Nucleic acid from beans extracted by ethanediamine magnetic particles

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Abstract Ethanediamine magnetite nanoparticles (EDAMPs) were used as adsorbents to isolate genomic DNA from various bean-species. A “single-pot” preparation of EDAMPs was described. Further characterization, including transmission electronic microscopy (TEM), scanning electronic microscopy (SEM), thermo-gravimetric analysis (TGA), X-ray powder diffraction (XRD) and Fourier Transform Infrared Spectrophotometer (FT-IR) were used to demonstrate the efficiency of this simple and general identification method. The EDAMPs provided excellent yields of genomic DNA. The isolated DNA was suitable for use in further applications by the Polymerase Chain Reaction (PCR) for the detection of Genetically Modified (GM) food and non-GM food. The EDAMPs are effective for bioseparation applications.

Keywords Genomic DNA · Electrophoresis · Beans · Ethanediamine magnetite nanoparticles

Introduction

The last two decades have seen considerable research and development efforts dedicated to new food technology, including the appearance of numerous genetically modified

(GM) crops (Kamle and Ali 2013). Their use in large-scale farm production in the past decade has created a demand for laboratory techniques that can reliably and consistently track plant genes and transgenes in the environment. Since GM crops find increasing use and applications, a more stringent quality control of foodstuffs is required. Consumers’ reactions to new food technologies are not a one-dimensional relationship, but involve several aspects such as risk-benefit perceptions, knowledge and information, trust and socio-demographic attributes (Gaskell 1999; Rollin 2011; Rowe 2004; Szwacka et al. 2012).

In order to guarantee food safety, several analytical methods for identification were developed and these were classified into different groups which focus on DNA, proteins, or other specific analysis (Elsanhoty 2011). Among these techniques, the polymerase chain reaction (PCR) has been widely used (Abdullah 2006; Greiner 2005; Jinxia 2011; Rodríguez-Lázaro et al. 2013). The successful extraction method plays a very important role in detecting GM organisms, with characteristics of quality, adequate amount, integrity, and high purity being of paramount importance.

Magnetic particles possess unique properties such as high surface to volume ratio, and an ability to absorb specific compounds (Rümenapp 2012). These characteristics make them attractive for many applications, including targeted delivery of drugs (Alexiou 2005; Häfeli 2004; Lübke 2001) and genes (McBain 2008); purification of RNA and DNA (Chiang 2006; Shan 2011), as well as immobilizing enzymes and proteins (Liburdi 2012; Qin et al. 2012; Zhou et al. 2012).

In order to apply this new methodological framework, surface modification need to be considered carefully. Matsunaga et al. (Yoza et al. 2003a, b) developed a DNA extraction method using PAMAM modified bacteria magnetic particles which extracted DNA from fluid suspensions and blood. Zhang et al. (Zhang et al. 2012) adopted hydrophilic magnetic nanoparticles with various surface functional groups

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Table 1 Primer used for PCR analysis^a

Primer	Sequence(5'-3')	Gene	Amplified Fragment(bp)
9 F	ATGGTTTCTCTTGGCTATATGG	Chloroplast ndhB	1000
13R	GCATACGTTTCATGCTTGTGTTGAG		
Lec1	GTGCTACTGACCAGCAAGGCAAACTCAGCG	Lectin	118
Lec2	GAGGGTTTTGGGGTGCCGTTTTTCGTC AAC		
RRS-F	CCTTTAGGATTTTCAGCATCAGTGG	CP4-EPSPS	121
RRS-R	GACTTGTCGCCGGGAATG		
35S-1	GCTCCTACAAAT GCCATCATTG	CaMV35S promoter	195
35S-2	GATAGTGGGATTGTGCGTCAT		
NOS-1	GAATCCTGTTGCCGGTCTTG	NOS terminator	180
NOS-2	TTATCCTAGTTTGCGCGCTA		

^a(Timmerman-Vaughan et al. 2005), Dong et al. (2005) and Yin et al. (2009)

and zeta values to extract DNA molecules from a model mixture of cell fractures. Qie (Qie et al. 2012) et al. developed a novel “single-pot” preparation of PAMAM magnetite nanoparticles and applied in screening GM and non-GM food. Bai et al. (Bai et al. 2013) adopted amino-modified silica-coated magnetic nanoparticles to adsorb a trace amount of genomic DNA of one or more types of pathogens from milk extracts under the optimized conditions and combining with PCR identification, single or multiple target pathogens could be detected sensitively and rapidly.

In the present work, ethanediamine magnetite nanoparticles (EDAMPs) were applied for DNA extraction from different bean-species. The structure of EDAMPs was confirmed and characterized by mainly Fourier transform infrared analysis (FT-IR), transmission electron microscopy (TEM) and X-ray diffractometer (XRD). The research is thereafter extended to study their absorption characteristics, and demonstrates that EDAMPs can potentially extract a large variety of nucleic acids.

Fig. 1 X-ray diffraction patterns, comparison with standard iron oxide

Experimental section

Materials

Four different beans were purchased at a local market, including soybeans (*Glycine max*(L) Merrile), mung beans (*Vigna radiata*), adzuki beans (*Abrus precatorius*) and runner beans (*Phaseolus coccineus*). GM soybeans were provided by the Chinese Academy of Inspection and Quarantine. RNase-A was purchased from Sigma. The 1 kb DNA ladder and DL2000 were purchased from New England Biolabs and Beijing QXTD-Biotechnology Go. Ltd, respectively. PCR primers, with sequences listed in Table 1, were synthesized by Sunbiotech (Beijing, China). All other chemicals and reagents were of analytical grade and obtained from the Beijing Chemical Reagent Factory.

Procedures for synthesis of EDAMPs

We applied a typical “single-pot” synthesis method (Deng et al. 2005). Firstly, 3.7 mmol ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, ~1.00 g) was added to 20 ml ethylene glycol (EG) at room temperature to form a clear solution by means of ultrasonication (40 KHz, 400 W). Secondly, 24.2 mmol anhydrous sodium acetate

Fig. 2 TEM (a) and SEM (b) images of EDAMPs, Bar = 100 nm

Fig. 3 The FT-IR analysis of the EDAMPs

(NaAc, ~2.00 g) was decanted into the previous mixture. Thirdly, 5 ml ethanediamine (EDA) was carefully added into the suspension; and the suspension was tightly sealed into a Teflon-lined autoclave with another 20 ml EG, heated at 200 °C for 24 h. The black or gray particles were separated under the effect of an external magnet, rinsed with de-ionized water in three cycles of centrifugation, washing and re-dispersion, and oven-dried at 60 °C for 4 h.

Isolation of genomic DNA (Taylor et al. 2000)

Different beans were smashed into powder and 30 mg of each was placed in an eppendorf tube and re-suspended by vortexing (2,800 rpm) in 0.1 M Tris–HCl buffer (pH 8.0) containing 0.05 M EDTA and 0.5 M NaCl (400 µl). Sodium dodecylsulfate (10 % (w/v), 16 µl) and RNase A (10 mg/ml,

Fig. 4 The TG characterization of the EDAMPs

Fig. 5 The differential TG analysis of the EDAMPs

89 µl) were subsequently added, and the sample was vortexed (2,800 rpm) and incubated at 65 °C for 10 min. The tube was thereafter placed on ice for 5 min.

5 M potassium acetate (13.5 µl) was added and the sample was vortexed (2,800 rpm) again. The tube was placed on ice for a further 20 min and the resulting suspension was centrifuged at 12,000 rpm for 20 min at 4 °C. The supernatant was removed and transferred to a fresh 1.5 ml microcentrifuge tube and an equal volume of binding buffer containing 20 % (w/v) polyethylene glycol-8000 and 2 M NaCl. Finally, 30 mg EDAMPs were added, after washing in PBS (pH 8.0).

The suspension was vortexed (2,800 rpm), then shaken on orbital mixer incubator at 110 rpm for 15 min and kept still for 10 min. The supernatant was discarded and the DNA absorbed particles were washed twice with binding buffer and 70 % ethanol. EDAMPs were dried in ambient air, and the DNA absorbed on the EDAMPs was eluted by TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0).

Fig. 6 1% agarose gel electrophoresis of amplified fragments corresponding to chloroplast *ndhB* gene: Lane 1, 1 Kb DNA marker; Lane 2, soybeans; Lane 3, mung beans; Lane 4, adzuki beans; Lane 5, runner beans; Lane 6, GM soybean (GTS-4032)

Fig. 7 2% agarose gel electrophoresis of PCR products corresponding to lectin gene: *Lane 1*, 50 bp DNA marker; *Lane 2*, soybeans; *Lane 3*, mung beans; *Lane 4*, adzuki beans; *Lane 5*, runner beans; *Lane 6*, GM soybean (GTS-4032)

Characterization of EDAMPs

Several characterization methods were applied, including:

Transmission electron microscopy (TEM-Hitachi H-800): a 3 μ l sample from the reaction batch was placed on a microporous carbon-coated copper TEM grid. Specimens were allowed to air-dry and were imaged at 200 kV.

Scanning electron microscopy (SEM-Zeiss SUPRA 55): the samples were first suspended in ethanol, and then poured onto a silica wafer previously treated with methanol, acetone and isopropanol before use. Specimen were air-dried and imaged.

Powder X-ray diffraction (XRD) data were taken on a D/max-Ultima (Rigaku) using Cu K_{α} radiation (40 kV, 30 mA, $\lambda=1.5418$ Å). The samples, as un-oriented powders, were scanned in steps of 0.02° (2θ) in the range $3-70^{\circ}$ using a count time of 4 s per step.

The *Zeta potential* (surface charge) of the samples was measured by Zetasizer (Malvern Instruments) after dispersing the solid samples in de-ionized water, while sonicating for over 10 min to form a transparent dispersion.

FTIR spectra (Varian 3100 FT-IR) were determined to study the surface composition of the nanoparticles in the

Fig. 9 2% agarose gel electrophoresis of PCR products corresponding to CaMV35S promoter gene: *Lane 1*, 50 bp DNA marker; *Lane 2*, soybeans; *Lane 3*, mung beans; *Lane 4*, adzuki beans; *Lane 5*, runner beans; *Lane 6*, GM soybean (GTS-4032)

$400-4,000$ cm^{-1} spectral range at a resolution of 2 cm^{-1} . The hydrophilic EDAMPs were mixed with KBr powder, then milled and compacted into thin disk-shaped pellets.

The thermal behavior of EDAMPs was determined by *thermogravimetry*, using a Thermo plus TG8120 (Rigaku), with Ar as carrier gas, and at a temperature ramp of $10^{\circ}\text{C}/\text{min}$ from room temperature to 800°C . They were allowed to regain room temperature by natural cooling.

Polymerase chain reaction (PCR) conditions and gel electrophoresis

The PCR was performed using a DNA Engine (PTC-200 Peltier Thermal Cycler, MJ Research, Waltham, MA). Amplifications were carried out with a final volume of 20 μ l containing 2.5 mM dNTPs, EasyTaq 10 $\times$ buffer, 10 μ mol/L of each primer. The DNA templates of the samples and 5 units/ μ l EasyTaq DNA Polymerase. PCR products were analyzed together with a molecular weight ladder (1 kb DNA ladder, DL2000) by electrophoresis on a 1–2 % agarose gel containing DNAGREEN which was diluted 20 times with de-ionized water.

Fig. 8 2% agarose gel electrophoresis of PCR products corresponding to CP4-EPSPS gene: *Lane 1*, 50 bp DNA marker; *Lane 2*, soybeans; *Lane 3*, mung beans; *Lane 4*, adzuki beans; *Lane 5*, runner beans; *Lane 6*, GM soybean (GTS-4032)

Fig. 10 2% agarose gel electrophoresis of PCR products corresponding to NOS terminator gene: *Lane 1*, 50 bp DNA marker; *Lane 2*, soybeans; *Lane 3*, mung beans; *Lane 4*, adzuki beans; *Lane 5*, runner beans; *Lane 6*, GM soybean (GTS-4032)

Results and discussion

Analysis of EDAMPs

The crystalline structure and phase purity were determined by power X-ray diffraction. As shown in Fig. 1, the sharp and strong peaks confirm that the products were well crystallized and presented the typical cubic iron oxide Fe_3O_4 (JCPDS, # 65–3107), which is indexed to (220), (311), (400), (422), (511) and (440) from left to the right. XRD hence confirms that the iron oxide remained as $\gamma\text{-Fe}_3\text{O}_4$ and did not form neither an iron salt, nor was subject to a change of the oxidation state of iron under the reaction conditions. The size and morphology of EDAMPs were studied by TEM and SEM. Figure 2a shows the typical TEM images of EDAMPs which indicate that the average diameter is between 150 nm and 200 nm, and the surface of the particles is well modified by EDA. Zeta potential measurement, as described further in the text, strongly supported that the magnetic nanoparticles are monodisperse and positively charged (zeta potential is 14.8 mV). SEM images of Fig. 2b coincide well with TEM images, which show that the surface morphology of particles corresponds to rough particles instead of smooth spheres, which can benefit bimolecular conjugation and bioseparation.

The IR spectroscopy was performed to reveal evidence of the modifying reagent present on the nanocrystalline surface. Figure 3 shows a typical IR spectrum for EDAMPs. Strong bands around 590 cm^{-1} correspond to the Fe–O stretching modes of the magnetite lattice (Roca et al. 2007). The intense band between $3,400$ and $3,500\text{ cm}^{-1}$ indicates the stretching vibration of free N–H. Bands around $1,576\text{ cm}^{-1}$ correspond to N–H bending vibration. A series of bands around $1,000$ – $1,200\text{ cm}^{-1}$ corresponds to the stretching vibration of the C–C bond. These results indicate that EDAMPs were successfully bound to Fe_3O_4 magnetite nanoparticles.

The TGA and DTGA profiles (Figs. 4 and 5) of EDAMPs further demonstrated that organics had been successfully bound onto the magnetic iron oxide nanoparticles, and mainly consisted of two typical stages, corresponding to 200°C and 600°C . The first sharp weight loss under high temperature probably corresponds to the loss of NH_3 converted from organics, the final loss may be due to the carbon-thermal reduction to form $\alpha\text{-Fe}$ or $\gamma\text{-Fe}$ (Hoch et al. 2008).

From the assessment by the different analytical techniques, it can be concluded that the “single-pot” preparation of EDAMPs is successful and provides a stable modified method with interesting properties and behavior.

Application of EDAMPs

The resulting EDAMPs were applied for DNA extraction from different beans including GM soybeans and other beans bought from market. The DNA templates extracted from beans

displayed high purity of the isolated DNA by UV absorbance ($A_{260}/A_{280}=1.7745$). Genomic DNA extracted from food-stuffs could not be detected directly by spectrometry or electrophoresis due to low DNA contents and high degradation of DNA during productive processes.

PCR of chloroplast sequences was employed as a functional test for the magnetic particles. As shown in Fig. 6, the amplicated products of 1,000 bp connecting to chloroplast *ndhB* gene were observed in all the samples tested.

Lecithine is also one of the housekeeping genes of soybean. The primer pairs, LEC-1 and LEC-2 (118 bp product), targeting the endogenous lecithine gene, were used to confirm the presence of amplifiable soy DNA. The expected fragment was observed in all the samples (Fig. 7).

The primer pairs, RRS-F and RRS-R, targeting the CP4-EPSPS gene were used to detect the presence of GM beans. After the PCR, the amplications of 121 bp was detected for the GM soybean, while none was present in the non-GM beans (Fig. 8). Meanwhile, the primer pairs for transgenes, CaMV35S promoter and NOS terminator were also viewed for the GM soybean and none in the non-GM beans (Figs. 9 and 10).

Conclusions

The present work applied a straightforward “single-pot” preparation method of EDAMPs. Different analytical methods were used to characterize the EDAMPs, thus confirming their stable structure and excellent properties.

The EDAMPs were thereafter applied in the adsorption and purification of DNA from various beans, and an appropriate gel-electrophoretic distinction between GM- and non-GM food was evidenced. This method is useful and important in detecting GM- and non-GM in the future.

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