

CONSTRUCTION AND CHARACTERIZATION OF SINGLE CHAIN VARIABLE FRAGMENT-ALKALINE PHOSPHATASE FOR RAPID DETECTION OF AFLATOXIN B₁ 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 AFB₁ was performed by ELISA. The result 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