CONSTRUCTION AND CHARACTERIZATION OF SINGLE CHAIN VARIABLE FRAGMENT-ALKALINE PHOSPHATASE FOR RAPID DETECTION OF AFLATOXIN B_1 IN AN ELISA-BASED ASSAY

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ABSTRACT

Aflatoxins are metabolites produced by *Aspergillus* spp. and can be found as contaminants in various food and agricultural products. A specific antibody is needed for the development of serological method, such as Enzyme-linked immunosorbent assay (ELISA), as a screening process to determine toxin contamination. In this study, nine clones of specific single chain variable fragments (scFv) were selected from naïve mouse phage display scFv library and their reactivity with aflatoxins were determined. The experiments were conducted in the Serology and Diagnostic Laboratory, Center of Agricultural Biotechnology, Kasetsart University from 2012-2013. The scFv gene from the recombinant phagemid clone 22A12 (scFv-22A12 gene), which gave the strongest reaction, was selected for further investigation on aflatoxin analysis. The recombinant protein product was 30 kDa. Concurrently, an alkaline phosphatase (AP) gene was amplified from *Escherichia coli* strain HB2151. The scFv-22A12 and the AP genes were ligated into pCANTAB-5E phagemid and transformed into *E. coli* TG1 and HB2151 to produce phage scFv-AP and soluble scFv-AP, respectively. Comparison on the efficiency of Phage scFv, Phage scFv-AP and soluble scFv-AP to whole molecule antibody for detecting AFB_1 was performed by ELISA. The resulst showed that the soluble scFv-AP gave highest reactivity and in accordance with those obtained from the whole molecule antibody. Cross reactivity with other aflatoxins (B2, G1 and G2) was reported to be 38.63%, 21.24 % and 9.64%, respectively. When using soluble scFv-AP to analyze ground samples of corn and groundnut spiked with 100 µg/kg of AFB1, acceptable results were obtained with 87.02 and 94.41% recovery, respectively. Analysis of the certified reference material (TMAF No.2 and TMAF No.3) showed comparable results with those analyzed through high performance liquid chromatography.

Keywords: mycotoxin, phage display, recombinant antibody, scFv, serological method

INTRODUCTION

Aflatoxins, a group of mycotoxins, are produced mainly by certain strains of *Aspergillus flavus* and *A. parasiticus*. They are found contaminated in a wide range of tropical and subtropical agricultural products, with commonly contaminated food commodities including cereals, oilseeds, spices, and tree

nuts. It is difficult to eliminate these toxins because of their heat stability, therefore heating or cooking cannot be relied on to destroy these toxins. Based on their fluorescent properties under ultraviolet light and chromatographic mobility, aflatoxins are divided into six major toxins, including aflatoxin B1 (AFB_1), B_2, G_1, G_2, M_1 and M_2. However, AFB_1 is the most toxic and the most prevalent and is categorized as Group 1 by the IARC (International Agency for Research on Cancer) (IARC, 1993; Rodrigues and Schuh, 2013). Concentrations of these toxins were limited in each country, with varying concentrations in each commodity. Since fungi can grow on a wide range of commodities and because of their stability in food, control is best achieved through methods designed to prevent the contamination of crops in the field and during storage, or early detection and removal of contaminated products from the food supply chain.

Analytical methods have been developed based on Thin layer chromatography (TLC), High performance liquid chromatography (HPLC) and Enzyme–linked immunosorbent assay (ELISA), as well as available rapid screening kits. Among these, serological method, especially ELISA, is preferred over other analytical methods because of their simplicity and cost-effectiveness. Production of high quality antibodies is required for the assay development of aflatoxin estimation. High-throughput phage display technology is currently an attractive and effective method of choice to obtain single chain variable fragment (scFv) antibody, wherein affinity maturation can be modified (Hoogenboom *et al*., 1991; Smith, 1985) and the molecule can be further engineered. In addition, several serological techniques require enzyme as a label to detect measurable signal, for example alkaline phosphatase (AP), which is one of the widely used enzymes. It has a wide range of applications in diagnostics, immunology and molecular biology, serving as a biochemical marker in the quantitative measurement of analytes. In this article, the scFv specific to AFB_1 was selected from the phage display naïve mouse scFv library (Koohapitagtam *et al*., 2010) and characterized, then the scFv gene was fused with alkaline phosphatase gene. Preliminary characterization of this fusion proteins was carried out for further application in aflatoxin determination.

MATERIALS AND METHODS

Bacterial culture and reagents

Aflatoxin B_1 conjugated with bovine serum albumin (AFB_1-BSA) and soluble aflatoxins B_1, B_2, G_1 and G_2 were purchased from Sigma (USA). DNA was extracted by using a QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany). *Escherichia coli* strains TG1 and HB2151 (Amersham Biosciences, Uppsala, Sweden) were used in phagemid manipulation and expression. PCR products of the scFv gene (light chain variable gene-heavy chain variable gene, V_L-V_H gene) were cloned into pCANTAB-5E phagemid (Amersham Biosciences, Uppsala, Sweden). The SOBAG medium (17 g tryptone, 10 g yeast extract, 5 g NaCl and 15 g bacto-agar in 1 L) plates supplemented with 100 µg/mL ampicillin and 2% (w/v) glucose were used for the selection of transformants. Anti-M13 polyclonal antibody (PAb) for phage library selection was previously produced in our laboratory (Koohapitagtam, 2010). Goat anti-rabbit antibody conjugated with alkaline phosphatase (GAR-AP) for anti-M13 detection was purchased from Sigma (USA). The detection reagents including *p*-nitrophenyl phosphate (*p*NPP), nitro blue tetrazolium (NBT), and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) for alkaline phosphatase assay were all purchased from Zymed (California, USA). Other reagents used in this study were at least of analytical grade.

Phage display scFv library (Phage library)

The naïve mouse scFv library in this experiment was previously constructed by Koohapitagtam (2010), which consists of fd phage carrying a second copy of phage gene VIII. The N-terminus of this gene contained a randomized sequence of cysteine codons, with a four or six codon deletion. The cysteine codons were designed to constrain by cross-linking the conformations which the peptides might adopt.

Affinity selection of phage display scFv antibody (phage scFv) from phage library by biopanning

Three rounds of biopanning were carried out on the library to select AFB_1-specific phage scFv, using AFB_1-BSA conjugate, by the method modified from Amersham Biosciences (Amersham, 1996). Four millilitre of AFB_1-BSA conjugate (10 µg/mL) in phosphate buffered saline (PBS, 137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.4) was coated in each immunotube. After overnight incubation (16–18 h) at 4ºC, the tubes were washed three times with PBS and blocked with PBS supplemented with 3% (w/v) skimmed milk (3% MPBS) for 1 h at room temperature. After removal of the blocking solution, the tubes were washed three times with PBS.

The library was pre-incubated with 2% (w/v) MPBS supplemented with 2% (w/v) BSA and 0.1% Triton-X100 for 1 h at room temperature, then added into the pre-coated immunotubes and continually incubated at room temperature for 2 h. Unbound phage was washed away 20 times with PBS and 3 times with PBS supplemented with 0.05% (v/v) Tween 20 (PBST). The eluted phage was recovered by infecting 1 mL of exponential growing *E. coli* TG1 and incubated at 37 ºC for 30 min without shaking. Infected cells were subjected to 10-fold-serial dilutions and spread onto SOBAG plates supplemented with 100 µg /mL ampicillin and 2% (w/v) glucose. The agar plates (inverted) were incubated overnight at 37 ºC.

Screening of AFB_1-specific phage scFv by ELISA

After the biopanning process, individual recombinant TG1 colonies were picked and grown at 37°C in a 96-well tissue culture plate (Corning, USA) for 1 h and rescued by M13KO7 helper phage to produce phage scFv for further screening. AFB_1-BSA at 5 µg/mL concentration was coated into a microtiter plate (Corning, USA) and incubated at 37°C for 1 h, while a negative control plate was coated with free BSA. Subsequently, the plates were washed three times with 200 µL of PBST and 150 µL of 3% MPBS was added to block non-specific binding. Fifty microlitre of each amplified phage was added to the plates and incubated for 2 h at 37°C. Plates were washed three times with PBST and indirect detection was carried out by adding 50 µL of rabbit anti-M13 antibody, followed by incubation with goat anti-mouse immunoglobulin G (IgG) conjugated with alkaline phosphatase (GAM-AP) at 1:30,000. In each step, the plate was incubated for 1 h at 37°C. The absorbance at 405 nm was recorded by a MultiscanEX ELISA reader (Labsystems, Finland) at the end of 1 h incubation period with 100 µL of enzyme substrate *p*NPP.

Cloning of an alkaline phosphatase gene

PCR primers were designed and synthesized for amplification of the alkaline phosphatase (AP) gene from *E. coli* HB2151. AP forward and reverse primers were as follows; 5'-GCGGCCGCTCGGACACCAGAAATGCCTGTTCTG-3' and 5'-GCGGCCGCTTTCAGCCCCAGAGCGGCTTTCAT-3', respectively. Each primer was modified to include a *Not*I restriction site (underlined). To amplify the AP gene by PCR method, 20 µL of the PCR reaction contained 2 µL of the bacterial cell suspension, 10 pmole of AP forward and reverse primers, 1x Phusion^R HP buffer, 25 mM dNTPs and 0.2U Phusion^R Hot Start II DNA polymerase. The reaction mixture was preheated at 98ºC for 30 s and 30 cycles of the amplification were carried out as follows; 98ºC for 10 s, 55ºC for 10 s, 72ºC for 45 s and then a final extension at 72ºC for seven minutes. The AP gene was purified using QIAquick Gel extraction kit according to the manufacturer and inserted into pJET1.2/blunt cloning vector (Qiagen, Valencia, USA) using T4 ligase. The ligated DNA population was introduced into *E. coli* DH5α by heat shock at 42ºC for 2 min. Transformants were grown overnight by culturing in 2YT supplemented with 100 µM ampicillin at 37ºC and the positive clones by PCR method using AP-specific primers were selected.

The positive recombinant plasmid DNA was sequenced and phylogenetic analysis was done by the distance method using CLC Main Workbench (version 5.5). The distance matrix for the aligned

sequences was clustered using Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Reliability of the inferred tree was estimated by 1000 bootstrap resampling using the same program.

Construction of the recombinant phagemid carrying scFv-AP genes

Single chain variable fragment-alkaline phosphatase fusion proteins (scFv-AP) for direct serological assay was constructed according to the protocol described previously (Wozniak *et al*., 2003). Briefly, 1 µg of DNA from the recombinant phagemid clone 22A12 carrying scFv gene and pJET1.2/blunt cloning vector containing AP gene were digested by *Not*I at 37ºC for 3 h. DNA fragments were purified from 1.5% agarose gel using QIAquick Gel extraction kit. The alkaline phosphatase gene was inserted into the scFv recombinant phagemid (ratio 3:1) using T4 ligase. The ligated DNA population was then introduced into *E. coli* TG1 and HB2151 by heat shock at 42ºC for 2 min (Sambrook and Russell, 2001).

Production of phage scFv-AP and soluble scFv-AP fusion proteins

Phage scFv-AP was produced by rescuing the recombinant phage using M13KO7 helper phage to infect the recombinant TG1 strain, and determined for their AFB_1 specificity by phage ELISA. To produce soluble anti-AFB_1 scFv-AP fusion proteins, the recombinant log-phase HB2151 culture was induced by isopropyl β-D-thiogalactopyranoside (IPTG) (Amersham, 1996). A further purification step of the soluble scFv-AP was carried out by gel filtration chromatography and the catalytic activity of each fraction was determined by dot immunobinding assay (DIBA) (Wozniak *et al*., 2003).

Characterization of the soluble scFv-AP by native polyacrylamide gel electrophoresis (native-PAGE) and Western Blotting

The integrity of the scFv-AP fusion proteins was examined by native-PAGE and Western blotting (Wozniak *et al*., 2003). Briefly, 10 µL of each soluble scFv-AP protein was mixed with 2x loading buffer and loaded into 10% native-PAGE (without SDS). The electrophoresis was run under the following conditions; 70 V for 30 min and then 120 V for 90 min (Laemmli, 1970). The protein bands were then transferred onto nitrocellulose membrane by electrophoresis at 40 V for 120 min. After washing the membrane in TBST, the scFv-AP band was allowed to react with AP substrates, NBT and BCIP, for a chromogenic reaction within 10 min.

Comparison of three scFv formats to whole molecule MAb on the detection of AFB_1 by plate-trapped antigen ELISA (PTA-ELISA)

Five µg/mL of AFB_1-BSA or 1% (w/v) BSA (control treatment) in 100 µL PBS were immobilized in ELISA wells and incubated for 1 h at 37ºC. The plates were washed three times with PBST and blocked with 3% MPBS for 1 h at room temperature. One hundred microlitres of each antibody format, including phage scFv-AP, soluble scFv-AP, phage scFv and whole molecule antibody [anti-AFB_1 monoclonal antibody (MAb) clone C10 (Kladpan *et al*., 2009)] at 10 µg/mL, was added into the ELISA wells with 1 h incubation. For AP-conjugated scFv formats, direct ELISA was performed and the AP substrate was added after the incubation period of the primary antibody, while the indirect ELISA method was applied when non-AP-conjugated scFv and MAb were in use according to the procedure described above. Absorbance values at 405 nm were measured after 1 h incubation at 37ºC with the substrate in both formats.

Determination on the Cross reactivity of the scFv-AP antibody by direct competitive ELISA (dcELISA)

Cross reactivity of the scFv-AP antibody was assayed against three aflatoxins, including B_2, G_1 and G_2. Stock solution of each aflatoxin was prepared in methanol and diluted in PBS-10% (v/v) methanol (PBS-met) with two-folded dilutions from 16 to 2 ng/mL. Wells in microtitre plates were coated with 50 µL of AFB_1-BSA conjugate dissolved in a carbonate coating buffer, pH 9.6 (CB). The plates were incubated for 1 h at 37°C, emptied and washed three times with PBST. Blocking solution (3% MPBS) was added at 200 µL per well and incubated for 1 h at 37°C, and washed three times with

PBST. A mixture of scFv-AP and the prepared standard aflatoxin (1:1 ratio) at varied concentrations was added to each pre-coated well and incubated for 1.30 h with 75 rpm shaking. Unbound antibody was removed by washing three times with PBST. Following this step, the *p*NPP substrate solution was added and incubated at room temperature for 60 min. Absorbance at 405 nm was read and degree of competition was calculated using the following a formula: % AFT binding = ($Absorption_{AFT}$/ $Absorption_{PBS}$) x 100. Relative cross-reactivity was determined as % cross-reactivity = [50% inhibitory concentration (AFB_1) / 50% inhibitory concentration (competitor)] x 100 (AOAC, 2002).

Sample preparation for aflatoxin determination

Groundnut and corn ground matrices were obtained in 50 g quantities. After a thorough mixing, sub-samples were drawn (20 g for each sample) and spiked with AFB_1 at 100 ppb, then the samples were incubated overnight at 4ºC. Each sub-sample was then extracted in 100 mL of 70% methanol while shaking at 250 rpm for 30 min. The extract was filtrated through Whatman no.1 filter paper and diluted 1:5 with PBS-met before analysis by ELISA (Technical Committee CEN/TC 275, 1999).

Determination of AFB_1 contamination in spiked groundnut, corn samples and certified reference materials (CRM) by competitive ELISA

The assay was performed by competitive ELISA protocol. Fifty microlitres of AFB_1-BSA conjugate (5 µg/mL) dissolved in carbonate coating buffer, pH 9.6 was pre-incubated in the ELISA wells for 1 h at 37ºC. The samples of spiked groundnut, corn samples and CRM (Total material aflatoxins; TMAF No.2 and TMAF No.3) were extracted as described above. For scFv-AP dcELISA, the extract was mixed with the scFv-AP at 1:1 ratio and transferred into the conjugate pre-coated wells. The plates were incubated for 1 h at 37°C, emptied and washed three times with PBST. Bound scFv-AP was detected by adding the substrate. In case of the MAb, indirect competitive ELISA (icELISA) was carried out by mixing the antibody with the extract at the same ratio, incubating for 1 h, then the GAM-AP was added and incubated. Reactivity was visualized by adding the enzyme substrate. Standard curve was obtained by plotting log_{10} values of AFB_1 standard at various concentrations ranging from 2 to 16 ng/mL, against the absorbance at 405 nanometer.

RESULTS AND DISCUSSION

Affinity selection of phage scFv from phage library by biopanning

The phage clones specific to AFB_1-BSA were first selected by biopanning method and screened by ELISA for three rounds. One hundred and fourteen clones were specific to AFB_1-BSA; however, only 9 clones (13C7, 15E4, 16C5, 17F4, 17F5, 18C3, 20C3, 21A1, 22A12) were positive with AFB_1 ($O.D._{405}$ ranging from 0.426 – 0.873) without cross reaction with BSA. The scFv gene from the recombinant phagemid clone 22A12 giving the strongest reaction (0.873) was chosen for the following experiments. During the biopanning step, we noticed that the selected phage scFv contained high ratio of anti-BSA phage antibody, therefore an attempt had been made to eliminate this background by incubating the phage scFv with 2% MPBS + 2%BSA + 0.1% Triton X-100 for 1 h prior to the addition into immunotubes. The satisfactory result was achieved.

Cloning of an alkaline phosphatase gene

The AP gene from *E. coli* HB2151 was successfully amplified and the result showed a band size of approximately 1,350 bp (Fig. 1). AP nucleotide phylogenetic tree sequence (KF387511) showed the similarity of our cloned AP gene with those reported in GenBank including 99% similarity with M29664, M29665, X04586, M13345, FJ546461, EU905389 and EU905386; 98% similarity with M29669, M29670; and 97% similarity with M29668 (Fig. 2A) supported by 100% bootstrap values. On the contrary, the AP gene was dissimilar to those belonging to *Aedes aegypti* (XM_001663484 and XM_001663428) by 100% bootstrap (Fig. 2B). In place of using the commercial vector which contained alkaline phosphatase gene (Kuntalee *et al*., 2011; Pershad *et al*., 2011; Wang *et al*., 2008), we

successfully cloned it from *E. coli* HB2151 and still maintained its catalytic activity as compared to the commercial one.

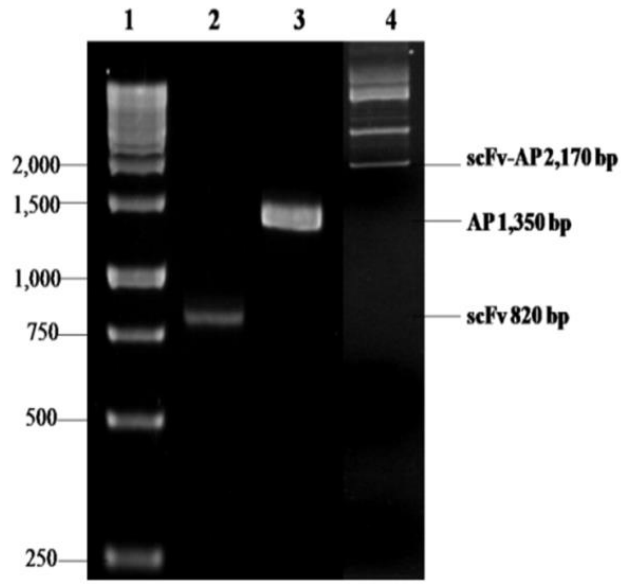

Fig. 1. Gel electrophoretic analysis of PCR fragments of scFv gene (lane 2), AP gene (lane 3), digested phagemid clone 22A12 with *Hind*III and *Not*I (lane 4) and molecular weight markers (lane 1).

Production and Characterization of the soluble scFv-AP by native-PAGE and Western Blotting

The insert carrying scFv (clone 22A12) and AP genes produced a DNA band of 2,170 bp by *Hind*III and *Not*I restriction digestion (Fig. 1). In this research, the fusion proteins were produced based on V_L-V_H construct; however, there is no consistent rule on the effect of direction, but rather it depends on the genes (Hu *et al*., 2005). The expressed recombinant fusion proteins provided a protein band of 72 kDa (Fig. 3). The purified protein from gel filtration chromatography (24 fractions) showed a catalytic activity of alkaline phosphatase in the fractions no. 4-18 by DIBA compared to phage scFv-AP and lysed cell extract of HB2151 strain.

Comparison of three scFv formats to whole molecule MAb on the detection of AFB_1 by plate-trapped antigen ELISA (PTA-ELISA)

After the successful generation of phage scFv from the recombinant phagemid clone 22A12 in *E.coli* TG1, the catalytic activity of the AP gene was determined by ELISA. Then, scFv-AP fusion proteins were produced by ligating the scFv and AP genes and subsequently transformed into *E. coli* TG1, as well as HB2151 strains. This led to the production of phage scFv-AP and soluble scFv-AP, respectively. Comparison on the efficiency of phage scFv, phage scFv-AP and soluble scFv-AP to the MAb in detection of AFB_1 were carried out by PTA-ELISA. Results showed that the soluble scFv-AP gave the highest $O.D._{405}$, which was comparable to the result obtained from the MAb. However, since the scFv-AP reaction was carried out by the direct method, therefore the consumed working period was reduced by one hour (Fig. 4). Thus, the scFv-AP could be useful and convenient as one-step detection probe for competitive ELISA. Since the scFv-AP was produced by gene fusion, its protein product was very stable and will be benefit for further use in other serological applications.

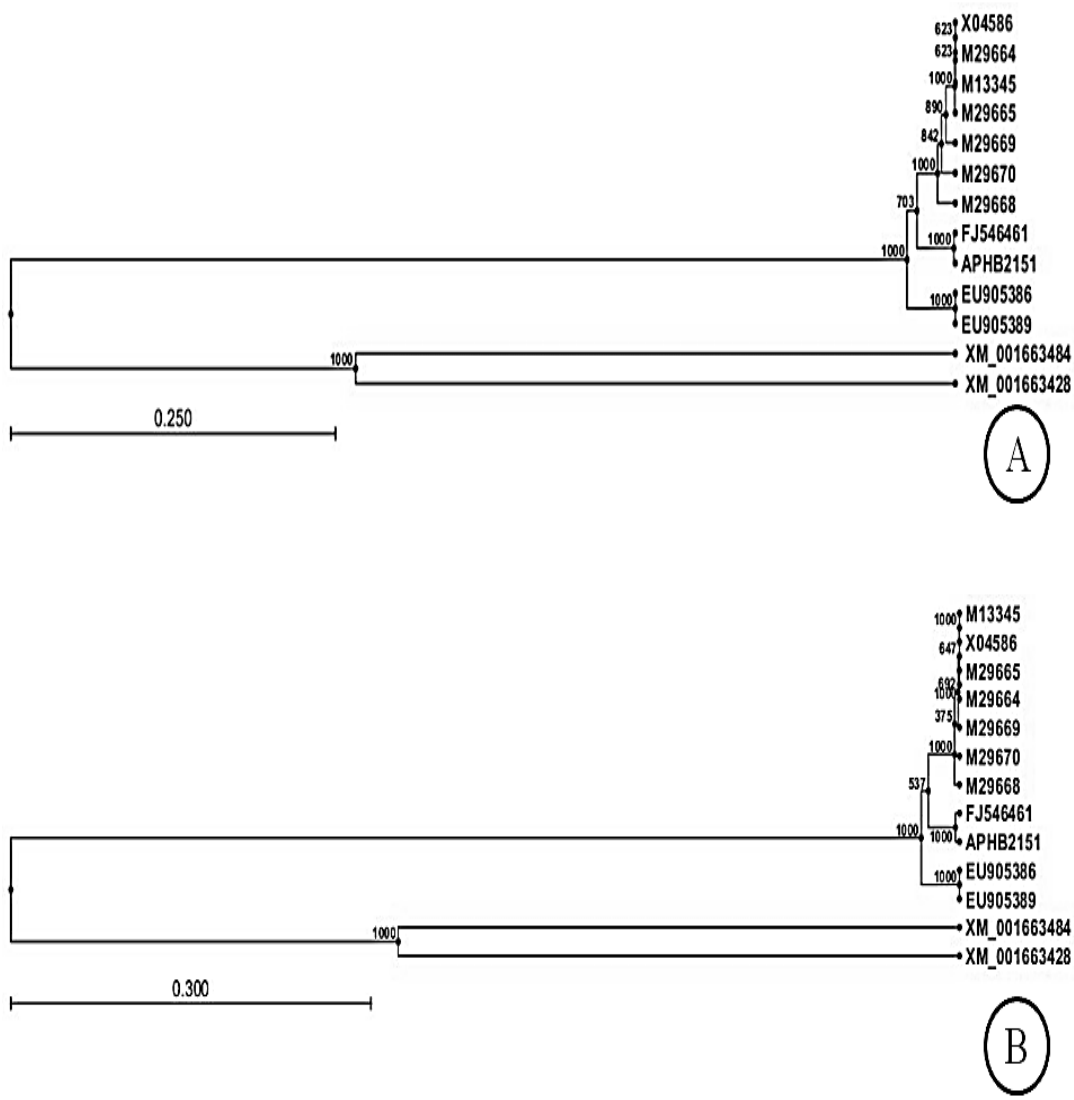

Fig. 2. Phylogenetic trees of alkaline phosphatase gene; nucleotide sequence analysis (A) and amino acid sequence analysis (B), compared with the AP genes from the GenBank databases, using UPGMA method at 1000 bootstrap replicates.

Determination of cross reactivity of the scFv-AP by dcELISA

Cross reactivity of the scFv-AP was determined by dcELISA and the results showed a degree of cross reaction with AFB_2, AFG_1 and AFG_2 at 38.6%, 21.2% and 9.6%, respectively. The competitive ELISA also showed promising outcome for using this scFv-AP raised from the naïve mouse library to detect AFB_1. In the previous report (Kuntalee *et al*., 2011), a unique human phage display library was used to generate scFvs to AFB_1 and the cross-reactivity test revealed that the scFv from the recombinant phages clones TomI-F6 cross-reacted with aflatoxins G1> B2 > G2 > M1 (68.75%, 16.92%, 13.75% and 12.94%, respectively), while YM1-C3 scFv could cross react with afaltoxins G1> G2 > B2 > M1 (70%, 29.17%, 26.92% and 0.88%, respectively). In our research, the advantage of cloning the scFv with the AP genes producing phage scFv-AP led to less time consumption in dcELISA, since the substrate could be added right after the addition of the phage scFv-AP.

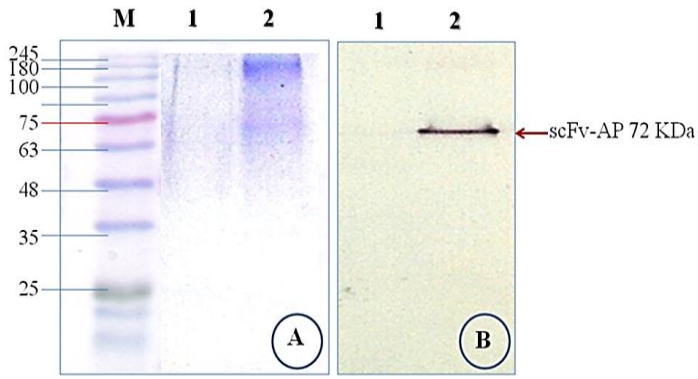

Fig. 3 Analysis of the expressed scFv-AP by native polyacrylamide gel electrophoresis (A) and Western blotting (B). Fraction no.6 from gel filtration chromatography (lane 2) showed strong reaction with alkaline phosphatase substrate compared to the lysed cell of *E.coli* HB2151 (lane 1) and molecular weight markers (M).

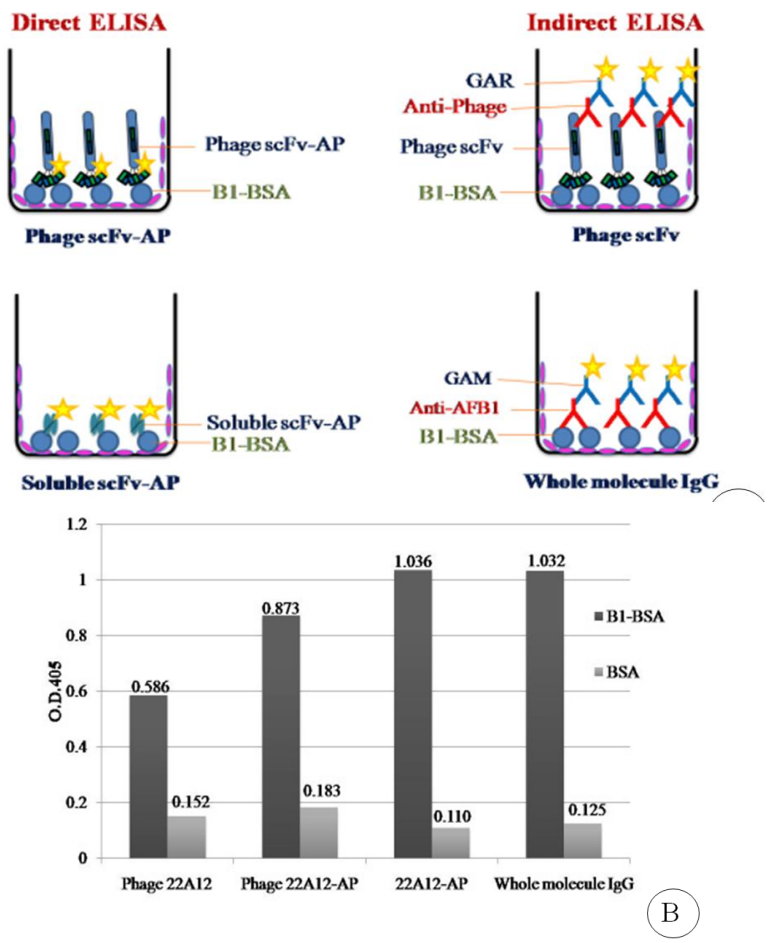

Fig. 4 Comparison on the efficiency of Phage scFv, Phage scFv-AP and soluble scFv-AP to whole molecule MAb for detecting AFB_1 by different ELISA formats (A). Result showed the absorbance values at 405 nm when reacting with AFB_1-BSA, compared to BSA negative control (B).

Determination of AFB_1 contamination in spiked groundnut, corn samples and certified reference material (CRM) by ELISA

When using soluble scFv-AP to analyze groundnut and corn spiked with 100 µg/kg of AFB_1, acceptable results were achieved with 87.02 and 94.41% recovery (Table 1), respectively. Analysis of the CRM (TMAF No.2 and TMAF No.3) showed comparable results with those analyzed by high performance liquid chromatography (Table 2).

Table 1 Analysis of groundnut and corn spiked with 100 µg/kg of AFB_1 by soluble scFv-AP antibody compared to whole molecule monoclonal antibody (MAb).

Sample	Analysis by scFv-AP		Analysis by MAb	
	AFB_1 (µg/kg)*	% Recovery	AFB_1 (µg/kg)	% Recovery
Blank corn	4.44±0.43	-	5.92±0.39	-
Corn spiked with AFB_1 100 ppb	91.46±0.44	87.02±0.07	91.19±0.17	85.27±0.22
Blank groundnut	16.44±0.43	-	18.18±0.55	-
Groundnut spiked with AFB_1 100 ppb	110.85±0.87	94.41±0.45	117.86±0.52	99.68±0.03

Note: * All samples were performed in triplicates and extracted as detailed under materials and methods. ± Standard error of each mean.

Table 2 Analysis of the certified reference materials TMAF No.2 and TMAF No.3 by competitive ELISA with HPLC.

Certified reference material	Total AFT (ppb)		
	scFv-AP/ dcELISA*	MAb/ Indirect C-ELISA*	HPLC**
TMAF No.2	58.25±0.91[a]	58.13±0.57[a]	57.11±1.18[a]
TMAF No.3	137.55±3.51[b]	133.36±0.63[b]	130.22±5.50[b]

Note: * All samples were extracted as detailed under materials and methods and performed in triplicates. ** Determined by Scientific Equipment Center, Kasetsart University Research and Development Institute. ± Standard error of each mean. Values in a column with different letter are significantly different at (P<0.05).

CONCLUSIONS

In conclusion, phage display technology has many advantages over the animal based antibody technology because it is much faster, more robust and consequent advantages are a large volume of antibody, and affinity maturation can be achieved. In our case, the scFv-AP fusion protein was successfully produced from naïve mouse scFv library and its specificity to AFB_1 is acceptable for further application in ELISA-based detection of the toxin contamination, which was focused on agricultural commodity, including corn and groundnut. Comparison of phage scFv, phage scFv-AP and scFv-AP with the whole molecule MAb showed that the efficiency of the scFv-AP was as good as MAb in binding to free AFB_1 in dcELISA. The soluble scFv-AP also gave high satisfaction for the detection of AFB_1 in spiked corn and groundnut as compared with the MAb. The results demonstrated that the soluble scFv-AP produced from this research is useful for developing rapid detection assays. Further study is required for large-scale production, and purification of the anti-AFB_1 scFv, as well as practical

application of detecting mycotoxins in food systems by serological methods, such as ELISA and lateral flow assay.

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