Annual Review & Research in Biology
3(4): 524-529, 2013

SCIENCEDOMAIN *international*
www.sciencedomain.org

Detection of Common Transgenic Elements from Soy Sauce Samples by PCRs

Ping Yu[1*] and Chunyan Yu[1]

[1]College of Food Science and Biotechnology, Zhejiang Gongshang University, Hangzhou 310035, Zhejiang Province, People's Republic of China.

Authors' contributions

This work was carried out in collaboration between all authors. Author PY designed and guided the experiments and wrote the manuscript. Author CY carried out the experiments. All authors read and approved the final manuscript.

Research Article

Received 7th March 2013
Accepted 25th May 2013
Published 15th June 2013

ABSTRACT

Aims: To establish PCR procedures for detecting common transgenic elements from soy sauce samples.
Methodology: Soy sauce samples, Haitian, Jiajia, Taitaile and Lijinji, and common transgenic elements from them, the CaMV35S promoter, the NOS terminator, and the Cp4-EPSPS, were chosen to establish PCR procedures. Genomic DNAs from soy sauce samples were extracted by the improved CTAB method. Primers for amplifying transgenic elements were designed and transgenic elements were amplified with extracted genomic DNAs as the templates. Amplified products were detected and analyzed using 1% agarose gel electrophoresis.
Results: The lectin gene from four samples, the NOS terminator from a sample and the Cp4 -EPSPS from two samples could be amplified by the established PCR procedure. The CaMV35S promoter sequence could be amplified from three soy sauce samples by the established nested PCR procedure.
Conclusion: This study lays a good foundation for developing the reagent kit for detecting common transgenic elements from soy sauce samples.

Keywords: Transgenic elements; soy sauce; nested PCR; detection.

**Corresponding author: Email: yup9202@yahoo.com.cn;*

1. INTRODUCTION

The use of genetically modified organisms (GMOs) as foodstuffs is becoming more and more widespread. The popularity of GMOs leads to the approval of making regulations in some countries which are intended to protect the consumer's rights. Two GMOs which are planted widely are maize and soybean [1,2]. According to the regulations of the European Union, they or their processed products are to be labeled specifically. Thus, it is necessary to establish the detection methods for detecting transgenic elements from them.

The detection methods of GMOs or their processed products can be established based on the detection of DNA, RNA or protein molecules. These methods specifically target to a particular sequence that is inserted into GMOs [3]. Most of the detection methods on GMOs which are currently used are based on DNA molecules [4-6]. To date, it has been unclear if PCR used widely is suitable for soy sauce samples because the DNA of soybean is degraded during the preparation of them. Thus, it is interesting to establish PCR procedures for detecting common transgenic elements from soy sauce samples.

In the present study, four soy sauce samples were chosen to investigate the feasibility to establish PCR procedures for detecting common transgenic elements from them. DNAs were efficiently extracted from them by the improved CTAB method. Common transgenic elements from soy sauce samples, including the CaMV35S promoter, the NOS terminator and the Cp4-EPSPS (herbicide resistant gene) were successfully amplified from the selected samples. To our knowledge, this is the first report on the detection of common transgenic elements from soy sauce samples.

2. MATERIALS AND METHODS

2.1 Samples

Four soy sauce samples, Haitian, Jiajia, Taitaile and Lijinji, were purchased from the Wumei supermarket in Hangzhou, Zhejiang Province, China. Standard GM soybean materials were purchased from the Hangzhou Quarantine of Imports and Exports of Zhejiang Province, China, and used as the control sample. PCR reagents were purchased from the TaKaRa Co. Ltd, Japan. The other reagents were analytical and used as the routine method.

2.2. DNA Extraction

The DNA extraction was performed as described by Lipp et al. [7] with minor modifications. In order to extract more genomic DNAs from them, samples were efficiently ground in a CTAB buffer (CTAB 20 g/l, 1.4M NaCl, 100 mmol/l Tris-HCl, 20 mmol/l EDTA) for at least 1h.

2.3 PCR Amplification

Primer sequences used are listed in Table 1, which were synthesized by the Sangon, Co. Ltd, Shanghai, China. PCR amplification was performed in a final volume of 50 µl containing 1×PCR buffer (20 mmol/l Tris-HCl,pH 8.4, 50 mmol/l KCl), 2.5 mmol/l $MgCl_2$, 0.2 mmol/l of each dNTP, 0.5 mM of each primer, 1 unit of *Taq* DNA polymerase (TaKaRa) and 2 µl of the extracted genomic DNA (maximum 50ng). For the lectin amplification, the PCR mixture was firstly denatured at 95ºC for 12 min, followed by 40 cycles of 95ºC for 1 min, 62ºC for 30 s and 72ºC for 30 s, and then further extension was performed at 72ºC for 10 min. For the

amplification of the CaMV35S promoter: the first amplification round was the same as that of lectin except for the annealing temperature changing from 62ºC to 59ºC and primers being replaced by P_3 and P_4. The resultant PCR product was diluted by 50-fold, and then 2 µl of the product were taken out and used as the template to undergo the second amplification round of the CaMV35S promoter using P_5 and P_6 according to the same procedure as that of lectin. For the NOS terminator and Cp4-EPSPS amplification: the same procedure was used as that of lectin except for the annealing temperature changing from 62ºC to 60ºC. PCR products were analyzed by the agarose gel electrophoresis, and photographed using the Syngene gel documentation system.

Table 1. PCR primers for detecting transgenic elements from soy sauce samples

Transgenic elements	Primer sequences (5'-3')	Amplified fragments (bp)
Lectin	P_1: GACGCTATTGTGACCTCCTC	122
	P_2: CGAAGCTGGCAACGCTACC	
CaMV35S1	P_3: GCTCCTACAAATGCCATCA	195
	P_4: GATAGTGGGATTGTGCGTCA	
CaMV35S2	P_5:TTGCGATAAAGGAAAGGC	117
	P_6:TTTGAAGACGTGGTTGGA	
NOS	P_7:GAATCCTGTTGCCGGTCTTG	180
	P_8:TTATCCTAGTTTGCGCGCTA	
Cp4-EPSPS	P_9:TGATGTGATATCTCCACTGACG	172
	P_{10}:TGTATCCCTTGAGCCATGTTGT	

3. RESULTS

Expected bands were amplified from the positive control and transgenic samples by established procedures. Fig. 1 showed that the amplified result of the lectin gene from four soy sauce samples using the GM soybean as the positive control. The lectin gene with the size of 122 bp was amplified from the positive control sample and four soy sauce samples by the established PCR procedure, whereas no band was found from the negative control sample. The nested PCR procedure was applied to amplify the CaMV35S promoter sequence by two sets of primers. Results are shown in Fig. 2. A 195-bp band from two samples was obtained in the first PCR amplification round using P_3 and P_4 as the primers. The CaMV35S promoter sequence with the size of 117bp was amplified from the positive control sample and three soy sauce samples using P_5 and P_6 as the primers and the first amplification product as the template. Fig. 3 showed that the NOS terminator sequence was amplified in the positive control sample and the sample 5 using the established PCR procedure, but no band was found from the negative control sample. Fig. 4 showed that the transgenic element Cp4-EPSPS was amplified in the positive control sample and samples 3 and 4 using the established PCR procedure. These results indicate that established standard and nested PCR procedures in our study are suitable for detecting common transgenic elements from soy sauce samples.

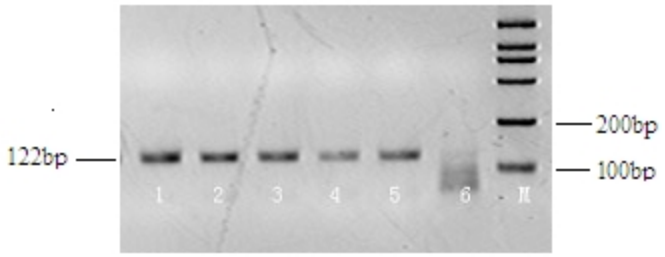

Fig. 1. The representative agarose gel electrophoresis of PCR products from GM soybean and soy sauce samples for analysis of the positive lectin gene

Lane 1: GM soybean (positive control); lanes 2-5: four soy sauce samples; lane 6: the negative control sample; lane M: DL2000 Marker

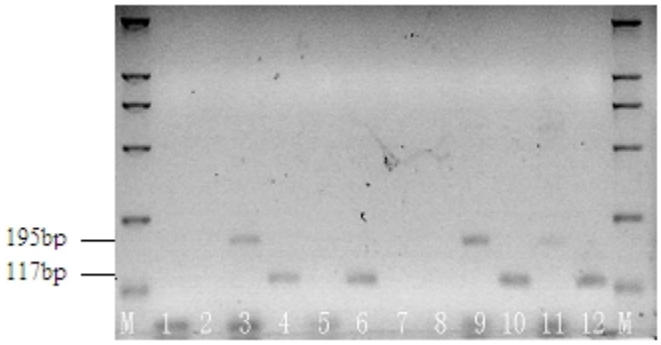

Fig. 2. The representative agarose gel electrophoresis of nested-PCR products from the GM soybean and soy sauce samples for analysis of the CaMV35S promoter

Lanes 1, 2: blank control; lanes 3, 4: GM soybean (positive control); lanes 5-12: four soy sauce samples; lane M: DL2000 Marker; lanes 1,3,5,7,9,11 showed results of the first amplification round; lane 2,4,6,8,10 and 12 showed results of the second amplification round.

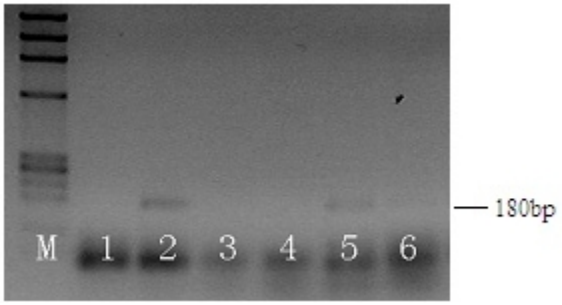

Fig. 3. The representative agarose gel electrophoresis of PCR products from GM soybean and soy sauce samples for analysis of the NOS terminator

Lane 1: blank control; lane 2: GM soybean (positive control); lanes 3-6: four soy sauce samples; lane M: Φx174-HaeⅢ digest.

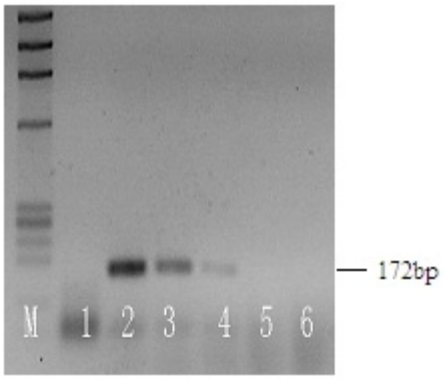

Fig. 4. The representative agarose gel electrophoresis of PCR products from GM soybean and soy sauce samples for analysis of the Cp4-EPSPS

Lane 1: blank control; lane 2: GM soybean (positive control); lanes 3-6: four soy sauce samples; lane M: Φx174-HaeⅢ digest.

4. DISCUSSION

In the experiments, we established PCR procedures to detect common transgenic elements from soy sauce samples. The genomic DNA extraction was carried out by the improved CTAB method. In order to eliminate the difference between negative and positive results due to the inhibition in the PCR amplification, the lectin gene that is a specific soybean sequence was firstly amplified. Common transgenic elements from soy sauce samples, including the CaMV35S promoter, the NOS terminator and the Cp4-EPSPS, were successfully amplified by established standard and nested PCR procedures.

It's very difficult to obtain the whole genomic DNA from soy sauce samples because it was highly fermented product and its genomic DNA has been degraded during fermentation. Thus, the nested PCR, instead of the standard PCR, was established to detect the CaMV35S promoter sequence. If the standard PCR amplification was used, it was not easy to observe amplified products. The CaMV35S promoter was amplified from 3 soy sauce samples by the established nested PCR procedure, whereas only 1 or 2 out of 4 samples were successful in the amplification of the CaMV35S promoter by the standard PCR procedure. Compared to standard and nested PCRs, the real-time PCR has higher specifity and sensitivity for detecting a particular DNA sequence from transgenic organisms. In the next step, we will establish real-time PCR procedures for detecting common transgenic elements from soy sauce samples.

5. CONCLUSION

In this paper, four soy sauce samples were chosen to establish PCR procedures for detecting common transgenic elements, the CaMV35S promoter, the NOS terminator and the Cp4-EPSPS (herbicide resistant gene). They were successfully amplified by established PCR procedures. To our knowledge, this is the first report on the establishment of PCR procedures to detect common transgenic elements from soy sauce samples. This study also lays a good foundation for the development of the reagent kit for detecting common transgenic elements from soy sauce samples.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gachet E, Martin GG, Vigneau F, Meyer G. Detection of genetically modified organism (GMOs) by PCR: a brief review of methodology available, Trends in Food Science and Technology. 1990;9:380-388.
2. Lin HY, Chiueh LC, Shih YC. Detection of genetically modified soy beans and maize by the polymerase chain reaction method, Journal of Food and Drug Analysis. 2000;8(3):200-207.
3. Forte VT, Pinto AD, Martino C, Tantillo GM, Grasso G, Schena FP. A general multiplex-PCR assay for the general detection of genetically modified soya and maize, Food Control. 2004;16(6):535-539.
4. Hübner P, Waiblinger HU, Pietsch K, Brodmann P. Validation of PCR methods for quantitation of genetically modified plants in food, Journal of the Association of Official Analytical Chemists. 2001;84:1855-1864.
5. Wiseman G. State of the art and limitations of quantitative polymerase chain reaction, Journal of the Association of Official Analytical Chemists. 2002;85:1-5.
6. Meyer R, Jaccaud E. Detection of genetically modified soya in processed food products: development and validation of a PCR assay for the specific detection of glyphosate-tolerant soybeans. In: Proceedings of the EURO FOOD CHEM IX conference (Vol. 1), Interlaken, Switzerland, Event No. 220. 1997;23-28.
7. Lipp M, Brodmann P, Pietsch K, Pauwels J, Anklam E. IUPAC collaborative trial study of a method to detect genetically modified soybeans and maize in dried powder, Journal of the Association of Official Analytical Chemists. 1999;82:923-928.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=239&id=9&aid=1478