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Optical thin-film biochips for multiplex detection of eight allergens in food

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ABSTRACT

An innovative method for the rapid detection of food allergens is developed and validated. Here we reported the development of silicon-based optical thin-film biochip technology that simultaneously permits visible detection of eight food allergens including celery, almond, oat, sesame, mustard, lupine, walnut and hazelnut on the basis of two tetraplex PCR systems. The biochip detection time was about 30 min after PCR amplification. Briefly, the optical thin-film biochip detects the presence of PCR fragment targets by enzymatically converting the formation of nucleic acid hybrids to molecular thin films. The mass contributed by the thin film alters the interference pattern of light on the biochip surface, resulting in a visible color change on the chip surface. Therefore, this assay permits sensitive, specific and high-throughput detection of allergens in food samples.

1. Introduction

Allergens in food have become an emerging public health issue affecting around 4% of the general population and 8% of infants and children population respectively in recent years (Helm & Burks, 2000; Sicherer, Muñoz-Furlong, & Sampson, 2004). In order to protect the health and safety of these allergic people, ingredients that cause allergic reactions must be labeled on retail foods. Currently, the United States, the European Union, Canada, Australia, New Zealand, Japan, Korea and Hong Kong have promulgated legislations related to food allergen labeling for retail food samples. Thus, efficiency and appropriate methods are required for detecting traces of allergenic ingredients in processed food. Currently, immunoassays offer the necessary sensitivity and specificity for detecting traces of protein sources in foods (Chambers, Lodi, Nicoletti, & Brett, 2004; Monaghan et al., 2008; Roux, Teuber, Robotham, & Sathe, 2001; Scheibe, Weiss, Ruëff, Przybilla, & Görg, 2001; Wei, Sathe, Teuber, & Roux, 2003; Yamashita et al., 2001; Yeung & Collins, 1999). However, food processing methods like heat treatment or baking may cause modification, denaturation and degradation of food protein, so it is possible for some food that can lead to unsatisfied results with immunoassays. More recently, DNA-based assays as an indirect approach also has been used for allergen detection due to the higher stability than the proteins (Brzezinski, 2007; Ehlert, Hupfer, Demmel, Engel, & Busch, 2008; Hai et al., 2009; Köppel et al., 2001; Scaravelli, Brohée, Marchelli, & Hengel, 2008; Zeltner, Glomb, & Maede, 2009). Since there are many different kinds of allergens in food products, it is necessary to establish the multiple detection methods. Among them, microarray method is one of the high-throughput methods for simultaneously detecting multiple proteins or DNA targets (Arenkov et al., 2000; Fukushima et al., 2003; Sergeev, Volokhov, Chizhikov, & Rasooly, 2004). Optical thin-film chip is one of microarray methods and has been used for detecting food-borne pathogens and genetically modified organisms (GMO) (Bai et al., 2010, 2007; Jenison, Rihanek, & Polisky, 2001; Zhao, Bai, Huang, & Chen, 2008). Compared with traditional biochips, the procedure of optical thin-film biochip is easy and simple only heat denatures, and few minutes hybridization steps are needed. Moreover, the visualized signals are able to be caught by unaided eyes without any specific equipment because the biotinylated amplification products combining with aldehyde-labeled probes deposit on the thin-film surface and change the interference pattern of light on the biochip surface.

Here, we developed an optical thin-film microarray method on a silicon-based surface on the basis of two multiplex PCR systems for simultaneously detecting eight food allergens including celery, almond, oat, sesame, mustard, lupine, walnut and hazelnut. In general, this assay decreases the test cost, exhibits high sensitivity and specificity, and remains very competitive with the other methods.

2. Materials and methods

2.1. Materials

Nuts samples walnut (*Juglans regia*), almond (*Hodgsonia macrocarpa*), hazelnut (*Corylus* spp.), and seed samples sesame (*Sesamum indicum*), celery (*Apium graveolens*), oat (*Avena sativa*), mustard (*Sinapis alba*), lupine (*Lupinus polyphyllus*), and wheat (*Triticum aestivum*) were

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obtained from local markets in Beijing, China. To dehydrate, all nut and seed materials were baked or roasted at 60 °C for 48 h in the lab.

2.2. DNA template preparation

In order to improve the yield of DNA extraction, five samples with high fat (walnut, almond, sesame and hazelnut) were pretreated with a blender and defatted three times with 4 volumes of ice-cold acetone and then air-dried at room temperature for two days before start extraction. DNA extraction was performed according to a CTAB protocol (Scaravelli et al., 2008).One hundred grams of sample was ground to 70 mesh using an IKA®A11 basic (IKA, Germany). From the resulting homogenized sample, all DNA extractions were done in duplicates. Two portions of 100 mg each of the homogenized sample were used for DNA extraction. Clean instruments were used for each sample to prevent cross contamination. Each sample was mixed with 1.5 ml of CTAB extraction buffer (2% CTAB, 1.4 mol/l NaCl, 0.1 mol/l Tris/HCl, 20 mmol/l Na_2EDTA, pH 8.0) and 10 μl of proteinase K solution (20 mg/ml) in an 2 ml tube and incubated at 60 °C overnight. The samples were centrifuged for 10 min at 13,000 *g*; the supernatant was transferred to a new 2 ml tube, mixed with 750 μl of chloroform, vortexed and centrifuged again for 10 min at 13,000 *g*. The upper phase was transferred into a new 2 ml tube, and the volume of the solution was determined. Two volumes of precipitation buffer (0.5% CTAB, 0.04 mol/l NaCl, pH 8.0) were added and incubated for 60 min at room temperature without agitation. The samples were centrifuged for 15 min at 13,000 *g*, the supernatant was discarded and the pellets were redissolved in 350 μl of NaCl solution (1.2 mol/l), vortexed and transferred to a new 2 ml tube and mixed with 350 μl of chloroform. After centrifugation for 10 min at 13000 *g*, the upper phase was transferred into a new 1.5 ml tube; 0.8 volumes of isopropanol were added for nucleic acid precipitation. After incubation at room temperature for 20 min, the samples were centrifuged at 13,000 *g* for 10 min, and the supernatant was discarded. The pellet was washed with 500 μl of 70% ethanol and resolved in 100 μl of TE buffer (10 mmol/l Tris, 1 mmol/l Na_2EDTA, pH 8.0) for further use. The DNA was quantified with a DU® 640 Nucleic acid and Protein Analyzer (Beckman, Germany) and diluted to 100 ng/μl. DNA Ladder marker (50 bp) (TaKaRa, Japan) was used for calibration.

2.3. Specific primers and probes design and synthesis

Oligonucleotides were purchased from Invitrogen (Shanghai, China). The primers and probes were established in this work or were taken from published single PCR systems (for details, see Table 1). To increase the specificity and sensitivity of the method, the sequences of primers and probes of Pru du1 gene from almond, Avenin gene from oat, Sin a I gene from mustard, ITS (18S–26S) gene from lupine and Oleosin gene from hazelnut were designed according to the optimum principle of primer and probe design, using Oligo 6 Demo software. During the design, all primers and probes were successfully checked for relevant homologies by BLASTNr search within GenBank databases. The reverse primers for PCR were synthesized with biotin at their 5′ ends for subsequent detection. The probes have 10 deoxyadenosine residues that constitute a "spacer" with an aldehyde group modification at their 5′ ends for conjugating to amino groups on the chip surface, followed by about 30 nucleotides complementary to the corresponding target sequence.

2.4. Multiplex PCR amplification

In order to improve the detection efficiency of biochip, two tetraplex PCR systems were designed and named reaction *A* and reaction *B*, respectively. Reaction *A* simultaneously determines the contents of DNA from celery, almond, oat and sesame, whereas reaction *B* is included to specifically amplify mustard, lupine, walnut and hazelnut. To obtain the optimized conditions of multiplex PCR, the reaction conditions of different annealing temperatures (50 °C, 52 °C, 54 °C, 56 °C, 58 °C, and 60 °C) and final primer concentrations (40 nmol/l, 80 nmol/l, 160 nmol/l, 320 nmol/l, 640 nmol/l, and 1280 nmol/l) were designed, and the optimized condition were applied in the following experiments.

Table 1
Sequences of PCR primers and capture probes for the detection of eight food allergens.

Species	Target gene	Sequence	Size (bp)	Accession number	Reference
Mustard	Sin a I	F[a]: 5′TGAGTTTGATTTTGAAGACGATATGG3′ R[b]: 5′biotin-TGTTTAACGGCTTTGGATGCTC3′ P[c]: 5′ALD[d]-aaaaaaaaaCAGGGACCACAGCAGAGGCCACC3′	147	S54101	This study
Lupine	ITS (18S–26S)	F: 5′CCTCACAAGCAGTGCGA3′ R: 5′biotin-TTGTTATTAGGCCAGGAGGA3′ P: 5′ALD-aaaaaaaaaaCGTGAATTTGTTTTACTACTCAGGGGTGGCT3'	129	GU058035	This study
Walnut	Jug r 2	F: 5′CGCGCAGAGAAAGCAGAG3′ R: 5′biotin-GACTCATGTCTCGACCTAATGCT3′ P: 5'ALD-aaaaaaaaaaGGGAAGAGGAGCAACAGAGGCACAA3′	91	AF066055	Hai et al. (2009)
Hazelnut	Oleosin	F: 5′CCCCGCTGTTTGTGATAT3′ R: 5′biotin-ATGATAATAAGCGATACTGTGAT3′ P: 5′ALD-aaaaaaaaaaTCCCGTTCTCGTCCCTGCGGT3′	67	AY224599	This study
Celery	Mtd	F: 5′TTTGATCCACCGACTTACAGCC3′ R: 5′biotin-ACAGATAACGCTGACTCATCAC3′ P: 5′ALD-aaaaaaaaaaATTACATGCTGAGTCACGATGAGCGTGTACTG3′	151	AF067082	Mustorp, Engdahl-Axelsson, Svensson, and Holck (2008)
Almond	Pru du1	F: 5′TTTGGTTGAAGGAGATGCTC3′ R: 5′biotin-TAGTTGCTGGTGCTCTTTATG3′ P:5′ALD-aaaaaaaaaaGTTGGTGGCATCTGCTGATGGA3′	108	EU424251	This study
Oat	Avenin	F: 5′CGGCGATGTGCGATGTATACG3′ R: 5′biotin-AGCCCTTGTAGTGTTCTTAGAAGC3′ P: 5′ALD-aaaaaaaaaaCCCACCGCACTGCCCTGTCGC3′	84	DQ370180	This study
Sesame	2S albumin mRNA	F: 5′CCAGAGGGCTAGGGACCTTC3′ R: 5′biotin-CTCGGAATTGGCATTGCTG3' P: 5′ALD-aaaaaaaaaaTCGCAGGTGCAACATGCGACC3′	62	FJ222625	Ma, Cao, Gao, and Ba (2009)

[a] Forward PCR primer.
[b] Reverse PCR primer.
[c] Probe.
[d] ALD, aldehyde modification.