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Solid-phase DNA isolation from food matrices using hydrophilic magnetic microspheres

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ABSTRACT

Dynamic light scattering (DLS) was used to monitor the coil–globule transition of calf thymus and bacterial DNAs by poly(ethylene glycol) (PEG 600 and PEG 6000) in aqueous NaCl solutions. The contribution of PEG 600 and PEG 6000 to DNA coil diameter change was investigated. Compaction of DNA molecules was observed for a PEG 6000 concentration ranging from 5.5 to 8%. Hydrophilic non-porous poly(2-hydroxyethyl methacrylate-*co*-glycidyl methacrylate) – P(HEMA-*co*-GMA), poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) – P(HEMA-*co*-EDMA), and poly(glycidyl methacrylate) (PGMA) microspheres containing carboxyl groups were used for DNA isolation. The highest DNA yield was achieved using 16% PEG 6000 and 2.0 M NaCl. The amount of isolated DNA correlated with the content of carboxyl groups on the microsphere surface. RNA adsorption on the surface of the microspheres tested was also studied. RNA recovery was more than one order lower than DNA recovery under the same conditions. All types of microspheres were tested for DNA isolation from crude cell lysates of different dairy products in solutions of 16% PEG 6000 and 2.0 M NaCl. Isolated DNAs were without PCR inhibitors, which was demonstrated using real-time PCR including melting analysis of the amplicons.

Keywords: DNA compaction; Magnetic microspheres; DNA isolation; RNA; Food matrices; Polymerase chain reaction (PCR)

1. Introduction

The polymerase chain reaction (PCR) has become a powerful tool for amplification and detection of nucleic acids from a number of real samples. The presence of interfering components in DNA isolated from real samples decreased PCR sensitivity, or led to false-negative results (Wilson, 1997; Al-Soud and Råndström, 1998; Kemp et al., 2006; Schrader et al., 2012). Pre-PCR processing procedures have been developed to reduce the effects of PCR inhibitors. The conventional DNA isolation using phenol and precipitation by ethanol (Sambrook and Russel, 2001) works with toxic organic solvents and is time-consuming. Different magnetically driven DNA separation techniques using micro- and nano-sized magnetic carriers have been used to speed up and facilitate DNA separation, purification and concentration procedures (Bruce et al., 2004; Aguilar-Arteaga et al., 2010). Magnetic microspheres in an appropriate buffer system can be used for nucleic acid extraction directly from crude cell lysates (He et al., 2013; Li et al., 2013; Ma et al., 2013; Rittich and Španová, 2013; Tang et al., 2013).

Duplex DNA carries two univalent negative charges per base pair at most pH values of the solution. The DNA–silica interaction is electrostatically impossible, since DNA and the silica surface are both negatively charged (Vandeventer et al., 2012) under usual experimental conditions. Silica-coated particles are required to be modified with functional

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Table 1 – The characteristics of magnetic microspheres.

Microsphere	Fe content (%)	COOH content (mM g^{-1})	Diameter (μm)	PDI[a]
P(HEMA-*co*-GMA) (A)	6.6	2.61	1.0	1.05
P(HEMA-*co*-GMA) (B)	10.0	0.76	2.2	1.09
P(HEMA-*co*-EDMA)	6.6	0.85	1.5	1.07
PGMA (A)	5.4	0.67	0.7	1.16
PGMA (B)	6.0	0.42	0.7	1.07
PGMA (C)	2.1	0.41	0.7	–

[a] PDI – polydispersity index (ratio of weight-to-number average particle diameter).

substituents such as amino groups for DNA coupling (Kang et al., 2009). Unmodified single-stranded oligonucleotide (ssDNA) can adsorb efficiently on negatively charged carboxylic acid-functionalised magnetic nanoparticles, whereas double-stranded oligonucleotide (dsDNA) does not possess this adsorbing property (Liang et al., 2012). Complexation of DNA with negatively charged sorbents can be achieved by an induced condensation mechanism (Yoshikawa, 2001) using a polymer (e.g., poly(ethylene glycol) – PEG) and monovalent salt (e.g., NaCl). The mechanism of DNA transition was discussed in previous papers (Vasilevskaya et al., 1995; Kleideiter and Nordmeier, 1999a,b).

Carboxyl-functionalised magnetic microspheres and a solution containing PEG 6000 (or PEG 8000) and NaCl were previously used for the isolation of short DNA fragments (Hawkins et al., 1994; de Angelis et al., 1995). Carboxyl group-containing hydrophilic magnetic P(HEMA-*co*-GMA) and PGMA microspheres were also used for the isolation of high-molecular-weight bacterial DNA from different complex samples containing PCR inhibitors (Špánová et al., 2006; Rittich et al., 2009; Trachtová et al., 2012). However, the mechanism of DNA interaction with the particle surface has not yet been fully understood (Eskilsson et al., 2001).

The aim of this work was to evaluate five different types of carboxyl-functionalised magnetic microspheres for the isolation of DNA from different food matrices containing PCR inhibitors. The quality of isolated DNA and the elimination of PCR inhibitors were examined by a quantitative real-time PCR (qPCR).

2. Methods

2.1. Chemicals and equipment

Calf thymus DNA and RNA from Sigma (USA) were used for evaluation of nucleic acid recovery. Agarose was purchased from Serva (Germany), ethidium bromide from Sigma. The PCR primers were synthesised by Generi Biotech (Czech Republic); *Taq*1.1 polymerase was from Top-Bio (Czech Republic). Poly(ethylene glycol) 600 and 6000 (M_w = 600 and 6000; PEG 600 and 6000) were purchased from Sigma. The real-time PCR was performed using the SYBR Green-q-PCR kit (Top-Bio, Czech Republic). Magnetic particles were separated on a Dynal MPC-M magnetic particle concentrator (Norway). DNA was amplified in a DNA thermal cycler Rotorgene 6000 (Corbett Research, Australia). Agarose gel electrophoreses were carried out using an electrophoresis unit (Bio-Rad, USA). The PCR products were visualised on an UltraLum EB-20E UV transilluminator (Paramount, USA) at 305 nm and photographed with a digital camera. A UV/Vis NanoPhotometer (Implen, Germany) was used for UV spectrophotometry. The particle size distribution of collapsed DNA was measured on a Zetasizer Nano ZS instrument, used Dispersion Technology Software v. 5.03 (Malvern Instruments, Worcestershire, UK) for the calculation.

2.2. Preparation of microspheres

Magnetic microspheres with different contents of COOH groups were used (Table 1). Poly(2-hydroxyethyl methacrylate-*co*-glycidyl methacrylate) – P(HEMA-*co*-GMA), poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) – P(HEMA-*co*-EDMA), and poly(glycidyl methacrylate) – PGMA microspheres were prepared by single-step dispersion polymerisation in the presence of sterically or electrostatically stabilised colloidal magnetite or maghemite (Horák et al., 2003, 2005). The hydroxy groups of the microspheres were oxidised with a 2% aqueous solution of potassium permanganate under acidic conditions (2 mol L^{-1} sulphuric acid) (Horák et al., 2005). The content of carboxyl groups in the microspheres was determined by titration using 0.1 mol L^{-1} NaOH on a 799 GPT Titrino titrator (Metrohm, Switzerland) after ion exchange with a 10% aqueous solution. Magnetic glass particles (5 μm) (MPG, USA) were used as a control for elution of nucleic acids from the particle surfaces.

2.3. Food samples and microorganisms

Food products, such as yoghurts, probiotic dairy drinks and instant complete initial food, were obtained from the market. The strain *Lactobacillus paracasei* ssp. *paracasei* CCDM 212 obtained from the Culture Collection of Dairy Microorganisms (CCDM, Czech Republic) and cultivated in MRS medium at 37 °C was used for DNA isolation and preparation of standard curves in real-time PCR as a positive control according to the procedure described (Trachtová et al., 2011). Calf thymus DNA and RNA from commercial sources were dissolved in TE buffer (10 mM Tris–HCl, 1 mM EDTA; pH 7.8; 1 mg mL^{-1}).

2.4. Preparation of crude cell lysates and DNA isolation from food samples

The food products (1 g in 1 ml of sterile water) or 1 ml of bacterial culture were separated by centrifugation (10,000 × *g* for 5 min), washed with buffer (10 mM Tris–HCl, pH 7.8; 5 mM EDTA, pH 8.0), and the cells were lysed in 500 μL of lysis buffer (10 mM Tris–HCl, pH 7.8; 5 mM EDTA, pH 8.0, and lysozyme 5 mg mL^{-1}). After 1 h of incubation at laboratory temperature, 10 μL of proteinase K (10 μg mL^{-1}) and 50 μL of SDS (20%) aqueous solutions were added and the mixture was incubated at 55 °C for 24 h.

DNA was extracted from the crude cell lysates of food products using two different procedures: by phenol extraction (control) according to the literature (Sambrook and Russel,