

Production of Single Chain Antibody against Mycotoxin Aflatoxin B1 in Recombinant *Escherichia coli*

민원기, 조영진, 최규호, 권대혁, 손동화¹, 박경문², 서진호*
서울대학교; ¹한국식품개발연구원; ²유진사이언스
(jhseo94@snu.ac.kr*)

Aflatoxin B₁ (AFB₁) is a secondary metabolite produced mostly by *Aspergillus flavus* strain and toxic to human and several animal species as food contaminant. The single chain variable fragment (scFv) genes of a monoclonal antibody (mAb) against AFB₁ were cloned and expressed in recombinant *Escherichia coli*. A fusion of myeloma cells and spleen cells from the immunized mice, hybridoma cells were cultured to obtain anti-AFB₁ mAb of 1.3 ng/ml IC₅₀ and 0.1 ng/ml detection limit. Complementary DNA was constructed by the reverse transcription-polymerase chain reaction and DNA sequence analysis identified that each variable region was composed of the heavy chain variable region (V_H) as a type of IgG1 and the light chain variable region (V_L) as a type of λ. Overlap-extension PCR using linker encoding polypeptide (Gly₄Ser)₃ led to combination of V_H and V_L genes and expression in recombinant *E. coli*. Co-expression of molecular chaperones enhanced soluble expression of anti-AFB₁ scFv and the ubiquitin fusion system increased expression levels. In vitro refolding was performed and competitive direct enzyme linked immunosorbent assay determined the optimized condition.