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Magnetic hydrophilic methacrylate-based polymer microspheres for genomic DNA isolation☆

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Abstract

Carboxyl groups containing magnetic and non-magnetic microspheres were used in solid-phase reversible immobilization (SPRI) of genomic DNA. Magnetic non-porous poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) — P(HEMA-*co*-EDMA), poly(glycidyl methacrylate) — PGMA and P(HEMA-*co*-GMA) microspheres with hydrophilic properties were prepared by dispersion copolymerization of the respective monomers in the presence of colloidal iron oxides. DNA from chicken erythrocytes and DNA isolated from bacterial cells of *Bifidobacterium longum* was used for testing of adsorption/desorption properties of magnetic microspheres. The occurrence of false negative results in polymerase chain reaction (PCR) caused by the presence of extracellular inhibitors in DNA samples has been solved using SPRI. The P(HEMA-*co*-EDMA) and P(HEMA-*co*-GMA) microspheres were used for isolation of DNA from different dairy products followed by PCR identification of *Bifidobacterium* strains.

Keywords: Magnetic and non-magnetic microspheres; Carboxyl groups; 2-Hydroxyethyl methacrylate; Ethylene dimethacrylate; Glycidyl methacrylate; DNA adsorption

1. Introduction

Fast and reliable genome typing of industrially important microorganisms is necessary for identification of closely related strains and for the detection of genetic changes. Genome analysis involves nucleic acid purification, amplification, labelling, and signal detection. Preparation of high-quality genomic DNA is an essential step in molecular diagnostics. The polymerase chain reaction (PCR) is a well-known specific and sensitive method for direct identification of microorganisms. The occurrence of false negative results is often caused by the presence of extracellular inhibitors in the samples [1–3]. The problem can be solved using various isolation and purification methods.

Chromatographic methods are routinely used in the purification of nucleic acids at laboratory scale including ion-exchange [4–8], affinity [9,10] or hydrophobic interaction [11] and size-exclusion chromatography [4,12,13]. Solid-phase systems based on silica and anion-exchange carriers became popular for DNA isolation. Nucleic acids interact with the silica surface under high concentration of chaotropic salts [14–18]. Many solid-phase techniques were adopted for use with magnetic particles. As an example, DNA fragments can be isolated by the solid-phase reversible immobilization method (SPRI) employing carboxyl groups on the surface of magnetic particles. These particles could reversibly bind DNA in the presence of poly(ethylene glycol) (PEG) and sodium chloride. The technique was adopted for isolation of PCR products [17,18].

In our work attention was given to the use of magnetic hydrophilic non-porous microspheres, ca. 1.0 μm in size, such as P(HEMA-*co*-EDMA), P(HEMA-*co*-GMA), and PGMA microspheres containing carboxyl groups. Carboxyl groups were introduced by oxidation of hydroxyl groups on the par-

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ticle surface. The microspheres were prepared by dispersion copolymerization of 2-hydroxyethyl methacrylate (HEMA) and ethylene dimethacrylate (EDMA) or glycidyl methacrylate (GMA) in the presence of colloidal iron oxides. Previously, magnetic non-porous P(HEMA-*co*-EDMA) microspheres with immobilized RNase A and DNase I were used for digestion of high-molecular mass RNA [19], chromosomal DNA, and for cleavage of plasmid DNA [20]. Such P(HEMA-*co*-EDMA) is known to be a highly hydrophilic and biocompatible hydrogel with a low non-specific protein adsorption and a good chemical stability. It imbibes a large amount of water, and its hydroxyl groups can be easily modified [19].

The aim of this work was to isolate high-molecular mass DNA using newly designed carboxyl group-containing magnetic P(HEMA-*co*-EDMA), P(HEMA-*co*-GMA), and PGMA microspheres. Different poly(ethylene glycol) and sodium chloride concentrations were used to optimize DNA adsorption and elution. The procedure developed was used for isolation of genomic DNA from dairy products. The quality of isolated DNA was tested by PCR amplification.

2. Materials and methods

2.1. Chemicals

DNA (Na salt) from chicken erythrocytes was from Reanal (Budapest, Hungary), or isolated from bacterial cells of *Bifidobacterium longum* (see Section 2.3.2). Agarose was purchased from Serva (Heidelberg, Germany), ethidium bromide from Sigma (St. Louis, USA). Silica microparticles were taken from Top-Bio (Prague, Czech Republic). Needle-like maghemite (γ-Fe_2O_3) was obtained from Société Française d'Electrométallurgie (Marseille, France), colloidal magnetite (Fe_3O_4) was prepared by co-precipitation of Fe^{2+} and Fe^{3+} salts in alkaline medium and stabilized by oleic acid [21] or electrostatically with perchloric acid (acid sol) or tetramethylammonium hydroxide (alkaline sol) [22]. Monomers, 2-hydroxyethyl and glycidyl methacrylate (HEMA; GMA), both from Röhm (Darmstadt, Germany), and ethylene dimethacrylate, Aldrich (Milwaukee, USA), were purified by distillation under reduced pressure in nitrogen atmosphere. Cellulose acetate butyrate (CAB) was a kind gift of Eastman (Kingsport, USA), poly(vinylpyrrolidone) (PVP) K 30 (M_w = 40,000) and poly(ethylene glycol) (PEG, M_w = 6000) were from Fluka (Buchs, Switzerland). The PbiF1 and PbiR2 primers [23] for PCR were synthesized by Generi-Biotech (Hradec Králové, Czech Republic); Taq 1.1. polymerase and DNA markers 100 bp ladder and 970–155 bp for gel electrophoresis were from Malamité (Moravské Prusy, Czech Republic) or Top-Bio (Prague, Czech Republic), respectively. 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 Genesys V (Spectronic Instruments, Rochester, USA), carboxyl group content was determined on a 799 GPT Titrino titrator, Metrohm (Herrisau, Switzerland), 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 electrophoreses were carried out using a 3000 Xi power supply (Bio-Rad Laboratories, Richmond, USA). PCR products were visualized on a UV transluminator 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 carrier

Magnetic non-porous P(HEMA-*co*-EDMA) (92/8, w/w) microspheres were prepared by cellulose acetate butyrate-stabilized and dibenzoyl peroxide (BPO)-initiated dispersion copolymerization of 2-hydroxyethyl methacrylate and ethylene dimethacrylate in a toluene/2-methylpropan-1-ol medium in the presence of needle-like maghemite (Fe_2O_3) according to the previously described procedure [20]. Magnetic P(HEMA-*co*-GMA) (1/1, w/w) microspheres were obtained in the same medium and under analogous conditions in the presence of colloidal oleic acid-coated magnetite (Fe_3O_4) particles [22]. Magnetic PGMA microspheres were prepared by poly(vinylpyrrolidone) K 30 stabilized and 2,2′-azobisisobutyronitrile-initiated dispersion polymerization of GMA in ethanol in the presence of colloidal magnetite treated with either perchloric acid or tetramethylammonium hydroxide (TMAH). In all cases, polymerizations were run at 70 °C for 16 h. Subsequently, the hydroxyl groups of the microspheres were oxidized with 2 wt.% aqueous solution of potassium permanganate under acidic conditions (2 M sulphuric acid) in the presence of a small amount of alkylbenzene sul-

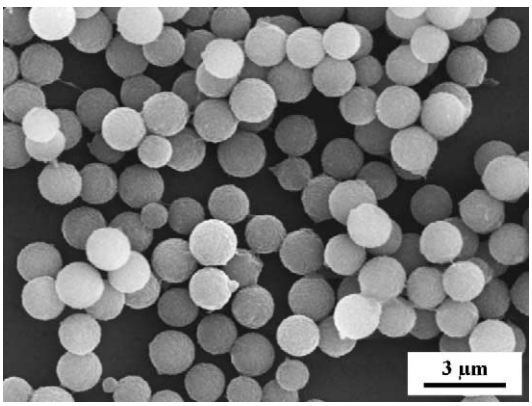
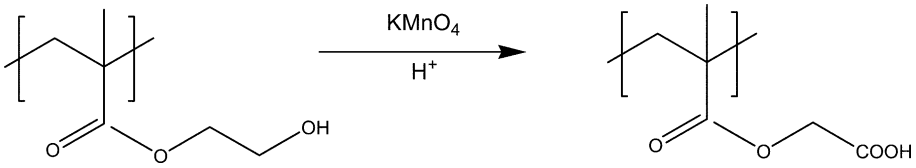


Fig. 1. Scanning electron micrograph of magnetic P(HEMA-*co*-EDMA) microspheres prepared in the presence of needle-like γ-Fe_2O_3.

phonic acid wetting agent (Abeson K; Pliva-Lachema, Brno, Czech Republic) for 3 h at room temperature to introduce carboxyl groups (see diagram).



The microspheres were carefully washed with 10 wt.% of potassium persulphate, water, hydrochloric acid, again water, and freeze-dried. The content of carboxyl groups was determined by alkaline titration after ion exchange with barium chloride [24]. Some properties of the synthesized microspheres are summarized in Table 1. A scanning electron micrograph of magnetic P(HEMA-*co*-EDMA) microspheres is given in Fig. 1.

2.3.2. DNA solid-phase reversible immobilization

Four types of carboxyl-coated microspheres were used in this study for genomic DNA isolation–three types were magnetic and one was non-magnetic (Table 1). Either PGMA and/or PHEMA based microspheres and silica (standard) were used in genomic DNA isolation experiments. An appropriate amount (30 μl) of DNA (1 mg/ml) in TE buffer, 40% PEG 6000 and 5 M NaCl solutions were mixed with 60 μl of microspheres (total volume 0.66 ml) and kept at laboratory temperature for 10 min, followed by magnetic separation of microspheres. Non-magnetic and silica particles were removed by centrifugation at 14,000 × *g*/min for 5 min. The NaCl concentration ranged between 0.5 and 3 M, PEG 6000 concentration ranged between 2 and 12% (reached to the whole sample volume). In the case of determination of DNA recovery the microspheres were washed twice with 200 μl of 70% ethanol and dried shortly. DNA elution from the microspheres was carried out at laboratory temperature using 0.66 ml of TE buffer. UV absorption was measured at 260 nm. The relative changes of UV absorbance were used to optimize DNA adsorption and to detect the process of DNA condensation. Recovery of eluted DNA was estimated from the difference of DNA absorbance in TE buffer before its adsorption and after its elution. At this wavelength PEG showed no absorption. Each experiment was repeated twice.

2.3.3. Cell cultivation and sample pretreatment

Bifidobacterial strain of *B. longum* CCM 3764 was obtained from the Czech Collection of Microorganisms—CCM (Brno, Czech Republic). Bacterial cells of *B. longum* were cultivated anaerobically on MRS medium (Oxoid, Great Britain) with cysteine (0.5 g/l) overnight (18 h). Altogether 1 ml of the cells was washed and resuspended in 100 μl lysis buffer (10 mM Tris–HCl, pH 7.8, 5 mM EDTA, pH 8.0, lysozyme 0.3 mg/ml) and incubated at 37 °C for 3 h; 10 μl proteinase K (10 mg/ml) and 2.5 μl SDS (20%) were then added and the mixture was incubated at 55 °C for 18 h. DNA was extracted from crude cell lysates by phenol [25] or separated using magnetic particles. 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 [26].

2.3.4. DNA separation from crude cell lysates using magnetic and non-magnetic microspheres

A total of 50 μl of crude cell lysate, 10 μl of particle suspension and 50 μl of the hybridization buffer (20% PEG, 2.5 M NaCl) was mixed and incubated 10 min at laboratory temperature. The microspheres with adsorbed DNA were then separated using a magnetic separator, the supernatant was discarded, and the microspheres were washed twice with 200 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 (2 μl) was used as DNA matrix in PCR amplification and for agarose gel electrophoresis. The same procedure was used for silica.

2.3.5. PCR amplification and detection of PCR products

Purified DNA (using the phenol extraction method) [25] or DNA separated using microspheres (after SPRI) was used as DNA matrix in PCR. PCR was performed using PbiF1 and PbiR2 primers which enable amplification of a 914 bp long DNA fragment specific to the *Bifidobacterium* genus [23]. Typically, the PCR mixture contained 0.5 μl of each 10 mM dNTP, 0.5 μl (10 pmol/μl) of each primer, 1–2 μl of DNA

Table 1
Characteristics of prepared magnetic microspheres

Polymer	Encapsulated iron oxide	Fe content (%, w/w)	–COOH content (mM/g)	Diameter (μm)	PDI
P(HEMA-*co*-EDMA) (92/8, w/w)–A1	Needle-like, γ-Fe_2O_3	9.9	0.85	1.5	1.04
P(HEMA-*co*-GMA) (1/1, w/w)–A2	Oleic acid-coated colloid Fe_3O_4	6.6	2.61	1.2	1.10
PGMA–A3	$(CH_3)_4NOH$-treated Fe_3O_4	2.9	0.41	0.66	1.11
P(HEMA-*co*-EDMA) (92/8, w/w)–A4	Non-magnetic	0.0	0.74	1.1	1.07

PDI: polydispersity index (ratio of weight to number – average particle diameter).

matrix, and 0.5 μl of Taq 1.1 polymerase (1 U/μl), 2.5 μl of buffer, and PCR water was added up 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 50 °C, 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 1.5% 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), decolorized 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 [27].

3. Results and discussion

Carboxyl group-containing microspheres are especially important for biological and biomedical applications. Carboxyl groups on the microsphere surface can be easily activated for covalent coupling of various ligands suitable for specific interactions with biological molecules [28]. In the 1990s, carboxyl group-coated magnetic particles were used for the isolation of PCR products (solid-phase reversible immobilization method, SPRI) [17,18]. We used newly designed carboxyl group-containing magnetic non-porous P(HEMA-*co*-EDMA), P(HEMA-*co*-GMA), and PGMA microspheres for the isolation of high-molecular genomic DNA. While P(HEMA-*co*-EDMA) and P(HEMA-*co*-GMA) microspheres were prepared by the BPO-initiated and CAB-stabilized dispersion polymerization in toluene/2-methylpropan-1-ol medium, their PGMA counterparts required AIBN initiation and PVP stabilization in ethanol [22]. The advantage of dispersion polymerization, which is a single-step procedure, consists in the production of microspheres in micron size range and a narrow size distribution characterized by a polydispersity index <1.11 (Table 1). As microspheres were obtained in the presence of various iron oxides in the feed, they contained up to ca. 10 wt.% of iron. The hydroxyl groups in the microspheres were oxidized with potassium permanganate to introduce carboxyl groups and up to ca. 2.6 mM of −COOH/g was found in the product (Table 1).

DNA from chicken erythrocytes was used first as a model sample. Various poly(ethylene glycol) (PEG 6000) and sodium chloride concentrations were tested to optimize DNA adsorption. The DNA absorbance changed in dependence on both PEG and NaCl concentrations. Thus, only a relative decrease of UV absorbance at 260 nm was used to optimize linkage conditions for constant PEG concentration. It is necessary to take account of the influence of both NaCl and PEG concentrations on DNA structure forms. The increasing concentration of chaotropic salt or PEG leads to reduced water activity and the helical structure of B-DNA is thus continuously changed to A-DNA structure [29]. DNA dehydration may contribute to a change of the driving forces for DNA adsorption. A more expressive DNA adsorption was achieved at a PEG concentration >5% and an NaCl concentration >1%. DNA was adsorbed in lower amounts (except for A4 microspheres) under 5% PEG concentration. The results are given in Table 2.

The mechanism of DNA interaction with the particle surface is evidently complicated because the DNA present in the solution does not interact only with the functional groups of the carrier. The use of poly(ethylene glycol) with different salts alone (without solid particles) for precipitation of

Table 2
Adsorption and elution of DNA isolated from chicken erythrocytes

Particle	NaCl (M)	Concentration of PEG 6000 (%)/DNA content (%)									
		5		6		7		9		12	
		A	B	A	B	A	B	A	B	A	B
P(HEMA-*co*-EDMA) (92/8, w/w)–A1	1.0	n.d.	3.1	5.6	4.2	15.5	3.4	20.4	6.0	5.5	32.9
	2.0	n.d.	3.7	6.2	4.4	19.3	5.1	23.9	21.8	8.6	35.0
	2.5	n.d.	9.4	17.1	15.3	31.2	24.5	25.1	25.6	12.6	27.4
	3.0	n.d.	3.9	12.1	6.6	22.8	14.9	22.3	23.3	7.7	33.4
P(HEMA-*co*-GMA) (1/1, w/w)–A2	1.0	n.d.	14.1	36.9	49.9	47.5	64.0	44.0	79.2	45.2	89.8
	2.0	n.d.	12.0	14.1	31.7	51.2	78.7	40.6	70.7	44.1	98.9
	2.5	n.d.	10.9	33.0	62.2	45.7	62.5	36.4	73.4	41.3	88.3
	3.0	n.d.	9.6	14.2	32.2	40.0	61.1	34.2	66.2	40.7	86.9
PGMA–A3	1.0	n.d.	12.6	n.d.	31.0	15.5	40.7	32.6	57.6	6.2	40.2
	2.0	n.d.	14.0	2.0	33.9	12.2	50.4	16.9	51.9	14.5	59.8
	2.5	n.d.	15.1	n.d.	35.9	22.2	58.0	22.8	48.7	13.1	59.9
	3.0	n.d.	12.3	n.d.	27.6	12.8	49.2	26.5	61.0	14.0	56.0
P(HEMA-*co*-EDMA) (92/8, w/w)–A4	1.0	57.9	79.1	69.7	85.7	70.3	84.7	64.9	87.9	34.1	78.2
	2.0	68.3	78.4	72.8	85.6	71.1	85.0	65.3	79.0	36.8	87.4
	2.5	62.8	84.3	72.9	84.7	70.8	83.6	64.9	87.2	32.9	81.5
	3.0	65.9	78.0	72.9	91.1	71.1	82.9	65.5	69.4	35.1	82.3

A: adsorbed DNA; B: eluted DNA.

DNA has been described in the literature [25,30]. Large DNA molecules were present in low salt concentration solutions in the form of random coils and changed their properties at critical PEG concentration. DNA coils swell and condense to a compact, relatively dense state [31–35]. DNA coil and compact DNA globules are different particles having a hydrodynamically different behavior [33]. At concentrations lower than critical, DNA molecules in the coil state do not deposit from the solution (do not sediment during centrifugation) and the absorbance does not change. On the contrary, condensed DNA molecules sediment on the bottom of the centrifuge tube, where they aggregate. The amount of DNA adsorbed depended on complete covering of iron oxides (maghemite or magnetite) with polymer in the case of use of magnetic microspheres (Table 2). Adsorption of DNA to iron oxide surfaces [36] is apparently stronger than on microspheres containing carboxyl groups only. The critical PEG concentration is a function of PEG molar mass and salt concentrations [33]. The value of critical concentration is reciprocally proportional to the molar mass and, according to the above-mentioned authors; it is equal to 57 g/l for PEG 6000. The region of coexistence of both coil and condensed DNAs is narrow; it has a width of 10 g/l. The amount of adsorbed DNA correlates namely with the content of carboxyl groups on the carrier surface (see Table 2). The highest adsorption has been determined in A2 microspheres with 2.61% of carboxyl groups. These results are comparable with the experimental data of other authors [32–34]. No decrease of the amount of adsorbed DNA was observed at higher PEG concentrations (>8%) for tested particles as in the case of silica and unmodified cobalt ferrite nanoparticles [36].

The decrease of adsorbed DNA was estimated at a PEG concentration of 12% (w/w). A two-phase aqueous system formed at a concentration of phase components higher than critical. No reliable data were so far published for the given system. The composition of the critical point for PEG 6000 and ammonium sulphate was 9.43% (w/w) and 9.21% (w/w), respectively [37]. Condensed DNA was apparently adsorbed at interphase in the two-phase aqueous system. This phenomenon was described for high-molecular mass RNA partitioning in the potassium phosphate–PEG system [38].

Subsequently, the elution of DNA from the microspheres was estimated. Concentrations of PEG 6000 and NaCl used for DNA attachment on carboxyl group-containing microspheres were the same as in the previous study. DNA elution from the microspheres was carried out at laboratory temperature using TE buffer. The results are given in Table 2. The highest recovery was achieved at higher PEG concentrations (7 and 9%). It can be stated that, in agreement with previously published results [39], the highest irreversible adsorption (and therefore the lowest elution) was estimated in A1 microspheres. The completeness of coverage of the iron oxides used was indirectly verified with the help of PCR inhibition. We concluded that a significant amount of DNA remained adsorbed to the uncovered maghemite. The elution efficiency (recovery) was rather small for this reason.

Table 3
Elution of DNA isolated from *B. longum* cells

Microspheres	NaCl (M)	Concentration of PEG 6000 (%)/DNA content (%)			
		5	6	7	9
P(HEMA-*co*-GMA) (1/1, w/w)–A2	2.0	15.3	16.9	17.1	20.1
	2.5	19.6	19.3	22.8	18.0
	3.0	19.8	19.3	31.6	23.9
P(HEMA-*co*-EDMA) (92/8, w/w)–A4	2.0	17.4	12.9	20.1	23.1
	2.5	13.1	21.5	20.6	19.0
	3.0	11.3	19.8	30.8	17.2

DNA isolated from *Bifidobacterium* cells (0.55 μg/μl) was also used to optimize DNA elution conditions. This DNA was chosen due to the following practical applications of isolated DNA. Microspheres P(HEMA-*co*-GMA, magnetic) and P(HEMA-*co*-EDMA, non-magnetic) were used with the same poly(ethylene glycol) (PEG 6000) and sodium chloride concentrations as in the case of DNA from chicken erythrocytes. The results are given in Table 3. The recovery was lower for DNA isolated from *Bifidobacterium* cells than for DNA from chicken erythrocytes. DNA isolated from *Bifidobacterium* cells was contaminated by bacterial RNA due to which spectrophotometric assessment of DNA concentration was overestimated. After RNA cleavage using RNase A, small RNA fragments were apparently not adsorbed to the microspheres and therefore were not eluted. According to authors [40], the adsorption of double-stranded DNA is thermodynamically favoured contrary to the adsorption of proteins and single-stranded RNA. The integrity of recovered DNA was not affected by the adsorption process (results not shown). The eluted DNA could be used directly for further applications. The quality of eluted DNA was tested in PCR. Bacterial cells of *B. longum* (0.55 μg/μl) were the source of DNA. All the studied microspheres (A1–A4) were used for the preparation of PCR-ready DNA. Silica-based particles were used as standard procedure (Fig. 2). Although the greatest amount of the amplified product was obtained in lane 8, it cannot be stated that these particles were the most convenient, as the amount of the silica used was unknown (only the volume). In comparison with silica particles the use of magnetic particles is easier and quicker as centrifugation is not necessary. The final concentrations of PEG and NaCl were 9.1% and 1.1 M, respectively, which were suitable conditions for DNA adsorption.

The microspheres A2 and A3 were used for isolation of DNA from different probiotic dairy products followed by PCR identification of *Bifidobacterium* strains. An example of amplification of DNA isolated from cheese, yoghurt, and a fermented dairy product is given in Fig. 3. The influence of PCR inhibitors was eliminated using tested magnetic particles. No PCR products were obtained from dairy product crude lysates without DNA separation (see lanes 2–4). In the case of identification of *Bifidobacterium* cells in the fermented dairy product sample (lane 5), no PCR product was obtained repeatedly even when phenol extraction was used

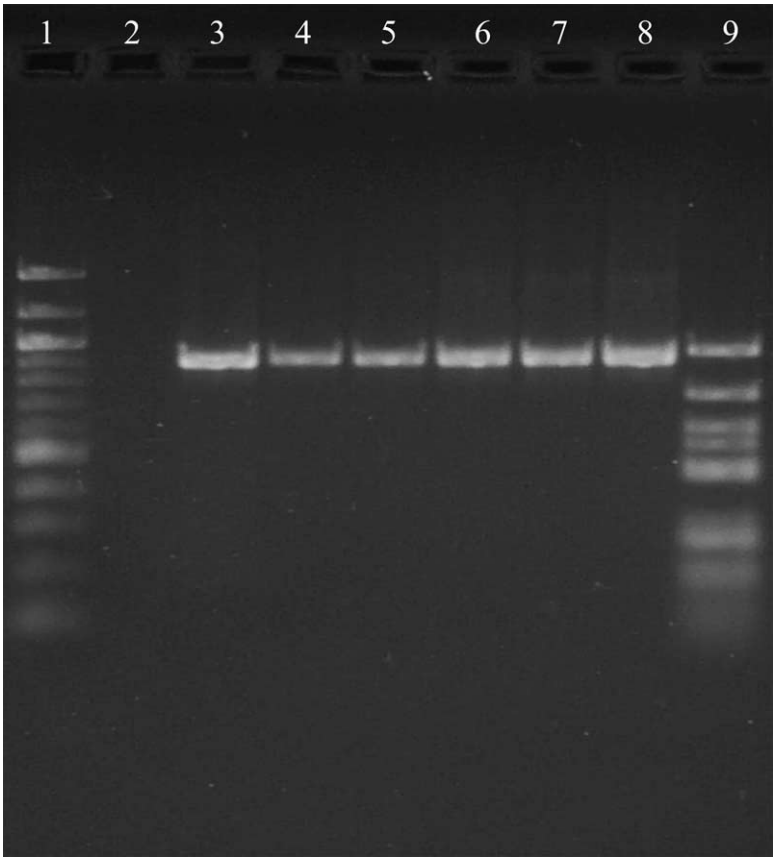


Fig. 2. Agarose gel electrophoresis of PCR products obtained after amplification of DNA isolated from *B. longum* by solid-phase reversible immobilization method employing A1–A4 microspheres. *Conditions:* 1.5% agarose gel, TBE buffer (45 mM boric acid, 45 mM Tris-base, 1 mM EDTA, pH 8.0). Lane 1: DNA standard (100 bp ladder); lane 2: negative control; lane 3: positive control (DNA from *B. longum*, phenol extraction); lanes 4–8: DNA separated using A1–A4 particles and/or silica; resp., lane 9: DNA standard (970–155 bp).

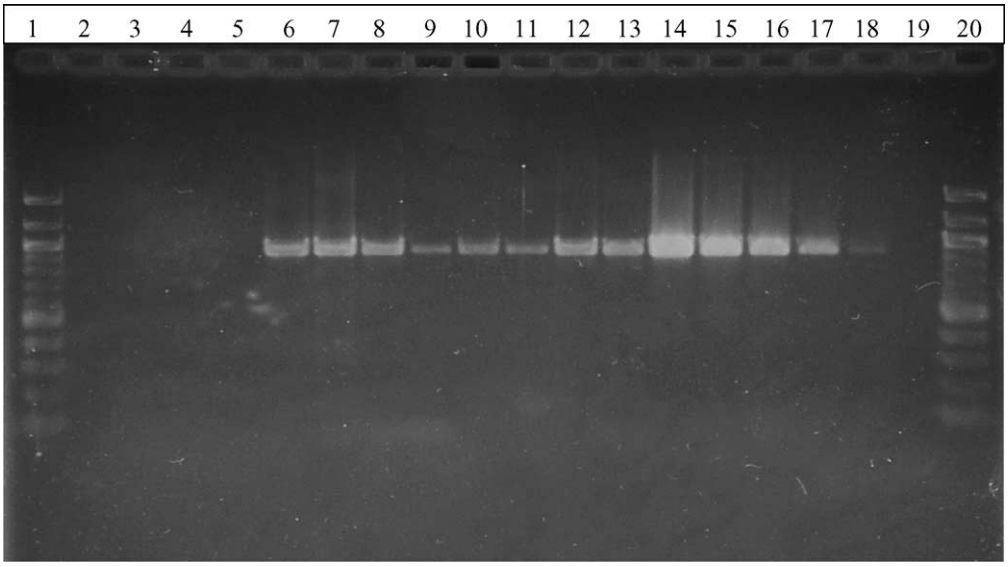


Fig. 3. Agarose gel electrophoresis of PCR products obtained after amplification of DNA isolated from probiotic dairy products by solid-phase reversible immobilization method employing A2 and A3 microspheres. *Conditions:* 1.5% agarose gel, TBE buffer (45 mM boric acid, 45 mM Tris-base, 1 mM EDTA, pH 8.0). Lane 1: DNA standard (100 bp ladder); lanes 2–4: DNA from crude lysates from fermented dairy product, yoghurt, and cheese; lanes 5–7: DNA (phenol extraction) from fermented dairy product, yoghurt, and cheese; lanes 8–13: DNA eluted from A2 and A3 microspheres from fermented dairy product, yoghurt, and cheese; resp., lanes 14–18: controls with 500, 100, 50, 10, and 1 pg of *B. longum* DNA (phenol extraction); lane 19: negative control without DNA; lane 20: DNA standard (100 bp ladder). Lanes 2–5—false negative results.

(which testifies to the presence of more types of inhibitors in the sample matrix. On the contrary, a positive result (PCR product) was obtained in the isolation of genomic DNA using tested particles. In the case of use of crude cell lysates, the phenol extraction step was omitted. DNA was adsorbed directly from cell lysates to microspheres and eluted DNA was used in PCR with success.

It can be summarized that the phenomenon of DNA collapse is of fundamental significance and has important applicability in molecular biology [41–45]. For example, a DNA molecule packed into a viral capsid is hundreds of times more compact than when it is free in solution. This compact in vivo configuration is stabilized by multivalent cations and by positively charged proteins. These conditions can be provoked in vitro by various chemical agents as spermidine [41], spermine [42], hexamine cobalt (Co^{3+}) [43], and poly(ethylene glycol) [34–36]. This phenomenon has been utilized in various practical applications, such as development of DNA delivery vehicles for gene therapy [43], or plasmid DNA purification [45].

4. Conclusion

The results presented in this report show that hydrophilic magnetic non-porous P(HEMA-*co*-EDMA), P(HEMA-*co*-GMA), and PGMA microspheres containing carboxyl groups are suitable for isolation and purification of genomic DNA. More pronounced DNA adsorption was achieved at critical and higher PEG concentrations. Condensed DNA was obviously adsorbed to microspheres. The amount of isolated DNA apparently depends on the content of carboxyl groups on particle surface and in the case of magnetic microspheres also on the complete (thorough) covering of magnetite.

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