Overexpression and Purification of Truncated Cry1C Protein, Equivalent to the Protein Expressed in Bt Cotton Event MLS9124

Metahelix Life Sciences Private Limited

Bangalore 560099

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Project Scientist : Dr. Tomal Dattaroy

Objective:

The objective of the project was to over-express and purify the truncated Cry1C protein in *E. coli* that is equivalent to the protein expressed in the MLS Bt cotton event 9124.

Cloning, Expression and Purification of the Recombinant Protein:

Cloning

The Cry1C gene was amplified from *Bacillus thuringiensis* sub species *galleriae* and cloned into pET41b vector. Primers were designed to amplify the portion of the sequence encoding only the N-terminal 630 amino acids of the Cry1C protein as this was the region known to confer toxicity. The amplified product of 1890 bp was cloned in pET41b vector, a plasmid that facilitates heterologous protein expression in *E. coli,* after confirming the sequence identity of the amplified fragment to correspond to a translated sequence of 630 amino acids that is identical to the protein expressed in the transgenic Bt cotton plant carrying the event MLS9124. This clone was used as the base plasmid and the Cry1C gene was excised out of the pET41b clone for cloning into the expression vector pRSET A.

The expression and purification of pRSET A-Cry1C protein is described below. A BamHI – XhoI fragment was cloned in the appropriate restriction sites of pRSET A. The fragment is shown Figure 1 below.

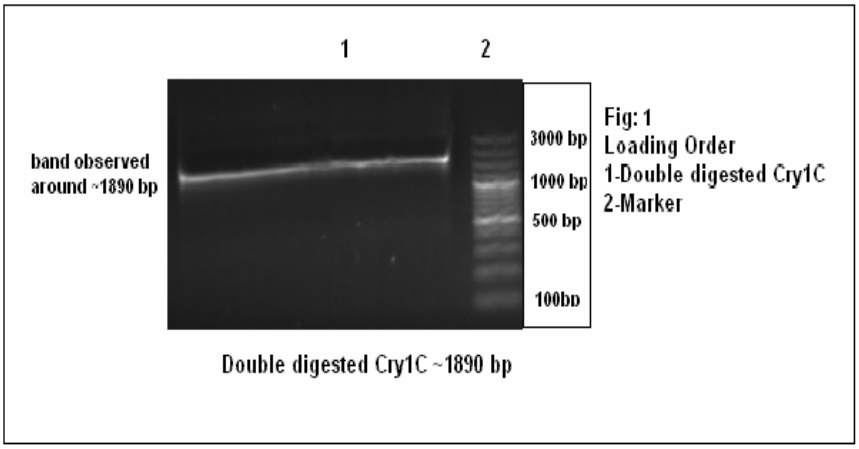


Figure 2 below describes the screening of the different colonies obtained by PCR.

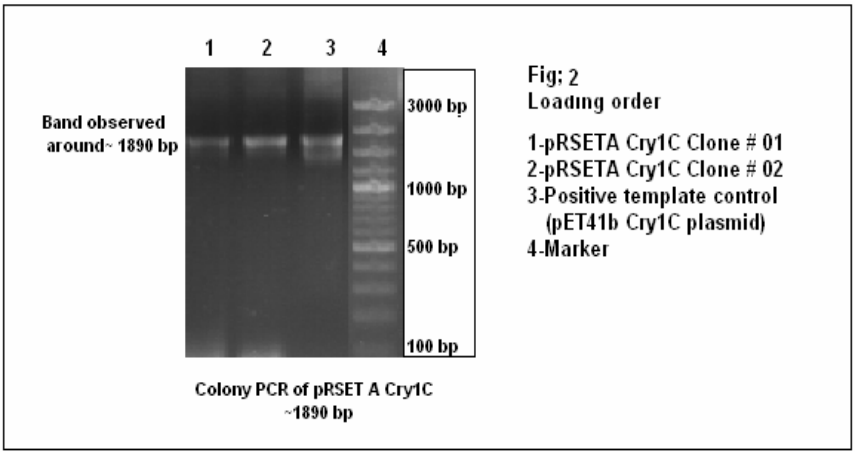
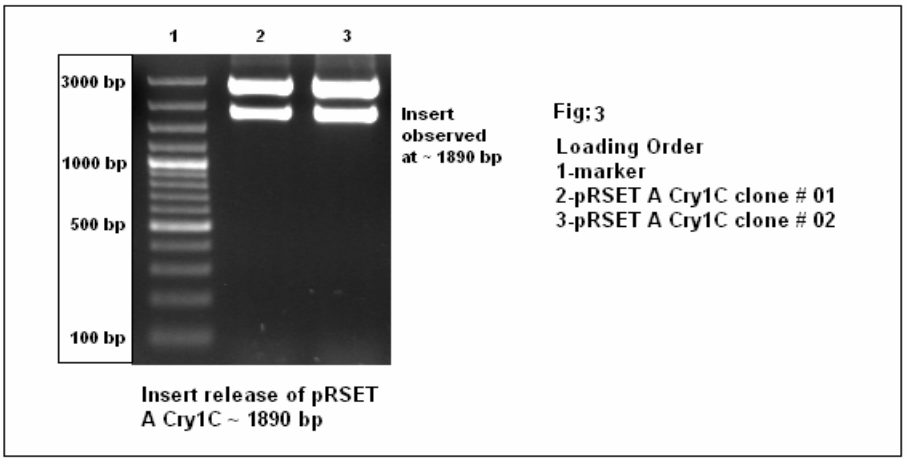
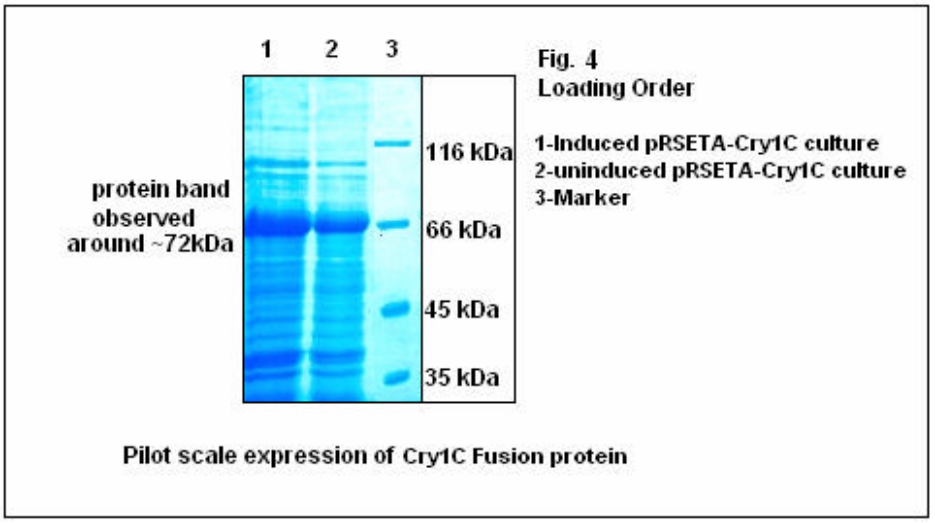


Figure 3 shows the release of the insert by BamHI-XhoI digestion.

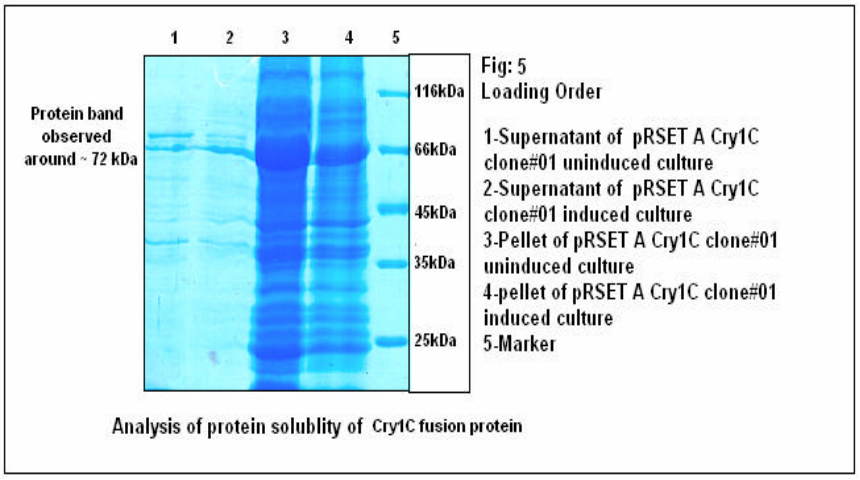


Pilot Expression:

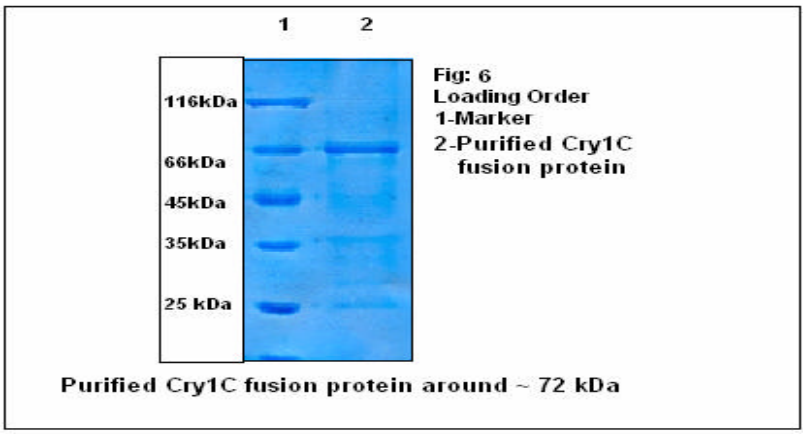
Pilot expression was carried at by inducing the plasmid with 0.4 mM IPTG for 4hours at 18?C. Figure 4 below shows the pilot expression of the protein. An expected ~ 72 kDa protein is observed. Solubility studies of the protein are displayed in Figure 5. The supernatant fraction has very less amount of Cry1C fusion protein. The major amount of the protein is found in the pellet as insoluble fraction in the form of inclusion bodies, and hence has to be purified from inclusion bodies under mild denaturing conditions and refolded to obtain soluble protein; or by direct inclusion body solubilization using buffers/detergents. Figure 6 displays the purification by denaturation and Figure 7 displays the purification by direct solubilization. The latter method required further purification using Ion Exchange chromatography.



Protein Solubility analysis of Cry1C fusion protein



Pilot scale purification of Cry1C fusion protein – Denaturation



Pilot scale purification of Cry1C fusion protein using inclusion bodies solubilization method

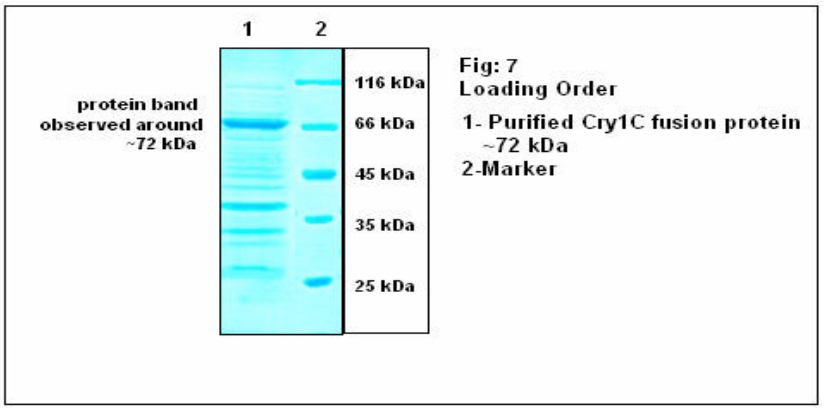


Figure 8 below shows a large-scale purification using denaturing conditions prior to dialysis, and 2 µg protein was loaded.

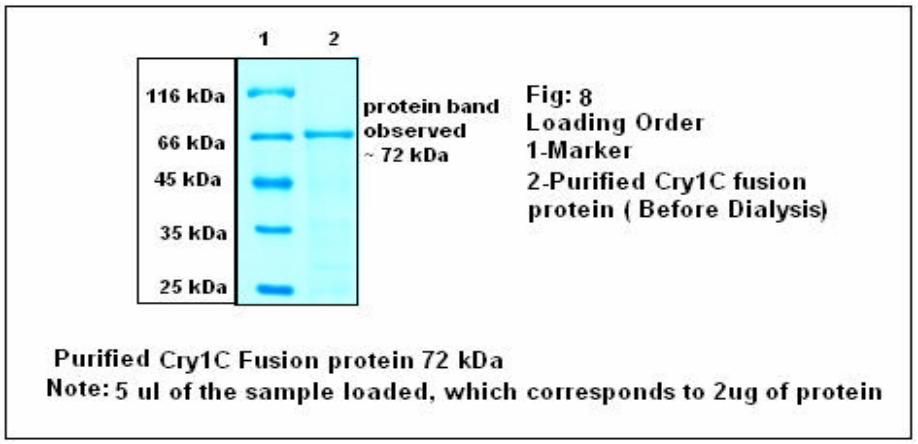
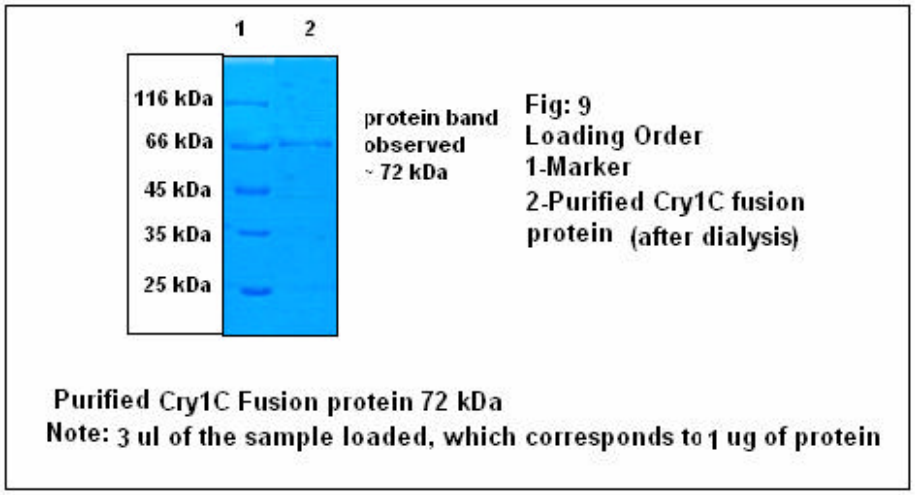


Figure 9 below displays 1 µg protein following dialysis.



Conclusion:

The single bands observed in Figures 8 and 9 are migrating around 72 kDa which is the predicted size of the truncated Cry1C protein. The Cry1C protein in the Bt cotton event MLS9124 is also expected to be of the same size of ~72kDa. This observation and the sequence verification of the clone used for over-expression conclusively shows that the purified truncated protein is indeed equivalent to the protein carried in the Bt cotton event MLS9124.