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ORIGINAL ARTICLE

Heterogenus expression of Cry 2Ax gene with his and fusion tags in *E.coli* and protein toxicity

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ABSTRACT

Background: Cry genes of *Bacillus thuruginensis* were widely used for development of biopesticide, transgenic crops in order to control insect pest, nematode and fungi.

Objective: The purpose of this study was to assess heterogeneous expression of tagged Cry 2Ax gene in *E.coli* and toxicity.

Methods: *Bt* (4Q7 strain) harboring Cry 2Ax gene was amplified with gene specific primers with and without tags. Restriction digestions were performed for amplified products in three ways viz., one with gene, second tagged gene and third pET 28(a) vector. Two sets of ligations and transformations were performed with *E.coli* strains (DH5 α) followed by BL 21(*E.coli*). Positive transformants were induced with IPTG based protein expression. The SDS page and bioassay were carried out.

Results: The colony PCR, plasmid PCR and restriction digestion conformed the vector band size (5.3 Kb) and insert band size (1.9 Kb). SDS page was confirmed with 65KDa band of cry 2Ax. Bioassay with heterogenous protein of cry 2Ax protein showed 100% mortality and the protein with tags showed 70% mortality towards *Helicoverpa armigera* and *Spodoptera litura*.

Conclusion: The study found that heterogenous expression of tagged gene in E. coli faced the problem of protein folding, transfer, and tRNA conversion. This disruption leads to increased protein accumulation instead of efficient chaperone –mediated transfer and solubility, consequently, the cry 2Ax protein with tags shows 30% reduction in toxicity.

KEYWORDS

• Heterogenous • Expression • Fusion Tags • Cry 2ax • Protein Expression • Toxicity • Bioassay

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INTRODUCTION

Cotton is one among the important cash crop in India (Haider *et al.,* 2015). Farmers are regretting to cultivate cotton crop due to serious pest damage by bollworm. In Africa several indigenous pest were available and bollworm is one among them. (Bennett, 2015).

As a devastating insect pest, *Helicoverpa armigera* Hübner is thought to cost the world's economy more than $3 billion a year (Townsend, T.2017). The most impacted crops are soybeans, cotton, tomatoes, chickpeas, and other pulses, as well as cereal crops including sorghum and corn. Common cutworms, or *Spodoptera litura*, are one of the most pervasive and damaging agricultural pests in the world. They feed on a broad range of food and economic crops (Song *et al*., 2024).

Numerous known entomopathogens, including *Bacillus thurungiensis (Bt)*, can be found in a wide range of environments (Jouzani *et al*., 2017). Numerous insect pests targeted by Bacillus thurungiensis live in environments, where Bt cannot establish itself, like the rhizosphere of plants. Several toxins produced by *Bacillus thurungiensis* have demonstrated insecticidal activity against nematodes and white grubs, among other subterranean pests (Bi *et al*., 2015; Ruan *et al*., 2015, Hussain, 2016). *Bacillus thuringiensis (Bt)* is a facultative anaerobic or aerobic rod-shaped bacterium that produces insecticidal crystal inclusions called Cry proteins or δ-endotoxins during its sporulation phase of growth. Bt-based solutions represents a good alternative to chemicals since they have a relatively low environmental impact and a high target specificity that make them eligible for the treatment of drinkable water due to its non-toxicity to humans and animals (Kache, 2020). For the preparation of fungicides, herbicides, and bioinsecticides, cry toxin has been employed. Transferring cry genes into rice, cotton, and brinjal plants to increase insect resistance has been the subject of numerous research projects. To specifically resist stem borer infestation, transgenic rice has been created. However, farmers are being successfully using transgenic cotton commercially since 2002. As of 2020, the Directorate of Economics and Statistics India reports that 117.47 lakh hectares of land in India are planted with Bt crops. Recombinant DNA technology is playing a vital role in improving health conditions by developing new vaccines and pharmaceuticals (Suliman Khan *et al*., 2016).

The treatment strategies are also improved by developing diagnostic kits, monitoring devices, and new therapeutic approaches. Synthesis of synthetic human insulin and erythropoietin by genetically modified bacteria *E. coli* is a popular way to produce recombinant proteins. It involves introducing a plasmid into *E. coli* cells to synthesize a protein that doesn't occur naturally. The process is used to produce a wide variety of proteins, including those with disulfide bonds.

The classic genetic engineering approaches in prokaryotes often use phage-derived RecET and lambda red recombinase-based recombineering (Tong *et al.,* 2021). *Escherichia coli* is the most preferred microorganism to express heterologous proteins for therapeutic use, as around 30% of the approved therapeutic proteins are currently being produced using it as a host. Owing to its rapid growth, high yield of the product, cost-effectiveness, and easy scale-up process, *E. coli* is an expression host of choice in the biotechnology industry for large-scale production of proteins, particularly non-glycosylated proteins, for therapeutic use. The availability of various *E. coli* expression vectors and strains, relatively easy protein folding mechanisms, and bioprocess technologies, makes it very attractive for industrial application (Baeshen *et al.,* 2015). *E. coli* is a typical prokaryotic expression system and one of the most attractive heterologous protein producer. The expression of proteins in this system is the easiest, quickest and cheapest. To date reformed *E. coli* is the extensively used cellular host for foreign protein expression because of its rapid growth rate which is as short as 20-30 minutes (Amitha Reena Gomes, 2016), capacity for continuous fermentation and relatively low cost. There are many commercial and non-commercial expression vectors available with different N and C terminal tags and many different strains are being optimized for special applications (Amitha Reena Gomes, 2016).

There are also problems related to the use of *E. coli* as production host. These problems can be grouped into two categories: those that are due to the sequence of the gene of interest and those that are due to the limitations of *E. coli* as a host (Amitha Reena Gomes, 2016).

In the first category again there are three ways in which the nucleotide sequence might prevent efficient expression of a foreign gene. Firstly, the foreign gene may contain introns which would be a major problem since *E. coli* genes do not contain introns and so the bacterium does not possess necessary machinery for removing introns from transcripts. Secondly, the foreign gene might contain sequences which act as termination signals in *E. coli*. These sequences are perfectly harmless in the normal host cell, but in the bacterium it results in the premature termination and a loss of gene expression (Amitha Reena Gomes, 2016).

Besides this, the heterogenous expression may leads to the problem of disulfide bond formation, absence of chaperone and miss match codon. To rectify these problems mutated strains, co-production of chaperone and lowering the growth temperature have been used either separately or by combination (Costa *et al*., 2014). With this background, In this experiment the recombinant chimeric cry 2Ax gene has been cloned with his and fusion tags, expressed in E. coli and toxicity assay was performed with *Helicoverpa armigera* and *Spodoptera litura*.

MATERIALS AND METHODS

The Department of Biotechnology, Centre for Plant Molecular Biology and Biotechnology, *Bacillus thurungiensis* lab, Tamil Nadu Agricultural University, Coimbatore, is where all the laboratory works has been carried out.

Reagents

We bought PCR purification kits and DNA isolation kits from Sigma (www.sigma-aldrich.com/genelutehp.) and Geni (http://geneilabs.com), respectively. T4 DNA ligase and restriction endonucleases were acquired from New England Biolabs (http://www.neb.com). Plasmid DNA isolation, plasmid DNA purification and gel DNA extraction kits were purchased from Sigma. 2-log DNA ladder was procured from NEB. All other reagents were purchased from Sigma.

Bt, E. coli strains and PET 28a vector

A recombinant Bt (4Q7) transformed with pHT vector of Cry 2Ax gene was utilized as a donor in this experiment. Novogen, USA (http://www.novogene.com) supplied pET 28 a, *E. coli* strain DH5 α, and BL 21.

Media, growth condition

Bt and *E. coli* strains were cultured using Luria-Bertani (LB) broth (Bertani, 2004) in accordance with standard procedure. *E. coli* harboring PET 28a and its derivatives were mass cultured using kanamycin marker (100µg/ml) as directed by manufacturer.

DNA isolation

The genomic DNA of the recombinant Bt (4Q7) strain was extracted using Genei kit. (Na *et al*., 2025). The quantity and quality of DNA was analyzed using the Agarose gel electrophoresis (Guijun Miao *et al*., 2020) and NanoDrop spectrophotometer 2000.

Gene, Primers and Vector selection

The Cry 2Ax gene (1.9 Kb) of 4Q7 *Bacillus thurungiensis* DNA was used as a template. Gene-specific primers (as given below) were used for amplification. Two sets of primers were designed along with restriction sites on the basis of vector (pET 28) map Figure 1. Primer sets with ECoR1 and Hind III and other with NCO 1 and Hind III. The second set of primers (NCO 1 and Hind III) was designed not to cut the fusion tags, while the first set of primers (Eco R I and Hind III) was designed to cut the fusion tags during PCR. The primer sequence details are as follows.

S. no. Primer	Primer Sequence with restriction enzyme	Restriction enzyme
1 Forward	5′ GCTCTAGATATTTAAGGAGGAATTTTATATGAA 3′	Eco R 1 (GCTCTAGA)
2 Forward	5′CCATGGGTAATGTATTGAATAGTGGAA3′	NCo 1(CATGGGT)
3 Reverse	5′ ACAAGCTTAGTTAATAAAGTGGTGGAAGATTA 3′	Hind III (AAGCTT)

PCR (Cry 2Ax), gene purification

PCR was done in an Eppendorf thermal cycler. The PCR mixture (25 µl) containing DNA (30 ng), PCR buffer (10X: 2.5µl), 75 µM each of dNTPs, 50 ng each of forward and reverse primers and 1.5 Units of *Taq* DNA polymerase have been used. The PCR was performed for 30 cycles at 60°C annealing

temperature and 2 minutes extension time. The PCR products were purified using ultraclean DNA purification kit (SIGMA USA, https://www.sigmaaldrich.com). The quantity and quality of amplified products was analyzed using the Agarose gel electrophoresis (Guijun Miao *et al.,* 2020) and NanoDrop spectrophotometer 2000.

Plasmid DNA (pET 28a)

pET 28a plasmid of E. coli (DH 5 α) strains was cultured in LB kanamycin broth at 37°C for overnight. Plasmid DNA was isolated using plasmid isolation kit (Sigma, USA) as per the instruction given by the manufacturer. Quantity and quality of DNA were analyzed through agarose gel electrophoresis (Guijun Miao *et al.,* 2020) and nanodrop reading (Thermo USA)

Restriction digestion (RD) of vector, gene

Two sets of RD were performed. Enzyme concentration was one unit for every µg of DNA. First RD set contains the restriction enzymes (Hind III and Eco R1), vector, insert, and buffer (BSA). The second RD set covers insert, vector, buffer (BSA), and restriction enzymes (Hind III and NCO 1). The restriction digestion was done in an Eppendorff tube at 4°C for one honor (Ramalakshmi *et al.,* 2022).

RD products clean up

Restriction digested products were purified by following the protocol provided by manufacturer Sigma Aldrich, Mumbai.

Ligation/cloning and Transformation

There were two sets of ligations performed. Cry 2Ax gene in one set, cry 2Ax gene with fusion tags in another. T4 DNA ligase (1µl), buffer (2 x), vector (75 ng in 4µl), insert (25 ng in 3 µl), and ligation water (10µl) were combined and stored at 22°C for one hour (Tianyuan Su *et al.,* 2019).

The ligated mixtures were transformed into E.coli (DH 5α) competent cells by heat shock method separately (Tianyuan Su *et al.,* 2019 and Ramalakshmi *et al.,* 2022).

Colony, Plasmid PCR and Restriction digestion (RD)

The transformed colonies of *E. coli* carrying the cry2Ax gene of pET 28 (a) were chosen based on the complementation test, and the colonies with self-ligated vector were removed (Ramalakshmi *et al.,* 2022). For verification, the transformed *E. coli* colonies and their plasmid DNA were subjected to PCR using primers specific to the cry2Ax gene.

pET 28a plasmid of *E. coli* transformants (DH 5α) were screened by plating the transformed colonies in LB kan (50 ppm) plate. Colony (Jamal Mahm, 2017), plasmid PCR and restriction digestion (Motohashi,2019) were done to confirm the positive transformants. Gene specific primers as mentioned above were used for colony and plasmid PCR (Horecka, 2014). Confirmed colonies with positive clones were used for restriction digestion. Digestion of positive clone was done with two set of restriction enzyme as per two types of clones. Eco R*1* and Hind III for Cry 2Ax gene, NCO 1 and Hind III for Cry 2Ax gene with fusion tags. Double digestion and single digestion were carried out. The results were confirmed by agarose gel electrophoresis.

Transfer of clone between *E.coli* strains

Positive clones (vector + insert) was isolated from recombinant DH 5α strains and purified using Sigma Kit, USA. The purified clone/ plasmid DNA was transferred into BL 21 (*E.coli*) expression strain. *E. coli* competent cells preparation and transformation were done by following standard protocol of Sambrook *et al.* (Ramalakshmi *et al.,* 2022 and Du *et al.,* 2021).

Expression of Cry 2Ax ORF of pET 28 (a) in *E.coli* (BL 21)

Cry 2Ax of pET 28a plasmid in BL 21 was cultured in LB broth (50 µg of kanamycin / ml) at 37°C overnight. BL 21 plain strain without plasmid was taken as a negative control. One percent mother culture of recombinant strains of two clones viz., Cry 2Ax with tag and without tag were inoculated into 25 ml LB broth under 37°C separately until to reach 0.6 OD at 600 nm. 1mM of IPTG (Isopropyl β-D-1-thiogalactopyranoside) was inoculated into the log phase culture for induction and kept under 30°C for 6 hrs. The broths were used for protein extraction by following the procedure of Ramalakshmi *et al.,* 2022.

SDS PAGE and Quantification of cry 2Ax protein

The gene expression/protein synthesis was analyzed by SDS-PAGE on separating gel of 9 percent w/vacrylamide (Nowakowski, 2014).

Proteins were quantified using known concentration of BSA as a standard. BSA Stock (10µg/µl) was diluted into 0.5, 1.0, 2.0 µg for SDS in such a way to calculate the concentration of recombinant protein after dilution. Dilutions were made based on protein expression in SDS PAGE after standardization. Since the Cry 2Ax protein with tags showed thick protein band, maximum dilutions viz., 1/7 and 1/10 and in case of Cry 2Ax protein without tags showed thin protein band 1/3, 1/4 and 1/5 dilutions were taken for comparison and quantification. Protein quantification was done by following the procedure described by Caballero *et al.* 2020.

Sequencing analysis

Positive clones of *E. coli* culture were sent for sequencing analysis. Homology search were conducted through Basic Local Alignment Search Tool (BLAST) at National Centre for Biotechnological Information (NCBI) (www.ncbi.nlm.nih.gov/Blast). The deduced amino acid sequence was generated by Bio Edit (Ramalakshmi *et al*., 2022).

Bioassay for insecticidal activity

Recombinant proteins (Cry 2Ax alone and 2Ax with fusion tags) were tested for toxicity assay. *E.coli* (BL 21) plain strain protein was used as absolute negative control. Semi-synthetic diet (Patel *et al*. 1968) dispensed inside the 1.8 ml cryovial (Tarson®; 1 cm dia.) were inoculated with 7 days old larva of *Helicoverpa armigera* and *Spodoptera litura*. Recombinant proteins (10 µl) were spread on the surface of the vial and dry for 30 min. Ten vials were maintained for each treatment with three replications. Larval mortality was recorded periodically (1-7 days) (Saleem F, Shakoori AR 2017).

RESULTS

PCR (Cry 2Ax) followed by sequencing of recombinant *Bt* 4Q7

PCR results confirmed that positive amplification of Cry 2Ax gene (1.9 Kb) from genomic DNA. The amplification product showed bright bands at 58◦C in 2% agarose gel (Figure 1). Cleanup product showed bright bands at 58◦C in 2% agarose gel (Figure 2). The sequencing result confirmed that cry 2Ax gene homology under NCBI (BLAST).

(Annealing temp: 60°C, Extension time: 2 min)

Primers: 2IF2: TGAATTCATGAATAATGT ATTGAATAGTGGAA (Eco R 1)

2IR$_2$a: ACAAGCTTAGTTAATAAAGTGGT GGAAGATTA (Hind III)

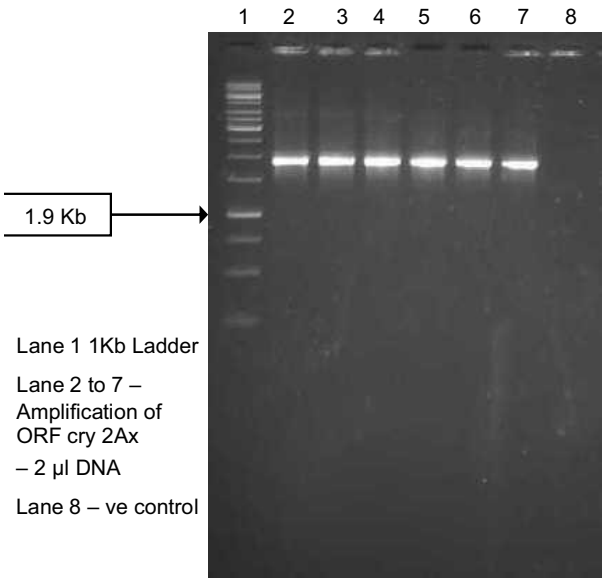


Figure 1: PCR amplification of the Bt 2Ax strain

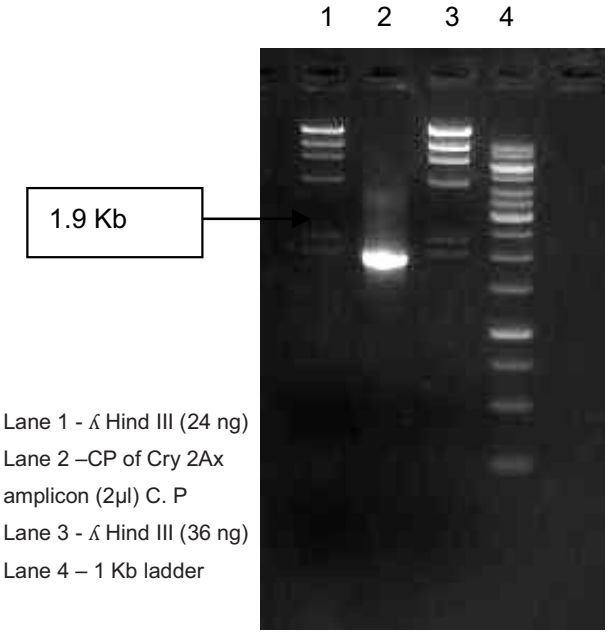


Figure 2: Column purification (CP) of PCR amplified products

Nanodrop: 242.0 ng/ul

Confirmation of Plasmid DNA

The concentration of plasmid DNA (100 ng/ ul) was confirmed by gel electrophoresis using ʎ Hind III (Figure 3). The RD of single and

double digestion of vector also confirmed the pET 28a (+) vector (5.3 Kb) in gel electrophoresis (Figure 4).

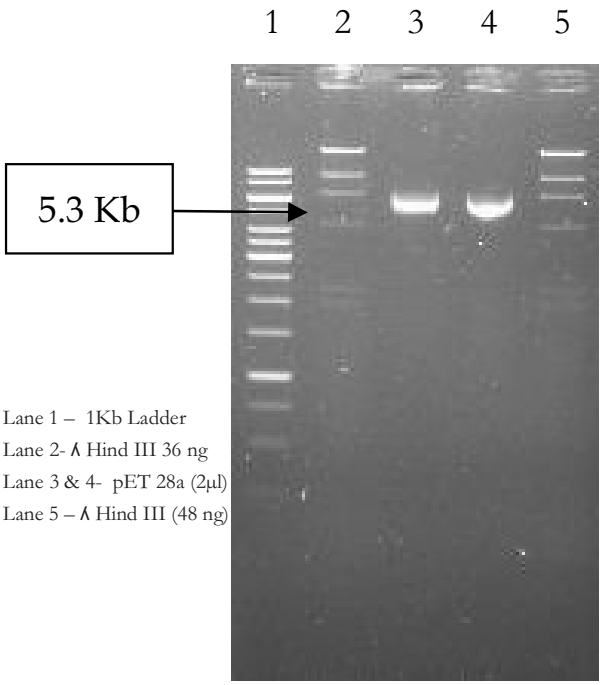


Figure 3: Concentration checking of isolated Plasmid DNA (pET 28a)

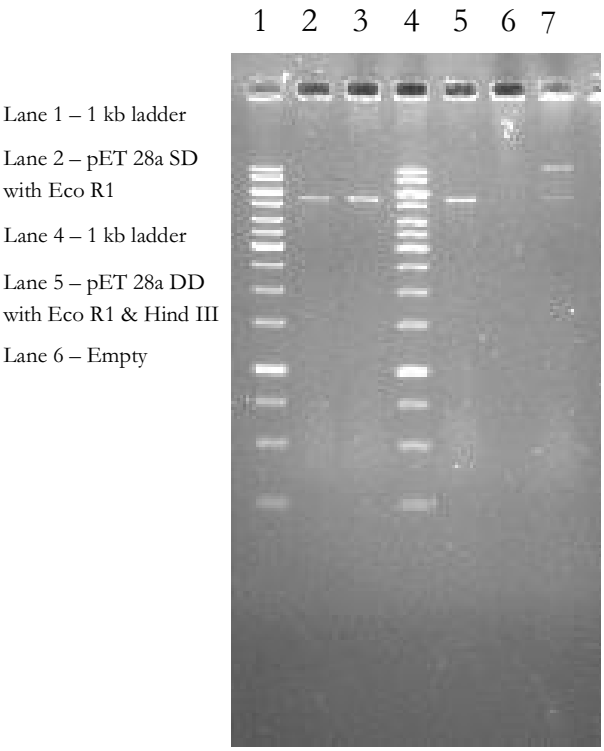


Figure 4: Confirmation of plasmid DNA through Restriction digestion

Concentration checking of Vector and insert after cleanup

Concentration of the Cleanup products viz., Vector (85 ng/ µl) and insert (135 ng/ µl) were confirmed by agarose gel electrophoresis (Figure 5)

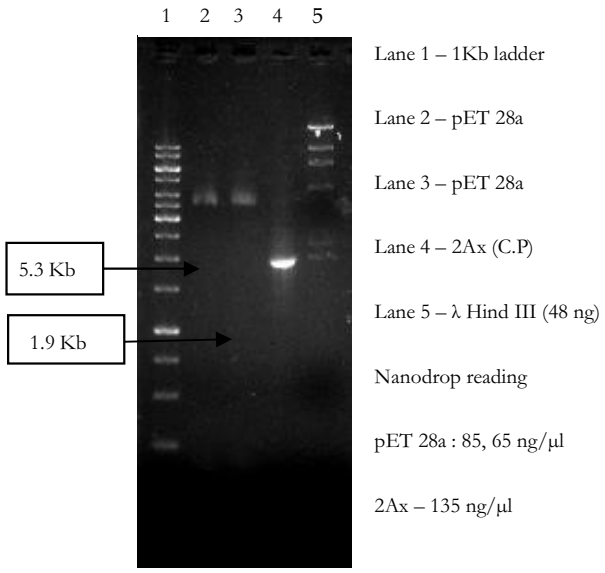


Figure 5: Concentration checking of vector and insert after

Confirmation of Ligation and transformation

White colour colonies of *E.coli* (DH 5 α) strain in Lb Kan plate confirmed the ligation. Blue colour colonies were confirmed as a non ligated one due to the expression Lac Z gene whereas the expression was masked by replacement of cry 2 Ax gene in white colonies.

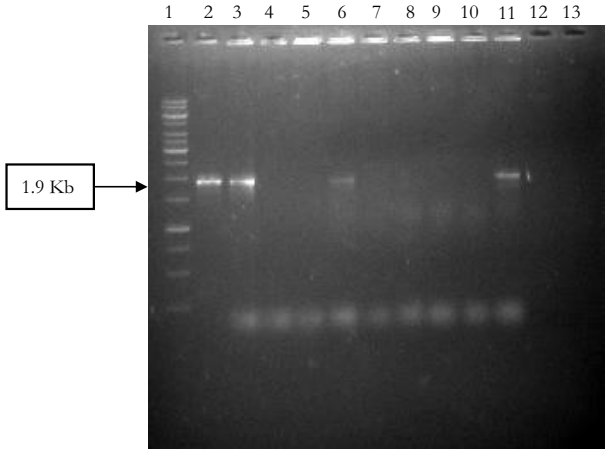


Figure 6: Colony PCR after transformation

Fragment size: 1.9 kb

Colony PCR, Plasmid PCR and Sequencing

Positive transformant containing clones were confirmed by colony PCR (Figure 6) with band size of 1.9 Kb (Cry 2Ax gene). The agarose gel electrophoresis result of Plasmid PCR also showed 1.9Kb band size, which confirmed that positive amplification of cry 2Ax gene from recombinant plasmid (Figure 7). PCR followed by the sequencing results also confirmed the 1.9 Kb size of cry 2Ax gene size.

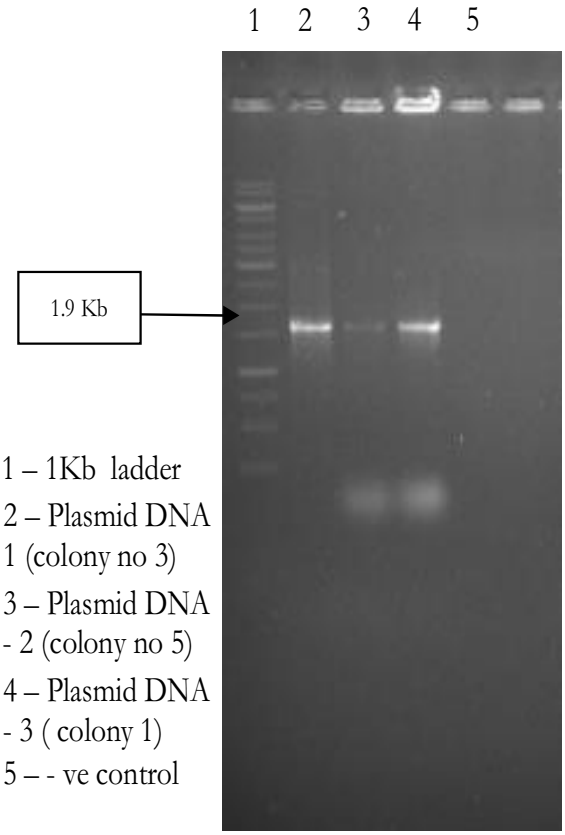
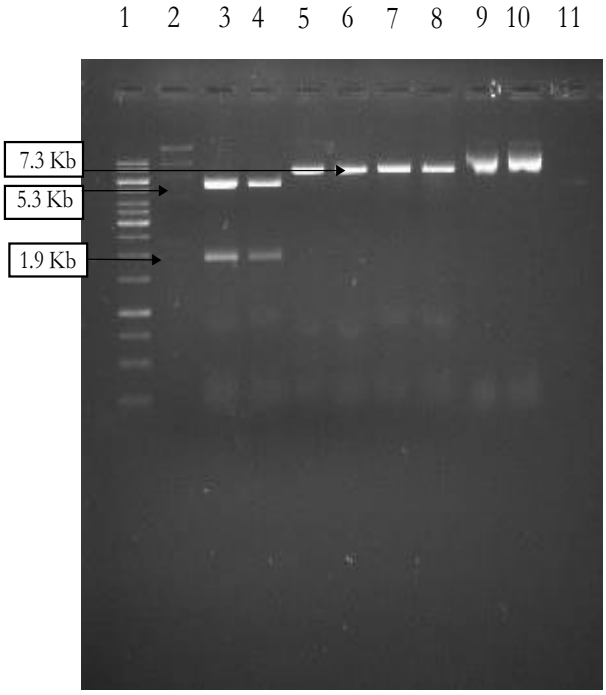


Figure 7: Plasmid PCR (pET 28a + 2ax)

RD of cry 2Ax in pET 28a

Ligation of the recombinant plasmid with insert (Cry 2Ax) was also confirmed by the restriction digestion. The band size in agarose gel electrophoresis confirmed the vector and insert size (Figure 8). The recombinant plasmid underwent double digestion, yielding two bands measuring 5.3 Kb and 1.9 Kb. where the insert region was indicated by 1.9 Kb and the vector size was indicated by 5.4 Kb. A single digestion resulted in a single band that was 7.2 Kb in size, including the insert and vector. The outcomes amply demonstrated that the cry2Ax gene was significantly ligated within the vector region.



1 – 1Kb ladder
2 – λ Hind III (36 ng)
3 – DD plasmid DNA (1)
4 – DD plasmid DNA (2)
5 – SD of plasmid DNA (1) with EcoR1
6 – SD of plasmid DNA (2) with EcoR1
7 – SD of plasmid DNA (1)with Hind III
8 – SD of plasmid DNA (2)with Hind III
9 – Plasmid DNA 1 (clone)
10 – Plasmid DNA 2 (clone)
11 – Linearized plasmid DNA

Figure 8: Restriction digestion of plasmid DNA (PET 28A+ 2AX)

Transfer of clone into E. coli expression strain (BL 21)

The growth of colonies in LB kan plate confirmed that the successful transfer of clone (pET 28 (a) + Cry 2Ax) from E.coli DH5 α to BL 21 strains.

Protein expression followed by quantification (Schlicke and Brakmann, 2005)

The SDS page result was 65 kDa, which was equivalent to the Bt protein cry 2Ax (Figure 10). The clone with tag had 2.0µg/µl of protein, while the clone without tag had 0.8µg/µl. It was evidenced that the recombinant *E. coli* strain (Figure 10) had substantially higher protein levels than Bt (0.4 µg/µl).

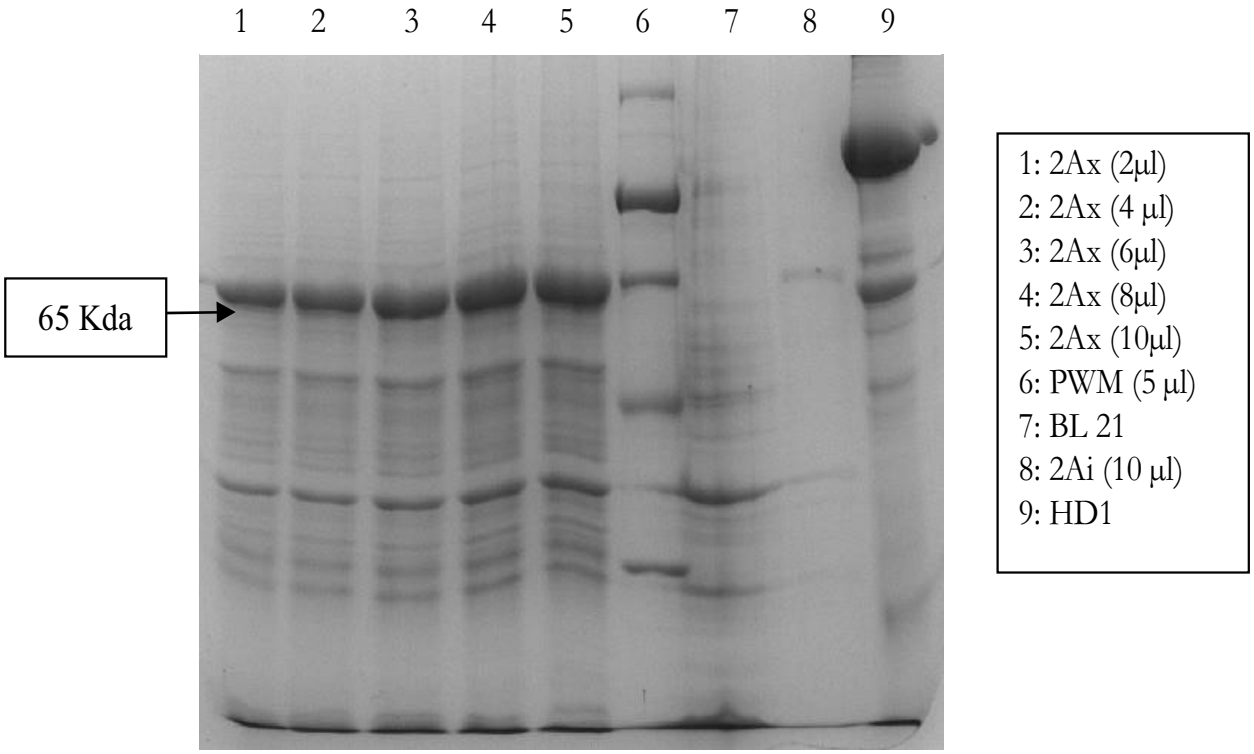


Figure 9: Protein harvesting through induction from BL 21 transformed with pET 28a + Cry 2Ax

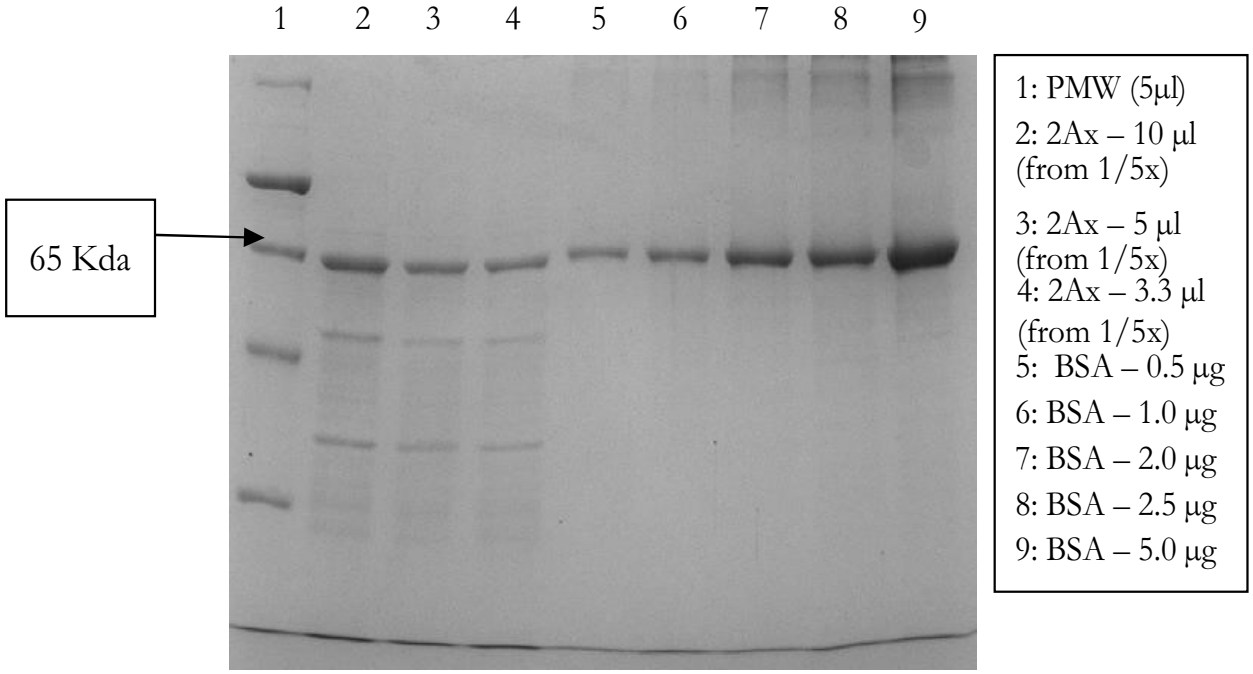


Figure 10: Quantification of 2Ax protein using BSA standard

Bioassay

The bioassay results for cry 2Ax protein without tag and cry 2Ax protein with tag showed 100% and 70% mortality, respectively (Table 1). The bioassay result's showed that there were notable differences between the recombinant proteins activity with and without tags.

Table 1: Bioassay of cry 2Ax protein against pink boll worm (Spodoptera litura)

Treatment	1 Day	2 Day	3 Day	4 Day	5 Day	6 Day	7 Day
Control	0	0	0	0	0	20 % 1 missing	20 % 1 missing
HD 1	10%	80%	100%	100%	100%	100%	100%
Cry 2Ax	0 %	30%	90%	100%	100%	100%	100%
Cry 2Ax with tags	0 %	30%	50%	50%	50%	60%	70%

Figure 11.1: Bioassay of pink pigmented boll worm (*Spodoptera litura*) without any protein (Control)

Figure 11.2: Bioassay of pink pigmented boll worm (Spodoptera litura) with Cry 2Ax alone (Heterologus E.coli)

DISCUSSION

Gene transfer from genome to genome is being leading practice in current science for the human welfare. Biopesticide development from recombinant cry gene was prolonged follow-up practice in biotechnological science to study gene cloning for the past several decades. Even though several decades' research had been undergone regarding heterogenous gene expression, there is a lack of study about the function of native chaperones. Apart from that spores (or) cry protein are expressed based on stress condition (or) whenever the signal molecules initiated. This may cause variation in the expression of recombinant cry gene. Cry gene expression was controlled by cry gene promoters and non cry gene promoters. The role of these cry gene and non cry gene promoter was mediated by stress depended and induced mechanism of the native strain which was a question mark in case of recombinant cry protein expression in *E.coli* strains.

All the human, animal and microbial cell depends on the chaperone mediated network to form successful protein transfer, placement and expression and their by preventing misfolded protein formation (Balchin *et al.,* 2016). Some chaperones use the ATP binding hydrolysis and some do not use for chaperone activity. In general ATP independent chaperone don't interact with native chaperone instead of that bind with partially folded client protein and try to avoid protein aggregation

(Suss and Reichmann, 2015 and Burmann and Hiller, 2015). Some exception like Spy is the ATP independent chaperone was incapable for facilitating their refolding also observed (Burmann and Hiller, 2015 and Suss and Reichmann, 2015).

Wu *et al.* (2019) reported that too tight binding inhibit protein folding and too loose binding favour protein aggregation. Cry gene transcriptional regulation has been divided into sporulation-dependent promoters and sporulation independent promoters. Sporulation depended promoters are classified into sporulation-specific sigma factors SigK and/or SigE. SigA factor controls the sporulation-independent promoters. Accessory factors also contribute to the transcriptional regulation of cry gene expression such as Spo0A, ORF2, and CcpA (Peng *et al.,* 2018).

E. coli expression systems are commonly used for the expression of cry genes. Tac promoters and T_7 are commonly used to for the expression of cry genes carried on pET or pGEX series vectors with His or GST tags(Qi peng *et al.,* 2019).

Even though, some cases *E. coli* fails to express eukaryotic genes due to the lack of disulphide bond formation, post translational modifications and fails to form codon. This may be due to the variation in the codon sequence of Eukaryotes and Prokaryotes. Fast expression of recombinant protein leads to formation of insoluble aggregates due to the failure of chaperone mediated network to prevent misfolded protein formation (Ajmal, 2023).

The experiential results revealed positive cloning of cry 2Ax gene in pET 28(a). There was a variation in the transgenic expression of cry 2Ax protein with and without tags. Experimental result revealed protein expression was higher (2.0 µg /µl) from transformed colonies with tag than without tags (0.8 µg /µl). Gene tagging help for expression but somehow affect the cofactor or chaperone binding into the fresh or newly released protein (Köppl *et al.,* 2022). Hence more proteins were accumulated in the cells. Cells rely on a comprehensive chaperone network to mediate protein folding and prevent protein aggregation (Balchin *et al.,* 2016). The chaperone specific to the native gene was missed in the case of chimeric cry 2Ax gene in recombinant *E.coli* DH 5 α strain. Hence there was more protein accumulation in the SDS page of recombinant tagged gene and the proper folding and release in appropriate place was absent, this will cause insoluble and non functional protein

There was low quantity of other extracellular protein along with 2Ax protein with fusion tags but high amount of extracellular protein were observed in case of 2Ax protein without tags. Tagging the proteins with tags during recombination increases protein aggregation and decreases the protein folding, toxicity and solubility. Cry 2Ax without fusion tags and expression in E.coli not disturb the protein folding and toxicity. The bioassay results confirmed that the cry 2Ax protein (without tags) results showed 100% mortality where as cry 2Ax protein (with tags) showed 80% mortality.

CONCLUSION

Complete expression of foreign genes in *E. coli* is dependent on a number of factors. If the heterogenous protein expression was perfect then scale up process will start. However efficient expression of heterogenous gene and scale up process depends similarity between donor and recipient genome. This experimental result concluded that his and fusion tags in pET 28a(+) vector decreases the positive way of protein folding, transport and release of cry 2Ax gene in *E.coli.*

Abbreviation: *Bt: Bacillus thurungiensis,* SD: Single digestion, DD: Double digestion

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.

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