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Isolation of genomic DNA using magnetic cobalt ferrite and silica particles

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Abstract

Adsorption separation techniques as an alternative to laborious traditional methods (e.g., based on phenol extraction procedure) have been applied for DNA purification. In this work we used two types of particles: silica and cobalt ferrite (unmodified or modified with a reagent containing weakly basic aminoethyl groups, aminophenyl groups, or alginic acid). DNA from chicken erythrocytes and DNA isolated from bacteria *Lactococcus lactis* were used for testing of adsorption/desorption properties of particles. The cobalt ferrite particles modified with different reagents were used for isolation of PCR-ready bacterial DNA from different dairy products.

Keywords: Silica particles; Cobalt ferrite particles; DNA; Polymerase chain reaction

1. Introduction

In DNA analysis and molecular diagnostics, DNA amplification by the polymerase chain reaction (PCR) or by related methods has been widely applied [1,2]. However, many organic and inorganic compounds inhibit the PCR course [3,4]. Great attention has therefore been paid to the development of fast, cost-effective and robust isolation methods for DNA analysis. Alternative separation techniques have been developed besides the laborious and time-consuming traditional methods (e.g., based on phenol extraction procedure). Thus, sorption procedures based on ion exchange, affinity, hydrophobic and size exclusion mechanisms were used for DNA purification. Solid phase systems which adsorb DNA – silica-based particles [5–8], glass fibres [9], anion-exchange carriers [10–12], and modified magnetic beads [13,14] – have been used for DNA purification. The advantage of such DNA binding is minimisation of DNA degradation during its purification. A key aspect of solid phase system development is the possibility of automating and miniaturising the process of DNA isolation and manipulation.

An alternative suitable method for both chromosomal and plasmid DNA separation from cell homogenates is adsorption of DNA to silica induced by chaotropic salts [8,9]. The binding step is carried out in 4 M sodium iodide [15] or sodium chloride [16] solution. Further progress is provided by application of the magnetically responsive particles in separation technology for the isolation of plasmid and genomic DNA [17,18]. The magnetic component is employed not only for magnetic separation of support from the reaction mixture but magnetite alone as solid adsorbent. This interaction was described already earlier [18]; DNA was adsorbed to the particles under high sodium chloride and PEG concentrations and recovered in water. These conditions are similar to those used for silica and silica–magnetite systems. Even though cobalt ferrite ($CoO{\cdot}Fe_2O_3$) particles have been intensively investigated in recent years for their magnetic properties [19–22], their application for nucleic acid separation was not published.

In this work we used two types of particles: either cobalt ferrite (unmodified or modified with a reagent containing weakly basic aminoethyl, aminophenyl groups; and/or with alginic acid—natural carboxylic polysaccharide), or silica. We describe the development of a methodology for reproducible adsorption and recovery of high-molecular mass

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DNA. The methodology developed was used for PCR-ready isolation of bacterial DNA in dairy products.

2. Materials and methods

2.1. Chemicals

DNA (Na salt) from chicken erythrocytes was from Reanal (Budapest, Hungary) or isolated from bacterial cells of *Lactococcus lactis* (see Section 2.3.2). Agarose was purchased from Top-Bio (Prague, Czech Republic), ethidium bromide from Sigma (St. Louis, USA). Silica microparticles were taken from Top-Bio (Prague, Czech Republic). Cobalt ferrite particles were prepared in the Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic (Prague, Czech Republic), see Section 2.3.1.

PCR primers specific to the species *L. lactis* [23], genus *Lactobacillus* [24], and genus *Bifidobacterium* [25] were synthesised by Generi-Biotech (Hradec Králové, Czech Republic); Taq 1.1. polymerase was from Top-Bio (Prague, Czech Republic), and DNA marker 100–1500 bp for gel electrophoresis was from Malamité (Moravské Prusy, Czech Republic). Other chemicals and solvents were of analytical grade and were taken from commercial sources.

2.2. Equipment

Spectrophotometric measurements were carried out on a UV spectrophotometer DMS 100 (Varian, Mulgrave, Australia). Magnetic particles were separated using an MPC-M magnetic particle concentrator, Dynal (Oslo, Norway). The PCR reaction mixture was amplified on an MJ Research Programme Cycler PTC-100 (Watertown, USA). Agarose gel electrophoresis was carried out using a 3000 Xi power supply (Bio-Rad Lab., Richmond, USA). PCR products were visualised on a UV transilluminator EB-20E from UltraLum (Paramount, USA), and photographed with a CD 34 Polaroid Camera (Polaroid, Cambridge, USA).

2.3. Methods

2.3.1. Preparation of carriers

Two types of particles were used in this study: either silica (standard) or cobalt ferrite. Unmodified cobalt ferrite A-101 was prepared by a modified method according to [26] using Co^{2+} instead of Fe^{2+} and sodium hydroxide instead of ammonia. A-110 is cobalt ferrite containing weakly basic aminoethyl groups (0.3 mM/g dry substance), A-109 is cobalt ferrite containing weakly basic aminophenyl groups (0.1 mM/g dry substance). They were prepared by coprecipitation of Fe^{3+} and Co^{2+} in the presence of bifunctional reagent containing a chelating group for binding to metal ions with free binding sites at the surface of oxide and functional groups for further interactions and modifications (aminoethyl, aminophenyl). A-112 is cobalt ferrite modified with alginic acid (natural carboxylic polysaccharide). Precipitate contains 7% alginic acid (0.1 mM –COOH/g dry substance). It was prepared by analogy with previous A-109 and A-110 by coprecipitation of cobalt ferrite with alginic acid. Nanoparticles of A-101, A-109 and A-110 are aggregates of 10–20 nm particles bound together by physical forces and give stable colloid solutions after ultrasound treatment; A-112 are microparticles (mean diameter 10 μm). Fig. 1 shows scanning electron micrograph typical precipitate of cobalt ferrite nanoparticles. Scanning electron micrograph of cobalt ferrite particle modified with alginic acid is given in Fig. 2.

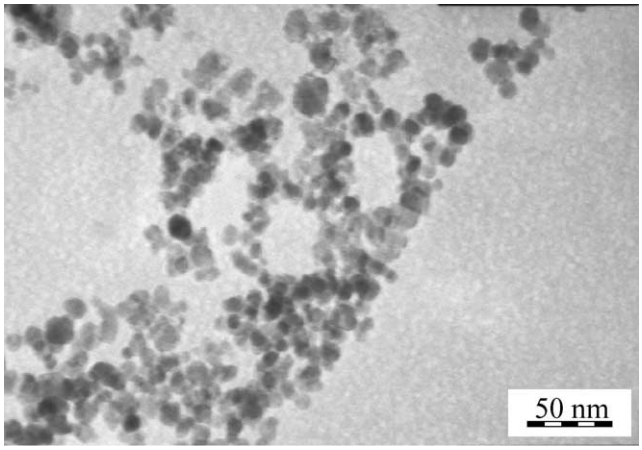

Fig. 1. Scanning electron micrograph of cobalt ferrite nanoparticles (aggregates) (A-101).

2.3.2. DNA reversible adsorption

An appropriate amount of DNA from chicken erythrocytes (1 mg/ml) and DNA isolated from *L. lactis* cells (0.28 mg/ml) in TE buffer (30 μl), 40% PEG 6000 and 5 M NaCl solutions were mixed with 60 μl of particles (total volume 0.66 ml) and kept at laboratory temperature for 10 min, followed by mag-

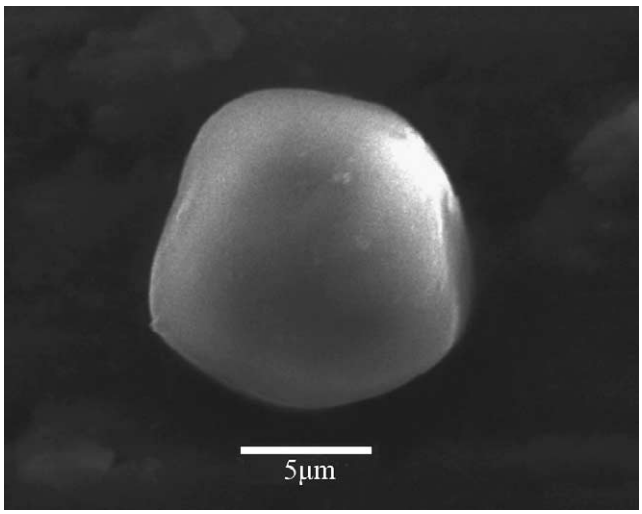

Fig. 2. Scanning electron micrograph of cobalt ferrite particles modified with alginic acid—natural carboxylic polysaccharide (A-112).

netic separation of particles. Silica particles were removed by centrifugation at 14 000 × *g*/min for 1 min. The NaCl concentration ranged between 0.5 and 3.0 M, PEG 6000 concentration ranged between 2 and 12% (reached to the whole sample volume). DNA elution from particles was carried out by TE buffer or sterile water and incubation with gentle agitation at laboratory or 55 °C temperature, respectively, for 5 min. The total volume of the eluted solution was 660 μl. UV absorption was measured at 260 nm. The amount of reversible adsorbed DNA was estimated from the difference of DNA absorbance before its adsorption and after its elution in the same buffer. The presence of eluted DNA in eluates was checked using agarose gel electrophoresis, too.

2.3.3. Cell cultivation and lysis

Bacterial cells of *L. lactis* (obtained from the Czech Collection of Microorganisms, CCM, Brno, Czech Republic) were grown overnight at 30 °C in MRS broth (Oxoid, UK) with 0.5% glucose. Altogether 1 ml of cells (OD_{600} = 0.75) was washed and resuspended in 100 μl of lysis buffer (10 mM Tris–HCl, pH 7.8, 5 mM EDTA, pH 8.0, lysozyme 0.3 mg/ml). Crude cell lysates were prepared using 12.5 μl of 20% SDS, 10 μl of proteinase K (1 mg/ml) and incubation at 57 °C for 2 h. DNA was extracted from crude cell lysates using the phenol extraction method [27], or it was separated using the tested carriers. The identity of nucleic acids was confirmed by gel electrophoresis and UV spectrophotometry. The ratio $A_{260\,nm}/A_{280\,nm}$ was used as a test of nucleic acid purity [28].

2.3.4. DNA isolation from crude cell lysates using silica and magnetic particles

A total of 50 μl of crude cell lysates, 10 μl of particles (10 mg/ml) and 50 μl of hybridisation buffer (20 wt.% PEG, 2.5 M NaCl) were mixed and incubated for 10 min at laboratory temperature. The particles with adsorbed DNA were then separated using a magnetic separator for 5 min, the supernatant was discarded, and the particles were washed with 500 μl of 70% ethanol and dried shortly. DNA captured to the particles was eluted into 50 μl of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.8). DNA in the eluate (1 μl) was used as DNA matrix in PCR amplification and for agarose gel electrophoresis.

2.3.5. PCR amplification and detection of PCR products

Bacterial DNA purified by phenol extraction [27] or DNA separated using silica and cobalt ferrite nanoparticles was used as DNA matrix in PCR. PCR was performed using PALA4 and PALA14 primers specific to the species *L. lactis* [23], R16-1, and LbLMA1-rev primers specific to the genus *Lactobacillus* [24], PbiF1 and PbiR2 primers specific to the genus *Bifidobacterium* [25], which enabled amplification of 1131, 250 or 914 bp long DNA fragments, respectively. Typically, the PCR mixture contained 1 μl of each 10 mM dNTP, 1 μl (10 pmol/μl) of each primer, 1 μl of DNA matrix, and 0.5 μl of Taq 1.1 polymerase (1 U/μl), 2.5 μl of buffer, and PCR water was added to a 25 μl volume. After 5 min of the initial denaturation period at 94 °C (hot start), amplification was carried out in 30 cycles of 60 s at 94 °C; 60 s at 45 °C for *Lactococcus*; 60 s at 55 °C for *Lactobacillus*; 60 s at 50 °C for *Bifidobacterium*, and 120 s at 72 °C. In the last cycle, the elongation step at 72 °C was prolonged to 10 min. PCR products were detected using agarose gel electrophoresis in 1.2 or 1.8% agarose gel in TBE buffer (45 mM boric acid, 45 mM Tris–base, 1 mM EDTA, pH 8.0). DNA was stained using ethidium bromide (0.5 μg/ml), decolorised in water, and photographed at 305 nm UV light on a TT667 film. The lengths of amplified DNA fragments were calculated using the Anagel programme [29].

3. Results and discussion

Silica particles – either nonmagnetic or magnetic – were used earlier for isolation of PCR products and genomic DNAs [5–9,15,16]. Magnetite and other metal oxides were also applied in the isolation of plasmid DNA. For this reason, newly designed functionalised cobalt ferrite nanoparticles (unmodified or modified with different reagents) were used for isolation of high-molecular mass DNA [17,18]. Silica-based particles were used as standard procedure. The results are given in Table 1. Expressive adsorption was estimated with all tested particles. There is no information on the adsorption mechanism of duplex DNA on metal oxides under high PEG and sodium chloride concentration. The mechanism of DNA adsorption on silica particles was used for comparison of DNA adsorption on cobalt ferrite particles. According to [6], the adsorption of highly charged duplex DNA to hydrophilic negatively charged silica is controlled by three competing effects: weak electrostatic repulsion forces, dehydration and hydrogen bond formation. It is known that PEG adsorbs on silica surface through hydrogen-bonding with surface silanol groups [30]. Therefore competitive displacement of DNA from the surface with increasing PEG concentration can be expected. A decrease of the amount of adsorbed DNA was observed at higher PEG concentrations (8%) for all types of tested particles. This decrease was striking for silica and unmodified cobalt ferrite nanoparticles.

The mechanism of DNA interaction with particle surface can be more complicated as DNA is precipitated by PEG and different salts already in the absence of solid particles [27,31]. Large DNA molecules are present in low-salt concentration solutions as random coils. DNA changes its properties at critical PEG concentration. The phenomenon of collapse of DNA macromolecules in aqueous solutions of polyethylene glycol (PEG) was described in the literature [32–34]. DNA coils swell and condense to a compact, relatively dense state at critical PEG concentration. At PEG concentrations lower than the critical one, DNA molecules in the coil state do not deposit from the solution, do not sediment during centrifugation, and the UV absorbance does not change. The critical PEG concentration is a function of PEG molar mass and salt

Table 1
Adsorption of DNA from chicken erythrocytes using tested particles

Particles	NaCl (M)	Concentration of PEG 6000 (%, w/w)/relative DNA content					
		4		5	6	8	
		A	B	A	A	A	B
Silica	1.0	n.d.	0.24	0.90	0.87	0.30	0.88
	1.5	0.42	0.30	0.88	0.90	0.43	0.87
	2.0	0.73	0.32	0.85	0.94	0.43	0.95
	2.5	0.65	0.16	0.80	0.88	0.43	0.93
	3.0	0.49	0.17	0.80	0.50	0.43	0.89
Cobalt ferrite unmodified A-101	1.0	1.00	0.18	1.00	0.98	0.45	0.14
	1.5	1.00	0.17	1.00	0.99	0.28	0.15
	2.0	1.00	0.16	1.00	0.99	0.62	0.23
	2.5	1.00	0.13	1.00	0.99	0.40	0.34
	3.0	1.00	0.16	1.00	0.99	0.79	0.53
Cobalt ferrite aminoethyl A-109	1.0	0.45	0.09	0.75	0.73	0.57	0.23
	1.5	0.24	0.07	0.70	0.78	0.61	0.23
	2.0	0.63	0.07	0.86	0.78	0.53	0.50
	2.5	0.63	0.05	0.86	0.78	0.60	0.53
	3.0	0.89	0.06	0.84	0.79	0.58	0.50
Cobalt ferrite aminophenyl A-110	1.0	0.83	0.10	0.96	0.96	0.82	0.26
	1.5	0.87	0.12	0.99	0.99	0.81	0.25
	2.0	0.90	0.13	0.99	0.97	0.89	0.40
	2.5	0.88	0.10	0.97	0.96	0.83	0.53
	3.0	0.56	0.13	0.97	0.95	0.81	0.58
Cobalt ferrite alginic acid A-112	1.0	0.27	0.07	0.68	0.85	0.64	0.17
	1.5	0.49	0.07	0.84	0.90	0.62	0.16
	2.0	0.62	0.07	0.81	0.87	0.70	0.44
	2.5	0.60	0.07	0.85	0.85	0.69	0.47
	3.0	0.44	0.07	0.86	0.92	0.75	0.40

Experimental conditions were described in Section 2. A: adsorbed, B: eluted, n.d.: not determined.

concentration [34]. The value of the critical concentration is reciprocally proportional to the molar mass and according to the above-mentioned authors is equal to 57 g/l for PEG 6000.

The highest recovery of DNA was achieved at 55 °C using TE buffer as the elution agent (the elution systems tested are given in Section 2.3.2), and this buffer was used in all experiments (Table 1). The highest recovery was achieved at PEG concentrations ≥5% (i.e., for condensed DNA). Thus, the mechanism of DNA interactions with particle surface is probably different for condensed and non condensed DNA molecules. From the results given in Table 1 it can be deduced that the interaction between DNA and cobalt ferrite surface is very strong. It is in agreement with the known high affinity of P–O^- (in phosphate, phosphonate, etc.) to iron oxides [35,36]. These interactions were also described by authors [18].

False negative results are an important problem in PCR identification of bacterial cells in real specimens. DNA isolated from *L. lactis* cells was used for verification of DNA elution conditions due to practical applications of isolated DNA (identification of lactic acid bacteria with probiotic properties in dairy products). DNA recoveries were similar for all tested cobalt ferrite particles. Therefore an additional criterion for choice of particles was used. In previous paper [37] was found that some magnetic carriers interfered with PCR. We stated that the interference of cobalt ferrite particles with alginic acid was the smallest in comparison with other cobalt ferrite particles tested. Therefore, cobalt ferrite particles modified with alginic acid were used for evaluation of DNA elution conditions. The amount of eluted DNA ranged from 5.2 to 6.0% (for 4% PEG) and from 7.8 to 8.8% (for 8% PEG). The recovery was lower for DNA isolated from *Lactococcus* cells than for DNA from chicken erythrocytes. DNA isolated from *Lactococcus* cells was partially contaminated by bacterial RNA due to which spectrophotometric assessment of DNA concentration was overestimated. According to authors [17], the adsorption of double-stranded DNA is thermodynamically favoured, while the adsorption of proteins and single-stranded RNA is not. A significant amount of DNA remained adsorbed to the tested particles. Small amounts of DNA could be eluted in the second elution step using the same conditions as in the first step. Agarose gel electrophoresis of *L. lactis* DNA after repeated elution is given in Fig. 3. The eluted amount of DNA was sufficient for PCR identification of tested strains (see later). The carrier modified by alginic acid resembled carboxyl coated polymeric methacrylate based microspheres with hydrophilic properties—P(HEMA-co-EDMA), P(HEMA-co-GMA), and PGMA [38].

The eluted DNA was used directly for PCR applications with success. All tested particles were used for isolation of

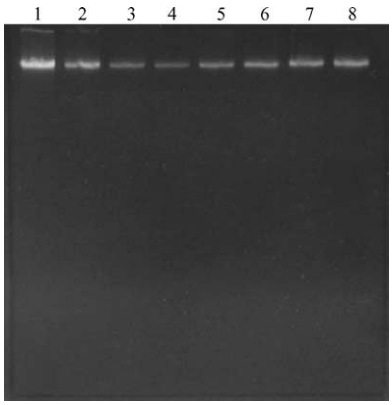

Fig. 3. Agarose gel electrophoresis of *Lactococcus lactis* DNA isolated using cobalt ferrite particles modified with alginic acid (A-112). Lane 1: crude cell lysate DNA; lane 2: DNA eluted from particles at 55 °C; lane 3: DNA after repeated elution; lanes 4–8: weight DNA standards—100, 200, 300, 400 and 500 ng, respectively.

DNA from different dairy products (butter milk, cheese, yoghurt) containing *Lactococcus*, *Lactobacillus* and *Bifidobacterium* cells followed by their PCR identification. Bacterial cells were lysed before DNA separation and particles were added to crude cell lysates (no phenol extraction was necessary). An example of amplification of *L. lactis* DNA isolated from butter milk is given in Fig. 4. The influence of PCR inhibitors was eliminated using tested magnetic particles as no PCR products were obtained from dairy product crude lysates without DNA separation (the results are not shown). Perfect separation of magnetic particles from DNA eluates was the assumption of high PCR sensitivity. In comparison with silica particles the use of magnetic particles is easier and quicker as centrifugation is not necessary.

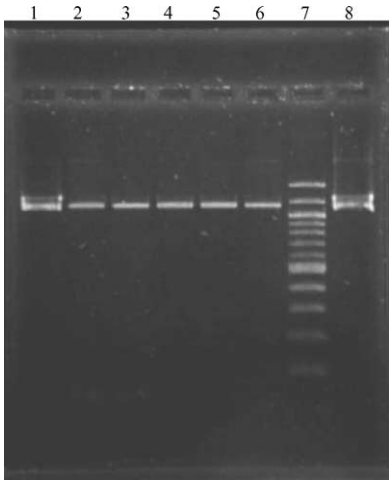

Fig. 4. Agarose gel electrophoresis of PCR products obtained after amplification of *Lactococcus lactis* DNA isolated from butter milk using cobalt ferrite particles. Lane 1: purified DNA from butter milk (phenol extraction); lanes 2–6: DNA isolated from butter milk using silica, A-101, A-109, A-110 and A-112 particles, respectively; lane 7: DNA standard (100–1500 bp); lane 8: positive control DNA (*L. lactis*).

4. Conclusion

The results presented in this report show that cobalt ferrite particles (unmodified and modified) are suitable for isolation and purification of genomic DNA. More pronounced DNA elution was achieved at PEG concentrations higher than the critical. Condensed DNA was obviously adsorbed to particles. It is possible to state that the mechanisms of DNA adsorption and desorption to modified cobalt ferrite surfaces are similar to those for silica-based supports.

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