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Membrane based detection of genetically modified organisms in some representatives food

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Abstract

Recently, DNA-based techniques became very common for the detection of genetically modified organisms (GMOs) in food products. For rapid and easy detection of GMOs, polymerase chain reaction (PCR) screening methods, which amplify common transgenic elements, are applied in routine analysis. Incorporation of PCR and membrane method introduced in this study offer an alternative detection of GMOs. In this study, a total of 32 samples and three certified reference materials were tested for the existence of the 35S promoter of cauliflower mosaic virus (CaMV) and 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS) gene residues. Dot blot screening system introduced in this study can be routinely used as a semi-quantitative screening of GMOs.
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Keywords: Cauliflower mosaic virus; 35S promoter; Genetically modified organisms; Polymerase chain reaction; Dot blot

1. Introduction

In this study, the segments concerned in the detection method were 35S promoter regions derived from the cauliflower mosaic virus (P35S) and the coding regions of the genes for the insert: 5-Enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS). As reported by Matsuoka et al. (2002), many foreign DNA segments including construct genes, promoter and terminator regions and intron sequences are introduced to confer new traits to crops. Moreover, Farid (2002) mentioned in his paper that cauliflower mosaic virus (CaMV) 35S promoter is one of the genetic elements that most currently detected.

Polymerase chain reaction (PCR) is widely used in many fields of analysis to detect even small amounts of DNA very specifically (Wolf et al., 2000). According to Brown (1995), dot blot analysis is used to determine the relative abundance of target sequences by hybridization of a specific gene probe to unfractionated DNA of various species. This method involves fixing isolated sample DNA onto nitrocellulose or nylon membrane, probing with double-stranded (ds)—labeled nucleic acid probes specific to the GMO, and detecting hybridization radiographically, fluoremetrically or by chemiluminescence. Many nonradioactive detection systems use biotinylated probes synthesized by nick translation and random primer-labeling. Besides DNA polymerase I and the Klenow fragment, *Taq* DNA polymerase can incorporate biotinylated nucleotides. In this chapter, incorporation of biotin-14-dCTP into amplification products by *Taq* DNA polymerase and the chemiluminescent detection of these

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probes after hybridization to target DNA on membranes were investigated.

2. Materials and methods

2.1. Testing samples

A total of 32 samples and three certified reference materials consisting of dried soya flour containing 0%, 2%, and 5% (w/w) Roundup Ready soya flour (Fluka, UK) as GMO standard (Tables 1 and 2) were used as testing samples in this study. Samples were comprised of raw soybean and processed food (Table 3). The texture of the samples includes solid, semi-solid and liquid. The standards used in this study are Certified Reference Material (IRMM, Geel, Belgium). The same standards were used in the study conducted by Vaitilingom, Pijnenburg, Gendre, and Brignon (1999).

2.2. DNA preparation

The extraction of DNA was carried out using DNeasy Plant Mini Kit (QIAGEN, Germany) for raw samples, modified QIAamp DNA Stool Mini Kit (QIAGEN, Germany) for highly processed food and Wizard® Magnetic DNA Purification System for Food (Promega, USA) for processed food especially with liquid matrices. The extraction procedure was according to the manufacturer's instructions. The DNA concentration of solutions was determined by measuring the UV absorption at 260 nm. The purity of the extracted DNA was evaluated by agarose gel electrophoresis using UV absorption ratios of 260/280 nm and 260/230 nm; in the majority of the samples studied, the absorption ratio at 260/230 nm was more than 1.7, and that at 260/280 nm was between 1.7 and 2.0.

2.3. Probe labeling

Prior to hybridization, labeling of the probes was carried out by using KPL Detector™ PCR DNA Biotinylation Kit (Catalog no: 60-01-01), the probes use incorporation of biotin-N4-dCTP. The probe labeling for the control DNA and control primers was carried out as follow; 10× PCR Buffer (5 μl), 25 mM $MgCl_2$ (4 μl), 10× labeling mix (5 μl), control primers (1μl), *Taq* polymerase (1 μl), control DNA template approximately 20 ng (1 μl) and DEPC treated water to make up 50 μl. The recommended cycling temperature for the control labeling reaction (control DNA and control primers) was pre-denaturation at 94 °C for 1 min, denaturation at 94 °C for 15 s, annealing at 68 °C for 1 min,

Table 2
Raw samples used in dot-blot analysis

Code	Description	Matrix
KLIA / ©	Raw soy bean	Solid
PU 0636 / 01	Raw soy bean	Solid
PU 0617 / 01	Raw soy bean	Solid
WP / 067 / 01	Raw soy bean	Solid
USS	Raw soy bean	Solid
SA1	Raw soy bean	Solid
SA2	Raw soy bean	Solid
SA3	Raw soy bean	Solid
SB1	Raw soy bean	Solid
SB2	Raw soy bean	Solid
SB3	Raw soy bean	Solid
SC1	Raw soy bean	Solid
SC2	Raw soy bean	Solid
SC3	Raw soy bean	Solid
SD1	Raw soy bean	Solid
SD2	Raw soy bean	Solid
SD3	Raw soy bean	Solid
0%	Standard soy bean powder	Solid
2%	Standard soy bean powder	Solid
5%	Standard soy bean powder	Solid

Table 3
Processed food samples used in dot-blot analysis

Code	Description	Matrix
SBDP	Soy bean drink powder	Solid
Choc	Chocolate (Butterfinger-US)	Semi-solid
GBC	Gerber baby food (paste)	Semi-liquid
GBF	Gerber baby food (powder)	Solid
TL	Tauhu lembut	Semi-solid
UT1	Unlabeled tauhu	Semi-solid
UT2	Unlabeled tauhu	Semi-solid
UT3	Unlabeled tauhu	Semi-solid
UTS1	Unlabeled tauhu	Semi-solid
VF	Vegetarian food	Solid
SNCD	Soy and corn drink	Liquid
JT	Japanese tauhu	Semi-solid
SRD	Soy rich drink	Liquid
BP	Bean paste (Tauchu)	Semi-solid
DSS	Dark soy sauce	Liquid

Table 1
PCR primers used in probe development

Name	Primer Sequence (5′–3′)	Sense/Antisense	Length	Reference
EPSPS	GTCTTCCCGTTACCTTGCGC	Sense	134	Redesigned from NCBI sequence
	CTCGATGACCGTCGTGATGC	Antisense		Accession no: I43998
P35S	ATTGATGTGATATCTCCACTGACGT	Sense	101	Matsuoka et al. (2002)
	CCTCTCCAAATGAAATGAACTTCCT	Antisense		

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