Production of the $^{13}C/^{15}N$ single-labeled insecticidal protein Cry1Ab/Ac for the assessment of metabolic fate using recombinant *Escherichia coli*

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Abstract

Stable isotope-labeled Cry1Ab/Ac protein is necessary for the metabolic study of exogenous insecticidal protein in soil using the stable isotope labeling technique, but no recombinant expression protocols for this protein have been reported. The artificially synthesized gene *Cry1Ab/Ac* of *Bt* rice Huahui No. 1, which obtained the safety certificate in China, was subcloned into pUC57 in this study, and the expression vector pET-28a-*CryAb/Ac* was constructed and transformed into *Escherichia coli* BL21 (DE3) competent cells. Next, 0.2 mM IPTG was added to these cells and cultured at 37°C for 4 h to induce the expressed protein to form inclusion bodies in the presence of M9 medium containing either [U-^{13}C] glucose (5% ^{13}C-enriched) or [^{15}N] ammonium chloride (5% ^{15}N-enriched). Then Cry inclusion bodies were dissolved in urea and purified by Ni column affinity chromatography under denaturing conditions, renatured by dialysis, and further detected by SDS-PAGE and Western blot. The purities of ^{13}C/^{15}N-labeled Cry proteins were each 99%, the amounts of which were 12.6 mg/L and 8.8 mg/L. The δ ^{13}C and δ ^{15}N values of ^{13}C-labeled Cry protein and ^{15}N-labeled Cry protein were 3268.68‰ and 2854.28‰, respectively. An insecticidal test showed that the prokaryotic expression of Cry1Ab/Ac protein had strong insecticidal activity. The stable isotope-labeled insecticidal Cry proteins produced for the first time in this study will provide an experimental basis for future metabolic studies of Cry protein in soil and the characteristics of nitrogen (N) and carbon (C) transformation. The findings will also provide a reference and basis for elucidating the environmental behaviors and ecological effects of *Cry* plants and expressed products.

Introduction

Cry proteins are a family of crystal-forming proteins that have specific insecticidal activity. They are produced by the proteolytic cleavage of protoxin from *Bacillus thuringiensis* in the early stages of spore formation[1]. At present, *Cry* genes have become some of the most widely used insecticidal genes in transgenic plant breeding. The *Cry1* gene exhibits highly specific toxicity to *Lepidoptera*, and the Cry protein encoded by the *Cry1ab/ac* gene, a fusion of cry1ab (GenBank Accession No. X54939) and cry1ac (GenBank Accession No. Y09787) into a single gene, is highly toxic to *Chilo suppressalis*, *Scirpophaga incertulas* and *Cnaphalocrocis medinalis*, three important lepidopteran pests of rice[2]. The codon-optimized *Cry* gene has been successfully transformed into a variety of plants, such as tobacco, corn and cotton, and a large number of transgenic plant varieties or germplasm resources with insecticidal traits have been obtained. With the rapid increase in the planting area of *Cry* crops, the potential environmental concerns for applications related to *Cry* crops have attracted wide attention. One of the major research areas is the effect of Cry proteins released by transgenic crops on soil ecosystems. Key to this research will be determining the metabolic fate of Cry proteins in different types of soil [3].

Cry proteins synthesized in the roots, stems and leaves of *Cry* crops are released into soil by decaying plant residues[4], root exudates[3, 5] or pollen[6]. For a *Cry3Bb1*-expressing Bt-maize, it was estimated that approximately 820 g of *Cry3Bb1* was synthesized by roots in 1 ha of soil[7, 8]. In order to improve the expression level of the insecticidal gene of transgenic plants, some researchers optimized *Cry* gene

codons artificially[9, 10], which probably caused the alteration of the structure and function of the Cry protein expressed by the *Cry* plants compared with that expressed by indigenous *Bacillus thuringiensis* in the soil. Therefore, the Cry protein released from the transgenic plants was regarded as a kind of exogenous environmental compound with insecticidal activity[11]. Consequently, it is of great scientific and practical significance to elucidate the degradation behaviors and the transformation processes of key elements of Cry proteins in the environment. Currently, the main method to quantify the content of Cry protein is enzyme-linked immunosorbent assay (ELISA), which is based on the complete extraction of Cry protein from a sample. Sims and Holden (1996) found that the Cry protein released from transgenic plants was bound tightly to the soil particles and thus difficult to isolate and purify. Therefore, the ELISA data could only indicate the decline of the initial compound without providing information on the metabolic transformations to intermediates, mineralization rates, adsorption, or incorporation into soil organic matter[12]. Accordingly, with regard to the environmental assessment of released Cry protein, the degradation procession of the initial Cry protein released into the soil and the transformation pathways of the main elements C and N should be paid more attention compared with the detection of low extractable Cry protein in soil by ELISA to characterize its absorbance and persistence. Stable isotopic mass spectrometry can be used to trace and quantitatively monitor the transformation, partitioning and dynamic change processes of the C and N elements of Cry protein in different carbon and nitrogen forms. This approach effectively circumvents the disturbance of indigenous Cry protein in the soil. However, the production of ^{13}C-labeled and ^{15}N-labeled Cry proteins has not yet been reported.

In this study, ^{13}C/^{15}N single-labeled Cry proteins with high purity and strong insecticidal activity were produced using recombinant *Escherichia coli* in M9 medium with either ^{13}C-labeled glucose as the sole carbon source or ^{15}N-labeled ammonium chloride as the sole nitrogen source, laying the experimental foundation for the evaluation of environmental safety and ecological effects of insecticidal proteins.

Materials And Methods

Strains and plasmids

E. coli (JM109 and BL21 (DE3)), prokaryotic clone vector pUC57 and expression vector pET-28a were preserved by our laboratory. Primers for DNA fragment amplification were purchased from Sangon Biotech (Shanghai) Co., Ltd. (Table 1).

Reaction reagents

High-fidelity DNA polymerase, *NcoI* and *XhoI* endonuclease, T4 DNA ligase, a MinElute Gel Extraction Kit and a Plasmid Extraction Kit were purchased from TaKaRa Bio Group (Dalian) Co., Ltd. The Ni-NTA Spin Kit for spin purification of His-tagged proteins was purchased from QIAGEN Sciences. Glucose (U-^{13}C6, 99%) for preparation of ^{13}C-glucose substrate with an isotopic purity of 5% and ammonium chloride (^{15}N, 99%) for preparation of ^{15}N-ammonium chloride substrate with an isotopic purity of 5% were purchased from Shanghai Research Institute of Chemical Industry. All other reagents were purchased from

Sinopharm Chemical Reagent Co., Ltd. LB liquid medium (g/L): peptone 10, yeast extract 5, NaCl 10, Wet heat sterilization at 121°C for 20 min. M9 medium (g/L): $Na_2HPO_4 \cdot 7H_2O$ 12.8, KH_2PO_4 3.0, NaCl 0.5, NH_4Cl 1.0, $MgSO_4 \cdot 7H_2O$ 0.492, $CaCl_2 \cdot 6H_2O$ 0.02191, glucose 0.08.

Expression vector construction

Chemical synthesis and cloning of *Cry1Ab/Ac* gene

The gene sequence of *Cry1Ab/Ac* (EU816953.1) was retrieved from the NCBI database, and the terminating codon was removed. *NcoI* and *XhoI* restriction sites were introduced at the 5' and 3' ends, respectively. The whole gene of *Cry1Ab/Ac* was synthesized by Sangon Biotech (Shanghai) Co., Ltd., cloned into plasmid pUC57 and transferred into competent JM109 cells. JM109 cells containing pUC57-*Cry1Ab/Ac* vector were grown on an LB agar plate overnight. The positive strain's plasmids were extracted and further verified after PCR, and the amplified fragments were sequenced by Sangon Biotech (Shanghai) Co., Ltd.

Construction and identification of *Cry1Ab/Ac* expression vector

First, pUC57-*Cry1Ab/Ac* and pET28a were digested with *NcoI* and *XhoI* restriction enzymes. Then *Cry1Ab/Ac* and pET28a were ligated by T4 DNA ligase at 22°C overnight. The prokaryotic expression vector pET28a-*Cry1Ab/Ac* was transformed into *E. coli* JM109 cells. The recombinant expression plasmids were checked by *NcoI* and *XhoI* double restriction digestion, and the positive ones were transformed into *E. coli* BL21 (DE3) to express the Cry1Ab/Ac protein.

Production and purification of Cry protein

Expression of Cry1Ab/Ac fusion protein in *E. coli*

Monoclones were inoculated into M9 medium with either glucose as the sole carbon source or ammonium chloride as the sole nitrogen source at 37°C until OD600 = 0.6–0.8. IPTG was added to a final concentration of 0.5 mM, and incubation was continued at 37°C for 4 h. Then the bacterial solution was collected for identification by SDS-PAGE (8% separation gel).

Induction conditions for optimization of Cry protein

The optimal conditions for protein expression with IPTG induction were investigated. The cells were oscillated at 37°C. When the growth culture reached an OD600 of 0.6–0.8, protein expression was induced with IPTG in the 0.2–1 mM range, temperature in the 15–37°C range and time in the 4–16 h range. The whole bacterial solution was collected for assay via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Amplified expression, denaturation dissolution, nickel column affinity chromatography purification and dialysis renaturation of $^{13}C/^{15}N$ single-labeled Cry proteins

Under the above-mentioned optimum conditions, the recombinant strains were inoculated in 1L M9 medium with either glucose as the sole carbon source ([U-^{13}C] glucose (5% ^{13}C-enriched)) or ammonium chloride as the sole nitrogen source ([^{15}N] ammonium chloride (5% ^{15}N-enriched)). The bacterial solution was collected, mixed with sterile phosphate buffer solution (PBS) and sonicated at 4°C over 30 cycles, with each cycle consisting of 10 s on and 10 s off. The precipitation (crude inclusion body) obtained by centrifugation was dissolved in the 8 mol/L urea denaturation solution. Then the supernatant was collected by centrifugation, which was purified using Qiagen Ni-NTA spin column under denaturing conditions according to the manufacturer's instructions. The amount of Cry protein relative to the total protein was assessed by SDS-PAGE.

The purified ^{13}C-labeled and ^{15}N-labeled Cry1Ab/Ac proteins were renatured by dialysis against 8 M urea, 50 mM Tris–HCl (pH 8.0) and 0.4 mol/L L-arginine with stepwise reduction of the urea concentration (6 M, 4 M, 2 M, 1 M, 0 M) at 4°C for 6 h. The refolded Cry1Ab/Ac in 50 mmol/L Tris-HCl buffer (pH 8.0) was centrifuged at 14,000 rpm for 15 min at 4°C to remove the aggregated protein. Finally, the solution was dialyzed overnight at 4°C in PBS (pH 7.2). The relative proportions of the purified ^{13}C-labeled and ^{15}N-labeled Cry proteins were analyzed using the grayscale scanning function of the gel image analyzer.

Western blot analysis and ELISA quantification of ^{13}C/^{15}N single-labeled Cry1Ab/Ac protein

The parted proteins were transferred onto a nitrocellulose membrane. Cry protein hybridization signals were detected by Western blot using His-labeled antibody (1:2000 dilution)[13]. The contents of ^{13}C-labeled and ^{15}N-labeled Cry proteins were determined by the assay kit (QuantiPlate™ Kit for *Cry1Ab/Cry1Ac*, EnviroLogix Inc., Portland, Maine, USA)[3].

δ ^{13}C and δ ^{15}N value of ^{13}C and ^{15}N single-labeled Cry proteins

^{13}C-labeled and ^{15}N-labeled Cry proteins in 1× PBS buffer were lyophilized to obtain dried Cry proteins using a CoolSafe Freeze Dryer (*LABOGENE* Co., *Ltd*). After the samples were packed with tin foil paper, the δ ^{13}C values of the ^{13}C-labeled Cry protein and the δ^{15}N values of the ^{15}N-labeled Cry protein were determined by element analyzer-isotope ratio mass spectrometry (EA-IRMS, deltaV advantage, Thermo Fisher Scientific, USA)

Insecticidal test and data analysis

The ^{13}C-labeled and ^{15}N-labeled Cry proteins were incorporated into conventional feeds of *Chilo suppressalis* with different addition gradient levels (1 μg/g, 5 μg/g, 10 μg/g, 15 μg/g, 20 μg/g, and 25 μg/g) with conventional feed as a control. Three replicates were performed for each gradient level. Twenty larvae were incubated in each culture tube for each replicate at 25±1°C [14]. All insects were checked for viability after 4 days. Based on the results of the insecticidal test, the semi-lethal concentrations (LC_{50}) of ^{13}C-labeled and ^{15}N-labeled Cry proteins were calculated by SPSS Statistics 19.0 (SPSS Institute, Inc., 2010).

Results

Cloning of recombinant plasmid

The results of PCR identification for plasmid pUC57-*Cry1Ab/Ac* showed that a 1.8 kb target band was confirmed in the most transformants (Fig. 1), which was consistent with the length of the *Cry1Ab/Ac* gene. This revealed that the *Cry1Ab/Ac* gene was successfully cloned. Positive clones were selected, sequenced and compared to the *Cry1Ab/Ac* gene in the GenBank database. The results indicated that the recombinant plasmid was constructed successfully and could be used in subsequent experiments.

Identification of prokaryotic expression recombinant plasmid

The constructed recombinant plasmid pET28a-*Cry1Ab/Ac* digested by *NcoI* and *XhoI* double enzymes produced two fragments of about 1.8 kb and 5.3 kb, the sizes of which were consistent with the length of *Cry1Ab/A*c and the expression vector pET28a fragment length (Fig. 2), indicating the successful construction of recombinant prokaryotic expression vector pET28a-*Cry1Ab/A*c.

Prokaryotic expression of *Cry1Ab/Ac* gene

The recombinant plasmid pET28a-*Cry1Ab/Ac* was transformed into *E. coli* BL21 (DE3), and *E. coli* BL21(DE3)/pET28a-*Cry1Ab/Ac* was induced by IPTG in M9 medium with either glucose as the sole carbon source or ammonium chloride as the sole nitrogen source. The bacterial solution was added into 2× SDS gel-loading buffer, which was boiled and centrifuged and then analyzed by SDS-PAGE. An apparent protein band was seen at the position of Cry1Ab/Ac protein (Fig. 3), suggesting that the protein was successfully expressed. The protein band markedly increased after IPTG addition, suggesting that *E. coli* with the recombinant plasmid was induced using IPTG.

Screening for the optimum conditions of induced expression

When the OD_{600} of the cell density of the recombinant strain reached 0.6–0.8, the IPTG addition amount, induction time and induction temperature were screened, and the whole bacterial solution was analyzed with SDS-PAGE. The maximum expression amount of protein was detected at 37°C, 4 h after the addition of 0.2 mM IPTG (Fig. 4). Then the bacteria solution according to the above-mentioned conditions of induced expression was sonicated and centrifuged, and the precipitate and the supernatant collected were analyzed by SDS-PAGE (Fig. 5), suggesting that the Cry protein existed in inclusion bodies.

Amplification expression and purification of $^{13}C/^{15}N$ single-labeled Cry1Ab/Ac

The recombinant strains were inoculated into either M9 medium with glucose as the sole carbon source (containing ^{13}C-labeled glucose ([U-^{13}C], 5%)) or M9 medium with ammonium chloride (containing ^{15}N-labeled ammonium chloride (^{15}N, 5%) as the sole nitrogen source, and the cultures were amplified at 37°C using the above-mentioned optimal induction conditions. Then, the cells were resuspended in 1× PBS buffer and subjected to ultrasonic treatment. Subsequently, the crude inclusion bodies obtained by

centrifugation were completely solubilized in 8 mol/L urea. The supernatant collected by centrifugation was purified by Ni-NTA affinity chromatography (Fig. 6).

Western blot analysis and content determination of $^{13}C/^{15}N$ single-labeled Cry1Ab/Ac

The denatured ^{13}C-labeled and ^{15}N-labeled Cry1Ab/Ac proteins were refolded with 0.4 mol/L L-arginine in a linear 8 to 0 mol/L urea gradient refolding buffer. SDS-PAGE revealed that the expressed and purified ^{13}C-labeled and ^{15}N-labeled Cry1Ab/Ac proteins each had a single band with relative molecular weight of approximately 66.2 kD (Fig. 7A, C). The purity of the Cry1Ab/Ac proteins obtained was above 99% with grayscale scanning. Western blotting confirmed that the stable isotope-labeled recombinant proteins were successfully expressed and purified (Fig. 7B, D). ELISA analysis showed the expression amounts of ^{13}C-labeled and ^{15}N-labeled Cry proteins were 12.6 mg/L M9 medium and 8.8 mg/L M9 medium, respectively.

Assay of δ ^{13}C and δ ^{15}N value of $^{13}C/^{15}N$ single-labeled Cry proteins

The δ ^{13}C value of the ^{13}C-labeled Cry protein and the δ ^{15}N value of the ^{15}N-labeled Cry protein determined by EA-IRMS were 3268.68‰ and 2854.28‰, respectively.

Insecticidal activity assay of the $^{13}C/^{15}N$ single-labeled Cry1Ab/Ac protein

Data obtained from the *C. suppressalis* assay are presented in Table 2. The mortality of the newly hatched larvae gradually increased with the increase of the application of insecticidal protein. When the protein content in the feed reached 25 μg/g, the mortality reached 100%. The LC_{50} values of $^{13}C/^{15}N$ single-labeled Cry proteins were 5.44 μg/g and 5.38 μg/g, indicating strong insecticidal activity of stable isotope-labeled protein.

Discussion

This study aimed to produce for the first time ^{13}C-labeled and ^{15}N-labeled Cry1Ab/A proteins suitable for assessing the metabolic fate of Cry protein in soil. Many studies have shown that various types of insecticidal proteins are expressed by recombinant strains of *E. coli*[12,15-18]. However, supplementation of growth with media-stable isotopes for generating labeled proteins has not been applied. Valldor et al. (2012) used radioactive isotope ^{14}C-labeled glycerol as a carbon source and cultured recombinant *E. coli* in small batch fermentation to obtain ^{14}C-labeled Cry1Ab protein. Although the radioisotopes tracer technique was simple, accurate and sensitive, it was harmful to health, which limited its research application [19]. The stable isotope labeling technique using stable isotopes as tracers utilizes a mass spectrometer to quantify the abundance of stable isotope tracers in biological samples, and it can be used to study the metabolic fate of a compound. More than 6,000 stable isotope-labeled compounds (tracers) are commercially available for use in metabolic studies. However, stable isotope-labeled Cry proteins are not currently available. Generally, stable isotope-labeled proteins can be produced by biosynthesis and chemical synthesis. Chemical synthesis based on the covalent attachment of stable

isotopes, however, may modify the protein structure and thus affect its biological activity and biodegradability[12]. Therefore, in this study, it was decided to produce the ^{13}C-labeled and ^{15}N-labeled Cry proteins by a recombinant Cry1Ab/Ab protein-synthesizing *E. coli* strain under optimum culture conditions. At first, ^{13}C-labeled glucose ([U-^{13}C], 99%) as a carbon source and ^{15}N-labeled ammonium chloride ([^{15}N], 99%) as a nitrogen source were used for producing Cry1Ab/Ac proteins. However, the δ ^{13}C and δ ^{15}N values of the proteins exceeded the upper limit of the analytical measurement range of EA-IRMS, so the stable isotopic purities of glucose and ammonium chloride were adjusted to 5% by the addition of corresponding unlabeled materials. Consequently, the δ ^{13}C value of the ^{13}C-labeled Cry protein and the δ ^{15}N value of the ^{15}N-labeled Cry protein were 3268.68‰ and 2854.28‰, respectively, which could be applied to future studies of biodegradation processes and metabolic pathways of Cry protein in soil.

Previous studies showed that the IPTG addition amount, induction time and induction temperature influenced the expression of exogenous protein [20,21]. Therefore, in order to enhance protein expression and save experimental costs, we optimized the prokaryotic expression conditions. Our results indicated that 0.2 mM IPTG treatment for 4 h at 37°C was most effective for expression, and the main reasons may be related to the host bacteria: the optimum growth temperature of *E.coli* is about 37°C [22]. Although some studies showed that low growth temperatures enhance protein folding and solubility [20,23], no protein was obtained at 15°C. The maximum expression amounts of the protein, which existed in the form of inclusion bodies, appeared at 37°C. Hence, 37°C was optimum for prokaryotic expression. Accordingly, in the present study, the insoluble inclusion body was denatured with urea, purified with Ni column affinity chromatography under denaturing conditions, and lastly renatured after dialysis with the phosphate buffer solution. Notably, there is a need to purify inclusion bodies prior to refolding, considering that the presence of inclusion body impurities could affect the refolding yield of recombinant proteins[24]. Furthermore, stable isotope-labeled protein revealed strong insecticidal activity after it was purified.

In this study, we produced ^{13}C/^{15}N single-labeled insecticidal protein Cry1Ab/Ac using a recombinant *E. coli* strain. Stable isotope-labeled Cry protein existing in the form of inclusion bodies was solubilized, purified and refolded successfully, revealing strong insecticidal activity. The results of this study lay a foundation for the further study of the biological function and safety evaluation of Cry proteins.

Declarations

Author contributions

P Li and XM Tang designed the experiments. ZB Wang performed most of the experiments. C Hu performed some of the experiments. Y Su contributed materials and analysis tools. W Jiang and GG Wu analyzed data and discussed the result. P Li wrote the manuscript. AH Pan revised the manuscript. All authors read and approved the manuscript.

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Conflict of interests

All authors declare that they have no conflict of interest.

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Tables

Table 1 Primers used in this study

Primers	Sequence	Fragment length
Cry1Ab/Ac-F	5’-CGGGATCCATGGACAACTGCCGTCCATACA-3’	1844 bp
Cry1Ab/Ac-R	5’-CCAAGCTTATTCAGCCTCGAGTGTTGCAGT-3’	

Table 2 Insecticidal Analysis of Cry1Ab/Ac Protein

Dosage/ (μg/g)	Replicates	Inoculation numbers	Average deaths
1 (^{13}C-labeled Cry protein)	3	20	3.2
1 (^{15}N-labeled Cry protein)	3	20	2.8
5 (^{13}C-labeled Cry protein)	3	20	7.5
5 (^{15}N-labeled Cry protein)	3	20	7.8
10 (^{13}C-labeled Cry protein)	3	20	12.2
10 (^{15}N-labeled Cry protein)	3	20	12.1
15 (^{13}C-labeled Cry protein)	3	20	15.5
15 (^{15}N-labeled Cry protein)	3	20	16.1
20 (^{13}C-labeled Cry protein)	3	20	19.5
20 (^{15}N-labeled Cry protein)	3	20	19.1
25 (^{13}C-labeled Cry protein)	3	20	20.0
25 (^{15}N-labeled Cry protein)	3	20	20.0

Figures

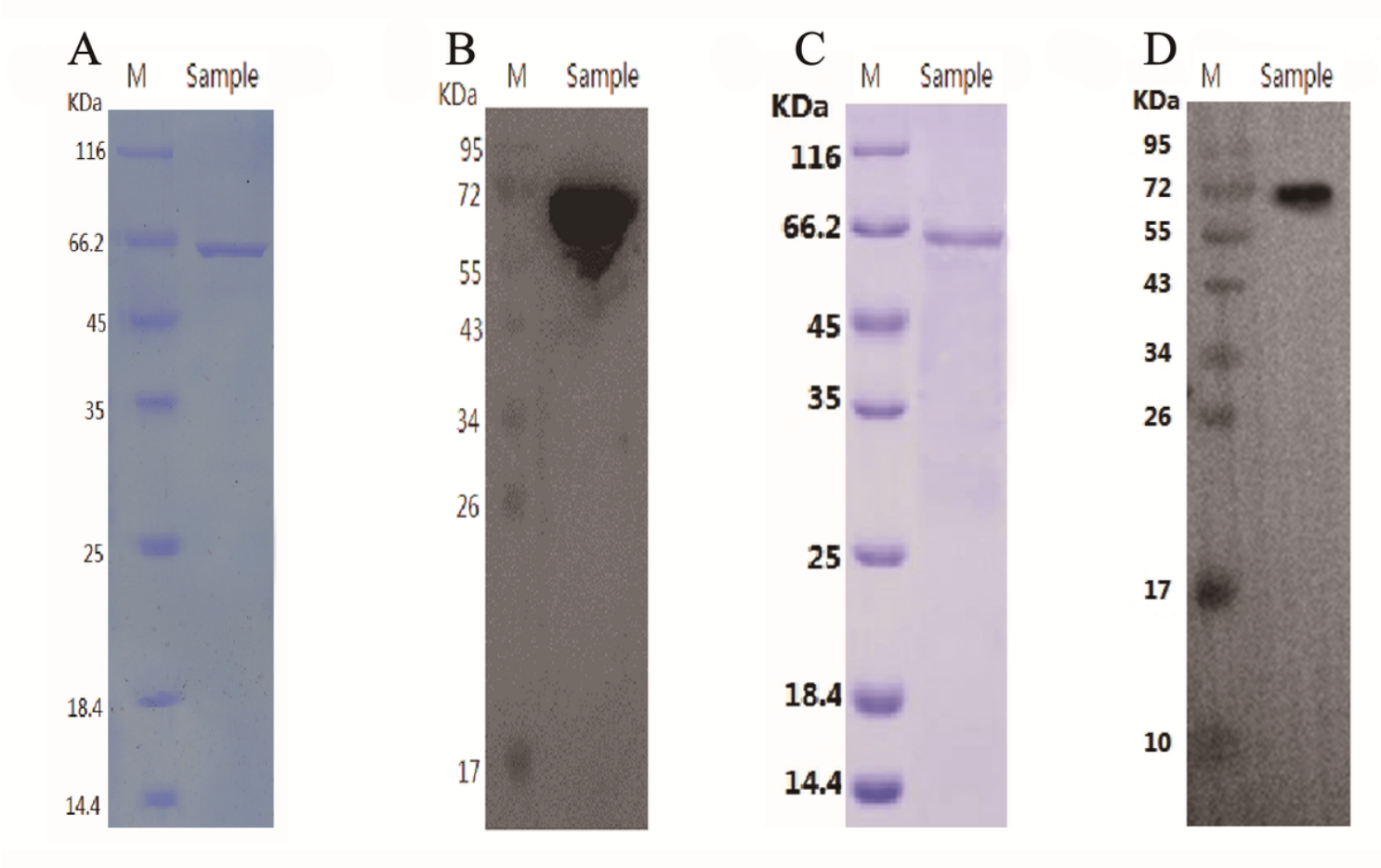

Figure 1

SDS-PAGE (A, C) and Western blot analysis (B, D) of Cry1Ab/Ac protein (prokaryotic expression of Cry1Ab/Ac protein in M9 medium containing 13C-labeled glucose (A, B) or 15N-labeled ammonium chloride (C, D)).

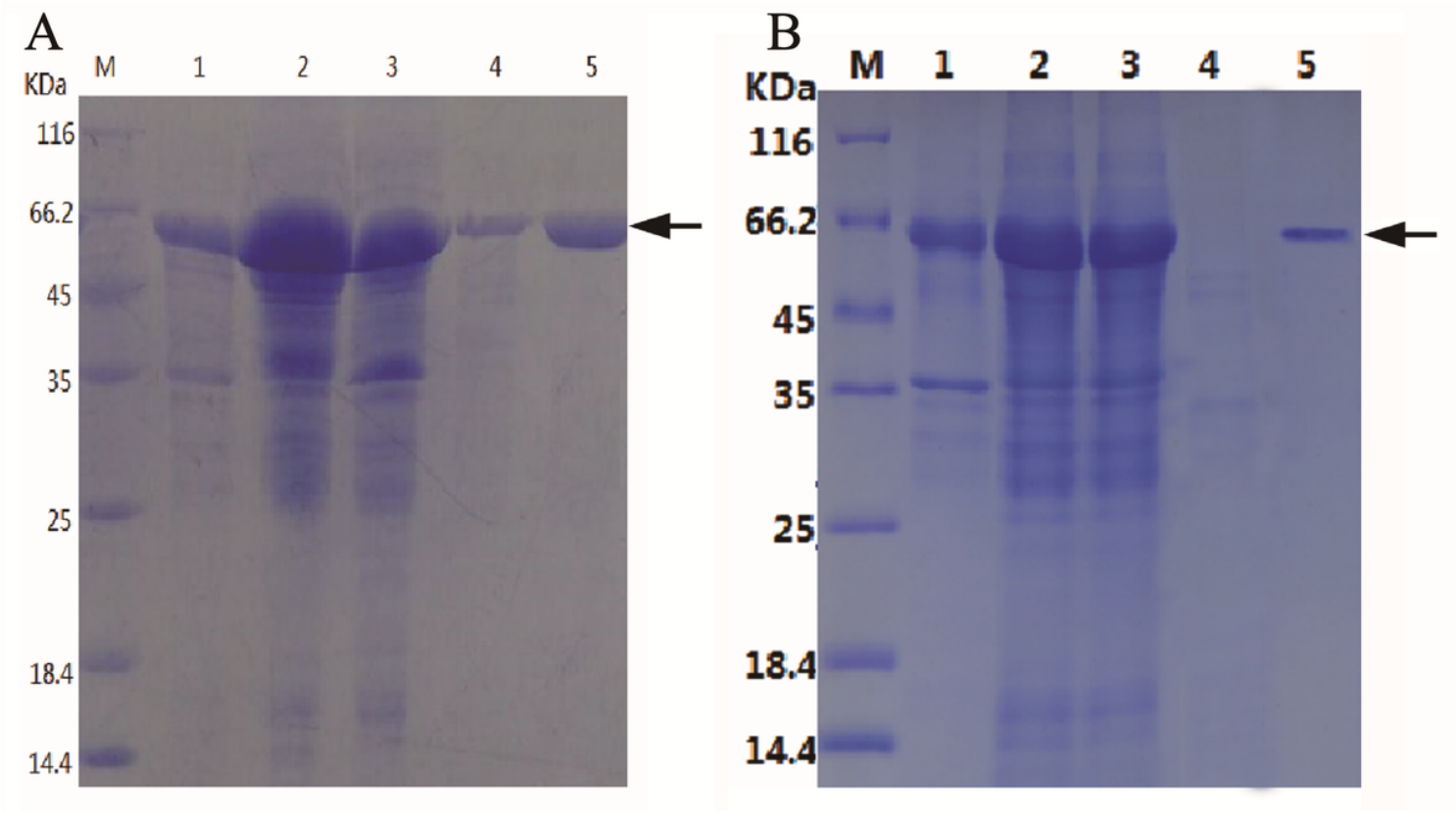

Figure 2

SDS-PAGE analysis of Cry1Ab/Ac protein purified by nickel column affinity chromatography (prokaryotic expression of Cry1Ab/Ac protein in M9 medium containing 13C-labeled glucose (A) and 15N-labeled ammonium chloride (B), respectively). M: Protein Marker; 1: Precipitate after lysis; 2: Supernatant after lysis; 3: Flow-through liquid; 4: Wash fraction; 5: Eluate.

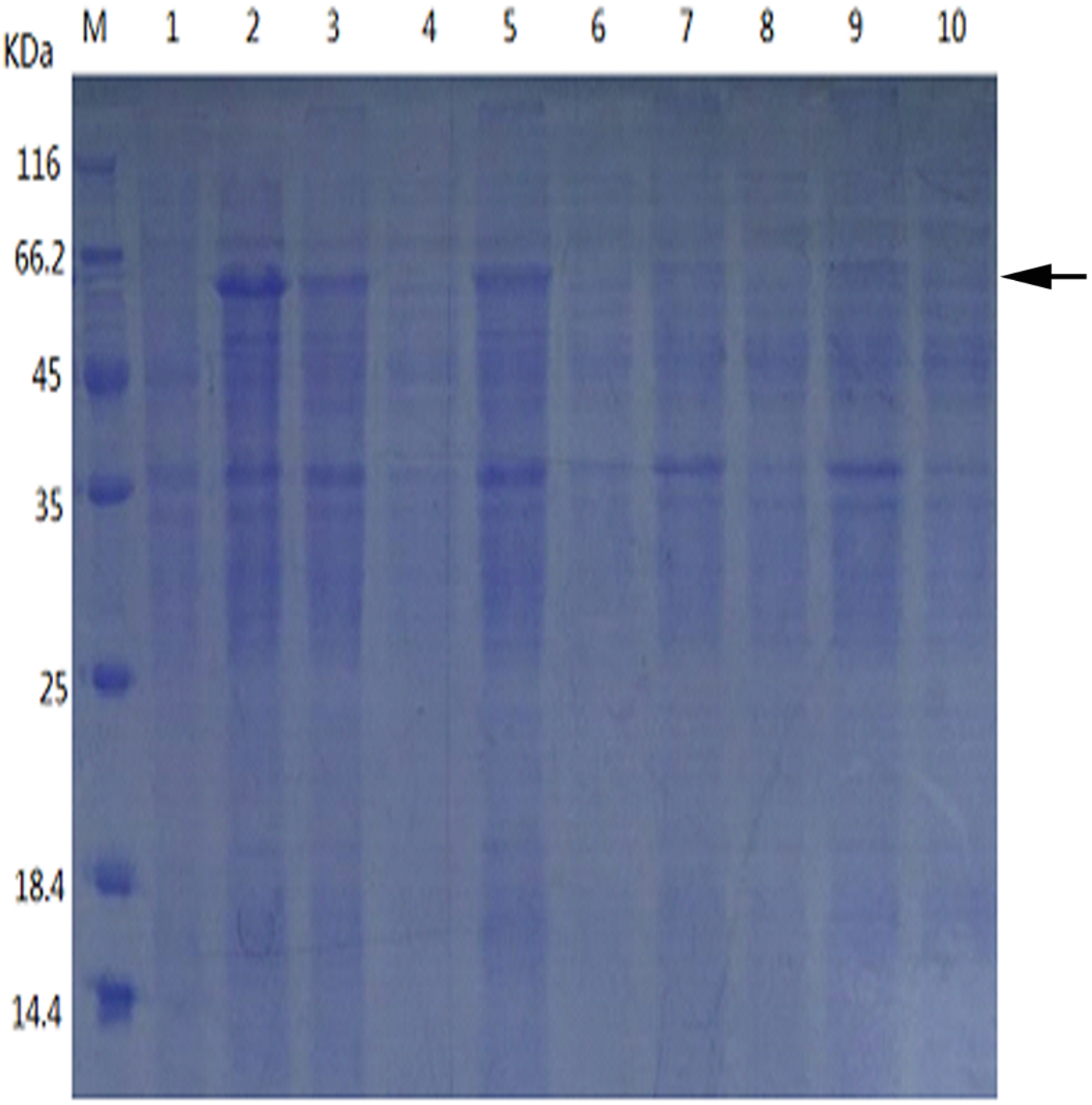

Figure 3

SDS-PAGE analysis of Cry1Ab/Ac protein solubility. M: Protein Marker; 1: Not induction bacterial solution; 2: Total bacterial solution after induction; 3, 4: Bacterial solution precipitate and supernatant after induction with 1.0 mM IPTG at 37°C for 4 h, respectively; 5, 6: Bacterial solution precipitate and supernatant after induction with 0.2 mM IPTG at 37°C for 4 h, respectively; 7, 8: Bacterial solution precipitate and supernatant after induction with 1.0 mM IPTG at 15°C for 16 h, respectively; 9, 10:

Bacterial solution precipitate and supernatant after induction with 0.2 mM IPTG at 15°C for 16 h, respectively.

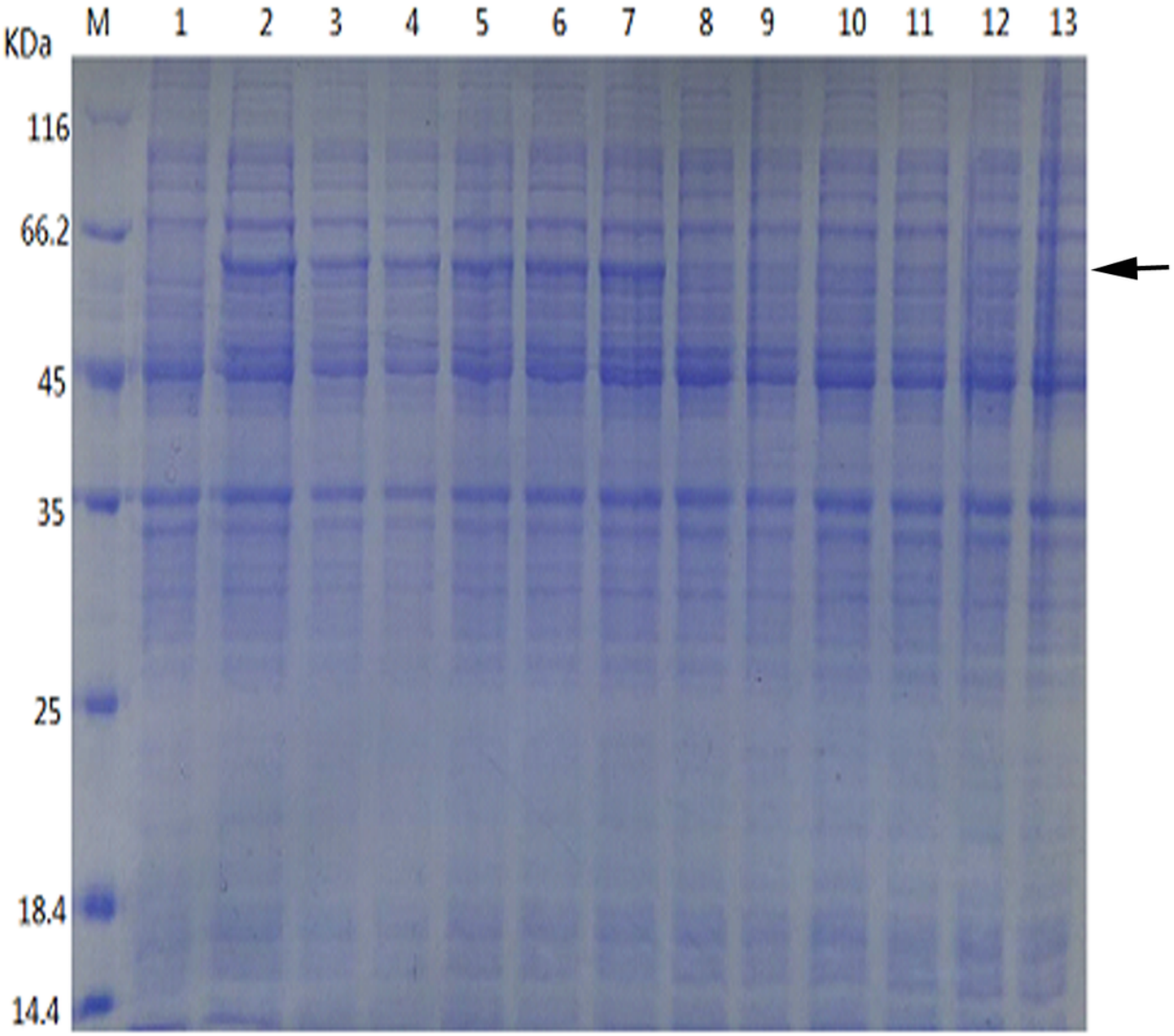

Figure 4

SDS-PAGE analysis of expression conditions optimization of Cry1Ab/Ac protein. M: Protein Marker; 1: Not induction bacterial solution; 2–4: Bacterial solution after induction with 1.0 mM IPTG at 37°C for 4 h; 5–7: Bacterial solution after induction with 0.2 mM IPTG at 37°C for 4 h; 8–10: Bacterial solution after induction with 1.0 mM IPTG at 15°C for 16 h; 11–13: Bacterial solution after induction with 0.2 mM IPTG at 15°C for 16 h.

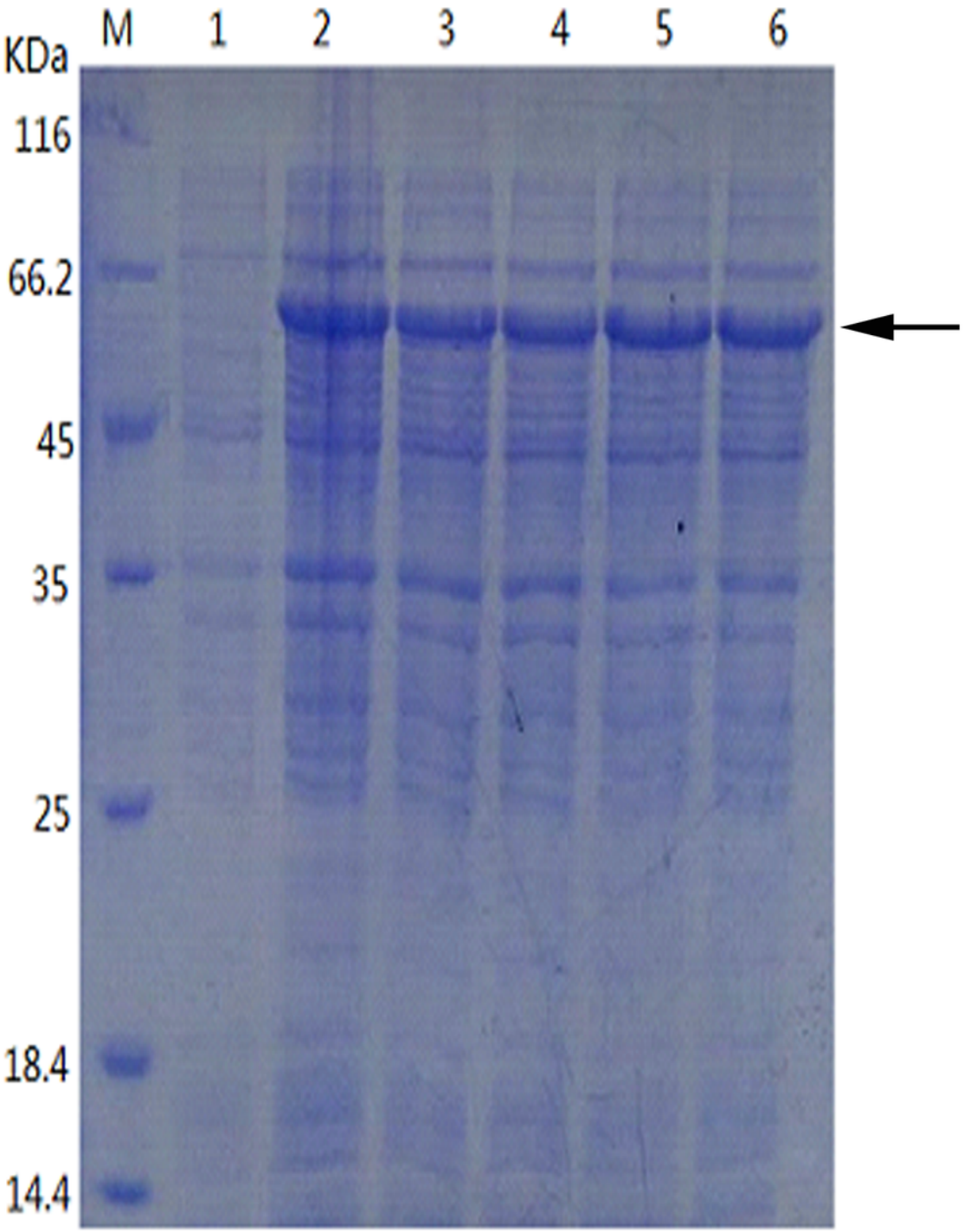

Figure 5

Expression of recombinant plasmid pET28a-Cry1Ab/Ac. M: Protein Marker; 1: Samples before induction; 2–6: Samples after induction.

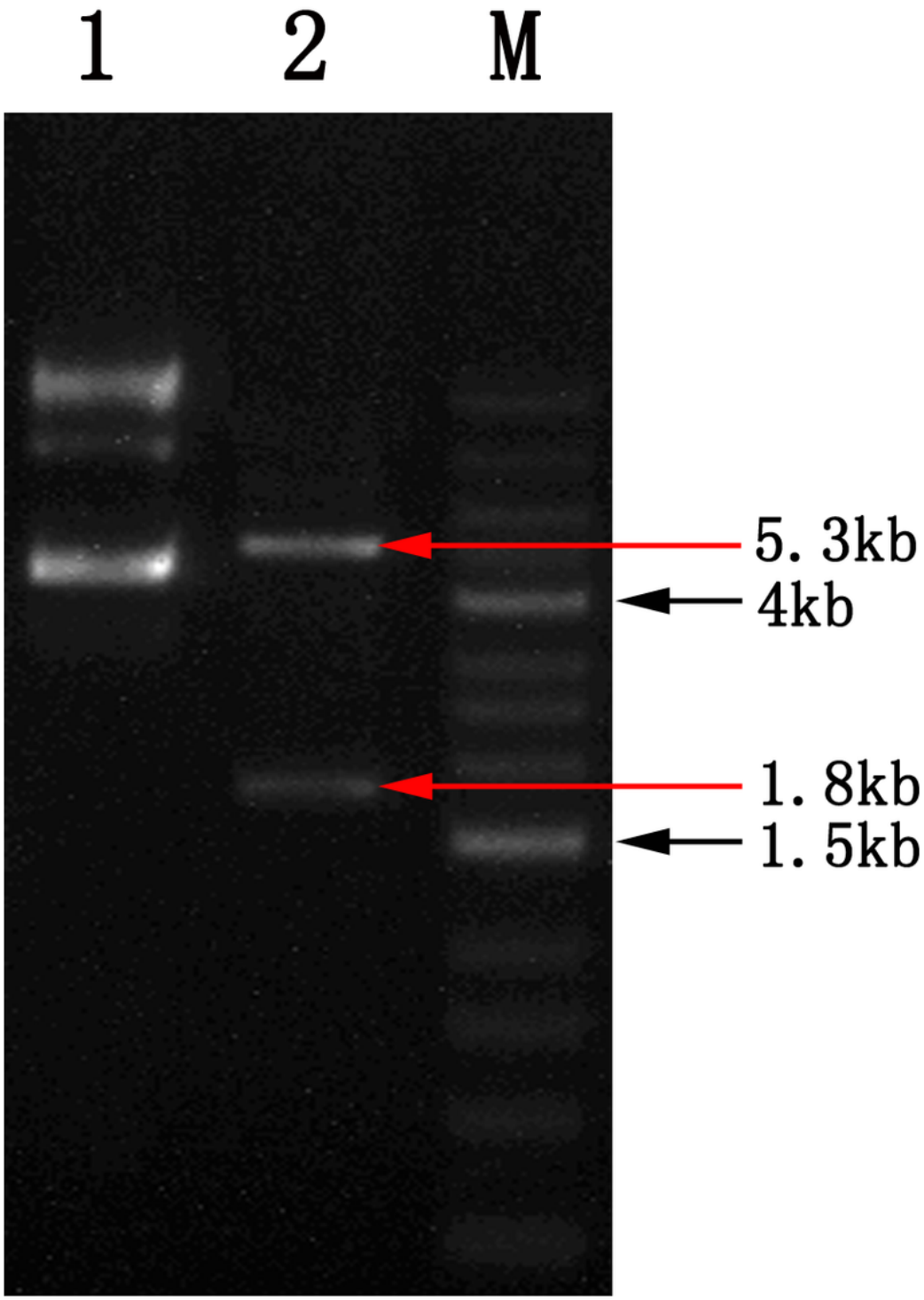

Figure 6

Identification of expression recombinant plasmid. 1: pET28a-Cry1Ab/Ac; 2: Double-restriction enzyme digestion of pET28a-Cry1Ab/Ac; M: DNA Marker.

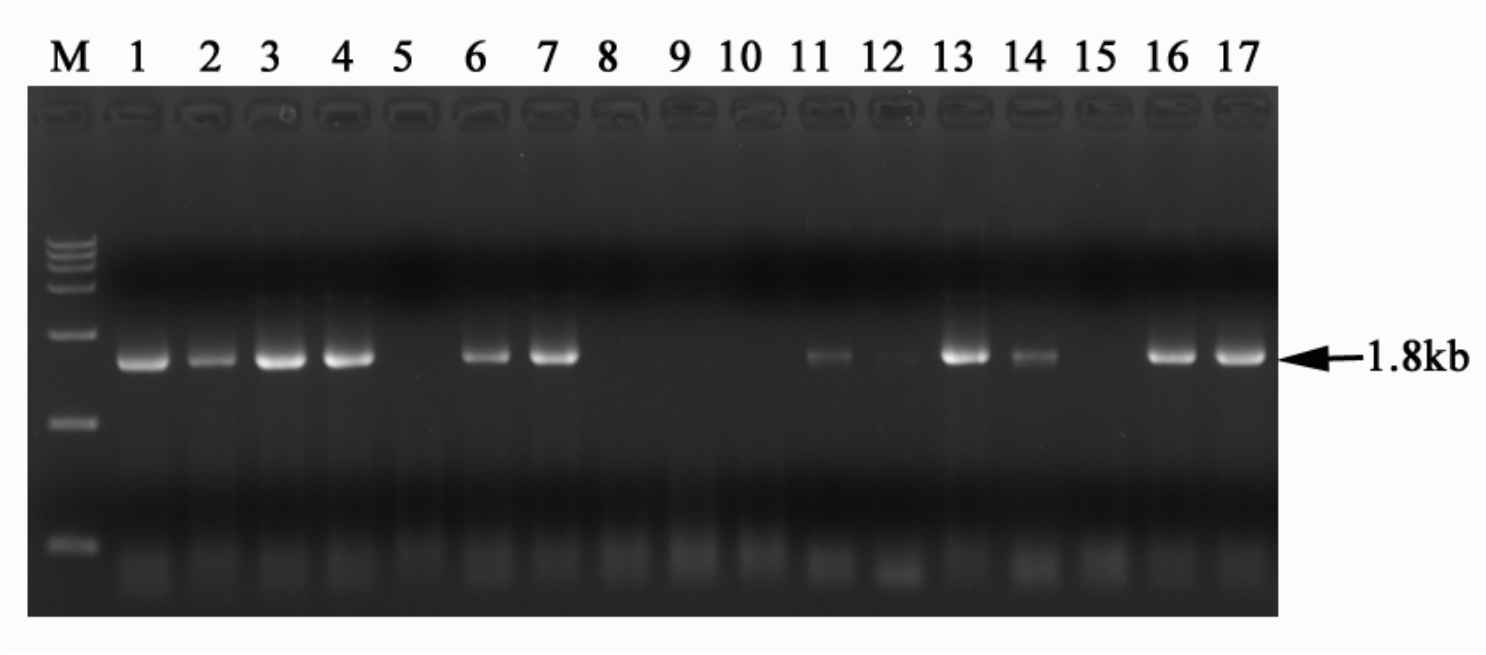

Figure 7

PCR identification of positive plasmid pUC57-Cry1Ab/Ac. M: DNA Marker; 1–17: PCR products of randomly selected plasmids.