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Microchip capillary gel electrophoresis using programmed field strength gradients for the ultra-fast analysis of genetically modified organisms in soybeans

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Abstract

We have developed a novel method for the ultra-fast analysis of genetically modified organisms (GMOs) in soybeans by microchip capillary gel electrophoresis (MCGE) using programmed field strength gradients (PFSG) in a conventional glass double-T microchip. Under the programmed electric field strength and 0.3% poly(ethylene oxide) sieving matrix, the GMO in soybeans was analyzed within only 11 s of the microchip. The MCGE-PFSG method was a program that changes the electric field strength during GMO analysis, and was also applied to the ultra-fast analysis of PCR products. Compared to MCGE using a conventional and constantly applied electric field, the MCGE-PFSG analysis generated faster results without the loss of resolving power and reproducibility for specific DNA fragments (100- and 250-bp DNA) of GM-soybeans. The MCGE-PFSG technique may prove to be a new tool in the GMO analysis due to its speed, simplicity, and high efficiency.

Keywords: Chip technology; GMO; Fast analysis; Programmed field strength gradients

1. Introduction

Biotechnology or gene transfer enables us to use important genes or characteristics from one organism and to place the genes with advantageous characteristics into another species. Transgenesis refers to the transfer of a gene or genes from one species into another host species. The newly introduced genes encode a new protein that gives the desired qualities in the host plant, animal or bacteria. Generally, this genetically modified organism (GMO) expresses new specific properties such as herbicide tolerance, insect resistance and productivity increase [1,2]. While the demand for commercial use of GMO has continuously increased due to its multiple advantages such as agricultural productivity, there is still a lot of controversy about GMO due to its potential risks to human health and world ecology. Thus, both European and Japanese legislation have recently introduced requirement of the obligatory labeling of foodstuffs produced from GMOs with a threshold of 1 and 5%, respectively, of GM material in a non-GM background [3–6]. Therefore, accurate and fast analytical methods for the quantification of GMOs in foodstuffs and/or for products containing GMO are required.

There are various detection methods for the identification of GMOs including protein-based methods, SDS gel electrophoresis, Western blot analysis, enzyme-linked immunosorbant assay (ELISA) [7,8], nucleotide-base amplification methods [9], detection of specific promoter and terminator sequences [10], capillary electrophoresis (CE) [11,12] and real-time PCR [9]. Among these techniques, slab gel electrophoresis, CE and real-time PCR method are most commonly used for the detection of GMO-specific amplification products. CE exhibits a faster separation with

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high resolution than the slab gel electrophoresis [11,12] and real-time PCR provides a quantitative analysis of GMO [9]. However, commercially available CE and real-time PCR systems are expensive.

Since the first demonstrations by Manz et al. [13] and Harrison et al. [14], microchip capillary electrophoresis (MCE) is fast becoming an important technique for the analysis of DNA fragments because of the analytical throughput, speed, small reagent volume, automation, miniaturization and high resolution [15–18]. One of the most significant advantages of MCE in DNA fragment analysis is its high speed compared to the traditional slab gel electrophoresis and CE. McDowell et al. reported that MCE was a fast and accurate alternative to PCR product quantification compared to the traditional slab gel electrophoresis method [23]. However, a major limitation of DNA fragment analysis by MCE is the use of a sieving matrix for the gel electrophoretic separation of DNA fragments. Because DNA fragment separation depends on the DNA size in the MCE separation, the separation of specific size DNA molecules does not easily acquire a short separation time without the loss of resolving power under constant electric field strength.

We recently reported the possibility of the fast separation method of DNA fragments by microchip capillary gel electrophoresis (MCGE) using programmed field strength gradients (PFSG) [19]. The PFSG allowed the fast separation of, and enhanced resolving power for target DNA fragments of long size (>1000 bp DNA). The method does not involve special requirements and/or devices. The results of MCGE-PFSG are based on electric field strength gradients that use a MCGE separation step in a sieving gel matrix. In this study, we have established a strategy for the ultra-fast analysis of GMO in soybeans, which have a relative short DNA fragment size (i.e., 100- and 250-bp DNA), by MCGE-PFSG in a microchip. The PFSG technique was able to decrease the analysis time for the detection of the CaMV 35S promoter sequence that is present in most GMOs [20]. The lectin, an endogenous and plant-specific gene, is also detected to prove that the extracted DNA from soybean. This paper also shows the ME-PFSG technique can be used for the ultra-fast analysis of all DNA fragments without considering the DNA fragment size.

2. Experimental

2.1. Chemical and reagents

1× TBE buffer (0.089 M Tris, 0.089 M borate and 0.002 M EDTA, pH 8.3) was prepared by dissolving pre-mixed powder (Amerosco, Solon, OH, USA) in deionized water. The dynamic coating matrix of the microchip was made by dissolving 0.5% (w/v) of M_r 1,000,000 polyvinylpyrrolidone (PVP) (Polyscience, Warrington, England) into the 1× TBE buffer with 0.5 μg/ml ethidium bromide (EtBr) (Sigma, St. Louis, MO, USA). The mixture was shaken for 2 min and left to stand for 2 h to remove any bubbles. The sieving matrix was made by dissolving 0.3% (w/v) of M_r 8,000,000 poly (ethyleneoxide) (PEO) (Sigma, St. Louis, MO, USA) into the 1× TBE buffer with 0.5 μg/ml EtBr, slowly stirring over night.

For the PCR analysis of soybean, PCR premix, Sapphire (components of 20 μl reaction: thermostable DNA polymerase 1 U, dNTPs 200 μM and $MgCl_2$ 1.5 mM) were purchased from Super-Bio (Suwon, Korea). A 100-bp DNA fragment from the GM soybean was amplified with forward primer for the CaMV 35S promoter, (35spF, 5′-TC GTTC AAGA TGCC TCTG CC-3′) and reverse primer, (35spR, 5′-TT GCTT TGAA GACG TGGT TGG-3′). A 250-bp DNA fragment from the GM soybean and the non-GM soybean were amplified with forward primer (Lec 250F, 5′-CT GACC AGCA AGGC AAAC TC-3′) and reverse primer (Lec 250R, 5′-GT GAAG TTGA AGGA AGCG GC-3′). All primers were synthesized by GenoTech (Daejen, Korea). DNA size markers, a 100-bp DNA ladder purchased from Genepia (Seoul, Korea) was diluted to 25 ng/μl with 1× TBE buffer before using.

2.2. PCR sample preparation

Soybean samples were acquired from the Bio-safety Research Institute at Chonbuk National University. The genomic DNA was extracted from 50 mg of soybean powder with or without GMO components through the CTAB (cetyltrimethyl-ammonium bromide) method [21,22]. Briefly, 600 μl of CTAB was added in an effendorf tube containing soybean powder and incubated at 55 °C for 2 h in 0.5 ml of extraction buffer (100 mM NaCl, 10 mM Tris–HCl, 1 mM EDTA, 1.0% SDS, pH 8) containing proteinase K. Proteins were extracted with 500 μl of phenol/chloroform/isoamylalcohol (25/24/1, v/v/v). Subsequently, 5 μl of RNase (10 mg/ml) was added, and incubated at 37 °C for 30 min. This sample was spun down (12,000 rpm) in a micro-centrifuge for 5 min at room temperature, and the top layer was discarded. Next, 600 μl of cold 70% ethanol was added and spun down for 30 s (12,000 rpm). The liquid was pipetted off in a tube and the DNA pellet was washed once with 600 μl of cold 70% ethanol. Then, the DNA pellet was washed with 600 μl of absolute alcohol. For elution, a supplementary treatment was carried out with the addition of nuclease free water.

The PCR reaction was performed in a thermal cycler (MJ Research PTC-200, USA) using the following temperature protocol: 5 min incubation at 95 °C; 40 cycles of denaturing at 95 °C for 30 s, annealing at 57 °C for 1 min, extension at 72 °C for 30 s; followed by a 7 min hold at 72 °C for 100-bp DNA fragment and 250-bp DNA fragment. The 20 μl PCR reaction mixture had the following final composition: 10 μl of PCR premix, Sapphire, 0.5 μl each of forward and reverse primer, and 2 μl of purified DNA. Finally, each amplified PCR product was introduced into the slab gel electrophoresis system and the MCGE system, respectively.