Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1080 (2005) 93–98
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Ferrite supports for isolation of DNA from complex samples and polymerase chain reaction amplification

Alena Španová [a], Bohuslav Rittich [a,*], Milan J. Beneš [b], Daniel Horák [b]

[a] *Masaryk University in Brno, Faculty of Science, CZ-602 00 Brno, Czech Republic*
[b] *Institute of Macromolecular Chemistry, AS CR, CZ-162 00 Prague, Czech Republic*

Abstract

The influence of cobalt ferrite particles, with non-modified or modified surface, on the course of polymerase chain reaction (PCR) was investigated. DNA isolated from bacterial cells of *Bifidobacterium bifidum* was used in PCR evaluation of magnetic microspheres. The presence of cobalt ferrite particles inhibits PCR amplification. The effect is not dependent on the functional groups of the modifying reagents used (none, amino, carboxyl). Amplification was improved after the magnetic separation of magnetic particles. Proposed indirect method enabled verification of the suitability of designed particles for their application in PCR assays. Magnetic particles coated with alginic acid under high PEG and sodium chloride concentration were used for the isolation of PCR-ready bacterial DNA from various dairy products. DNA was isolated from crude bacterial cell lysates without phenol extraction of samples. *Bifidobacterium* and *Lactobacillus* DNAs were identified in dairy products using PCR.

Keywords: Cobalt ferrite magnetic particles; DNA; Polymerase chain reaction; Dairy products

1. Introduction

Identification of bacteria using polymerase chain reaction (PCR) primer pairs is a rapid and accurate method based on specific nucleic acid sequences. The occurrence of falsely negative results is a problem in the routine identification of various microorganisms in real samples by PCR. Falsely negative results can be caused by the presence of extracellular or intracellular PCR inhibitors [1–3]. For this reason, great attention has been focused on the development of fast and robust DNA isolation methods.

A classical procedure for the isolation of genomic DNA involves phenol/chloroform extraction and DNA precipitation in ethanol [4]. The method, however, requires application of toxic compounds (phenol, chloroform) and is rather complicated. Alternative isolation methods were therefore developed and several strategies used:

- various chromatographic methods [5–14],
- solid phase systems selectively or non-selectively adsorbing target cells or genomic DNA [15–24].

Particles carrying specific ligands were used for the capturing of either target cells or specific DNA sequences from competitive microflora or from the environment containing extracellular PCR inhibitors [1,2,4]. Different solid phase systems, such as silica-based particles [15–18], glass fibres, and anion-exchange carriers were investigated for DNA adsorption [19–22]. Further progress is achieved by the application of magnetically responsive particles in the separation of plasmid and genomic DNAs [23–26]. A key aspect of this system consists in the possibility of easy manipulation, automation, and miniaturisation of the process of DNA isolation. The magnetic particle components are employed not only for magnetic separation of support from the reaction mixture but they alone are used as a solid adsorbent [26]. Their sorption behaviour is determined by the oxide type, particle size, and also by coating. The presence of magnetic particles in the separation system can, however, decrease PCR sensitivity or lead to false negative PCR results. This can be caused by the adsorption of some components from the solution or by

* Corresponding author.
E-mail address: rittich@sci.muni.cz (B. Rittich).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.05.006

the leakage of metal ions or stabilisers and rests of reagents during preparation. This interaction was described already earlier [27].

Most biological and biomedical applications of magnetic particles are based on magnetite (Fe_3O_4) or maghemite (γFe_2O_3). Cobalt ferrite ($CoO{\cdot}Fe_2O_3$) particles have recently been intensively investigated for their potential application in high-density magnetic recording, microwave devices, and magnetic fluids [28–30]. Cobalt ferrite particles in the nanometre range are suitable for biotechnology and biomedical applications [31–33].

The aim of this report was to study the influence of different cobalt ferrite particles on the PCR course (PCR sensitivity) using DNA isolated from lactic acid bacteria (*Bifidobacterium bifidum*) cells. Selected types of proposed magnetic particles were used for isolation of PCR-ready bacterial DNA from different dairy products.

2. Material and methods

2.1. Chemicals

Agarose was purchased from Top-Bio (Prague, Czech Republic), ethidium bromide from Sigma (St. Louis, USA). Cobalt ferrites, magnetite and P(HEMA-*co*-EDMA) 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 *Lactobacillus* [34] and *Bifidobacterium* [35] genera 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

The spectra were recorded on a UV spectrophotometer DMS 100 (Varian Techtron, Mulgrave, Australia). Magnetic particles were separated on 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

Unmodified cobalt ferrite (A-101) was prepared by co-precipitation of a mixture of Co^{2+} and Fe^{3+} salts according to the method described for precipitation of magnetite [36]; Co^{2+} was used instead of Fe^{2+} and sodium hydroxide instead of ammonia. The modified cobalt ferrite particles were prepared by co-precipitation of a mixture of Co^{2+} and Fe^{3+} salts in the presence of complexing bifunctional reagent containing a chelating group for binding to metal ions with free binding sites at the surface of oxide and functional group for further modification. Two types of chelating reagents were used: a low-molecular-weight strongly chelating bisphosphonate of the general formula $R–C(OH)[PO(OH)_2]_2$, where R is $H_2N–CH_2CH_2–$ (denoted as A-110) or $H_2N–C_6H_4–$ (A-109), and a natural polysaccharide with multiple complexing carboxylic groups—alginic acid (A-112, A-114) [37]. The carriers A-101, A-109, and A-110 are aggregates of 10–20 nm nanoparticles bound together by physical forces giving stable colloids under sonication; A-112 consists of microparticles of 10 μm diameter [38]. Particles A-109 contain 0.3 mmol of weakly basic aminoethyl groups/g of dry substance and A-110 contains 0.1 mmol of weakly basic aminophenyl groups/g of dry substance. Particles A-112 contain 4.3 mol% of alginic acid (0.22 mmol COOH/g dry substance). For comparison, magnetite particles coated with alginic acid (A-114) were analogously prepared, too. The characteristics of ferrite particles are given in Table 1.

Magnetic nonporous P(HEMA-*co*-EDMA) (92/8, w/w) microspheres were prepared by cellulose acetate butyrate-stabilised and dibenzoyl peroxide (BPO)-initiated dispersion copolymerisation of 2-hydroxyethyl methacrylate (HEMA) and ethylene dimethacrylate (EDMA) in a toluene/2-methylpropan-1-ol medium in the presence of needle-like maghemite (γFe_2O_3) according to the previously described procedure [39]. Hydroxyl groups of the microspheres were oxidised to introduce carboxyl groups [40]. The diameter of the particles was 1.5 μm and the –COOH groups content was 0.85 mmol/g. They were used as a control.

2.3.2. Bacterial cells and sample pretreatment

B. bifidum microorganisms (Laktoflora, Prague, Czech Republic) were used for DNA isolation and the study of the effect of particles or components on the PCR course [41]. They were anaerobically cultivated on MRS agar supplemented by 0.05% (w/w) of cysteine. Crude cell lysates were prepared from pure bacterial cultures and from dairy products. In the case of bacterial cultures altogether 1 ml of the cells

Table 1
Characteristics of prepared magnetic particles

Particles	Functional group		Diameter (nm)
	Type	Concentration (mmol/g)	
A-101[a]	None	–	10–20
A-109[a]	4-Aminophenyl	0.30	10–20
A-110[a]	2-Aminoethyl	0.10	10–20
A-112[a]	Carboxyl	0.22	1×10^4
A-114[b]	Carboxyl	0.35	1×10^4

[a] Magnetic oxide: cobalt ferrite.
[b] Magnetic oxide: magnetite.

was washed and resuspended in 100 μl lysis buffer (10 mM Tris–HCl, pH 7.8, 5 mM EDTA, pH 8.0, lysozyme 3 mg/ml) and incubated at laboratory temperature for 1 h. To crude cell lysates 10 μl proteinase K (1 mg/ml) and 50 μl SDS (20%) were then added and the mixture was incubated at 55 °C for 18 h. DNA was extracted from crude cell lysates using phenol procedure [4]. 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 [42]. DNA prepared in this way was used for PCR inhibition studies. Crude cell lysates from dairy products were prepared following way: in the case of fluid products 1ml of sample was centrifugated (10 000 × *g*/5 min), sediment was resuspended in 1 ml of sterile water and again centrifugated. To the sediment was added 1 ml of lysis buffer and treated as above. Yoghurt samples were resuspendet in water (10 g/10 ml) and 1 ml of suspension was used for centrifugation and lysis procedure as above. Solid samples were cut into small pieces and 1 g of sample was used for lysis as above. Real dairy products samples were from market.

2.3.3. DNA isolation from crude cell lysates using magnetic particles

A total of 50 μl of crude cell lysates, 10 μl of tested particles (10 mg/ml), and 50 μl of capturing buffer (20% (w/w) polyethylene glycol (PEG), 2.5 M NaCl) were mixed and incubated for 10 min at laboratory temperature. The particles with adsorbed DNA were then separated on a magnetic separator for 5 min, the supernatant was discarded, and the particles were washed twice with 500 μl of 70% ethanol, separated again for 3 min using magnetic separator, and dried at laboratory temperature. DNA captured to the particles was eluted 10 min at 55 °C in 50 μl of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 7.8). DNA in the eluate (magnetic particles were removed) was used as DNA matrix in PCR amplification.

2.3.4. PCR amplification and detection of PCR products

DNA purified by phenol extraction and DNA isolated from crude cell lysates using the magnetic supports tested served as DNA matrix in PCR. PCR was performed using R16-1 and LbLMA1-rev primers specific to the *Lactobacillus* genus [34], PbiF1 and PbiR2 primers specific to the *Bifidobacterium* genus [35], which enabled the amplification of 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 1.1 (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 55 °C for *Lactobacillus* or 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 electrophoresis in 1.2% agarose gel in TBE buffer (45 mM boric acid, 45 mM Tris-base, 1 mM EDTA, pH 8.0). DNA was stained with ethidium bromide (0.5 μg/ml), decolourised in water, and photographed at 305 nm UV light on a TT667 film.

2.3.5. Influence of particles on the PCR course

The influence of particles on the PCR course was studied according to the following procedure. Appropriate amount of particles (10–60 mg) were mixed with 1 ml of sterile distilled water, 10 μl of the suspension was added to 10 μl of tested DNA, mixed, and 1 μl of the mixture was used in PCR. Supernatants were obtained by magnetic separation of magnetic particles. Altogether four different DNA amounts (2 ng, 200, 20, and 2 pg/25 μl of PCR mixture) were used for PCR amplification. The use of different DNA amounts enabled to determine PCR sensitivity.

3. Results and discussion

In a previous paper [27] magnetic particles were used for the immobilisation of polyclonal *Salmonella* antibodies. These particles were used for the selective attachment of target cells and their identification using PCR (IMS-PCR). In this case particles must not be detached from microbial cells and the complex microorganism – the particle can be directly used for microbial cell identification. The presence of interfering compounds released from magnetic particles decreased PCR sensitivity or led to false negative results, too [27]. The aim of this work was to prepare magnetic particles with low interference efficiency in PCR. We used the technique which was successfully applied in the evaluation of magnetic hydrophilic methacrylate-based microspheres [41]. DNA isolated from *B. bifidum* cells has been used as template in PCR.

At first, the influence of ferrite particles presented in PCR mixture, unmodified and modified with different compounds, on the PCR course (sensitivity) was investigated. We proceeded from the fact that IMS-PCR assay is one of the possible applications of magnetic particles. The effect of ferrite particles on the PCR course is given in Table 2. From results it follows that the PCR course was influenced by particles present in the PCR mixture. Inhibition of PCR by magnetic particles decreased with the dilution of the samples. Dilution of the sample with interfering particles represents the simplest method that can facilitate the amplification (Fig. 1). Thus, particles with lower inhibition effect can be used in higher concentration. Inhibition also decreased by separation of magnetic particles from the amplification mixture using a permanent magnet. The increase of PCR sensitivity after magnetic separation thus confirmed the inhibitory effect of magnetic particles. These results are in agreement with previously described observations [41].

The influence of the modification of the particle surface on PCR sensitivity was also evaluated. The coating of A-101 particles with 2-aminoethyl (A-110) or 4-aminophenyl (A-109) groups have been effected PCR sensitivity slightly. The coating of particles with alginic acid (A-112) did not

Table 2
Effect of ferrite particles on PCR course

Particles	Particles/PCR mixture (μg/25 μl)	Magnetic separation	*B. bifidum* DNA/PCR mixture (pg/25 μl)/PCR product			
			2000	200	20	2
Control	0	N	+++	++	+	–
A-101	3	N	–**	–**	–**	–
	0.03	N	+++	++	–**	–
	3	Y	+++	++	++	+
A-109	5	N	–**	–**	–**	–
	0.5	N	+++	–**	–**	–
	0.05	N	+++	++	++	+
	5	Y	–**	–**	–**	–
	0.5	Y	+++	++	–**	–
	0.05	Y	+++	++	++	+
A-110	2	N	–**	–**	–**	–
	0.2	N	+++	++	+	+/–
	2	Y	++	–**	–**	–
	0.2	Y	+++	++	++	+
A-112	2	N	–**	–**	–**	–
	0.2	N	+++	++	+	–
	2	Y	+	–**	–**	–
	0.2	Y	+++	++	+	–
A-114*	0.2	N	–**	–**	–**	–
	0.02	N	+++	++	+	–

Y—yes; N—no. PCR product: +++, ++, +, +/–: band of very strong, strong, mean, and weak intensity; –: no band. (*) Magnetite; (**) interference with PCR.

markedly enhanced PCR sensitivity in comparison with bare (non-modified) particles. It can be stated that the inhibition of PCR amplification was not dependent on the functional groups of the modifying reagents used (none, amino, carboxyl) in our experimental arrangement. Particles A-114 could not be fully separated from PCR mixture using our permanent magnet. From this reason the PCR sensitivity was studied for nonseparated A-114 particles only (Table 2).

The cobalt ferrite particles coated with alginic acid (A-112) were applied for PCR-ready bacterial DNA isolation from crude cell lysates of different dairy products (kefir drink, yoghurt, cheese, tablets). These particles were used due to their easier separation from sample mixtures in comparison of all tested particles. The particles modified by alginic acid resembled hydrophilic methacrylate P(HEMA-*co*-EDMA) microspheres containing carboxyl groups [40]. Bacterial cells were lysed before DNA separation and the particles were added to crude cell lysates (phenol extraction was not necessary). Adsorption of bacterial DNA on cobalt ferrite particles was carried out under high PEG and sodium chloride concentration. In a previous paper [38], optimization of conditions for the isolation of genomic DNA on ferrite particles was described. Magnetic nonporous P(HEMA-*co*-EDMA) microspheres were used as control in the separation of DNA from dairy products. The DNA eluted from particles (supernatant) was directly used for PCR applications. The amount and the quality of isolated DNA was sufficient for DNA amplification. The results are given in Table 3. In dairy products claimed to contain *Lactobacillus* and *Bifidobacterium* strains the target bacteria were identificated. A growing research interest has been focused on the incorporation of probiotic bacteria into cultured dairy products. Not all foods provide the optimal environment for the growth of lactic acid bacteria (especially bifidobacteria). Nowadays, cheeses are under great interest in addition to kefir and yoghurts. Cheese does provide an environment that is suitable for a long-term survival of bifidobacteria. The A-112 particles tested are suitable for isolation and identification of target cells in dairy products as follows from a comparison of the results obtained using ferrite particles coated with alginic acid and P(HEMA-*co*-EDMA) microspheres coated with carboxyl groups and the phenol extraction method. In conclusion it can be stated that

Table 3
Identification of *Bifidobacterium* and *Lactobacillus* DNA in dairy products using PCR

Product	Carrier/bacteria/PCR product					
	A-112		P(HEMA-*co*-EDMA)[a]		Phenol extraction	
	A	B	A	B	A	B
Kefir milk ABT	+	+	+	+	+	+
Yoghurt Activia	+	+	+	+	+	+
Yoghurt Hollandia	+	+	+	+	+	+
Bifido yoghurt Yoplait	+	+	+	+	+	+
Bifido cheese	+	+	+	+	+	+
Probiotic cheese	+	+	+	+	+	+
Probiotic tablets	+	+	+	+	+	+

Altogether 5 μl of DNA separated using different carriers or after phenol extraction (control) were used as DNA matrix.

[a] Coated with carboxyl groups, A—*Bifidobacterium*, B—*Lactobacillus*, + PCR product was detected.

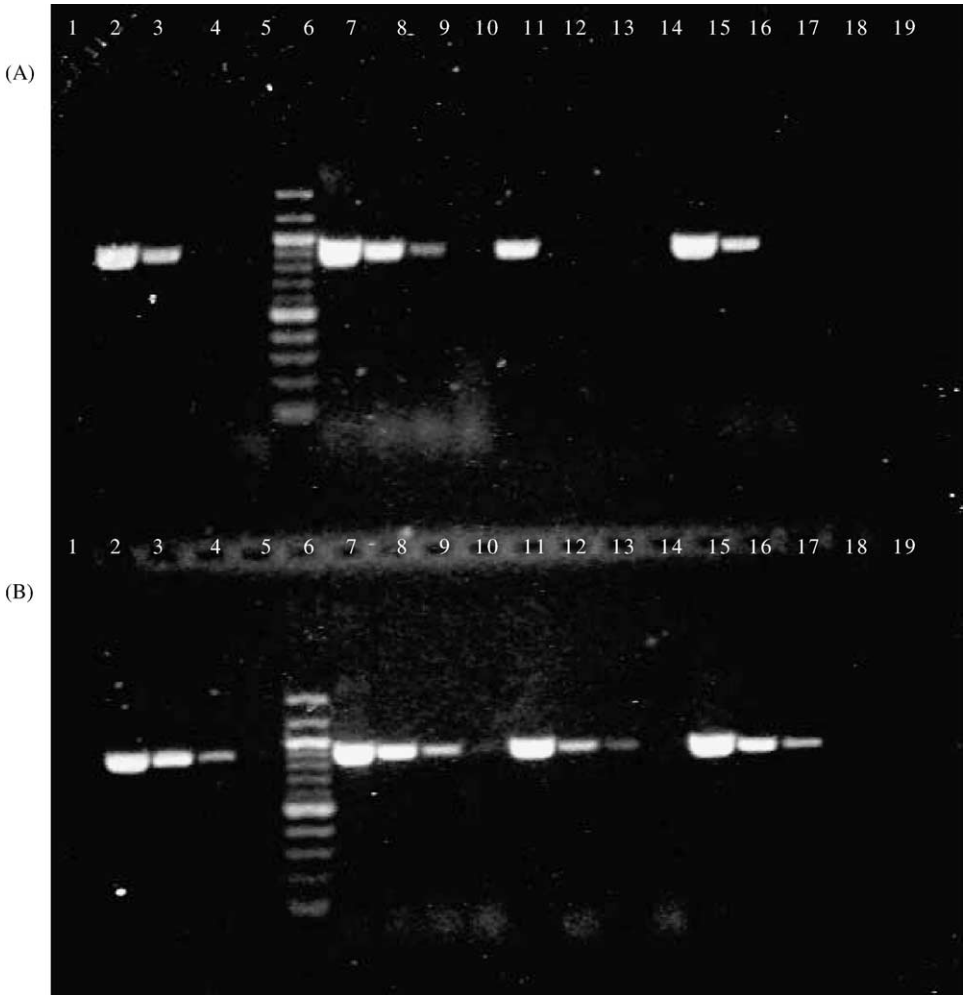

Fig. 1. Agarose gel electrophoresis of PCR products obtained after amplification of *B. bifidum* DNA (2 ng, 200, 20, and 2 pg/25 μl of PCR mixture, respectively) in the presence of cobalt ferrite particles. (A) Lane 1: negative control—without DNA; lanes 2–5: A-101 particles (0.3 μg/25 μl)—without magnetic separation; lane 6: DNA standard (100–1500 bp); lanes 7–10: controls—without particles; lanes 11–14: A-109 particles (0.5 μg/25 μl)—without magnetic separation; lanes 15–18: A-109 particles (0.5 μg/25 μl)—with magnetic separation. (B) Lane 1: negative control—without DNA; lanes 2–5: A-114 particles (0.02 μg/25 μl)—without magnetic separation; lane 6: DNA standard (100–1500 bp); lanes 7–10: controls—without particles; lanes 11–14: A-112 particles (0.2 μg/25 μl)—without magnetic separation; lanes 15–18: A-112 particles (0.2 μg/25 μl)—with magnetic separation.

the evaluated method allows a rapid identification of target lactic acid bacteria from commercial dairy products. In the literature the use of PCR for identification of DNA-damaging agents was published [43], too.

There is no information on the adsorption mechanism of duplex DNA on metal oxides under high PEG and sodium chloride concentration. It is known that PEG (neutral hydrophilic polymer) adsorbs on silica surface through hydrogen-bonding with surface silanol groups. Same mechanism of PEG adsorption on ferrite particles can be supposed. In low-salt solution concentrations and at PEG concentrations lower than the critical one, large DNA molecules are present as random coils and do not deposit from the solution. DNA changes its properties at critical PEG concentration (or higher): coils swell and condense to a compact, relatively dense state. The phenomenon of collapse of DNA macromolecules in aqueous solutions of polyethylene glycol was described in the literature [44]. Competetive displacement of DNA from the surface with increasing PEG concentration can be expected [45]. The addition of salt promotes interactions between condensed DNA and solid particles (salting out of condensed DNA from aqueous phase to PEG coated solid phase). Although, DNA as highly negatively charged macromolecule it readily adsorbed to surfaces. The increasing concentration of salt or PEG leads to reduced water activity and the helical structure of B-DNA is thus continuously changed to A-DNA structure [46]. DNA dehydration may contribute to a change of the driving forces for DNA adsorption.

4. Conclusion

In the present paper, utilisation of a methodology capable of testing the applicability of magnetic carriers in PCR was

designed. The presence of cobalt ferrite – with non-modified or modified surface – inhibits PCR amplification. The effect was not dependent on the functional groups of the modifying reagents used (none, amino, carboxyl) in our experimental arrangement. Amplification was slightly improved after magnetic separation of magnetic particles and after their dilution. Particles coated with alginic acid were used for DNA isolation from dairy products under high PEG and sodium chloride concentrations. *Bifidobacterium* and *Lactobacillus* DNAs were identified in whole DNA eluated from particles using PCR.

Acknowledgements

Supported by the Grant Agency of the Czech Republic, grant No. 202/02/1361, and by the National Grant Agency for Agricultural Research (NAZV), grants No. QD 1377 and 1G46045. We thank Mr. Ladislav Červený for kind language improvement.

References

[1] N. Rijpens, L. Herman, F. Verecken, G. Jannes, J. DeSmedt, L. DeZutter, Int. J. Food Microbiol. 46 (1999) 37.
[2] A. Španová, B. Rittich, R. Karpíšková, L. Čechová, D. Škapová, Bioseparation 9 (2001) 379.
[3] G. Wilson, Appl. Environ. Microbiol. 63 (1997) 3741.
[4] J. Sambrook, D.W. Russel, Molecular Cloning, third ed., Cold Spring Harbor Laboratory Press, New York, 2001.
[5] D.M.F. Prazeres, T. Schluep, Ch. Cooney, J. Chromatogr. A 806 (1998) 31.
[6] D. Sykora, F. Svec, J.M.J. Fréchet, J. Chromatogr. A 852 (1999) 297.
[7] Y. Kato, Y. Yamasaki, A. Onaka, T. Kitamura, T. Hashimoto, J. Chromatogr. 478 (1989) 264.
[8] E. Coto, T.E. Hugli, R.D. Ye, R.G. DiScipio, Anal. Biochem. 209 (1993) 199.
[9] H.W. Jarrett, J. Chromatogr. 618 (1993) 315.
[10] M.M. Diogo, J.A. Queiroz, G.A. Monteiro, D.M.F. Prazeres, Anal. Biochem. 275 (1999) 122.
[11] A. González, J. Gómez-Márquez, Genet. Anal. Techn. Appl. 7 (1990) 2.
[12] A. Španová, B. Rittich, I. Vinš, E. Lukášová, S. Kozubek, Int. J. Bio-Chromatogr. 2 (1996) 49.
[13] P.R. Levison, S.E. Badger, J. Dennis, P. Hathi, M.J. Davies, I.J. Bruce, D. Schimkat, J. Chromatogr. A 816 (1998) 107.
[14] B. Vogelstein, D. Gillespie, Proc. Natl. Acad. Sci. U.S.A. 76 (1979) 615.
[15] R. Boom, C.J.A. Sol, M.M.M. Salimans, C.L. Jansen, P.M.E. Wertheim-van Dillen, J. van der Noorda, J. Clin. Microb. 28 (1990) 495.
[16] K.A. Melzak, Ch.S. Sherwood, R.F.B. Turner, Ch.A. Haynes, J. Colloid Interface Sci. 181 (1996) 635.
[17] H. Tian, A.F.R. Huhmer, J.P. Landers, Anal. Biochem. 283 (2000) 175.
[18] M.C. Breadmore, K.A. Wolfe, I.G. Arcibal, W.K. Leung, D. Dickson, B.C. Giordno, M.E. Power, J.P. Ferrance, S.H. Feldman, P.M. Norris, J.P. Landers, Anal. Biochem. 75 (2003) 1880.
[19] G.N.M. Ferreira, J.M.S. Cabral, D.M.F. Prazeres, Biotechnol. Prog. 16 (2000) 416.
[20] E. Thwaites, S.C. Burton, A. Lyddiatt, J. Chromatogr. A 943 (2002) 77.
[21] H.N. Endres, J.A.C. Johnson, C.A. Ross, J.K. Welp, Biotechnol. Appl. Biochem. 37 (2003) 259.
[22] M.A. Teeters, S.E. Conrardy, B.L. Thomas, T.W. Root, E.N. Lightfoot, J. Chromatogr. A 989 (2003) 165.
[23] M.M. deAngelis, D.G. Wang, T.L. Hawkins, Nucleic Acids Res. 23 (1995) 4742.
[24] P.R. Levison, S.E. Badger, J. Dennis, P. Hathi, M.J. Davies, I.J. Bruce, D. Schimkat, J. Chromatogr. A 816 (1998) 107.
[25] P.R. Levison, S.E. Badger, P. Hathi, M.J. Davies, I.J. Bruce, V. Grimm, J. Chromatogr. A 827 (1998) 337.
[26] J.I. Taylor, C.D. Hurst, M.J. Davies, N. Sachsinger, I.J. Bruce, J. Chromatogr. A 890 (2000) 159.
[27] A. Španová, B. Rittich, D. Horák, J. Lenfeld, J. Prodělalová, J. Sučiková, S. Štrumcová, J. Chromatogr. A 1009 (2003) 215.
[28] C.-W. Kim, E.F. Funkenbush, K.H. Moh, Patent WO9218514A1 (1992).
[29] Y.I. Kim, D. Kim, Ch.S. Lee, Physica B 337 (2003) 42.
[30] T. Tago, T. Hatsuta, K. Miyajima, M. Kishida, S. Tashiro, K. Wakabayashi, J. Am. Ceram. Soc. 85 (2002) 2188.
[31] S. Kuckelhaus, V.A.P. Garcia, L.M. Lacava, R.B. Azevedo, Z.G.M. Lacava, E.C.D. Lima, F. Figueiredo, A.C. Tedesco, P.C. Morais, J. Appl. Phys. 93 (2003) 6707.
[32] S. Kuckelhaus, S.C. Reis, M.F. Carneiro, A.C. Tedesco, D.M. Oliveira, E.C.D. Lima, P.C. Morais, R.B. Azevedo, Z.G.M. Lacava, J. Magn. Magn. Mater. 272 (2004) 2402.
[33] A.C. Tedesco, D.M. Oliveira, Z.G.M. Lacava, R.B. Azevedo, E.C.D. Lima, P.C. Morais, J. Magn. Magn. Mater. 272 (2004) 2404.
[34] S. Dubernet, N. Desmasures, M. Guéguen, FEMS Microbiol. Lett. 214 (2002) 271.
[35] D. Roy, S. Sirois, FEMS Microbiol. Lett. 191 (2000) 17.
[36] A. Bee, R. Massart, S. Neveu, J. Magn. Magn. Mater. 149 (1995) 6.
[37] M. Beneš, M. Hrubý, B. Rittich, A. Španová, Czech Patent Application PV 2004-392 (2004).
[38] J. Prodělalová, B. Rittich, A. Španová, K. Petrová, M.J. Beneš, J. Chromatogr. A 1056 (2004) 43.
[39] B. Rittich, A. Španová, Yu. Ohlashennyy, J. Lenfeld, I. Rudolf, D. Horák, M.J. Beneš, J. Chromatogr. B 774 (2002) 25.
[40] J. Křížová, A. Španová, B. Rittich, D. Horák, J. Chromatogr. A 1064 (2005) 247.
[41] A. Španová, D. Horák, E. Soudková, B. Rittich, J. Chromatogr. B 800 (2004) 27.
[42] R.R. Sinden, DNA Structure and Function, Academic Press, San Diego, 1994, p. 34.
[43] G.J.S. Jenkins, B. Burlinson, J.M. Parry, Mol. Carcinog. 27 (2000) 289.
[44] V.V. Vasilevskaya, A.R. Khokhlov, Y. Matsuzawa, K. Yoshikawa, J. Chem. Phys. 102 (1995) 6595.
[45] I.J. Bruce, J. Taylor, M. Todd, M.J. Davies, E. Borioni, C. Sangregorio, T. Sen, J. Magn. Magn. Mater. 284 (2004) 145.
[46] S.C. Harvey, Nucleic Acids Res. 11 (1983) 4867.