conditions i.e. IPTG concentration, temperature, and incubation time for good expression of Cry11 protein were 1 mM, 37 °C, and 3.5–4 h, respectively.

Alvaro et al. (2018) reported three discrete structural domains of Cry11 family which goes to large group of δ-endotoxins, Domain I has α-helical roll with 6 helices frame involve in membrane insertion by Li et al. (2001). Domain II consists of three antiparallel sheets specificity is apex loops and its variability due to variable length, conformation and sequence. Domain III is less variable and the difference is due to length, conformation, orientation of loop binding pocket associated with receptor joining. Domain III of different toxins was observed in microbial lyases, esterases, and glycoside hydrolases (Wang et al., 2019).

The present study describes that all Cry11 proteins have 97% homology with Cry11 insecticidal protein (novel OUB27301.1) with three domains. Domain 1 has α helices and has a prominent loop in domain II having β sheets. Domain 111 is a completely conserved region with β plated sheets, both involve in receptor binding and recognition (Fig.). The *cry11* gene encodes 65–85 kDa protein which is highly active to control VBD like dengue, encephalitis, malaria and yellow fever.

The bioassays of positive clones *E. coli* BL21(DE3) harboring *cry11* full length gene in plasmid (pET30 a +) against third instar larvae of *A. aegypti* showed that *cry11*gene of 9NF was highly toxic having LC_{50} 42.883 ± 6 μg/ml as compared to HD500 having LC_{50} 103.6 ± 47ug/ml. The purified expressed recombinant Cry11 (ICP) is highly toxic counter to 3rd instar larvae of *A. aegypti* having LC_{50} 42.883 ± 6 and showed 100% mortality at dose 140 μg/ml as compared to the crude recombinant protein having LC_{50} 237.09 ± 22 and showed 100% mortality at dose of 450ug/ml. So, it is concluded that *cry11* gene is expressed by using the machinery of host *BL21D3* and mosquitocidal delta endotoxin is expressed as ICP.

5. Conclusions

In the present study *Bt*. local isolates harboring 99% homology with *Bti.* prototoxin *Bacillus thuringiensis* (AXJ97553.1) were isolated and characterized. *Cry 11* gene from the most toxic *Bt*. strain 9NF was cloned and sub-cloned in pTZ57R/T and pET30a(+) for expression. The optimized conditions for good expression of *cry11* gene were found 1 mM IPTG, 3.5–4 h incubation time, and 37 °C. Biotoxicity assays revealed that partially purified *Bt.* protein is highly toxic against *A. aegypti* larvae with LC_{50} value of 42.883 ± 6 μg/ml. Further screening from different ecological habitats must be necessary to search for a novel *cry11* gene to form biopesticide and to overcome resistance in insects. This research may lead to applications in the field to control insects.

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.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2022.103463.

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