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Expression and Purification *cry*1Aa Gene

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***Abstract*— The *cry* gene encodes a crystal protein of *Bacillus thuringiensis* known as Cry toxin, which is toxic to major Lepidopteran insect pest. The effectiveness of Cry toxin against the rice yellow stem borer (YSB, *Scirpophaga incertulas* Wlk) has been reported. Transgenic *Bacillus thuringiensis* (*Bt*) rice has been developed by introducing the fusion gene *cry*1B::*cry*1Aa gene driven by the *CaMV35S* promoter to Javanica rice cv. Rojolele to improve its resistance against rice YSB. Resistance to YSB at a greenhouse and multi-site scales have been conducted and reported. Food and feed safety studies for the release and commercialization of genetically modified crops require a sufficient amount of purified protein sample. Therefore, a strategy to produce Cry1Aa protein in a short time with high productivity needs to be developed. In this study, the *cry*1Aa gene (1857 bp) was cloned into pRHA-SDM expression vector under the control of the pRHA promoter fused to a 6xHis tag on the C-terminal to produce pRHA-SDM-*cry*1Aa. The expression host used was *Escherichia coli* strain NiCo21, and protein purification was performed using IMAC Co^{2+} on the TALON® matrix. The results showed that the recombinant Cry1Aa protein, approximately 69 kDa, was detected using Western blot performed using anti-rabbit Cry1Aa polyclonal antibody and His detector nickel-NTA conjugated with HRP reporter enzyme. Expression and purification protocols that have been developed can be used to produce Cry1Aa protein which can be utilized for further protein studies.**

***Keywords*— *Bacillus thuringiensis*; cloning; *cry*1Aa; expression; purification.**

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I. INTRODUCTION

Bacillus spp is one of the most widely used microbial groups for the biological control of pathogens and pests. *Bacillus* spp are known to produce antimicrobial compounds, enzymes, or toxins that can inhibit the growth and function of bacteria, fungi, insects, nematodes, and cellular organisms such as viruses. The *cry* gene from *Bacillus thuringiensis* (*Bt*) encodes a crystal protein (δ-endotoxin) known as Cry or Cry toxin. Cry toxin is the largest group of insecticidal protein produced by *Bt*. The *Bt* toxin nomenclature committee reports, till date 78 different Cry toxins have been studied and explored, and Cry1 toxin is the most widely reported [1]. The Cry1 toxin group is toxic to Dipteran, Lepidopteran, and Coleopteran insects, while the Cry2 toxin group is active against Dipteran, Lepidopteran, and Hemipteran insects. Despite having a wide variety of Cry toxins, Cry1 toxin is the most predominantly exploited commercially. Several commercial *Bt* bioinsecticide products have been marketed, including Dipel, Thuricide, Biobit, Gnatrol, VectoBac [1].

Owing to its status as a safe and environmentally friendly biopesticide, *Bt* has also been developed in biotechnology. *Bt* is the most widely explored and studied bacterial system for developing pest-resistant crops [1], [2]. Several *Bt* crop commodities that have been developed include cotton, potato, maize, rice, canola, soybean, chickpea, tomato, brinjal, and alfalfa [3], [4]. Several studies have reported the development of *Bt* rice which expresses the *cry* genes, such as *cry*1Ab [5]–[7], *cry*1Ac [8], *cry*1Ca [9] *cry*1C [10], *cry*2A [11], *cry*2Aa [12], fused gene *cry*1Ab/*cry*1Ac [13], *cry*1Ac::ASAL [14].

Research Center for Genetic Engineering, National Research and Innovation Agency has developed *Bt* rice resistant to the rice yellow stem borer (*Scirpophaga incertulas* Wlk) using several *cry* gene variants with different combinations of promoters [15]. Among others are *cry*1B driven by wound-induced through stem borer bites using the *mpi* promoter (maize proteinase inhibitor) [16], free-marker *cry*1Ab gene with the *CaMV35S* promoter [17], and *cry*1B::*cry*1Aa fusion gene controlled by the *ubiquitin* promoter from maize [18], on Indica cultivars IR64, Ciherang,

Cisadane, and a tropical Japonica cultivar Rojolele backgrounds. Resistance to yellow stem borer at a greenhouse and multi-site scales has been tested and reported [19]–[21].

Nevertheless, the safety of genetically modified crops must be ascertained before they are released and commercialized. Multiple approaches are available for assessing the safety of genetically modified crops, including the use of bioinformatics, stability testing for the protein in the gastrointestinal tract, and allergenicity examining using an animal model. Questions about safety issues, including toxicity, allergenicity, and environmental impacts, are frequently raised. The best approach to dealing with allergies is to avoid allergens because allergies cannot be cured permanently. Therefore, it is important to assess the potential of the genetically modified plant for allergens.

The safety assessment includes question of whether the genetically modified crops have a lower, equal, or higher protein allergy potential than their non-transgenic counterparts. Basic information regarding proteins such as resistance to heat, mechanical aspects involved during food preparation and processing, and the effect of gastric and intestinal fluids simulations are in vitro studies for allergenicity assessment [22]. All these tests require adequate amounts of protein produced in a short time with high productivity. Many hosts' choices can be used to produce recombinant proteins such as mammalian cells, yeast, algae, or plant [23]. However, *Escherichia coli* is preferred because of its faster and easier to grow in relatively cheaper media, relatively high expression levels, a larger selection of suitable expression vectors, and being easier to genetically manipulate. Additionally, the whole genomic sequence has been mapped [24]. In this report, the cloning, expression, and purification of *cry*1Aa protein in *E. coli* strain NiCo21 to obtain recombinant *cry*1Aa protein with high quality, purity, and productivity for in vitro allergenic studies were reported.

II. Materials and Methods

A. Materials

Plasmid pCAMBIA1300-Ubi-*cry*1B::*cry*1Aa, which has a pCAMBIA1300 [25] backbone carrying *cry*1B::*cry*1Aa gene fused to *Ubiquitin* (*Ubi*) promoter from maize, was obtained from CIRAD (Agricultural Research for Development), France. The expression vector used in this study is pRHA-SDM, which is a modification of pJExpress804::77539 with the QuickChange™ site-directed mutagenesis (SDM) method [26]. The pRHA-SDM plasmid uses a pHRA promoter that is strictly regulated by an L-rhamnose inducer and a D-glucose repressor with an ampicillin selection marker. *E. coli* strains used were DH5α and NiCo21 for expression. Western blot analyses were performed using the anti-rabbit CryIAa polyclonal antibody (1st BASE antibody production service) and HisDetector™ Ni-HRP Conjugate kit (KPL).

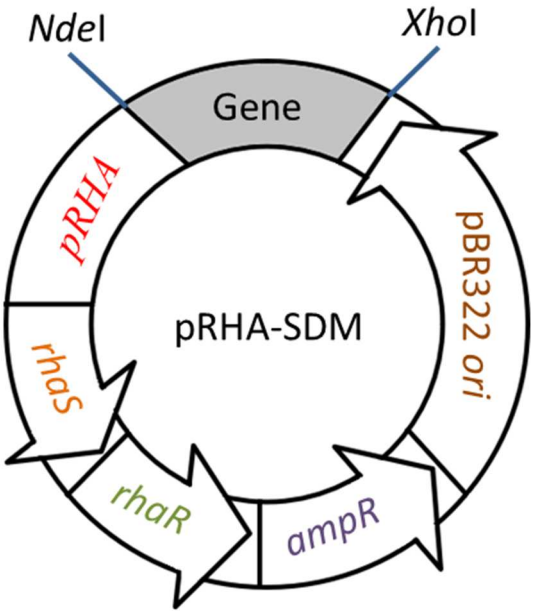

Fig. 1 Vector pRHA-SDM

B. Methods

1) Cloning of the cry1Aa gene: The *cry*1Aa gene fragment was isolated from plasmid pCAMBIA1300-Ubi-*cry*1B::*cry*1Aa using PCR with *cry*1Aa specific primers were synthesized (1st Base) based on the *cry*1Aa (1857 bp) sequence provided by CIRAD by adding a *Nde*I site in the forward primer (For1Aa: CCACATATGGACAACAACCCAAACATCAACGAGTGC) and an *Xho*I and 6xHis sequence in the reverse primer (Rev1B: ATTCTCGAGTTAATGGTGATGGTGATGATGCCTCTCCAAGTCGTACTCGGCCTCGAA). The PCR reaction was performed using DreamTaq Green PCR Master Mix (Thermo Fischer Scientific) following the manufacturer recommendation, with initial denaturation for 3 minutes at 95 °C, followed by 35 amplification cycles (denaturation at 95 °C, annealing at 55 °C, and elongation at 72 °C for 1 minute each) and final extension for 10 minutes at 72 °C. Visualization of PCR in 0.8% agarose gel with EtBr staining and UV illumination. PCR fragment (1857 bp) was isolated and purified using Silica Bead DNA Gel Extraction kit (Thermo Fischer Scientific) and checked in 0.8% agarose gel. Purified fragments were double digested with *Nde*I (Thermo Fischer Scientific) and *Xho*I (Thermo Fisher Scientific) at 37 °C following manufacturer recommendation, visualized in 0.8% agarose gel electrophoresis. The purified fragment were ligated with T4 DNA ligase (Promega) onto plasmid pRHA-SDM, which had been double digested with *Nde*I (Thermo Fischer Scientific) and *Xho*I (Thermo Fisher Scientific) to produce plasmid pRHA-SDM-*cry*1Aa plasmid in *E. coli* DH5α.

2) Production of the Cry1Aa Protein: Transformation of plasmid pRHA-SDM-*cry*1Aa to *E. coli* strain NiCo21 was conducted by heat shock method [27]. The transformant was selected on LB solid media containing 100 µg/mL ampicillin after incubation at room temperature (RT) for 16 hours. A single colony was grown in 5 mL of LB liquid media

containing 100 µg/mL ampicillin, incubated, and shaken at 150 rpm at RT for 16 hours until OD_{600} 0.6 – 0.8. Four mL of preculture was added to 200 mL of LB liquid media containing 0.2% D-glucose and 100 µg/mL ampicillin, incubated, and shaken at 150 rpm 6 hours until the initial exponential phase (OD_{600} 0.3 – 0.4). The culture was induced by adding L-rhamnose until final concentration of 1000 mM. Growth was continued in a shaker incubator at 150 rpm at RT for 16 hours until OD_{600} 0.8 – 1.0 [28].

3) Protein Extraction and Purification: Cells were harvested by centrifugation at 4000 × g at 4 °C for 10 minutes, then the cell pellets were resuspended with 10 mL of lysis buffer (50 mM NaH_2PO_4; 300 mM NaCl; 10 mM Imidazole) containing Phenyl Methane Sulfonyl Fluoride (PMSF) solution at a final concentration of 1 mM [28]. Extraction of the cell cytoplasm fraction was conducted by sonication for 30 seconds 3 times with a 1 minute pause between each run [29]. The supernatant was collected by centrifugation at 25,000 × g at 4 °C for 10 minutes. The protein was then purified with IMAC Co^{2+} on the TALON® (Clontech Laboratories) matrix according to the manufacturer's protocol.

4) SDS-PAGE and Visualization: Twenty-five µl protein samples were loaded on SDS PAGE (4% stacking gel and 10% separating gel). Staining was performed by soaking in PAGE Blue™ Protein solution (Thermo Fischer Scientific) for overnight with agitation at RT. The gel was washed with a destaining solution (40% methanol; 7% glacial acetic acid; 53% aquadest) and aquadest until the background disappeared and the protein band was evident. Then the gel was scanned for documentation.

5) Western Blot: The presence of recombinant Cry1Aa protein was verified by Western blot using a specific polyclonal antibody, anti-rabbit Cry1Aa. The proteins were transferred from SDS PAGE onto a 0.45 µm nitrocellulose membrane using Trans-Blot® SD Semi-Dry Transfer Cell (BioRad) for 30 minutes at 10 volts. The membrane was then blocked using 5% non-fat skim milk solution in Tris-buffered saline overnight with agitation. The membrane was washed three times in Tris-buffered saline tween-20 with agitation for 5 minutes each wash. The polyclonal antibody anti-rabbit Cry1Aa (1:500, v/v) was then added and incubated at RT for 2.5 hours with agitation. The membrane was then washed three times in Tris-buffered saline tween-20 with agitation for 5 minutes each wash. The anti-rabbit alkaline phosphatase-conjugated antibody (Santa Cruz) was then added (1:1000, v/v) and incubated at RT for 2.5 hours with agitation. The membrane was then washed three times in Tris-buffered saline tween-20 with agitation for 5 minutes each wash. Detection was performed in the dark at RT for 15-30 minutes after adding 2 mL BCIP/NBT substrate (Promega).

6) His Detector: Verification of recombinant Cry1Aa protein was also performed with HisDetector™ Ni-HRP Conjugate kit (KPL) following the manufacturer's instructions. The proteins were transferred from SDS PAGE onto a 0.45 µm nitrocellulose membrane using Trans-Blot® SD Semi-Dry Transfer Cell (BioRad) for 30 minutes at 10 volts. The membrane was then blocked with agitation with a 1% BSA blocking solution at RT overnight. The nickel-NTA conjugated with HRP reporter enzyme (1: 5000, v/v) was then added and incubated at RT for 5 hours with agitation. The membrane was then washed three times in Tris-buffered saline tween-20 with agitation for 5 minutes each wash. Detection was performed in the dark at RT for 30 minutes after the addition of 4 mL TMB membrane substrate (KPL).

III. Results and Discussions

A. Cloning of cry1Aa Gene

Selection of the expression vector is one of the key factors for expressing target proteins in *E. coli*. Various expression vectors suitable for the *E. coli* host are available, but those commonly used contain some sequence elements such as replication origin, promoter, multi-cloning site, an affinity tag, terminator, and selection marker (Figure 2). One important parameter to consider is the plasmid copy number. However, a high copy number plasmid might result in high recombinant protein production. In different instances, sometimes, a high number of copies can reduce the growth rate of the host owing to metabolic load, which causes plasmid instability and decreased protein synthesis [30].

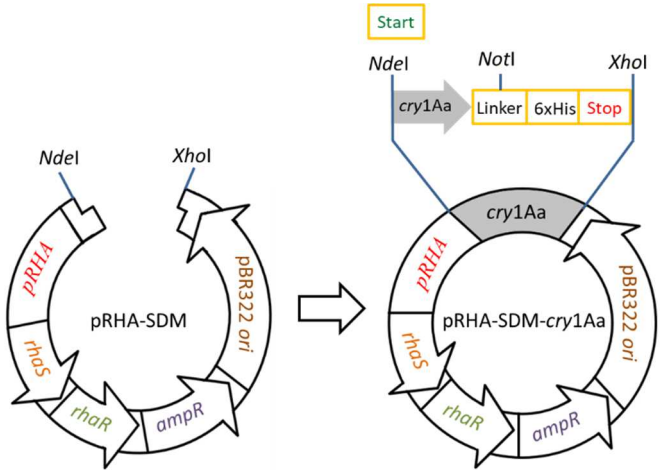

Fig. 2 Schematic of cloning the *cry*1Aa gene into pRHA-SDM to make plasmid pRHA-SDM-*cry*1Aa

The pRHA-SDM vector was chosen because it has been successfully constructed to express genes and produce a stable functional recombinant protein [26]. This vector has a pRHA promoter tightly regulated by an L-rhamnose inducer and a D-glucose repressor, preventing expression leakage [31]. The ideal expression system is an expression vector with a sufficiently strong promoter to accumulate recombinant protein with minimal basal transcriptional activity. In some studies, it has been reported that the use of influential promoters inactivates the transcriptional host and tends to leakage of expression and result in the formation of an inclusion body. This system is superior as compared to other vectors like T7 promoters, especially if gene expression leads to inclusion bodies (low solubility) due to improper protein folding and leakage of expression. The main drawback of the T7 promoter with IPTG inducer is that the expression leaks and, consequently, is not suitable for expressing a protein that is toxic for the host cell [30]. The number of copies of the pRHA-SDM is 2.0 (medium copy). A medium copy of the pRHA-SDM is vital to maintain metabolic load during protein synthesis in the cell hosts.

In this study, the widely used affinity tag to facilitate recombinant protein purification from crude extracts is the 6xHis tag. Moreover, the 6xHis tag acts as a recombinant protein detection epitope using nickel-NTA conjugated with the HRP reporter enzyme. A small tag such as hexahistidine (usually 6 or 10 histidines) is fused to N or C terminal of the target protein, enabling capture by metal ions coordinated on a variety of resins. Hexahistidine is less likely to affect recombinant protein properties, but some other tags can harm the biological activity or protein structure [30].

Cloning of the *cry*1Aa gene fragment into the expression vector pRHA-SDM was confirmed by restriction analyses using *Nde*I and *Xho*I (Figure 3), which showed 2 DNA bands of 4177 bp and 1857 bp. DNA band of 4177 bp corresponds to the size of plasmid pRHA-SDM previously published [26], and the DNA band of 1857 bp corresponds to the predicted DNA fragment of the *cry*1Aa gene insert. The experimental results showed that DNA fragments of the *cry*1Aa gene were successfully cloned to the expression vector pRHA-SDM.

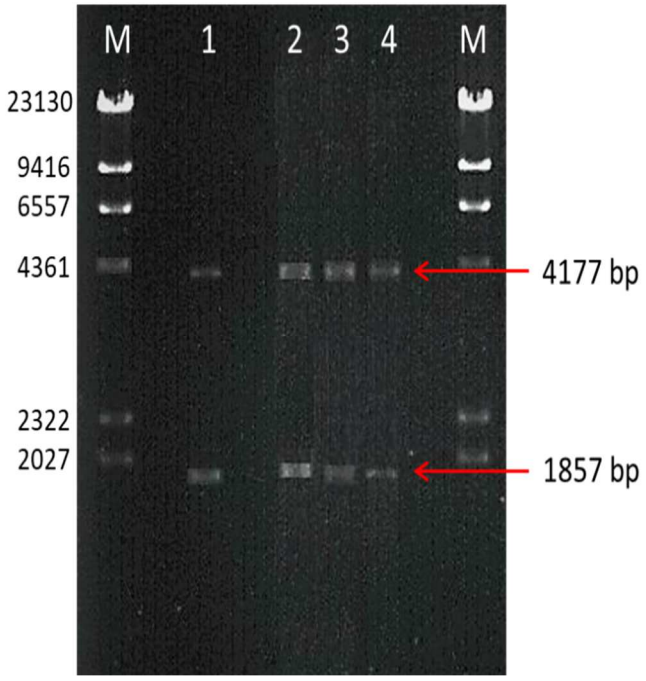

Fig. 3 Double digest of plasmid pRHA-SDM-*cry*1Aa with restriction enzymes *Nde*I and *Xho*I. M. Lambda DNA/*Hind*III; 1. Plasmid pRHA-SDM-*cry*1Aa in *E. coli* strain DH5α; 2 – 4. Plasmid pRHA-SDM-*cry*1Aa in *E. coli* strain NiCo21.

B. Production and Purification of Cry1Aa Protein

Usually, the expression level of a native protein is low, and therefore the production of recombinant protein is carried out. *E. coli* is still a widely used host in genetic manipulation to produce recombinant proteins. Theoretically, the production of recombinant protein in *E. coli* is convenient, starting from the identification, and cloning of the target gene in the appropriate vector, transforming the vector construction in the appropriate host strain, induction to strengthen the production of the target protein, purification, and characterization of the target protein like sequencing, purity, structural integrity, stability, and activity. Nevertheless, in practice, some constraints can occur at each phase in the production of the recombinant, such as poor host growth, instability, and toxicity of the recombinant protein, aggregation, and formation of inclusion bodies (low solubility), incompatibility with the environment like temperature, pH, salt concentration, and low expression. This research will describe the strategy in developing the production of the recombinant Cry1Aa protein using *E. coli* [30].

The recombinant protein expressed in *E. coli* often has low solubility, and a better solution is achieved by lowering the culture temperature during expression. One of the advantages of this system is the ability of induction by lowering the culture temperature of *E. coli* to induce the recombinant protein to fold correctly [32]. Expression of the recombinant protein can be increased by optimizing the growing conditions for *E. coli*. Several parameters can be changed and modified. (i) *Compound of host medium* – a complex or specific medium and other supplements. (ii) *Temperature* – affects speed cell growth, which impacts toxicity, stability, and aggregation of the recombinant protein, especially post-induction. (iii) *Induction* – cell intensiveness at induction needs to be considered; this usually occurs during the log phase of growth and OD_{600} from ~0.6 – 0.7. (iv) *Dose of the inducer* – controls the production of recombinant protein. (v) *Induction period* –affects the level of expression, toxicity, stability, and aggregation of the recombinant protein; the harvest is usually done before reaching the stationary phase [30].

Interestingly, every protein's characteristic is different, unique, and it is very challenging to create a suitable system for the many recombinant proteins to achieve high quality of expression, optimal yield, and stable protein. All of the things mentioned above can be optimation on cell culture flask-scale expression experiments to recognize optimal conditions for each recombinant protein [30].

Production of the Cry1Aa protein was conditioned by growing *E. coli* NiCo21 at RT with the addition of 0.2% D-glucose as a repressor before induction with L-rhamnose to produce high expression. Growth at RT (below the optimum growth temperature of *E. coli* was to optimize the protein folding process so that the expression and localization of the target proteins are efficient [32]. Additionally, it could maintain plasmid stability to minimize plasmid loss from cells. The repression process by adding of 0.2% D-glucose to the culture before the application of L-rhamnose inducer was intended to optimize the early growth of the host. Moreover, a repressor's addition effectively blocks expression leakage and produces high expression [31]. The addition of PMSF as a protease inhibitor before protein extraction was intended to maintain the stability of recombinant protein against enzymatic degradation (in this case proteases) during extraction, purification, and protein analysis processes [33].

The host used for expression in this study was *E. coli* strain NiCo21. Strain NiCo21 was an engineered form of the *E. coli* strain of BL21. Engineering was performed by tagging the chitin-binding domain (CBD) to endogenous proteins that often contaminate such as SlyD, Can, ArnA, and AceE proteins, so that during purification with IMAC, these endogenous proteins can be removed easily by flowing through a buffer containing chitin on the sample column [34]. Another modification was the GlmS protein, where histidine was replaced with alanine to lose its affinity to the metal ion IMAC. The glmS protein functions in cell wall synthesis. This

combination makes the NiCo21 strain superior in minimizing endogenous *E. coli* proteins' contamination during purification of the recombinant proteins fused His tag [34]. The NiCo21 strain has been widely used in protein expression studies [35]–[38].

Purification of the Cry1Aa protein was performed using IMAC Co^{2+} on the TALON® matrix. In principle, tagged purification is incorporated genetically between the 6xHis tag with targeted protein or peptide. Separation method based on the binding affinity of metal ions to proteins fused 6xHis tag in solution at neutral pH environment. The amino acids histidine, tryptophan, and cysteine can form complexes with bivalent metal ions such as Ni^{2+}, Co^{2+}, Cu^{2+}, or Zn^{2+} to form a solid matrix [33]. Even though this method has a very high affinity and is specific to protein, it is influenced by various factors such as column chromatography structure, metal ions, buffers, pH, protein molecular structure, number, and position of his tag [33]. IMAC Co2+ was used in this research because of its advantages over Ni^{2+}, Cu^{2+}, or Zn^{2+} metal ions affinity for his tag recombinant protein. The binding capacity of Co^{2+} is 30-50% higher than that of the Ni^{2+} metal ion [33].

Purification of 6xHis tagged Cry1Aa protein was monitored with SDS PAGE. The presence of the Cry1Aa protein was shown as ~69 kDa band. Visualization of SDS PAGE (Figure 4) showed that most contaminants of the crude extract in lane 1 was removed in the flow-through fractions (lane 2 – 3), and the rest of the contaminants were removed by washing buffer (line 4 – 5). The purified proteins, including the predicted Cry1Aa protein, were obtained in 10 elution fractions as shown in lanes 6 – 15. One dominant protein band, approximately 69 kDa molecular weight, was predicted as the Cry1Aa overexpressed protein, which was later proven by the Western Blot analyses.

Unlike the glutathione S-transferase (GST) tag, the His-tag tends to form dimers that affect protein properties. Lower imidazole concentrations in both the flow-through and washing buffers (50 mM NaH_2PO_4; 300 mM NaCl; 10 mM Imidazole) can remove the weakly bound proteins. The target protein can be harvested at higher imidazole concentrations with an elution buffer (50 mM NaH_2PO_4; 300 mM NaCl; 300 mM Imidazole) [39].

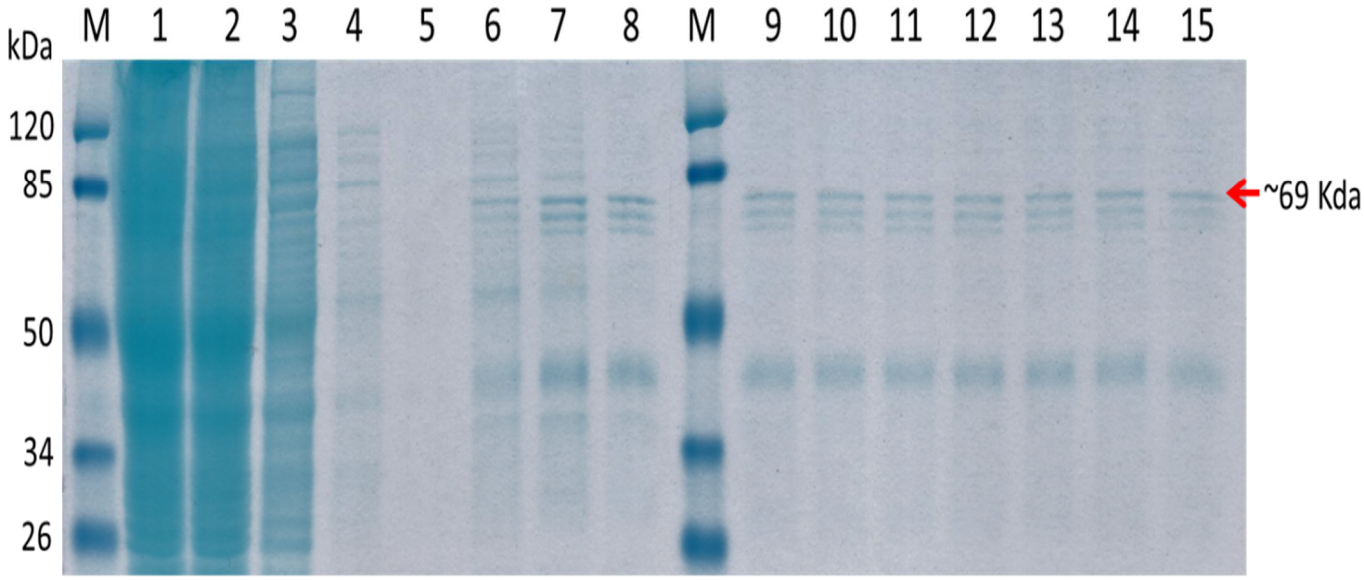

Fig. 4 SDS-PAGE purification of the Cry1Aa protein using IMAC Co^{2+} on the TALON® matrix. M. Pre-stained Protein MW Marker (Thermo Fisher Scientific); 1. Crude protein; 2 – 3. Flow-through fraction; 4 – 5. Washing fraction; 6 – 15. Elution fraction (1st to 10th elution fraction)

C. Verification of Cry1Aa Protein

Confirmation of the presence of Cry1Aa protein was performed with the immunoblotting technique (Figure 5 and 6). Western blotting is a procedure that has been used widely to determine the presence, relative abundance, and relative mass of proteins. This Western blot application relies on the antibody's specific interaction with the target antigen/protein in the sample mixture. After the sample protein is separated on the polyacrylamide gel, the protein is transferred to the membrane and the primary and secondary antibodies are used to bind and visualize the target protein [40].

Western blot analysis (figure 5) showed that the protein target bands were detected at approximately 69 kDa molecular weight; Western blot analysis using anti-rabbit Cry1Aa polyclonal antibody specifically detected the Cry1Aa protein band. The cry1Aa gene (1857 bp) was predicted approximately 69 kDa molecular weight protein is consistent with the calculation of molecular weight based on the calculator from the translation of the base sequence or in silico using ProtParam tool. Detection of the Cry1Aa protein at approximately 69 kDa molecular weight agrees with the size of the cry1Aa protein in previous studies reported earlier [41], [42] showed approximately 65 kDa molecular weight. The difference in the molecular weight of the Cry1Aa protein in this study with Cry1Aa protein in the earlier report is due to differences in the *cry*1Aa gene sequence (provided by CIRAD).

The primary antibody has two keys properties to improve the data's accuracy and reliability resulting from the Western blotting technique. (i) *Specificity* – the ability of the antibody to recognize and bind to the target antigen/protein. The

specificity of the primary antibody in the Western blot technique means that the antibody recognizes the target protein, either as a single distinct band or a set of molecular mass bands. The detection of a single band at the expected molecular weight is an essential but insufficient first step to prove antibody specificity. Antibodies can be validated by testing antibodies' performance for knockout, knockdown, positive, and negative genetic protein samples. (ii) *Selectivity* – preference of antibodies to bind to target antigen/protein in the presence of a heterogeneous mixture of protein samples. In Western blot data analysis, the target protein's abundance may be lower than the non-target protein in the samples. Antibodies must overcome this imbalance and selectively bind to the target antigen/protein in a complex mixture. Selectivity must be verified with various levels of endogenous protein expression in the sample. Purified or overexpressed target protein alters the balance of an abundance of proteins in the sample that may not reflect actual antibody selectivity and expected non-target binding [40].

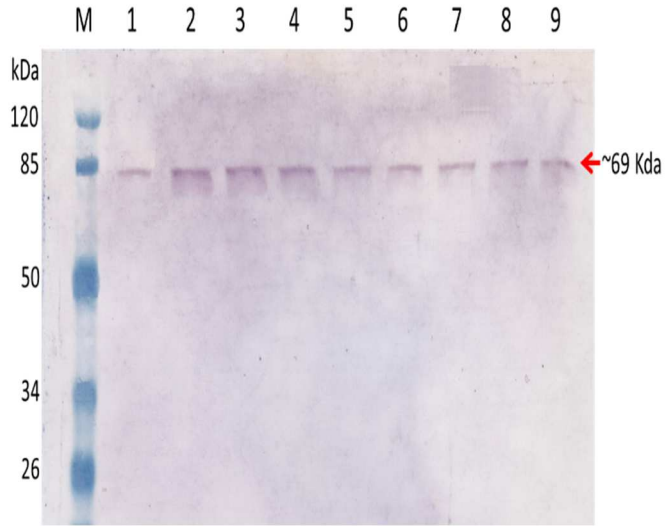

Fig. 5 Western blot the Cry1Aa protein purified using the anti-rabbit Cry1Aa polyclonal antibody. M. Pre-stained Protein MW Marker (Thermo Fisher Scientific); 1 – 9. Elution fraction

The Cry1Aa protein has been fused to the 6xHis tag to facilitate purification. Moreover, the 6xHis tag was able to detect target proteins using HisDetector™ Ni-HRP Conjugate kit (KPL). The ability of this detector to verify the Cry1Aa protein fused to the 6xHis tag because 6xHis can act as an epitope recognized by nickel-NTA conjugated with the HRP reporter enzyme. The nickel-NTA detector technique conjugated with the HRP reporter enzyme can be used if time and funds are limited to produce primary antibodies specific to the target protein because not all antibodies are commercially available. In this study, HisDetector™ Ni-HRP Conjugate kit (KPL) was able to verify the Cry1Aa protein fused to the 6xHis of the same molecular weight as a western blot testing using the Cry1Aa anti-rabbit antibody, which is about 69 kDa (Figure 6).

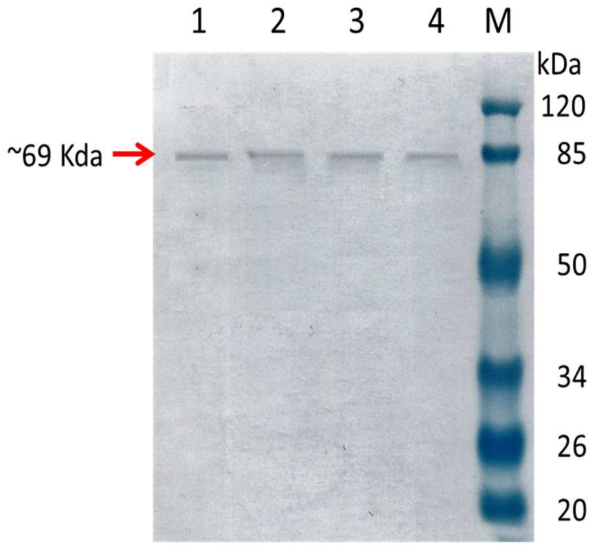

Fig. 6 His Detector the Cry1Aa protein purified using nickel-NTA conjugated with HRP reporter enzyme. 1 – 4. Elution fraction; M. Pre-stained Protein MW Marker (Thermo Fisher Scientific)

The results showed that the process of cloning, transformation, production, and purification of the recombinant Cry1Aa protein using the pRHA-SDM vector on the NiCo21 expression host of E. coli strain was in line with expectations. Results also showed that expression of *cry*1Aa gene fused to the 6xHis tag was driven by the pRHA promoter tightly induced by L-rhamnose and suppressed by D-glucose as expected. The size of the *cry*1Aa gene and the molecular weight of Cry1Aa protein obtained was as predicted.

IV. Conclusion

The 1857 bp *cry*1Aa gene was successfully cloned and fused to 6xHis, driven by pRHA promoter tightly induced by L-rhamnose and repressed by D-glucose in the pRHA-SDM vector, in the *E. coli* strain NiCo21. The resulting plasmid pRHA-SDM-*cry*1Aa were confirmed by restriction digestion. The purified 69 kDa Cry1Aa protein purified with IMAC Co^{2+} on the TALON® matrix as shown in SDS PAGE was able to be consistently detected with both. Western blot using anti-rabbit Cry1Aa polyclonal antibody, and His detector using nickel-NTA conjugated with HRP reporter enzyme as predicted. The results indicated that the protocol for the production of the Cry1Aa protein has been established and can be used for further research.

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