

เอกสารอ้างอิง

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นอกจากแอนติบอดีต้นแบบที่ได้พัฒนาขึ้น วิธีการดำเนินการวิจัย และผลการทดลองที่ได้จากโครงการวิจัยนี้ ยังได้นำไปเผยแพร่ในรูปแบบผลงานตีพิมพ์ในวารสารวิชาการระดับนานาชาติ ที่ต้องผ่านการประเมินโดยผู้ทรงคุณวุฒิ คือ Molecular Biotechnology ซึ่งมีค่า impact factor 2.444 ดังนี้

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ส่วนรายละเอียดเกี่ยวกับคลัง และวิธีการสร้างคลังเฟจ YAMO-I สามารถดูได้จากรายงานการวิจัยฉบับสมบูรณ์เรื่อง “การพัฒนาเทคโนโลยีการแสดงโปรตีนบนผิวฟาจเพื่อการผลิตโมโนโคลนอล แอนติบอดี” รหัสโครงการ SUT3-304-47-24-11

One-Step Detection of Aflatoxin-B₁ Using scFv-Alkaline Phosphatase-Fusion Selected from Human Phage Display Antibody Library

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Abstract A unique human phage display library was used to successfully generate a scFv to the highly carcinogenic toxin aflatoxin B₁. Such an antibody has major potential applications in therapy and diagnostics. To further exploit its analytical capacity, the scFv was genetically fused to alkaline phosphatase, thereby generating a novel and highly sensitive self-indicating reagent. The performance of this reagent was further characterized, demonstrating its efficacy. The sensitivity of scFv-AP fusion was three-fold better than that of the scFv form. The ability of this human library to generate antibodies to a small hapten was clearly demonstrated and this is linked to its intrinsic diversity, which exceeds many existing conventional human libraries. Our results indicate that demography may influence the diversity of the repertoire of the library in

terms of its capacity to generate antibodies to specific targets. Equally, the approach demonstrated should also be applicable for other haptens and larger antigens.

Keywords Phage display · scFv · Antibody engineering · Alkaline phosphatase · ELISA · Aflatoxin · Human · Hapten · Competitive · Recombinant antibody

Abbreviations

AFB	Aflatoxin B
AFG	Aflatoxin G
AFM	Aflatoxin M
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
scFv	Single chain fragment variable
AP	Alkaline phosphatase
BAP	Bacterial alkaline phosphatase
IC ₅₀	Inhibitory concentration at 50%
ELISA	Enzyme-linked immunosorbent assay

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Introduction

Haptens are low molecular weight molecules that cannot elicit an immune response unless they are coupled to appropriate carriers [1]. Examples of common haptens include pesticides, herbicides, insecticides, drugs, vitamins, steroids, hormones, explosives, dyes, and toxins. Other haptens are reagents commonly used in biological research such as fluorescein, biotin, digoxigenin, and dinitrophenol [2]. Thus, obtaining antibodies that can interact specifically with these haptens will be very important and beneficial for various applications, such as immunoassays, immunobiosensors, immunotherapy, and affinity purification. The major difficulty related to generation of an antibody to haptens is to find the right conjugate, as the hapten is not intrinsically

immunogenic [3]. Moreover, many antibodies that can bind very well to conjugated haptens, cannot recognize the soluble free form of the haptens, and this is required for diagnostic tests and certain therapeutic applications [4].

Aflatoxin B₁ or AFB₁ is an extremely potent and carcinogenic mycotoxin that is produced by *Aspergillus flavus* and *Aspergillus parasiticus*. These two strains of *Aspergillus* also produce other aflatoxins such as AFB₂, AFG₁, and AFG₂. However, they are not as abundant and toxic as AFB₁ [5]. Aflatoxin M₁ is a metabolite of AFB₁, which can be found in milk of animals that consume aflatoxin-contaminated feeds [6]. The structures of various aflatoxins are illustrated in Fig. 1. Aflatoxins are often found to contaminate agricultural products in Thailand and other countries in tropical and subtropical regions, where the surroundings are warm and humid, favoring the growth of aflatoxin-producing *Aspergillus* both during and post harvesting period [7–10]. AFB₁ has been classified as Group 1 carcinogen by WHO-IARC (World Health Organization and International Agency for Research on Cancer) as it can lead to mutation in the gene that can result in the development of liver cancer [11]. Therefore, aflatoxin contamination is a serious problem and there is a very urgent need to develop a rapid, highly sensitive, reliable, and inexpensive assay to monitor agricultural products, to protect consumers and animals, and to meet regulatory requirements. There is also potential to use such antibodies in analyses of complex matrices where preliminary sample 'clean-up' and concentration is needed prior to analysis.

Many techniques have been developed to analyze aflatoxins such as thin-layer chromatography (TLC),

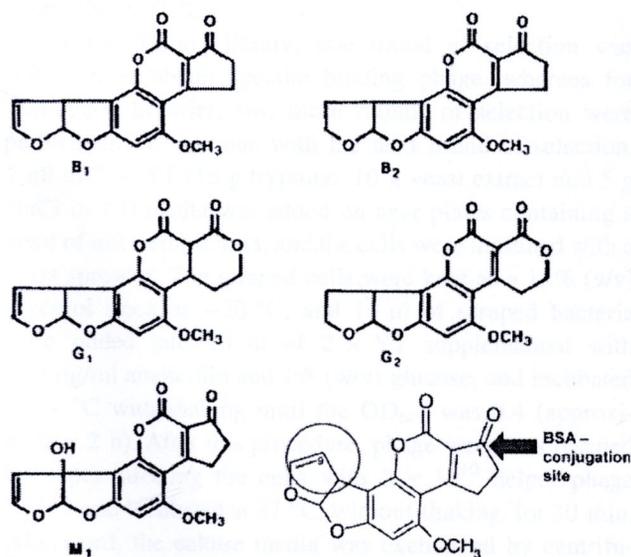


Fig. 1 Structures of different types of aflatoxins. C1 position used for conjugation of AFB₁ to BSA is indicated. The area around C9 where the scFv antibodies bind is highlighted with a circle. AFM₁ and AFM₂ are metabolic products of AFB₁ and AFB₂, respectively

high-performance liquid chromatography (HPLC), near infrared spectroscopy (NIR), and immunoassay [12]. Among these, immunological methods that use polyclonal or monoclonal antibody are appropriate and necessary for rapid, high-throughput, highly sensitive, and specific analysis.

This research reports the engineering and characterization of human single chain variable fragment (scFv) antibodies against AFB₁ from two human phage display antibody libraries. The first library is Yamo1, a compact human non-immunized phage display library generated from 140 healthy individuals in Northeastern Thailand [13], and the second library is Tomlinson I&J, a semi-synthetic library that is based on one framework but has different amino acids on 18 positions that are diverse in the primary repertoire. Binding properties of antibody selected from these two libraries were studied. In addition, selected scFv antibodies were further engineered to create scFv-alkaline phosphatase fusions (scFv-AP) and used as convenient one-step detection probes for competitive ELISA. The sensitivity and specificity of scFv and scFv-AP to different aflatoxins were determined.

Materials and Methods

Materials

Yamo 1, a human non-immunized scFv library, was constructed in our laboratory using B-lymphocytes from 140 healthy individuals in the Northeastern Thailand [13]. Tomlinson I&J, two similar semi-synthetic libraries, were kindly provided by the MRC, Cambridge. They are based on a single human framework and variation at 18 residues (H50, H52, H52a, H53, H55, H56, H58, H95, H96, H97, H98, L50, L53, L91, L92, L93, L94 and L96) that are responsible for antigen binding. KM13 helper phage was provided with the Tomlinson libraries and was propagated as described in the MRC phage display protocols. Aflatoxins B₁ conjugated with BSA (AFB₁-BSA), and soluble aflatoxins B₁, B₂, G₁, G₂, and M₁ were obtained from Sigma, Germany. *E. coli* TG1 and HB2151 were obtained from the MRC, Cambridge, UK, and used for cloning and amplification of phage, or production of soluble scFv fragments, respectively. The anti-M13/HRP detection kit was purchased from Amersham-Pharmacia Biotech (Uppsala, Sweden), and Protein L peroxidase HRP was from Sigma. *E. coli* TG1 was used for cloning and expression of scFv-AP fusions.

Biopanning

The selection was performed on Nunc Maxisorp immunotubes (Nunc, Denmark). Various concentrations of

aflatoxin B1-BSA conjugate, in 100 μ l phosphate buffered saline (PBS, 137 mM NaCl, 3 mM KCl, 8 mM Na₂HPO₄, 1.5 mM KH₂PO₄, pH 7.4) were used. The amounts of AFB₁-BSA conjugate used for the first, second, and third rounds of selection were 50 μ g, 15 μ g, and 5 μ g per tube (4 ml), respectively. After overnight incubation (16–18 h) at 4 °C, the immune tubes were washed three times with PBS and blocked with PBS supplemented with 4% (w/v) skimmed milk (4%, w/v, MPBS) for 2 h at room temperature. After removal of the blocking solution, the tubes were washed three times with PBS. The libraries were pre-incubated with 4% (w/v) MPBS and 1% (w/v) BSA for 30 min at room temperature. The libraries were then added into the immuno-tubes and incubated at room temperature for 2 h. Unbound phage was washed away three times with PBS supplemented with 0.05% (v/v) Tween 20 (PBST) and three times with PBS. For the Tomlinson library, bound phage was eluted by adding 500 μ l of 1 μ g/ml trypsin buffer (in PBS) and incubated at room temperature for 20 min with rotation. For the Yamol library, an additional acid elution step was carried out after treatment with trypsin. Five hundred μ l of 50 mM glycine-HCl, pH 2.0 was added into the tube and immediately after incubation for 10 min, 500 μ l of neutralization buffer (200 mM NaHPO₄, pH 7.5) was added. The eluted phage was recovered by infecting 350 μ l of exponentially growing *E. coli* TG1 and by incubation at 37 °C for 30 min without shaking. Infected cells were subjected to 10-fold-serial dilution (10²–10⁶) and spread on TYE (10 g tryptone, 5 g yeast extract, 8 g NaCl and 15 g bacto-agar in 1 L) agar plates supplemented with 100 μ g/ml ampicillin and 1% (w/v) glucose. The agar plates (inverted) were incubated overnight at 37 °C.

For the Yamol library, one round of selection was sufficient to obtain specific binding phage, whereas for Tomlinson libraries, two more rounds of selection were performed. To continue with the next round of selection, 1 ml of 2 \times YT (16 g tryptone, 10 g yeast extract and 5 g NaCl in 1 l) media was added on agar plates containing a lawn of infected bacteria, and the cells were loosened with a glass spreader. The scraped cells were kept as a 15% (v/v) glycerol stock at –70 °C, and 10 μ l of scraped bacteria were added into 10 ml of 2 \times YT supplemented with 100 μ g/ml ampicillin and 1% (w/v) glucose, and incubated at 37 °C with shaking until the OD₆₀₀ was 0.4 (approximately 2 h). After this procedure, phage were rescued by super-infecting the cells with 5 \times 10¹⁰ helper phage KM13 and incubated at 37 °C, without shaking, for 30 min. Afterward, the culture media was exchanged by centrifugation at 4,000 rpm at 4 °C for 15 min, the supernatant was removed, and the pelleted bacteria were resuspended in 5 ml of 2 \times YT containing 100 μ g/ml ampicillin, 50 μ g/ml kanamycin, and 0.1% (w/v) glucose. Later, it was incubated

at 30 °C with shaking overnight. On the following day, the overnight culture was centrifuged at 4,000 rpm and 4 °C for 15 min. Phage was precipitated by adding 1 ml of PEG/NaCl (20% (v/v) polyethylene glycol 6000 in 2.5 M NaCl) into 4 ml of the supernatant and kept on ice for 1 h, and later centrifuged at 4,000 rpm, at 4 °C for 30 min. The supernatant was removed and the pellet was resuspended in 100 μ l PBS for the next round of selection.

Individual Phage Rescue

Individual phage-infected colonies were randomly picked from the TYE plate and grown in wells of a 96-well plate (Nunc, Denmark) containing 100 μ l 2 \times YT plus 100 μ g/ml ampicillin and 1% (w/v) glucose. After overnight incubation at 37 °C, small inocula (5 μ l) from each well were transferred to a second 96-well plate containing 200 μ l of 2 \times YT plus 100 μ g/ml ampicillin and 1% (w/v) glucose. The first plate was kept as master stock by adding glycerol to a final concentration of 20% (v/v) and kept at –20 °C. The second plate was incubated with shaking at 37 °C for 2 h, and later phage was rescued by adding 10¹⁰ helper phage to each well. Following this they were then incubated at 37 °C for 1 h before centrifugation of the plate at 4,000 rpm for 10 min. The supernatant was discarded and the pellet was resuspended in 200 μ l of 2 \times YT containing 100 μ g/ml ampicillin and 50 μ g/ml kanamycin, and cultured at 30 °C overnight (20 h) with shaking (250 rpm). The overnight culture was spun at 4,000 rpm for 10 min, and 50 μ l of the supernatant-containing phage was used in monoclonal phage ELISA.

Phage ELISA

The wells of 96 MicroWell™ plates (Nunc, Denmark) were coated with 5 μ g of aflatoxin B1-BSA in PBS buffer, or 1% (w/v) bovine serum albumin (BSA) in 100 μ l PBS (control). After incubation at 4 °C overnight, the plates were washed three times with PBS. The wells were then blocked with 4% (w/v) MPBS for 2 h at room temperature. The wells were re-washed three times with PBS. Fifty μ l of phage supernatant and 50 μ l of 4% (w/v) MPBS were added to each well and incubated at room temperature for 2 h. Unbound phage was removed by washing three times with PBST and 3 times with PBS. Subsequently, 100 μ l of HRP-labeled anti-M13 (1:5000 dilution in 2% (w/v) MPBS) was added into each well. After incubation for an additional 1 h at room temperature, the wells were washed again, as described previously, and 100 μ l of substrate solutions (TMB or ABTS, Sigma) were added into each well and incubated at 37 °C for 30 min. The resulting absorbance was read at 450 nm for TMB or 405 nm for ABTS.

Expression and Purification of scFv Antibody in Microtiter Plates

Escherichia coli non-suppressor strain HB2151, which reads the amber codon as a stop signal, thereby solubly expressing the antibody fragments, was infected with individual phage clones to express soluble scFvs. Five μl of individual phage particles from the master glycerol stock were mixed with 150 μl of exponentially growing *E. coli* HB2151 ($\text{OD}_{600} = 0.4$), and incubated at 37 °C for 30 min, without shaking. The solution. Infected bacteria were then streaked onto TYE agar plates containing 100 $\mu\text{g}/\text{ml}$ ampicillin and 1% (w/v) glucose. The plates were incubated at 37 °C overnight. On the next day, single colonies were picked and cultured in the wells of a 96-well plate containing 100 μl of 2 \times YT plus 100 $\mu\text{g}/\text{ml}$ ampicillin and 1% (w/v) glucose. The culture was incubated with shaking at 37 °C overnight. On the next day, 8 μl of culture was transferred to another 96 deep-well plate containing 800 μl of 2 \times YT plus 100 $\mu\text{g}/\text{ml}$ ampicillin and 0.1% (w/v) glucose, and incubated with shaking at 37 °C until the OD_{600} reached 0.9. Later, 100 μl of 2 \times YT containing 100 $\mu\text{g}/\text{ml}$ ampicillin and 9 mM isopropyl- β -D-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM IPTG. The incubation was continued at 30 °C with shaking overnight (18–20 h). The secreted antibody could be found in the supernatant. The first plate was kept as a master stock by adding glycerol to a final concentration of 15% (v/v) and was then stored at -70 °C.

Large-Scale Production of Soluble scFv

Production of soluble scFv antibodies was carried out as previously described [13]. In some cases, the crude scFv products were purified by immobilized metal affinity chromatography (IMAC) according to the manufacturer's protocol (Qiagen, Germany). The resins were washed twice with washing buffer 1 (20 mM Tris; pH 8.0, 150 mM NaCl, 5 mM imidazole) and washing buffer 2 containing 20 mM imidazole before scFv fragments were collected with elution buffer (20 mM Tris; pH 8.0, 150 mM NaCl, 250 mM imidazole).

ScFv Antibody ELISA

The procedure was similar to the phage ELISA method described previously, except that 50 μl of culture supernatant containing the scFv antibody and 50 μl 3% (w/v) BSA were added into each of the wells instead of individual phage clones. Bound scFv was detected by addition of 100 μl of peroxidase-labeled Protein L (1:5000 dilution in 3% (w/v) BSA) using TMB as substrate.

Cloning and Expression of scFv-AP Fusion

The scFv gene from the phagemid vector was cut with *Nco* I and *Xho* I restriction enzymes and subcloned into pKP300 Δ III, an alkaline phosphatase fusion vector (Kay, B.K. unpublished). The integrity of the constructs was confirmed by automated DNA sequencing. To prepare scFv-alkaline phosphatase (scFv-AP) fusions, a single colony of *E. coli* TG1 harboring recombinant plasmid was inoculated into 10 ml of low phos media (3.75 g ammonium sulphate, 0.71 g sodium citrate dehydrate, 1.07 g KCl, 5.36 g Yeast extract, 5.36 g Hy-case SF casein hydrolysate, 7 mM MgSO_4 , 14 mM glucose per liter, pH 7.3 (adjusted by addition of KOH)). The culture was allowed to grow for 20 h before the culture supernatant containing the secreted scFv-AP fusion product was collected and kept at 4 °C for no more than 1 week. No further purification is required for the assay.

scFv-AP ELISA

Five μg of aflatoxin B1-BSA or 1% (w/v) BSA (control) in 100 μl PBS were immobilized into wells of an immuno 96 WellsTM plate. The plate was washed three times with Tris-buffered saline (TBS; 25 mM Tris-HCl, pH 7.5, 140 mM NaCl, 3 mM KCl) supplemented with 0.05% (v/v) Tween 20 (TBST) and blocked with 3% (w/v) BSA in TBS. After incubation for 1 h at room temperature, the wells were washed 5 times with TBST. Later, 50 μl of scFv-AP supernatant and 50 μl of 3% (w/v) BSA were added into each well and incubated at room temperature for 2 h. The plate was then washed five times with TBST. Subsequently, 100 μl *p*-Nitrophenyl phosphate substrate (pNPP; SIGMA, USA) was added to each well. After incubation for 20 min at room temperature, absorbance was measured at 405 nm.

Competitive ELISA

Competitive or inhibition ELISA was performed as described in the normal ELISA methods, except that optimal amount of the phage particle, scFv or scFv-AP were pre-incubated with increasing amount of soluble AFB₁ ranging from 0.019 to 5.0 $\mu\text{g}/\text{ml}$ before adding into previously coated, blocked, and washed wells of ELISA plates. For every assay, appropriate dilutions of scFv or scFv-AP that showed a linear relationship by direct ELISA (Fig. 3), was used.

Determination of Cross-Reactivity

Selected scFv or scFv-AP antibodies were assayed for binding against a range of soluble aflatoxins; B₁, B₂, G₁,

G₂, and M₁. Stock solutions of aflatoxins were prepared in 100% (v/v) methanol and diluted using TBST containing 5% (v/v) methanol. The assays were performed following the competitive ELISA protocol, as described above, for each of the aflatoxins tested.

Results

Bio-Panning of Aflatoxin-Specific scFv Antibodies

A simple bio-panning procedure was carried out to select phage that displays specific antibody to soluble AFB₁ from two human phage display scFv libraries. The libraries were pre-incubated with 3% (w/v) BSA to minimize the possibility of non-specific binding to BSA since the AFB₁-BSA conjugate was used as an affinity selection target. For semi-synthetic libraries (Tomlinson I & J), three rounds of bio-panning was performed according to the manufacturer's protocol, whereas for a compact non-immunized Yamo1 library, we have previously reported that only one round of bio-panning was sufficient to obtain specific phage [13]. A summary of bio-panning results is listed in Table 1. Enrichment of AFB₁-specific phage after each round of affinity selection was observed for the Tomlinson libraries. After three rounds of affinity selection, 2.2×10^5 and 2×10^6 phage clones could be obtained from Tomlinson I and J, respectively. For Yamo1 library, 2.5×10^2 phage clones could be obtained after one round of selection. Confirmation by ELISA revealed that 71, 32, and 4 phage clones from Tomlinson I, J, and Yamo1, respectively, were authentic binders to AFB₁-BSA. The frequency of specific binders from Tomlinson and Yamo1 libraries was the same after one round of selection; however, none of the clones from round one of Tomlinson bio-panning could bind to soluble AFB₁ in the next step.

Specific Binding of scFv Antibody to Soluble AFB₁

For diagnostic or therapeutic purposes, it is necessary to obtain antibody that can bind to free (soluble) AFB₁, and thus we performed competitive ELISAs to identify these scFv-secreting clones. Soluble scFv fragments were first generated by infecting selected phage clones into *E. coli* HB2151, which reads the amber stop codon (TAG) as a stop signal, allowing secretion of soluble scFv into the periplasm, upon induction with IPTG. From the selected phage clones tested, 19, 2, and 3 clones from Tomlinson I, J, and Yamo1, respectively, could be successfully induced for expression, and from these soluble scFv clones, only 3 and 1 clone from Tomlinson I, and Yamo1, respectively, showed specific competitive binding to soluble AFB₁ by competitive ELISA. Amino acid sequence analysis of selected scFv clones revealed that the three clones that were isolated from Tomlinson I (TomI-F6, TomI-H2, and TomI-E9) have an identical DNA sequence, thus there was one scFv fragment from each Tomlinson I and Yamo1 library that could be selected by our bio-panning procedure. These two clones (TomI-F6 and YMO1-C3) were used for further study. Amino acid sequence analyses of these two scFv fragments are shown in Fig. 2. The TomI-F6 scFv antibody was based on a single human framework for VH (IGHV3-23*05, DP47) and V κ (IGKVID-39*01, DPK9), which belong to subgroup VH3 and VK1, respectively. The positions of amino acid different from the germ lines are located in CDR2 and CDR3 regions of the VH and VL, respectively. The second scFv antibody, YMO1-C3 was obtained from Yamo I. The VH and VL fragments were derived from germlines IGHVI-3*01 (DP25) and IGKVID-39*01 (DPK9), which belong to subgroup VH1 and VK1, respectively. There is no amino acid different from the germline gene in VH fragment, whereas there are four amino acid differences from germline gene in the framework region 1, 2, and 3 of VL fragment.

Table 1 Overview of selection of antibodies against aflatoxin B₁-BSA

Results from each step	Tomlinson I	Tomlinson J	YAMO 1
Number of clone after 1st panning	3.2×10^3	4×10^2	2.5×10^2
Number of clone after 2nd panning	1×10^5	1.4×10^5	–
Number of clone after 3rd panning	2.2×10^5	2×10^6	–
Number of positive phage clones after 1st round from ELISA	6/96 ^a	8/96 ^a	4/56 ^a
Number of positive phage clones after 3rd round from ELISA	71/96 ^a	32/96 ^a	–
Number of expressed soluble scFv	19/62 ^a	2/34 ^a	3/4 ^a
Number of scFv fragments that can bind soluble Aflatoxin B ₁	3/8 ^a	0	1/3 ^a
Number of different scFv clones	1	0	1

^a The number of positive clones/the number of tested clones

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                                VH/CDR1                    -VH/CDR2-
TomI-aft6  MAEVQLLESGGGLVQGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSISNAGTYT 60
Ym1-aft3   MAQVQLVQSGAEVKKPGASVKVSKASGYTFTSYAMHWVRQAPGQRLIEWMWINAGNGNT 60
          **::***::** . : **::**::** ***::**::*** *****: ***. . * . . *

          -VH/CDR2                    -VH/CDR3--
TomI-aft6  YYVDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKN--YTF---FDYWGQGLTV 114
Ym1-aft3   KYSQKPGQRVITITRDTSASTAYMELSSLRSEDTAVYYCARADDYGSGSYGFDYWGQGLTV 120
          * :.::**::**::** . * *:::..*****: * : *****

          linker sequence                    --VL/CDR1--
TomI-aft6  TVSSGGGSGGGSGGGSGGGSTDIQMTQSPSSLSASVGRVITITCRASQSISSYLNWYQKP 174
Ym1-aft3   TVSSGGGSGGGSGGGSGGGS-DTVMTQSPSSLSASVGRVITITCRASQSISSYLNWYQK 179
          *****:***** * *****:*****:*****

          VL/CDR2                    VL/CDR3
TomI-aft6  GKAPKLLIYSASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQGANYPNTFGQ 234
Ym1-aft3   GKAPRLLIYASASSLQSGVPSRFSGNGSGTDFTLTISLQPEDFATYYCQQSYSTPYAFGQ 239
          *****:*****:*****.*****:*****. . * :***

TomI-aft6  GTKVEIKRAAAHHHHHHGAA--EQKLISEEDLNGAA 268
Ym1-aft3   GTKVEIKRAAAHHHHHHGAAGPEQKLISEEDLNGTA 275
          *****:***** * *****:*****
    
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Fig. 2 Amino acids sequence analysis The amino acid sequence of the whole scFv antibody fragments of TomI-F6 (TomI-aft6, upper panel) and Ym1-C3 (Ym1-aft3, lower panel) are shown. The GC-rich sequence that links V_H and V_L segments is indicated. The three complementarity determining regions (CDRs) are shown. The underlined letters are amino acids that are different from the germ

line. Structure analysis was carried out based on IgBLAST software (<http://www.ncbi.nlm.nih.gov/igblast/>) and reconfirmed by DNA-PLOT from V BASE. (<http://vbase.mrc-cpe.cam.ac.uk/>). Sequence alignment of the two scFv antibodies was done using CLUSTALW 2.1 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>)

Cloning and Characterization of scFv-Alkaline Phosphatase (scFv-AP) Fusions

To create a convenient one-step detection reagent for diagnostic purposes, we cloned the TomI-F6 and Ym1-C3 scFv DNA fragments into an alkaline phosphatase (AP) expression vector. The scFv-AP fusions could be induced for expression in *E. coli* and secretion into the periplasmic space. Culture supernatant can then be used directly in ELISA as illustrated in Fig. 3. The signal was excellent and the background was low. Directed ELISA using AFB₁-BSA conjugated as a target revealed that the optical density using Ym1-C3-AP fusion at 405 nm was 3-fold higher than that of the TomI-F6-AP. This result suggested that scFv-AP could be used as a convenient reagent for detecting soluble AFB₁ by competitive ELISA.

To compare the binding properties of soluble scFv and scFv-AP, competitive ELISA was performed on the two forms of the Ym1-C3 antibody. Various concentrations of free AFB₁ were pre-incubated with appropriate dilutions of scFv or scFv-AP before adding into well of an ELISA plate coated with the 3–5 µg of AFB₁-BSA conjugate. Bound scFv or scFv-AP was detected by use of a protein-L-peroxidase, followed by TMB substrate, or pNP substrate, as appropriate. The IC₅₀ values (Fig. 4), being the concentration of soluble AFB₁ that results in 50% inhibition, for the scFv and scFv-AP fusion, were 0.12 and 0.04 µg/ml, respectively (Fig. 4). This result indicated that scFv-AP fusion has 3-fold higher sensitivity than soluble scFv form. Therefore, the scFv-AP fusion format was subsequently

used to characterize the binding properties of the two scFv antibodies in the next step.

Ym1-C3 scFv-AP has Higher Binding Sensitivity than TomI-F6 scFv-AP

Competitive or inhibition ELISA was successfully used as an indirect method to estimate the binding affinity of an antibody [14]. In addition, it has also been used to determine binding constant (IC₅₀) of modular binding domain-AP fusions to peptide [15]. Thus, we performed competitive ELISA to estimate the binding affinity and compare the binding sensitivity of the two scFv fragments that were isolated from two different phage libraries, as shown in Fig. 5. Various concentrations of free AFB₁ were pre-incubated with the appropriate dilution of scFv-AP fusions and the ratio of OD of bound scFv-AP over the OD in an absence of free AFB₁ was plotted against the log of concentration of free AFB₁. The IC₅₀ values for Ym1-C3 and TomI-F6 were 0.034 and 0.14 µg/ml, respectively, suggesting that the binding sensitivity of Ym1-C3 is approximately 4 fold higher than that of TomI-F6. The useful assay range for Ym1-C3 and TomI-F6 was between 0.007–0.2 and 0.04–1.0 µg/ml.

Cross-Reactivity to Various Aflatoxins

To evaluate the binding specificity of the selected scFv antibody, we compared IC₅₀ values derived from inhibition ELISA of structurally related aflatoxins, i.e., B₂, G₁, G₂,

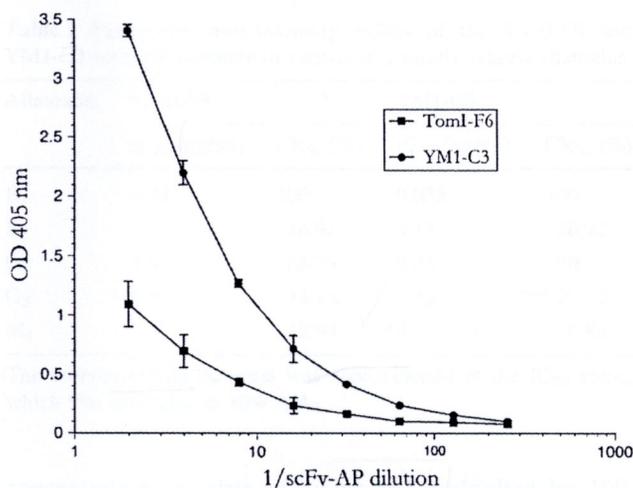


Fig. 3 Direct ELISA of scFv-AP antibody fusions. Various 2-fold dilutions of culture supernatant containing scFv-AP fusions from $1/2$ to $1/256$ were assayed on an ELISA plate coated with AFB₁-BSA. Bound antibodies were detected by colorimetric assay with pNPP (p-Nitrophenyl Phosphate) as substrate. The average OD₄₀₅ values and standard errors are shown. Antibody dilutions of $1/10$ (TomI-F6) and $1/20$ (YM1-C3), which was in the linear range of the curve, were chosen for competitive ELISA

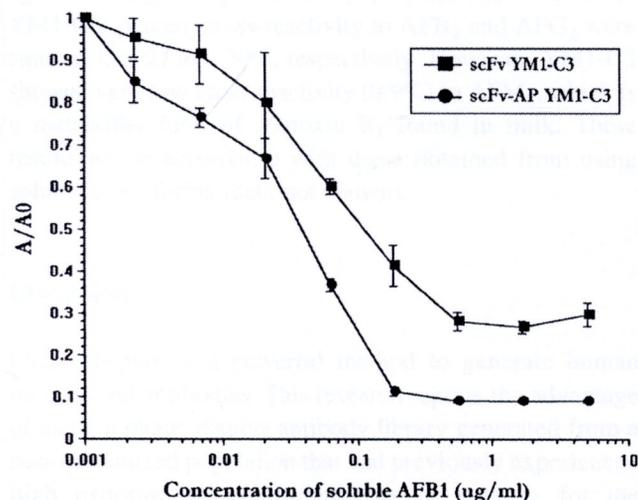


Fig. 4 Comparison of binding properties of soluble scFv and scFv-AP antibody. Two formats of YM1-C3 scFv antibody were compared by competitive ELISA. Optimal dilutions of soluble scFv or scFv-AP were incubated with equal volumes of various amounts of free AFB₁ in TBST containing 5% (v/v) methanol to reach a final concentration ranging from 2.3 ng to 5,000 ng/ml. The IC₅₀ values for the scFv YM1-C3, and YM1-C3-AP were found to be 0.12 and 0.04 μ g/ml, respectively. The scFv-AP fusions showed improved binding sensitivity of approximately 3 fold better than the soluble scFv format

and M1 as demonstrated for YM1-C3 scFv-AP in Fig. 6. Standards of each potential cross-reactive aflatoxins, ranging from 2.3 to 5,000 ng/ml in TBST containing 5% (v/v) methanol, were mixed with an equal volume of scFv-AP antibody at the optimal concentration, before

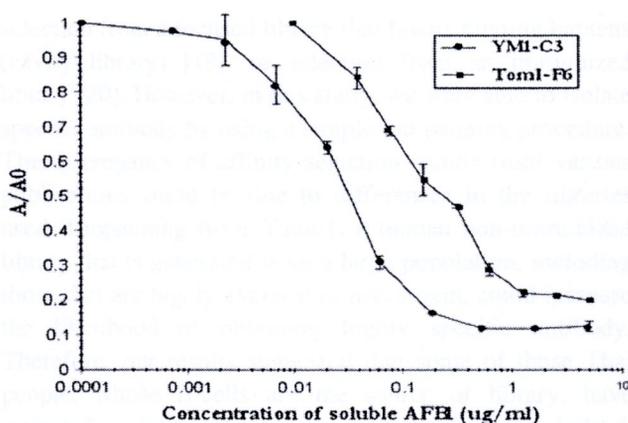


Fig. 5 Comparison of binding properties of TomI-F6 and YM1-C3 scFv-AP antibodies. Various concentration of soluble AFB₁ from 5.0 μ g/ml to 0.762 ng/ml were incubated with two scFv-AP (TomI-F6, YM1-C3) antibodies at 37 °C for 30 min before addition to wells of Immuno 96 MicroWell™ plates, coated with 5 μ g AFB₁-BSA. The plates were washed five times with TBS-T after a 1 h incubation. Bound antibodies were demonstrated by colorimetric detection using the AP substrate, pNPP. The average absorbance at 405 nm and S.Ds are shown. Normalized absorbance values (expressed as A/A₀) were plotted against the logarithm of AFB₁ concentration. The IC₅₀ values for the TomI-F6-AP and YM1-C3-AP were found to be 0.14 and 0.034 μ g/ml, respectively

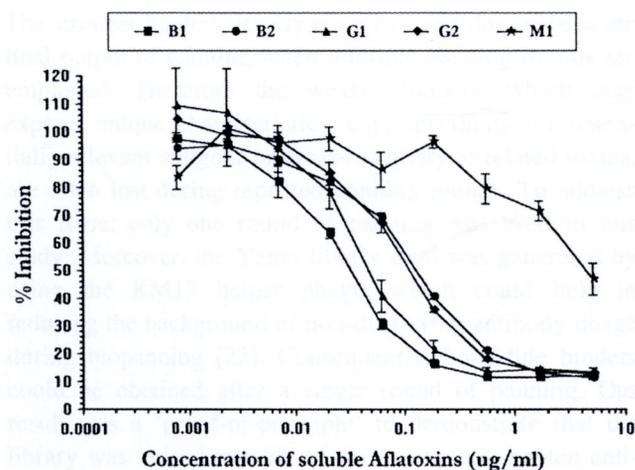


Fig. 6 Cross-reactivity against different aflatoxins. Competitive ELISA of YM1-C3 scFv-AP antibody with various structurally related aflatoxins (AFB₁, AFB₂, AFG₁, AFG₂, AFM₁) is demonstrated. Results were plotted as the mean percent inhibition vs. concentration of soluble aflatoxins. Percent inhibition of aflatoxin antibody was calculated from this type of competitive ELISA

incubation on wells of an ELISA plate that were coated with AFB₁-BSA conjugates. The cross-reactivity potential was approximated as the percent inhibition according to the equation: % inhibition = [(OD sample/OD control)] \times 100. The percentage cross-reactivity determined at IC₅₀ (%CR₅₀) was expressed as 50% inhibitory concentration of aflatoxin B1 divided by the 50% inhibitory

Table 2 Percentage cross-reactivity values of the TomI-F6 and YM1-C3 scFv-AP antibody to various structurally related aflatoxins

Aflatoxins	TomI-F6		YM1-C3	
	IC ₅₀ (ug/ml)	CR ₅₀ (%)	IC ₅₀ (ug/ml)	CR ₅₀ (%)
B ₁	0.22	100	0.035	100
B ₂	1.3	16.92	0.13	26.92
G ₁	0.32	68.75	0.05	70
G ₂	1.6	13.75	0.12	29.17
M ₁	1.7	12.94	4	0.88

The cross-reactivity potential was approximated at the IC₅₀ value, which was estimated at 50% A/A₀

concentration of other aflatoxins and multiplied by 100. Percentage cross-reactivity values of the TomI-F6 and YM1-C3 scFv-AP antibodies to various potential cross-reactive aflatoxins are reported in Table 2. Both scFv-AP antibodies showed a high degree of cross-reactivity, especially to AFG₁ > AFB₂ > AFG₂ > M₁ (in decreasing order). The highest levels of cross-reactivity with aflatoxin G₁ were 68.75% and 70% for TomI-F6 and YM1-C3, respectively. There is no significant difference in percent cross-reactivity against AFB₂, AFG₂, and AFM₁ for TomI-F6, whereas for YM1-C3, percent cross-reactivity to AFB₂ and AFG₂ were similar, i.e., 27 and 30%, respectively. However, YM1-C3 showed very low cross-reactivity (0.9%) to AFM₁, which is a metabolite form of aflatoxin B₁ found in milk. These results are in accordance with those obtained from using soluble scFv forms (data not shown).

Discussion

Phage display is a powerful method to generate human monoclonal antibodies. This research reports the advantage of using a phage display antibody library generated from a non-immunized population that had previously experienced high exposure to certain antigens of interest, for the selection of a specific and sensitive antibody. In addition, we also demonstrated that the alkaline phosphatase fusion of scFv antibody (scFv-AP) is an attractive format for the detection of hapten by inhibition ELISA. Since the antibody is human, its potential application encompasses therapeutic purposes, such as the treatment of acute toxicity or drug addiction [16, 17].

Since obtaining specific antibody to free hapten is often very difficult with either naïve libraries or libraries derived from immunized animals, many strategies have been developed to increase the chance of selecting specific antibodies from a phage display antibody library. These include modification of the elution step by competing with free hapten [18], alternative hapten-carrier conjugates [6],

selection from a focused library that favors binding haptens (cavity library) [19], or selection from an immunized library [20]. However, in this study, we were able to isolate specific antibody by using a simple bio-panning procedure. The discrepancy of affinity selection results from various publications could be due to differences in the libraries used. Biopanning from Yamo1, a human non-immunized library that is generated from a large population, including those that are highly exposed to mycotoxin, could increase the likelihood of obtaining highly specific antibody. Therefore, our results suggested that some of these Thai people, whose B-cells are the source of library, have mounted an immune response against this toxin. Indeed, there have been two previous reports indicating antibody activity against aflatoxin B₁ in serum from individuals in Kenya who experience high exposure to AFB₁ [21], and in Danish workers who are laboring in animal-feed processing plants [22]. Therefore, this finding should be applicable for other antigens such as other mycotoxins, pesticides, and viruses, to which certain populations are commonly exposed.

In general, the main reason for performing only a single round of biopanning is to maximize the diversity of binders selected. Increasing the number of panning rounds affects the outcome with regard to the number of weaker binders. The stronger binders usually overgrow and dominate in the final output of panning when multiple panning rounds are employed. Therefore the weaker binders, which may express unique characteristics, e.g., specificity for potentially relevant antigens or cross-reactivity to related toxins, are often lost during repeated panning rounds. To address this issue, only one round of panning was used in this study. Moreover, the Yamo library used was generated by using the KM13 helper phage, which could help in reducing the background of non-displaying antibody phage during biopanning [23]. Consequently, bona fide binders could be obtained after a single round of panning. Our result was a 'proof-of-principle' to demonstrate that the library was indeed capable of generating anti-hapten antibodies, and that the population used to make the library would have had exposure to the toxin. In addition, another advantage of obtaining antibody from a single round of panning is that the isolated antibody may be able to cross react with other related toxins (aflatoxin G₁, B₂, G₂) with similar structures more so than antibodies from the TomI library obtained after three rounds of panning. While it is possible to obtain the highest affinity and most specific scFvs by performing more rounds of biopanning, the affinity, stability, or specificity of the isolated antibody from a single round of panning can also be further improved by affinity maturation [4].

The scFv format has many advantages over other antibody formats, i.e., it is more efficient for display on phage

coats [24], it may pass through the blood–brain-barrier [25], and the library is more stable [26]. However, one key disadvantage of this format is the formation of dimers or higher order multimers [27]. This complicates standard affinity measurement by systems such as surface plasmon resonance (SPR) analysis. The formation of multimeric antibody molecules depends on the amino acid sequence of individual clones and the linker length, and can be unpredictable. Indeed, both of the two scFv antibodies described in this study did form several multimers as observed by SDS-PAGE analysis (data not shown). Purified monomeric scFv antibodies isolated by gel filtration chromatography could re-aggregate when left in solution.

Analysis of binding sensitivity by competitive ELISA revealed that the IC_{50} of YM1-C3 was approximately 0.04 $\mu\text{g/ml}$ or 40 ppb, whereas, the IC_{50} of TomI-F6 was approximately 0.14 $\mu\text{g/ml}$ or 140 ppb. These values are in the same range as previously published clones that were isolated from pre-immunized or naïve libraries [6, 18, 28]. The detection limit of YM1-C3 was 0.007 $\mu\text{g/ml}$ (7 ppb). In several regions where aflatoxin contamination is prevalent, the acceptable limits of aflatoxin contamination in agricultural products are 20 ppb [29], thus, this YM1-C3-AP fusion could be applicable for analytical purposes.

Determination of cross-reactivity to structurally related aflatoxin (B2, G1, G2, and M1) revealed that YM1-C3 scFv could cross react with AFG₁ (70%), with slight cross-reactivity (~30%) with AFB₂ and AFG₂, but showed almost no binding to AFM₁. Judging from the structure of these aflatoxins (Fig. 1), it seems that the antibody interacts near the C9 position. These data are in accordance with previous reports that also used AFB₁-BSA conjugate as a target for affinity selection [18, 28], confirming that the type of conjugates used could dictate the outcome of affinity selection results. A wide specificity of antibody could be very useful in the following cases: (a) extraction of toxins from complex food or environmental matrices for analysis or in the purification of expensive food ingredients, (b) application as an antidote in food to sequester the toxin or to reduce potential toxicity, and (c) broad specificity toxin detection e.g., on an array.

One important outcome from this study is that we have demonstrated that the isolated antibodies could be further engineered to create a scFv-alkaline phosphatase fusion (scFv-AP) protein and this could be used as a convenient one-step detection probe for competitive ELISA. Bacterial alkaline phosphatase (BAP) is a homodimeric enzyme and fusions of various proteins or peptides to this enzyme have been previously demonstrated to be useful reagents for various detection formats [30]. Our results indicated that the sensitivity of scFv-AP fusion was approximately 3-fold better than the soluble scFv form and 20-fold better than the phage-displayed format [13]. This result is in

accordance with previous reports that demonstrate higher specificity and sensitivity of peptide-AP fusion when compared to free biotinylated peptides or peptides that display on phage [31]. It is possible that the increased affinity is due to a higher proportion of the secreted AP-fused scFv being present in a correctly folded—and therefore active format than in the case of soluble scFv. The AP-fused proteins are likely to be dimeric, because AP itself is a homodimer, hence the binding avidity is increased. Ease of engineering, expression, and purification makes scFv-AP fusion antibodies attractive for large-scale production for the diagnostics industry.

Although the IC_{50} or detection limit of the antibody in this study is comparable to other antibodies isolated from a naïve library, it is approximately ten times less sensitive than antibodies from some immunized mouse libraries [12]. However, it is important to note that this antibody is 100% human and it is not possible to directly immunize humans with toxins for ethical and safety reasons. Nevertheless, the sensitivity of these antibodies can be further improved by various methods of affinity maturation [4].

Conclusions

Our results demonstrate the value of phage display antibody technology to engineer human recombinant antibodies for carcinogenic haptens such as aflatoxins. This knowledge should be applicable to a host of other haptens or antigens to which human populations may have been exposed. The library and approach used should be advantageous over other human library-based systems.

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ข้อวิจารณ์

ผลลัพธ์ที่ได้จากโครงการวิจัยนี้แสดงให้เห็นว่าสามารถนำเทคโนโลยีการแสดงโปรตีนบนผิวพองไปประยุกต์ใช้ในการผลิตแอนติบอดีต่อสารปนเปื้อนโมเลกุลเล็กทางการเกษตร ได้เป็นอย่างดี โดยในกรณีนี้ได้ใช้สารอะฟลาทอกซิน เป็นตัวแทนสารปนเปื้อนโมเลกุลเล็กทางการเกษตร (hapten) เพราะเป็นสารพิษก่อมะเร็งร้ายแรง และเป็นปัญหาต่อผลผลิตทางการเกษตรมานานทั่วโลก โดยเฉพาะประเทศในแถบร้อนชื้น จึงได้มีการศึกษาวิจัย และมีข้อมูลเชิงวิชาการเกี่ยวกับสารพิษนี้ เพื่อใช้ในการวิเคราะห์ ประเมินผลการวิจัย ได้เป็นอย่างดี ซึ่งจากผลการวิจัยทั้งหมดสามารถสรุปได้ว่า สามารถประยุกต์ใช้เทคโนโลยีแอนติบอดีบนผิวพอง ในการค้นหาแอนติบอดีส่วน scFv ของมนุษย์ แล้วนำไปทำวิศวกรรมแอนติบอดี ด้วยวิธีการทางพันธุวิศวกรรมให้ยีนของแอนติบอดีส่วน scFv เชื่อมต่อกับยีนของเอนไซม์ alkaline phosphatase เพื่อใช้เป็น ตัวตรวจสอบขั้นเดียว (one-step detection probe) ที่สะดวกต่อการใช้งาน และที่สำคัญคือ แอนติบอดีในรูปแบบ scFv-AP นั้น มีความไวในการตรวจสอบอะฟลาทอกซินมากกว่าแบบ scFv เปล่าๆ ถึง ๓ เท่า ดังนั้นแนวทางการทำวิศวกรรมแอนติบอดีที่ได้พัฒนาขึ้นมา นี้ จะสามารถนำไปประยุกต์ใช้สำหรับการผลิตแอนติบอดีต่อสารปนเปื้อนโมเลกุลเล็กอย่างอื่นได้ดี

เหตุผลสำคัญประการหนึ่งที่ทำให้โครงการวิจัยนี้ได้ผลสำเร็จเป็นอย่างดี น่าจะเป็นเพราะแหล่งที่มาของแอนติบอดีหรือคลังของแอนติบอดีนั้น ได้ถูกสร้างขึ้นมาจากเม็ดเลือดขาวของประชาชนที่มีสุขภาพดีในเขตจังหวัดนครราชสีมา ประมาณ ๑๕๐ คน (คลังย่ำโม ๑) ซึ่งประชากรเหล่านี้ น่าจะมีจำนวนค่อนข้างสูง ที่เคยสัมผัสกับอาหารที่ปนเปื้อน ด้วยอะฟลาทอกซินมาก่อน ทำให้ร่างกายได้ผลิตแอนติบอดีขึ้นด้านทานไว้แล้ว ดังนั้น คลังที่สร้างขึ้นจากประชากรกลุ่มนี้จึงมีแอนติบอดีที่มีคุณสมบัติ เมื่อเทียบกับคลังอื่น เช่นคลังกึ่งสังเคราะห์ หรือคลังที่สร้างจากประชากรในทวีปอเมริกาเหนือ ดังที่ได้วิจารณ์ไว้โดยละเอียดในบทความที่ได้ตีพิมพ์ไปแล้ว เพราะสามารถค้นหาแอนติบอดีที่มีคุณภาพดีได้จาก การทำ biopanning จากคลังย่ำโม ๑ เพียงครั้งเดียว ดังนั้นจึงน่าจะสามารถนำคลังนี้ไปใช้ในการค้นหา แอนติบอดีต่อ สารปนเปื้อนโมเลกุลเล็กทางการเกษตรอื่นๆ ได้อีก โดยเฉพาะที่มักปนเปื้อนในผลผลิตทางการเกษตรของประเทศ

อย่างไรก็ตาม ถึงแม้ แอนติบอดี scFv-AP ต่ออะฟลาทอกซิน ที่พัฒนาขึ้นมาได้จากโครงการนี้จะสามารถจับกับ สารพิษ อะฟลาทอกซินในรูปแบบโมเลกุลเดี่ยว (soluble) ซึ่งเป็นรูปแบบที่ปนเปื้อนมาในอาหาร ได้ดี และมีความไวในระดับที่ดีกว่า หรือเท่ากับแอนติบอดี อื่นๆ ที่ผลิตได้ด้วยเทคโนโลยีนี้ และมีความไวสูงพอที่จะนำไปใช้ในการตรวจวิเคราะห์การปนเปื้อนในอาหาร ได้ เพราะปริมาณขั้นต่ำของการปนเปื้อน อะฟลาทอกซินในอาหารจะอนุญาตให้มีได้ถึงระดับ 20 ppb แต่แอนติบอดีที่พัฒนาขึ้นมาได้นี้ก็ยังมีค่าไวต่ำกว่า แอนติบอดีจากชุดตรวจสอบที่มีขายในท้องตลาด และที่เคยมีรายงานมาก่อน ทั้งที่เป็น polyclonal antibody และ monoclonal antibody ถึง ๑๐ เท่า คือ scFv-AP สามารถตรวจสอบความปนเปื้อนที่ระดับ 10 ppb แต่ แอนติบอดีแบบดั้งเดิมที่ใช้ในชุดตรวจสอบที่มีคุณภาพดีมักตรวจได้ถึงระดับ 1 ppb ดังนั้นจึงมีความจำเป็นที่จะต้องพัฒนาความไวของแอนติบอดีนี้ให้ขึ้น ด้วยกระบวนการ affinity maturation ซึ่งเป็นวิธีการทางวิศวกรรมแอนติบอดี ที่มีประสิทธิภาพ ดังที่ได้กล่าวไว้แล้วโดยละเอียดเนื้อหาของรายงานการวิจัยนี้ ต่อไป

สรุป และข้อเสนอแนะ

ผู้วิจัยประสบความสำเร็จในการประยุกต์ใช้เทคโนโลยีการแสดงโปรตีนบนผิวเฟจในการผลิตแอนติบอดีต่อสารอะฟลาทอกซิน ซึ่งเป็นสารพิษปนเปื้อนโมเลกุลเล็กจากเชื้อรา ที่เป็นสารก่อมะเร็งร้ายแรง และเป็นปัญหาต่อผลผลิตทางการเกษตรของประเทศไทย และประเทศอื่นๆ ที่อยู่ในสภาวะร้อนชื้น ทั่วโลก โดยสามารถทำการคัดหาแอนติบอดี ส่วน scFv ที่ความสามารถสูงในการจับกับ อะฟลาทอกซิน จากคลังแอนติบอดี ของมนุษย์ ชื่อคลัง “ยาโม ๑” ซึ่งเป็นคลังที่ได้สร้างขึ้นเองในห้องปฏิบัติการของผู้วิจัย โดยแอนติบอดีที่คัดหาได้นี้ มีศักยภาพในการนำไปพัฒนาต่อ ทั้งเพื่อการรักษา และการตรวจวิเคราะห์ โดยในโครงการวิจัยนี้ ผู้วิจัยได้แสดงถึงศักยภาพของการนำไปใช้ในการตรวจวิเคราะห์ การปนเปื้อน ด้วยการนำแอนติบอดีที่คัดหาได้ ไปพัฒนาให้มีคุณสมบัติเหมาะสมยิ่งขึ้น ด้วยกระบวนการวิศวกรรมแอนติบอดี โดยการนำยีนของแอนติบอดีส่วน scFv ที่ได้คัดหาจากคลังยาโม ๑ ไปเชื่อมต่อยีนของเอนไซม์ อัลคาไล ฟอสฟาเทส แล้วนำมาแสดงออกในระบบการผลิตโปรตีนของแบคทีเรีย อีโคไล เพื่อสร้างให้เป็นตัวตรวจสอบแบบสำเร็จในขั้นเดียว ที่สะดวกต่อการนำไปใช้ในการตรวจวิเคราะห์ การปนเปื้อนผลิตภัณฑ์ทางการเกษตร โดยจากการวิเคราะห์คุณสมบัติ และประสิทธิภาพของ แอนติบอดี แบบ scFv-AP ที่ได้พัฒนาขึ้นนั้นพบว่า แอนติบอดีชนิด scFv-AP ที่ได้พัฒนาขึ้นมาี้ มีความไวในการตรวจวัดสาร อะฟลาทอกซิน มากกว่าแบบ scFv เปล่าๆ ถึง ๓ เท่า นอกจากนั้นแล้ว ผลจากโครงการวิจัยนี้ยังได้แสดงให้เห็นว่า คลังของแอนติบอดี ยาโม ๑ ที่ผู้วิจัยได้พัฒนาขึ้นมาั้น มีคุณสมบัติเด่นกว่าคลังทั่วไป เพราะสามารถใช้เป็นแหล่งในการคัดหาแอนติบอดีต่อสารปนเปื้อนโมเลกุลเล็กทางการเกษตรที่มีคุณสมบัติดี ซึ่งอาจเป็นเพราะเป็นคลังที่สร้างขึ้นจากตัวอย่างที่เป็นคนไทยในแถบจังหวัดนครราชสีมา ซึ่งมีอัตราเสี่ยงต่อการบริโภคอาหารที่มี อะฟลาทอกซิน ปนเปื้อนสูง จึงอาจมีแอนติบอดีต่อสารนี้อยู่มาก กว่าคลังแอนติบอดีที่สร้างขึ้นจากประชากรในแถบอื่น หรือแบบกึ่งสังเคราะห์ ผลสำเร็จจากโครงการวิจัยนี้ นอกจากจะเป็นนวัตกรรม ต่างๆ ได้แก่ ยีน และ แอนติบอดีที่จำเพาะเจาะจงต่อ อะฟลาทอกซิน วิธีการในการคัดหาแอนติบอดีจากคลัง และรวมทั้งวิธีการทางวิศวกรรมแอนติบอดี เพื่อพัฒนาคุณสมบัติให้มีประสิทธิภาพดี ในการนำไปประยุกต์ใช้ในการตรวจวิเคราะห์สารปนเปื้อนทางการเกษตร อื่นๆ ที่มีประสิทธิภาพสูง แล้ว ยังได้สร้างองค์ความรู้ใหม่ ซึ่งสามารถนำไป ตีพิมพ์ในวารสารวิชาการระดับนานาชาติ ที่ต้องผ่านการประเมินโดยผู้ทรงคุณวุฒิอีกด้วย ซึ่งผลสำเร็จที่ได้ทั้งหมดนี้ สามารถนำไปพัฒนาต่อยอด ในการตรวจสอบสารปนเปื้อนทางการเกษตรอื่นๆ ทั้งที่เป็นสารโมเลกุลเล็ก และโมเลกุลใหญ่ ที่มีความสำคัญต่อผลผลิตทางการเกษตร ในระดับอุตสาหกรรมต่อไป

ภาคผนวก

ก. การเผยแพร่ผลงานทางวิชาการจากโครงการวิจัยนี้

ก.๑ การตีพิมพ์ในวารสารทางวิชาการระดับนานาชาติที่มี peer review (impact factor 2.444)

Rangnoi K, Jaruseranee N, O'Kennedy R, Pansri P, Yamabhai M. **One-Step Detection of Aflatoxin-B(1) Using scFv-Alkaline Phosphatase-Fusion Selected from Human Phage Display Antibody Library.** Mol Biotechnol. 2011.

ก.๒ การนำเสนอผลงานในการประชุมวิชาการระดับนานาชาติ

Rangnoi K, Pansri P, Kennedy RO, Yamabhai M. Selection of scFv antibody fragment specific to Aflatoxin by using phage display technology. Agricultural Biotechnology for Better Living and a Clean Environment. Queen Sirikit National Convention Center, 22-25 September 2009.

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ประวัตินักวิจัย

มณฑลพายัพ ย้ายเกิดเมื่อวันที่ ๘ มกราคม ๒๕๑๐ เป็นบุตรของ รศ.ดร. สวนิต และ ผศ. อำไพ ย้าย จบการศึกษาทั้งในระดับประถม และ มัธยมศึกษาจากโรงเรียนสาธิตจุฬาลงกรณ์มหาวิทยาลัย จากนั้นเข้าศึกษาต่อที่ คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล ได้รับปริญญาตรีเภสัชศาสตร์บัณฑิตเกียรตินิยม เมื่อปี พ.ศ. ๒๕๓๒ แล้วได้เข้ารับราชการเป็นเภสัชกรประจำโรงพยาบาลหัวตะพานเป็นเวลา ๑ ปี ก่อนจะมาเป็นอาจารย์ประจำคณะเภสัชศาสตร์ มหาวิทยาลัยรังสิต ในปี ๒๕๓๖ ได้รับทุน Fulbright Pre-doctoral Fellowship ไปทำงานวิจัยที่ University of Minnesota เป็นเวลา ๕ เดือน แล้วจึงได้รับทุนรัฐบาลไทยไปเรียนต่อในระดับปริญญาเอกที่ University of North Carolina at Chapel Hill ประเทศสหรัฐอเมริกา โดยมี Prof. Dr. Brian K. Kay เป็นอาจารย์ที่ปรึกษา จบการศึกษาระดับปริญญาเอกด้าน molecular biology ในปี พ.ศ. ๒๕๔๑ จากนั้นในปี พ.ศ. ๒๕๔๓-๒๕๔๕ ได้ทุนไปทำ post-doctoral research ที่ ห้องปฏิบัติการของ Prof. Dr. Richard G.W. Anderson ณ. University of Texas, Southwestern Medical Center, Dallas และในปี พ.ศ. ๒๕๔๖-๒๕๔๗ ได้รับทุน Alexander von Humboldt Fellowship ไปทำการวิจัยที่ห้องปฏิบัติการของ Prof. Dr. Kai Simons ณ. Max Planck Institute for Molecular Biology and Genetics กรุง Dresden ประเทศ สหพันธรัฐเยอรมัน สมรสกับ ศ.ดร. ศิณัฐมา หาลทิช เมื่อวันที่ ๑๖ สิงหาคม ๒๕๔๗ และมีบุตร ๑ คน ชื่อ คณ. ฐานิกา ย้าย หาลทิช ปัจจุบันเป็นรองศาสตราจารย์ และหัวหน้าสาขาวิชาเทคโนโลยีชีวภาพ สำนักวิชาเทคโนโลยีการเกษตร มหาวิทยาลัยเทคโนโลยีสุรนารี งานวิจัยในปัจจุบันเป็นงานด้านเทคโนโลยีชีวภาพเชิงอนุ (molecular biotechnology) มุ่งเน้นการใช้เทคโนโลยีเฟจ (phage display technology) และ เทคนิคอนุวิวัฒนาการ (molecular evolution) ในงานทางวิศวกรรมแอนติบอดี (antibody engineering) และวิศวกรรมเอนไซม์ (enzyme engineering) จนถึงปัจจุบันมีผลงานวิจัยที่ได้รับการยอมรับการตีพิมพ์ในวารสารทางวิชาการระดับนานาชาติ ๓๖ เรื่อง เป็นอาจารย์ที่ปรึกษาวิทยานิพนธ์หลักของมหาบัณฑิต ๔ คน และคุษฎีบัณฑิต ๔ คน และเป็นหัวหน้าโครงการวิจัยทั้งหมด ๒๒ โครงการ แล้วเสร็จ ๑๘ โครงการ

ที่อยู่ที่สามารถติดต่อได้

สาขาวิชาเทคโนโลยีชีวภาพ สำนักวิชาเทคโนโลยีการเกษตร

มหาวิทยาลัยเทคโนโลยีสุรนารี

ถ. มหาวิทยาลัย อ. เมือง จ. นครราชสีมา ๓๐๐๐๐

โทร ๐๔๔ ๒๒๔๑๕๒-๔ ๒๒๔๒๓๔ หรือ ๒๔๔๓๘๘ โทรสาร ๐๔๔ ๒๒๔๑๕๐

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