Production of GMO markers by genetic recombination and their characterization toward immuno-analytical reagents

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Abstract

Legislation enacted worldwide to regulate the content of genetically modified organisms (GMOs) in crops, foods, and ingredients, reliable and sensitive methods for GMO detection have been developed. Proteins produced in GMO plants can be determined by qualitative and quantitative analyses and thus GMO designation has performed exactly. Target proteins selected in this study were neomycin phosphotransferase II (NPTII), 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS), cucumber mosaic virus (CMV), and phosphinothricin acetyltransferase (PAT). Analytical method employing western blotting was used for final characterization..

Introduction

In a few years, worldwide agricultural enterprises have developed new plant varieties by adopting modern biotechnology including genetic transformation. Several transgenic crop species have spreaded rapidly to more than 40 million ha in the world, and at least 60% of food products in US supermarkets could contain GMO (Farid E. Ahmed, 2002; Elke Anklam, 2002). Up to now, these modified crops have developed to retain a resistance to herbicides and other characteristics needed in, for instances, agriculture, fruit growing, and floriculture. However, these modified foods have not gained worldwide acceptance because of a problems related to the environmental and public health safety issues. To ensure that consumers were informed of the content of the food they purchased, an accurate information has been provided on the product labels (Lipp M., 2000). Generally, the GMO analysis has focused on the development of an efficient, accurate, and cost effective methods.

In this paper, both Western blotting has developed for the qualitative analyses of

proteins contained in transgenic products. The targets for detection were NPTII protein, a selectable marker of genetically modified species (Roy L. Fuchs, 1993), EPSPS protein, that expressed in Round-up ready soybean product of Monsanto (G. J. Rogan, 1999), CMV coat protein, a representative of cucumovirus (Wei Sun, 2001), and PAT protein, a resistant marker for the herbicide phosphinothricin (Jan Peter Nap, 1999).

Material and Methods

Polymerase chain reaction (PCR) was used to amplify DNA coding the four genes from each plant. The DNA amplified fragments were ligated to pET28a vector using T4 DNA ligase. These recombinant DNA plasmid were separately transformed into *E. coli* strain BL21 (Sambrook, 1989). The recombinant proteins were produced in high levels using Isopropyl-beta-D-thiogalactopyranoside (IPTG). The cells were then harvested by centrifugation, and lysed by sonication. Soluble proteins were separated from the cell debris by centrifugation, and the target protein was purified on a affinity column (Qiagene, 2002) using a protocol provided by the manufacture.

The target proteins were characterized comparing to reference proteins based on immunological reactions with antibodies produced in this laboratory. Antibodies against the recombinant proteins produced from New Zealand White rabbit by immunizing with the protein. Western blotting was used for qualitative analyses of the proteins.

Results and Discussion

Production and Characterization of GMO Marker Proteins. The target proteins from recombinant organisms were produced in large quantities by IPTG induction. After affinity purification, each target protein were in a high purity (higher than 95% based on the area of protein bands stained). The recombinant proteins were characterized regarding to the binding affinity to a reference antibody and structural conformation of each protein molecule. From the results of Western blotting, the antibodies specifically bound to each target locating at positions of 29 kDa for NPTII, 50 kDa for EPSPS, 27kDa for CMV, and 25kDa for PAT. We confirmed that the recombinant and reference proteins were reacted with each specific antibody to the same degree, which indicated the epitopes of each recombinant were identical to those intact.

Qualitative immunoassay of GMO plants. Antisera were raised from animal by immunizing with the recombinant proteins. With the antisera, the constitutive proteins contained in GMO seeds and leaves were analyzed on Western blotting. Although a number of proteins were present in the genetically modified plants, we were able to specifically detect.

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