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# Isolation of single chain variable fragment (scFv) specific for Cry1C toxin from human single fold scFv libraries

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ABSTRACT

As bioinsecticides *Bacillus thuringiensis* Cry1C δ-endotoxins also have been used in genetically modified crops worldwide since last century. In this study, single chain variable fragments (scFvs), which could specifically recognize and detect Cry1C in food samples, were isolated from naive phage displayed human antibody libraries (Tomlinson I + J) by iterative affinity selection procedure instead of immunization process. With increasing selection pressure, after four rounds of panning, three individual scFvs were obtained and sequenced. The antibodies were characterized by enzyme-linked immunosorbent assay (ELISA). Thereafter, a conformed novel anti-Cry1C scFv, namely scFv-H6, was expressed in *Escherichia coli* (*E. coli*) HB2151 and purified by Ni metal ion affinity chromatography. An indirect competitive ELISA assay (ic-ELISA) of scFv-H6 was developed for the determination of Cry1C toxin in the range from 0.023 μg $mL^{-1}$ to 4.35 μg $mL^{-1}$, and 50% inhibition concentration ($IC_{50}$) was 0.39 μg $mL^{-1}$. This approach showed ignorable cross-reactivity with toxin Cry1Ac and Cry1B (3.51% and 7.28%, respectively). This ic-ELISA approach was exploited for the determination of Cry1C in spiked ground rice samples with a mean recovery rate of 92.5% and coefficient of variation (C.V.) less than 5.0%. This study proves that phage display libraries provide a valuable system for the low-cost, rapid and continuous generation of specific antibody fragments directed against toxin targets and develop a simple detection method. Our results show that anti-Cry1C scFv could be a valuable tool for detection of Cry1C in food and agricultural samples.



## 1. Introduction

As a Gram-positive, spore-forming bacterium, *Bacillus thuringiensis* (Bt) was initially characterized by its production of insecticidal parasporal crystals during sporulation (Abdul-Rauf and Ellar, 1999), which has become a dominating bioinsecticide and commercial genetically modified (GM) crops worldwide (Bravo et al., 2011; James, 2009). However, some reports showed that Bt bioinsecticides would infect non-target species (Yuan et al., 2011) and induce resistance to target insects (Heckel et al., 2007). In addition, many factors need to be verified before GM crops enter the market, such as their potential allergenicity and environment residual persistence (Randhawa et al., 2011). Therefore, it is necessary to establish a convenient method for detecting Bt insecticidal Cry toxins.

Active Cry toxins are approximately 60 kD proteins (Gómez et al., 2007) that belong to the group of pore-forming toxins (PFT) (Bravo et al., 2007). It is widely acknowledged that the toxic effect is due to the formation

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of ionic pores in the membrane of the midgut epithelial cells, which leads to pest death (Fernández et al., 2008). The Cry1C toxin is effective in controlling a variety of Lepidoptera pests (Avisar et al., 2009; Alcantara et al., 2004). Because it does not share the same binding sites with the Cry1A toxins (Alcantara et al., 2004), the Cry1C toxin can be a potential supplement to the Cry1A toxin. Strizhov et al. (1996) produced Cry1Ca-transgenic alfalfa and tobacco that exhibited enhanced resistance to the Egyptian cotton leaf worm and the beet armyworm. In addition, cry1C also can be combined with cry1A genes to develop two-toxin Bt crops (Tang et al., 2006). Cao et al. (2005) generated cry1Ac/cry1C-transgenic collard lines that have the potential to be used to control Lepidoptera. Currently, the detection of Bt-GM crops and Bt residue are mainly performed by instrument analysis, DNA-based and protein-based methods. Rodríguez-Nogales et al. (2008) first introduced reversed-phased high-performance liquid chromatography (RP-HPLC) to distinguish the Bt-transgenic and non-transgenic corps. However because of its high cost and professional requirement for its operators, this method is not suited for most testing agency. The DNA-based method based on polymerase chain reaction (PCR) focuses on the Bt DNA whether inserted into crops (Cankar et al., 2006; Margarit et al., 2006). Although PCR assay is highly sensitive, the complicated process, high cost and difficulty for a high-throughput analysis restrict its application. On the other hand, the immunoassay has been recognized as the most successful method, such as ELISA and colloidal gold strip (Holst-Jensen, 2009). Nonetheless, it requires specific antibodies for binding targets. Antibody phage display technology is an attractive alternative to hybridoma technology. It reduces batch to batch variation and the antibody protein (i.e. scFvs) can be produced using prokaryotic or eukaryotic expression system (Wu et al., 2007). This technology has been widely used to select antibodies against biotoxin. Garet et al. (2010) have panned the scFv anti-palytoxin with 0.0005–500 ng $mL^{-1}$ working range and could be used to quantify in shellfish samples. Pansri et al. (2009) constructed a native human library and obtained scFv against snake venom and Aflatoxin B1. Furthermore, a protective human scFv has showed to partially neutralize the major toxin of the Brazilian scorpion *Tityus serrulatus*-Ts1 (Amaro et al., 2011).

Here, we described an optimized protocol to generate monoclonal scFvs against Cry1C toxin from naive human single fold scFv libraries (Tomlinson I + J) without immunization. The detection of Cry1C toxin is developed by ic-ELISA. Our report shows that the phage antibody technology allows the rapid, simple and economical production scFv against Cry1C toxin as well as quantification of Cry1C toxin from food and agricultural samples.

## 2. Materials and methods

### 2.1. Chemicals

Cry1C and Cry1Ac were purchased from YouLong Bio. Co. Ltd (Shanghai, China) and dissolved in sodium carbonate buffer (CBS, pH 9.6), stored at −20 °C. Other reagents were purchased from GE Healthcare (Beijing, China).

### 2.2. Libraries, helper phage and bacterial strains

The Tomlinson I + J human single fold synthetic native phage display single chain antibody fragment libraries (in phagemid/scFv format—fused to the pIII minor coat protein of M13 bacteriophage), helper phage KM13, *Escherichia coli* strains TG1 and HB2151 for selection of specific antibody clones and for production of soluble scFvs were obtained from MRC (Cambridge, England), respectively. This scFv phagemid library contains synthetic V-gene ($V_H$–$V_L$) of human immunoglobulin recloned in the pIT2 phagemid vector. The library size of Tomlinson I and J is $1.47 \times 10^8$ and $1.37 \times 10^8$, respectively.

### 2.3. Subtractive selection of anti-Cry1C scFvs clones

After growing the library stock in fresh medium, the phage particles were rescued by superinfection with helper phage (Marks et al., 1991) and incubated at 30 °C overnight. Next day resuspended the pellet in PBS. The bio-panning procedure was essentially the same as described previously (Garet et al., 2010; Eteshola, 2010) with some modifications. In brief, $1 \times 10^{11}$ phage units of the library were mixed together with four times volume of 2% dried milk in PBS in 25 $cm^2$ polypropylene cell culturing flasks coated with 2 μg $mL^{-1}$ Cry1Ac for 1 h at room temperature. Then the phage supernatant was transferred to new cell culturing flasks coated with Cry1C. Subtractive screening can be used to eliminate non-specific phage. The concentration of the coated antigen was 75, 50, 25 and 10 μg $mL^{-1}$ for the first, second, third and fourth round of selection, respectively. Limiting the amount of antigen used for coating can progressively increase detection of scFv molecules with higher binding affinity. The binding phages were eluted by Cry1C (with the same concentration as the coating toxin) and the eluted fraction was treated with trypsin (1 mg $mL^{-1}$) for 30 min with end-over-end mixing at room temperature. The eluted phages were used to infect fresh exponentially growing culture of *E. coli* TG1 cells ($O.D._{600}$ = 0.4) and incubated at 37 °C in a water bath (without shaking) for 30 min to allow optimal infection. Phage particles were rescued by helper phage, and were amplified for further rounds of panning. Four rounds of bio-panning experiments were carried out for selection of scFv-phage clones with specific binding to Cry1C.

### 2.4. Screening of antibodies against Cry1C

The binding of phages to Cry1C toxin was assessed by polyclonal phage ELISA as described by Garet et al. (2010) with some modification. Briefly, 96-well plate (Corning, USA) was coated with 100 μL of Cry1C toxin (2 μg $mL^{-1}$) in CBS overnight at 4 °C and blocked for 2 h at room temperature with (200 μL) 5% skim-milk in phosphate buffer (MPBS). Phages precipitated at each round of panning (100 μL) were added and incubated for 2 h at room temperature. The plate was washed with PBST (contain 0.1% Tween-20) prior to adding (100 μL) 1:5000 dilution of

anti-M13 antibody/Horseradish Peroxidase (HRP) conjugate and incubated at room temperature for 1 h. After added tetramethylbenzidine (TMB) substrate solution, color development was performed for 15 min at room temperature and stopped with (50 μL) 2 mol $L^{-1}$ sulfuric acid. All experiments were performed in triplicate. $O.D._{450}$ was measured with an automatic microplate reader (Thermo, USA).

### 2.5. Regular monoclonal ELISA for fusion scFv-phage

Individual colonies from the fourth round were picked and inoculated into 2 × TY with 1% glucose and 100 μg $mL^{-1}$ ampicillin, grown shaking at 250 rpm overnight at 37 °C. 2 μL of each culture from plates was grown in a second plate of 2 × TY (200 μL) with ampicillin and 1% glucose shaking until $O.D._{600}$ of 0.4. Each culture was infected with KM13 helper phage, incubated for 30 min at 37 °C, spun down at 1800 *g* for 10 min, resuspended in 2 × TY of ampicillin/kanamycin/0.1% glucose, and shaked overnight at 30 °C. Next day, the supernatant was obtained to use in monoclonal fusion phage-scFv ELISA as described above. Negative controls on each ELISA plate included one buffer blank (no Ab applied) and negative Ag (with Cry1Ac coated). KM13 Helper phage ($1 \times 10^9$ pfu) was coated to the ELISA plates as a positive control for the anti-M13 Ab reactivity.

### 2.6. Colony PCR and DNA sequencing

The selected phage particles were amplified by PCR (94 °C 4 min, then 94 °C 1 min, 55 °C 1 min, 72 °C 2 min for 30 cycles, and final extension at 72 °C for 10 min) to check whether the clones contain full length $V_H$ and $V_\kappa$ insert (935 bp), without insert only have 329 bp. Two primers used in PCR reaction are: LMB3 (5′-CAG GAA ACA GCT ATG AC-3′) and pHEN seq. (5′-CTA TGC GGC CCC ATT CA-3′). The PCR products were examined by electrophoresis on 1.5% agarose gel. The selected monoclonal phages were sequenced by Invitrogen (Shanghai, China). Sequences of scFvs were blast on NCBI Website and compared by clustalx software.

### 2.7. Small-scale expression and purification of soluble scFv fragments

For the production of soluble scFv antibody fragments from selected positive Cry1C clones, phage clones were infected into *E. coli* HB2151 non-suppressor strain. The cultures were grown with shaking (250 rpm) at 37 °C until the $O.D._{600}$ was approximately 0.9. At this stage, the transcription of scFv cassette was driven with a lacZ promoter by inducing with isopropyl β-D-thiogalactopyranoside (IPTG), which was added to a final concentration of 0.2–1 mmol $L^{-1}$. Shaking was continued at 200 rpm, 20–37 °C for 6–20 h. The antibody fragments that have secreted into culture supernatant and the *E. coli* periplasm were harvested. Thus, soluble scFvs were yield, which contained the His tags at the C-terminus.

To purify the scFv fragment, the induced bacterial culture was centrifuged at 3300 *g* at 4 °C for 30 min and the supernatant is collected by filtration through disposable 0.45 μm filters. The resulting supernatant was concentrated by ultrafiltration (10 kD cut dialysis system). To extract scFv secreted into the *E. coli* cell periplasm, the bacterial pellets from the above centrifugation step were resuspended in 1/50 the original culture volume of ice-cold TES (50 mmol $L^{-1}$ Tris-HCl, pH 8.0, containing 20% sucrose, 1 mmol $L^{-1}$ EDTA). The suspension was agitated gently for 20 min in ice bath, centrifuged for 30 min at 30,000 *g* at 4 °C and the supernatant maintained at 4 °C. Soluble scFv antibodies obtained from culture supernatants and periplasm were purified using Ni metal ion affinity chromatography (GE, USA). The purity of the eluted fraction was checked by12% SDS–PAGE.

### 2.8. Indirect competitive ELISA to estimate working range and specificity of the purified scFv

Wells were coated overnight at 4 °C with 2 μg $mL^{-1}$ Cry1C. After washing and blocking, they were incubated for 2 h at 30 °C with a pre-incubated mixture of equal volumes of serial dilutions of Cry1C (10–0.005 μg $mL^{-1}$) and purified scFv. scFv bound to Cry1C was detected by incubation with 100 μL of anti-His-HRP antibody and revealed by adding TMB substrate solution. All analyses were carried out in triplicate. The 50% inhibitory concentration ($IC_{50}$) of the purified anti-Cry1C scFv was calculated by using the formula $[(P - S - N)/(P - N)] \times 100\%$, where *P* is the absorption value of the positive control (50 μL of scFv + 50 μL of PBS), *N* is the absorption value of the negative control (100 μL of PBS), and *S* is the absorption value of the standard (50 μL of scFv + 50 μL of serial Cry1C toxin standard).

Specificity was defined as the ability of the scFv to distinguish other kinds of Cry1 toxins (Cry1Ac and Cry1B). Cross-reactivity (CR) was used to describe the specificity: $CR\ (\%) = IC_{50,\ Cry1C}/IC_{50,\ x} \times 100\%$, where $IC_{50,\ Cry1C}$ is the concentration of standard Cry1C toxin at 50% inhibition (μg $mL^{-1}$) and $IC_{50,\ x}$ is the concentration of Cry1Ac or Cry1B at 50% inhibition (μg $mL^{-1}$).

### 2.9. Spiked rice samples

After grinding, rice samples were spiked with Cry1C toxin at three concentration levels (0.2, 0.5, 1.0 mg $kg^{-1}$), and 2 mL of protein extraction solution (0.1 mol $L^{-1}$ PBS pH 7.4, containing 0.1% BSA and 0.05% Tween-20) was added and extracted by shaking 4 h, then the suspension was centrifuged for 10 min at 10,000 *g*. Each concentration was replicated three times. To reduction the unspecific absorbance due to the rise powder matrix, samples without toxin were processed in the same way. Extracts were diluted stepwise 1-fold, 2-fold, 5-fold, 10-fold and 50-fold then analyzed by ic-ELISA.

## 3. Results

### 3.1. Isolation of phage-scFvs anti-Cry1C

The Tomlinson I + J libraries were used to obtain phage scFvs against Cry1C. In this experiment, binding efficiency was measured as the output fraction of input phage that bound with the Cry1C. The phage titer was quantified after

**Table 1**
Recovery of phages during selection against Cry1C.

| Selected round | Input[a] | Output[a,b] | Phage bound[c] |
|---|---|---|---|
| 1 | $2.0 \times 10^{11}$ | $2.0 \times 10^{4}$ | $1.0 \times 10^{-7}$ |
| 2 | $1.5 \times 10^{11}$ | $3.0 \times 10^{5}$ | $2.0 \times 10^{-6}$ |
| 3 | $1.1 \times 10^{11}$ | $5.0 \times 10^{5}$ | $4.5 \times 10^{-6}$ |
| 4 | $1.2 \times 10^{11}$ | $6.0 \times 10^{7}$ | $5.0 \times 10^{-4}$ |

[a] Phage inputs and outputs were calculated by transduction of *E. coli* TG1.
[b] Number of eluted scFv phages expressed as colony forming unit per milliliter (cfu $mL^{-1}$).
[c] Phage bound = output/input.

each round of the four bio-panning experiments. Approximately $10^{11}$ phage particles were added to each round of selection. The results showed that the relative output/input of binding phage particles was increased approximately 5000-fold from the first to the fourth round of subtractive selection as shown in Table 1. This was taken as initial evidence of the progressive isolation and enrichment of phages with the capacity to bind to Cry1C.

### 3.2. Specificity of scFv phages anti-Cry1C by polyclone ELISA

The presence of positive binding phages after each round of panning was proved by polyclone ELISA as shown in Fig. 1(A). Few scFv phages from the unselected libraries were able to recognize Cry1C and presented a weak signal. However, clones selected in third round displayed binding for Cry1C toxin with $O.D._{450}$ standing around 0.3. After round four of panning, ELISA results showed an increase in binding ($O.D._{450} = 0.84$).

### 3.3. Monoclonal scFv ELISA

Eluted phages from the fourth round selected from the libraries were used to infect *E. coli* TG1 and rescued by M13KO7. 192 phage clones randomly picked for monoclonal ELISA, 24 clones showed positive binding to Cry1C (absorbance against Cry1C/absorbance against negative coated >3). Only three of these clones, designated as A2, C11 and H6, showed great binding ability to the antigen (above 0.9 absorbance in ELISA assay), as indicated in Fig. 1(B). Therefore, these three clones were selected for further analysis.

### 3.4. Sequence of the positive clones

PCR amplification (using LMB3 and pHEN primers) of the scFv genes yielded products approximately 935 bp showed in Fig. 2(A). When compared to published NCBI

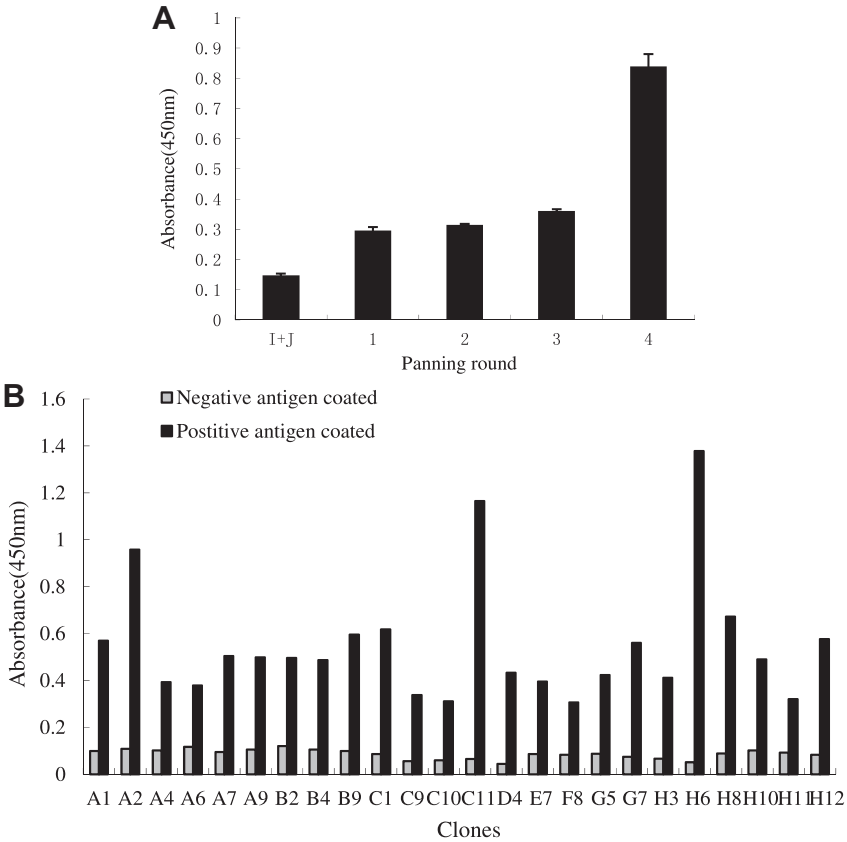


**Fig. 1.** Polyclonal and monoclonal phage ELISA against Cry1C. (A) Polyclonal phages ELISA rescued at each round of panning against Cry1C. Culture supernatants containing approximately $10^{11}$ (pfu $mL^{-1}$) were analyzed by polyclonal phages ELISA. Absorbance values are the mean of three independent determinations. Error bars show the mean ± standard deviation from triplicate measurements. (B) The binding to Cry1C of 24 selected clones expressing scFv fragments was determined by ELISA. In the selected libraries, absorbance values of specific scFv phages were obvious higher than negative antigen coated.

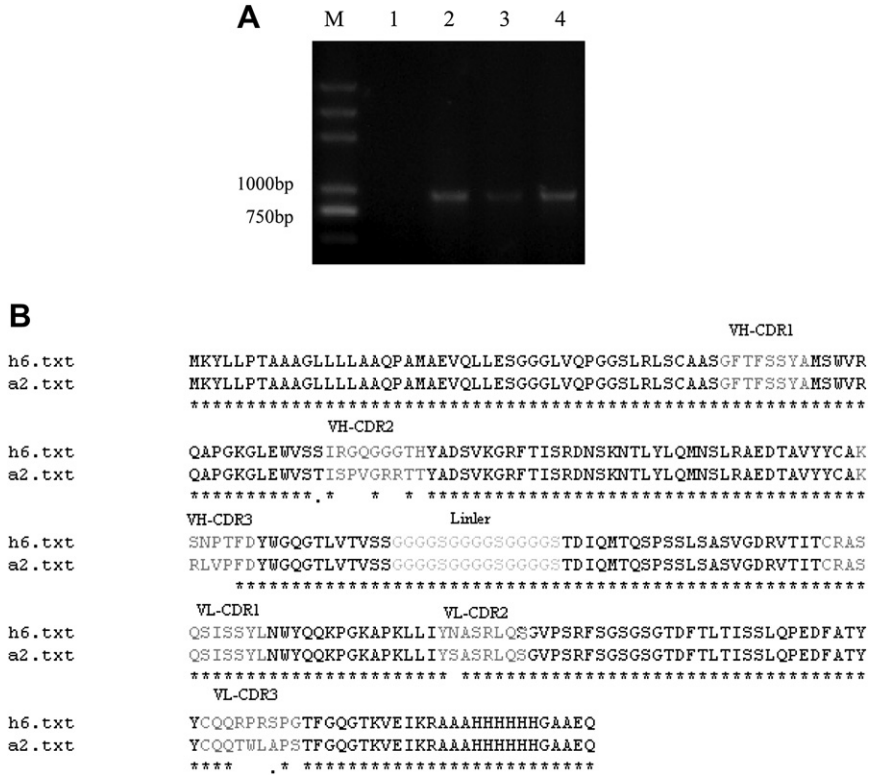


**Fig. 2.** PCR amplification and sequencing of the clones. (A) Clones with full length scFv gene had bands at 935 bp. Lane M: DNA marker. Lanes 1–4: empty TG1, A2, C11, H6. (B) Amino acid sequences were deduced from the nucleotide sequences by Expasy website. The difference between A2 and H6 display in the CDR2 and CDR3 regions of both heavy and light chain were indicated by underline. The symbol (*) indicates that the aligned residues are identical; (:) indicates conservative and (.) means semi-conservative.

database, the selected positive clones were revealed to be human $V_H$ and $V_\kappa$ inserts and similar to other reports utilizing Tomlinson phage libraries (Wen et al., 2010; Zhang et al., 2012). Three positive clones deduced amino acid sequences with complementarity determining region (CDRs) and immunoglobulin frameworks (FRs) are shown in Fig. 2(B). The results indicated that the coding sequences of A2 and C11 share an identical amino acid sequence. The differences between A2 and H6 were included in the CDR2 and CDR3 regions of both $V_H$ and $V_\kappa$. These regions indicated probably the most important contact-associated epitopes.

### 3.5. Expression and purification of H6

Because low expression level of A2, meanwhile it has higher $IC_{50}$ than H6. Based on the above results, we chose the scFv molecule that showed highest apparent binding to Cry1C (H6) to produce the soluble scFv antibody fragments in the nonsuppressor *E. coli* HB2151 strain. The results would imply that the optimized soluble expression condition was culturing 16 h with 1 mmol $L^{-1}$ IPTG at 30 °C. Since the soluble scFv contain a His tag, so it could be purified using Ni ion affinity chromatography. After purification, SDS–PAGE analysis for H6 showed a band corresponding approximately to 29 kD molecular weight (Fig. 3). Soluble scFv-H6 fragments protein expression level of supernatant

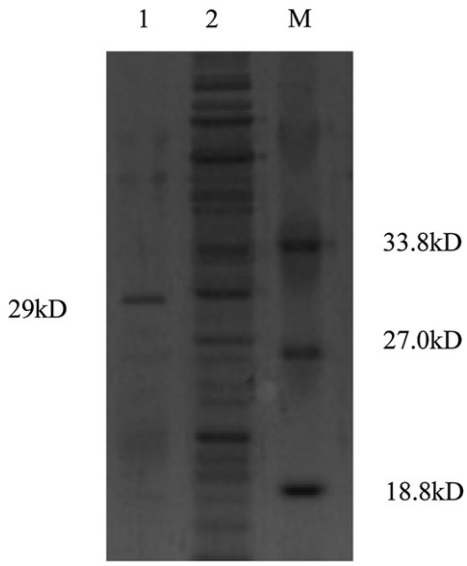


**Fig. 3.** SDS–PAGE analysis soluble expression and purified scFv. SDS–PAGE analysis of anti-Cry1C scFv after affinity purification from supernatant and periplasmic extract. Lane 1: Washing with elution buffer (including 500 mM imidazole), lane 2: Crude extract, M: A protein mass marker served as a standard.

and periplasm together was 0.8 mg $L^{-1}$ of culture. Some reports showed that the yields from *E. coli* culture for clones isolated from the Tomlinson libraries were in the range of 0.1–5.0 mg $L^{-1}$ (Kennel et al., 2004; Wu et al., 2007; Lobova et al., 2008).

### 3.6. Indirect competitive ELISA of the soluble scFv-H6

Indirect competitive ELISA can calculate the working range and specificity of scFv-H6. The concentration of antibody binding to Cry1C causing 50% inhibition ($IC_{50}$) was 0.39 μg $mL^{-1}$. The working calibration range, conventionally defined as the standard toxin concentration, of between $IC_{80}$ and $IC_{20}$ was 0.023–4.35 μg $mL^{-1}$ as shown Fig. 4. Cross-reactivity of scFv-H6 to the other Cry1 toxins was examined, results indicated that the Cry1Ac and Cry1B was 3.51% and 7.28%, respectively, as shown in Table 2.

### 3.7. Measurement of Cry1C in spiked rice samples

Rice powder samples were spiked with Cry1C at different concentrations and assayed by ic-ELISA. The average recoveries ranged from 89.8% to 97.2%, with a C.V. less than 5.0% as shown in Table 3, which demonstrated a good accuracy for the quantitative detection of Cry1C toxin in agricultural samples. Samples matrix effects were eliminated by sample dilution 10-fold with buffer (data not shown).

## 4. Discussion

Antibodies were widely utilized for rapid detection of food borne pathogens, adulterants, toxins and pesticide residues in food and environmental sample analysis or monitoring (Ayyar et al., 2012). The approach of convenient and stable production antibodies against interest antigen has become much more important. Significantly, phage displayed technique has met our goals rapidly without immunization (Golchin and Aitken, 2008). There are a few examples of production anti-Bt toxin scFv (Fernández et al., 2008; Gómez et al., 2006), which laid a foundation for our research. In this report, we describe the selection and functional analysis of Cry1C toxin specifically binding scFv antibodies, which may be used as the monitor of GM crops and environment residual. False positive phage clones posed a big disadvantage in phage display technique. To reduce them, we modified the protocol. The antibody library was pre-adsorbed analog toxin to reduce nonspecific binding. We also reduced the coated antigen concentration and increased washing pressure at each round. The monoclonal ELISA results confirmed that our improved panning protocol was successful in reducing false positive clones. After four rounds of selection, three representative positive clones, namely A2, C11 and H6 were obtained, but A2 and C11 had identical sequence but with low expression level, so H6 was chosen for further detection.

The scFv-H6 can be expressed and produced in *E. coli* with a considerably lower cost and shorter process than that used to generate traditional antibodies against Bt toxins (Walschus et al., 2002). These properties make H6 suitable for the development of simple and economic detection method. Soluble scFv fragment protein was purified by Ni metal ion affinity chromatography with a yield of about 0.8 mg $L^{-1}$ of culture and the molecular weight was approximately 29 kD. Following successful soluble expression and purification, we proceeded to further test the scFv in ic-ELISA and cross-reactivity tests. The results showed that this scFv was able to specifically detect Cry1C, within a working range of 0.023–4.35 μg $mL^{-1}$ and had ignorable cross-reactivity with other Cry toxin. In addition, the good range of recoveries was obtained in rice samples (Table 3). These properties make scFv-H6 suitable for the development of simple and economic detection for food samples. As to Cry1C, this study took some advantages from previous works. On one hand, it is the first time to use phage display technique to pan scFv while avoiding animal immunization. Moreover,

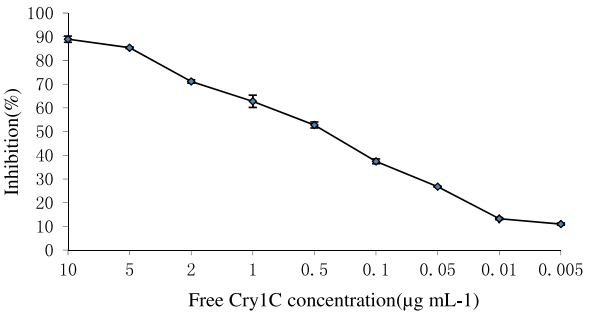


**Fig. 4.** scFv-H6-based inhibition curve by ic-ELISA. Descending dose-dependent inhibition curves of scFv(H6) by increasing Cry1C concentrations (0.005 and 10 μg $mL^{-1}$) were obtained. The linear range of detection was between 0.19 and 1.1 μg $mL^{-1}$ and the calculated 50% inhibition of control ($IC_{50}$) valued 0.39 μg $mL^{-1}$. The data represent mean ± standard deviation from triplicate measurements.

**Table 2**
Cross-reaction of soluble and purified scFv-H6 in ELISA with selected Cry1 toxins.

| Toxins | scFv-H6 | |
|---|---|---|
| | $IC_{50}$ (μg $mL^{-1}$) | CR (%) |
| Cry1C | 0.39 | 100 |
| Cry1B | 5.36 | 7.28 |
| Cry1Ac | 11.1 | 3.51 |

The cross-reactivity potential was approximated at the $IC_{50}$ value, which was estimated at 50% inhibition.

**Table 3**
Recoveries of Cry1C toxin from spiked samples by ic-ELISA.

| Sample | Spiked Cry1C (mg $kg^{-1}$) | Mean recovery ± SD (%)[a] | C.V. (%) |
|---|---|---|---|
| Rice powder | 0.2 | 89.8 ± 3.8 | 4.6 |
| | 0.5 | 90.6 ± 2.9 | 3.5 |
| | 1.0 | 97.2 ± 1.7 | 3.6 |

[a] Data from ELISA are the means of triplicate measurements.

the target-selected phage can be stored and used on demand to generate stable antibodies batch by batch. On the other hand, it offers a tool to study Cry1C receptor for reducing insect resistance (Fernández et al., 2008; Rodríguez-Cabrera et al., 2008).

Although phage display screening is frequently utilized to identify high-affinity antibodies (Hoen et al., 2012), it appears that scFvs often possess lower affinity than corresponding monoclonal antibodies (Walschus et al., 2002). In our previous studies, the anti-Cry1B scFv, which isolated from the Tomlinson J libraries, showed $10^7$ $M^{-1}$ binding activity (Zhang et al., 2012). The reason may be that the library we used is non-immunized human library and the scFv is less stable than normal antibody with bivalent nature (Wörn and Plückthun, 2001). Using the online bioinformatics to predict the structure of scFv, it can help to forecast the site of affinity maturation (Sheedy et al., 2007).

Monoclonal scFv antibodies are necessary reagents to develop immunoassays based on antibody protein, as well as immunobiosensor technologies and immunoaffinity chromatography purification column (Sheedy et al., 2007). According to the phage display technique, the physical link between the scFv gene and the expressed protein, the coding regions for scFv can be sequenced easily, which facilitating studies on binding regions structure, antigen–antibody interactions (Fernández et al., 2008) and antibody affinity maturation (Meng et al., 1995). We have generated a fast and efficient protocol to select and produce anti-Cry1C scFv from phage display libraries, which will be helpful to a wide range of researches as well as practical applications.

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## Conflict of interest statement

The authors declare that they have no conflict of interest.

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