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Magnetic Hydrophilic Poly(2-Hydroxyethyl Methacrylate-*co*-Glycidyl Methacrylate) Microspheres for DNA Isolation from Faeces

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*Magnetic non-porous hydrophilic poly(2-hydroxyethyl methacrylate-*co*-glycidyl methacrylate)-P(HEMA-*co*-GMA) microspheres containing carboxyl groups were used for DNA isolation from mouse faeces spiked by a probiotic* Lactobacillus gasseri *K7 strain. The quality of isolated DNA and the presence of target DNA were verified by PCR and real time PCR using primers specific for* Lactobacillus *genus or primers targeting gassericin A gene of the* Lactobacillus gasseri *K7 strain. For comparison, two other DNA extraction procedures were used. It was shown that DNA extracted by carboxyl-coated P(HEMA-*co*-GMA) microspheres were sufficient for the amplification of target DNA using PCR and real-time PCR.*

Keywords DNA isolation; magnetic microspheres; mouse faeces; P(HEMA-*co*-GMA); polymerase chain reaction (PCR)

1. Introduction

Probiotic lactic acid bacteria (LAB) play an important role in health maintenance of the human gastrointestinal tract. Probiotic characteristics are strain-specific and therefore each new probiotic candidate has to be tested individually [1]. *Lactobacillus gasseri* K7 is a probiotic strain which was isolated from the faeces of one-week-old breast-fed baby [2–4]. Due to bacteriocin production it is also a very good candidate as a food preservative [5].

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Its resistance to low pH as well as bile salts is important for the survival during transport through the gastrointestinal tract. Possible beneficial effects on the host have already been tested in pigs and mice [2,6] and are also being tested in humans.

A mouse model has been widely used for in vivo studies of lactic acid bacteria influence on the host organism's health. The study of the ability of probiotic strains to survive the gastrointestinal passage is dependent on the appropriate detection of the investigated probiotic cells in the faeces. The polymerase chain reaction (PCR) has become a powerful diagnostic tool for the analysis of microorganisms in different types of samples including faeces. Faecal samples are a difficult specimen for the application in PCR due to the presence of PCR inhibitors [7]. For this reason, an optimal DNA extraction method in PCR-ready quality is crucial for the target cell detection in faecal samples.

Various procedures have been employed to overcome the above-mentioned problems with the aim to standardise the DNA extraction procedure. Phenol-chloroform extraction [8] is a very common procedure for DNA isolation. Since this method is time-consuming and requires the use of toxic phenol, alternative methods were developed as well. Among them there are methods based on the adsorption of DNA on solid phase carriers. Silica gel and anion-exchange carriers have become popular for DNA isolation [9–12]. Further progress was achieved by the application of magnetically responsive nano- [13–15] and microparticles [16–18]. Magnetic particles coated with silica have been applied for the elimination of inhibitory substances from heavily contaminated samples, including stool samples [19]. Great attention has been paid to the application of magnetic particles in automated systems for nucleic acid isolation [20]. Recently, we have used carboxyl-functionalised magnetic non-porous P(HEMA-*co*-GMA) microspheres for DNA isolation from different types of complex food and environmental samples containing PCR inhibitors [17,21,22]. They have not yet been tested for DNA isolation from faeces.

The aim of this study was to evaluate carboxyl-functionalised magnetic non-porous poly(2-hydroxyethyl methacrylate-*co*-glycidyl methacrylate)-P(HEMA-*co*-GMA) microspheres for DNA isolation from mouse faeces. The quality and quantity of isolated DNA were compared with the phenol extraction method and with commercial kit. The quality of extracted DNA was checked by PCR and real-time PCR using primers specific to the *Lactobacillus* genus and primers targeting the gassericin A gene of a probiotic *Lactobacillus gasseri* K7 strain.

2. Experiments

2.1. Chemicals

2.1.1. Chemicals, Microorganisms and Equipment. Agarose was purchased from Serva (Heidelberg, Germany), ethidium bromide from Sigma (St. Louis, Missouri, USA). The PCR primers were synthesised by Generi Biotech (Hradec Králové, Czech Republic); *Taq*1.1 polymerase was from Top-Bio (Prague, Czech Republic), and the DNA marker (100 bp ladder) for gel electrophoresis was from Malamité (Moravské Prusy, Czech Republic). PEG 6000 was purchased from Sigma (St. Louis, USA). Real-time PCR was performed using the SYBR Green-q-PCR kit (Top-Bio, Prague, Czech Republic). The strain *Lactobacillus gasseri* K7 used as a positive control of DNA isolation was obtained from a culture collection of the Institute of Dairy Science and Biotechology Faculty, University of Ljubljana (IM 105), Slovenia. Magnetic particles were separated on an MPC-M magnetic particle concentrator Dynal (Oslo, Norway). DNA was amplified in a DNA thermal cycler Rotorgene 6000 (Corbett Research, Mortlake, Australia). Agarose gel electrophoreses

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were carried out using an electrophoresis unit (Bio-Rad, Richmond, USA). The PCR products were visualised on an UltraLum EB-20E UV transilluminator (Paramount, USA) at 305 nm and photographed with a digital camera. UV/Vis NanoPhotometer (Implen, München, Germany) was used for UV spectrophotometry.

2.1.2. Magnetic Microspheres and Commercial Kit. Magnetic non-porous P(HEMA-*co*-GMA) (1:1 w/w) microspheres were prepared by cellulose acetate butyrate-stabilised and dibenzoyl peroxide-initiated dispersion copolymerisation of 2-hydroxyethyl methacrylate (HEMA) and glycidyl methacrylate (GMA) in a toluene/2-methylpropan-1-ol mixture in the presence of colloidal oleic acid-coated magnetite (Fe_3O_4) particles as described earlier [23]. Subsequently, the hydroxy groups of the microspheres were oxidised with a 2 wt.% aqueous solution of potassium permanganate under acidic conditions (2 M sulphuric acid) yielding −COOH contents of 0.764 mM/g [24]. The content of carboxyl groups in the microspheres was determined by titration using 0.1 M NaOH on a 799 GPT Titrino (Metrohm, Herrisau, Switzerland) after ion exchange with a 10 wt.% aqueous solution of $BaCl_2$ [25]. The microspheres were 2.2 μm in diameter with a rather narrow size distribution characterised by a polydispersity index PDI = 1.1 (PDI = D_w/D_n, where D_w and D_n are the weight- and number-average particle diameters, respectively, determined from the measurement of at least 500 microspheres on scanning electron micrographs). Commercial kit, QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) was also used.

2.2. Methods

2.2.1. Bacterial Cell Cultivation, Preparation of Spiked Faeces and DNA Isolation. Lactobacillus gasseri K7 cells were cultivated at 37°C aerobically in a liquid Man, Rogosa, Sharp (MRS) medium for 24 h. The bacterial cells from 1 ml of MRS culture were washed by buffer solution (10 mM Tris-HCl, pH 7.8; 5 mM EDTA, pH 8.0) and resuspended in 500 μl of lysis buffer (10 mM Tris-HCl, pH 7.8; 5 mM EDTA, pH 8.0) containing lysozyme (5 mg/ml) and 2 μl mutanolysin (5 U/μl). After 1 h incubation at laboratory temperature, 10 μl of proteinase K (100 μg/ml) and 50 μl of SDS (20%) aqueous solution was added and the mixture was incubated at 55°C for 24 h.

Mouse faeces samples containing the probiotic strain *Lactobacillus gasseri* K7 were prepared by spiking the faeces by the cells of this strain. Crude cell lysates of the spiked faeces samples were prepared in the following way: the faeces (50 mg) were 2 times washed in 1 ml PBS buffer (137 mM NaCl, 137 mM KCl, 4.3 mM Na_2HPO_4, 1.47 mM KH_2PO_4, pH 7.4), the bacterial cells were collected by centrifugation (12,000 *g* for 3 min), washed in buffer solution (10 mM Tris-HCl, pH 7.8; 5 mM EDTA, pH 8.0), and collected by centrifugation again. One ml of *Lactobacillus gasseri* K7 overnight culture (diluted 1:10; *ca* 6×10^8 cfu) was added and centrifuged again. The pellet obtained was resuspended in 940 μl of lysis buffer (10 mM Tris-HCl, pH 7.8; 5 mM EDTA, pH 8.0, lysozyme 10 mg/ml) with 2 μl mutanolysin (5 U/μl). After incubation for 1 h at 37°C, the bacterial cells were destroyed by sonication (3 × 30 s, 1 × 15 min) and 10 μl proteinase K (end concentration 10 μg/ml) and 50 μl SDS aqueous solution (end concentration 1 wt.%) was added. The mixture was incubated at 55°C for 24 h to prepare crude cell lysates (1 ml).

DNA was extracted from crude cell lysates using three different procedures: by phenol extraction (method I, control) according to the literature [8], by carboxyl-functionalised magnetic P(HEMA-*co*-GMA) microspheres (method II)—see Chapter 2.1.2; and using commercial kit–QIAamp DNA Stool Mini Kit (method III) according to the recommendation of the producer. The concentration and the purity of isolated DNA were estimated

spectrophotometrically at 260 nm and 260/280 nm, respectively [26]. The integrity of nucleic acid was confirmed by gel electrophoresis (0.8% agarose) in 0.5 x TBE buffer (45 mM boric acid, 45 mM Tris-base, 1 mM EDTA, pH 8.0). The DNA was stained with ethidium bromide (0.5 μg/ml), decolourised in water, and observed on a UV transilluminator.

In method II, DNA was isolated using carboxyl-functionalised magnetic P(HEMA-*co*-GMA) microspheres (2 mg/ml). A mixture of crude cell lysates (200 μl), microsphere suspension (100 μl; 2 mg/ml) and binding buffer (700 μl; end concentration 16% PEG 6,000 (w/v) and 2M NaCl) was kept at laboratory temperature for 15 min. Magnetic microspheres with adsorbed DNA were separated in a magnetic concentrator for 15 min, the supernatant was discarded, and the microspheres were washed twice with 1,000 μl of 70% ethanol and dried shortly at laboratory temperature. The DNA absorbed on the microspheres was eluted in 200 μl of TE buffer (10 mM Tris-HCl, pH 7.8; 1 mM EDTA, pH 8.0) for 15 min. Experiments were carried out duplicate.

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2.2.2. PCR Amplification and Detection of PCR Products. DNAs isolated using the methods tested were applied in PCR with the *Lactobacillus* genus-specific primers LbLMA 1-rev and R16-1 [27] and gassericin K7 A-specific primers LFA 185 and LFA 268 [2]. The resulting DNA fragments had approximately 250 bp or 84 bp length, respectively. Briefly, the PCR mixture contained 0.5 μl of 10 mM dNTP, 0.5 μl (10 pmol/μl) of each primer, 1 μl of Taq 1.1 polymerase (1 U/μl), 2.5 μl of PCR buffer with 1.5 mM Mg^{2+} ions, and 1 μl of DNA matrix (10 ng/μl); PCR water was added to 25 μl volume. The amplification reactions were carried out using the following cycle parameters: 5 min of the initial denaturation period at 95°C (hot start), 30 s of denaturation at 95°C, 30 s of primer annealing at 55°C, and 30 s of extension at 72°C for LbLMA 1-rev and R16-1 primers. For LFA 185 and LFA 268 primers, denaturation at 94°C and primer annealing at 54°C lasted for 60 s and 30 s, respectively. The final polymerisation step was prolonged to 10 min; the number of cycles was 35. The PCR products were detected using agarose gel electrophoresis (1.8%).

The DNA samples isolated from mouse faeces by different methods were used as DNA template in real-time PCR. Amplification was performed with the same primers and under the same conditions as above (see Chapter 2.3.4.) using a SYBR Green–q-PCR kit. The reaction consisted of 12.5 μl of the mixture supplied, 1 μl of each primer, 9.5 μl of PCR water, and 1 μl of template DNA. The last polymerisation step was followed by a melting curve analysis from 50 to 99°C (held 1 s in the 1st step and 5 s in next steps). A non-template control (NTC) and a positive control (10 ng/μl of DNA from *Lactobacillus gasseri* K7) were included in each plate. The software obtained with a real-time PCR Rotorgene 6000 cycler was used for statistical evaluation of the results.

The reaction efficiency (r.e.) was determined as follows. At first, the linear regression was applied to the experimental dependence of Ct vs. logarithm of the concentration of nucleic acid providing the slope (M) of the respective plot. The latter was used to calculate the r.e. value according to the equation:

$$\text{r.e.} = 10^{(-1/\text{M})} - 1 \tag{1}$$

The dilution method was used for the study of the influence of the DNA extraction procedures tested on the efficiency of amplification. The robustness of the method was estimated from the correlation coefficient of the linear regression (R^2). The corresponding amount of DNA was calculated from the Ct values using a standard curve obtained by dilutions of DNA isolated from *Lactobacillus gasseri* K7.

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3. Results

Phenol extraction method, commercial kit and carboxyl-functionalised magnetic non-porous P(HEMA-*co*-GMA) microspheres were used for DNA isolation from mouse faeces. The last method has been successfully used for the isolation of total DNA from different matrixes containing PCR inhibitors but not from faeces samples yet [17,21].

3.1. Concentration and Purity of Isolated DNA and PCR Amplification

The concentration and purity of total DNA isolated from mouse faeces by different methods are shown in Table 1. The magnetic P(HEMA-*co*-GMA) microspheres are non-porous with a lower area of specific surface and thus the amount of isolated DNA is lower in comparison with other tested method. It should be noted that the concentration of DNA isolated using magnetic P(HEMA-*co*-GMA) microspheres (method II) was also the lowest because only 200 μl of crude cell lysate was used for DNA isolation, while 1 ml or 1.2 ml was used in methods I and III. For commercially available DNA purification system, the amount of starting material is limited by the fixed size of the sample. The advantage of magnetic particles is their possibility of application using variously sized volumes of initial samples.

The amplificability of isolated DNA was proved by genus-specific PCR using LbLMA 1-rev and R16-1 primers [27]. DNA of sufficient quality for PCR was isolated with all methods. No apparent PCR inhibition was observed in DNA isolated by methods I, II, and III in non-diluted samples (results are not shown). The presence of DNA of the strain *Lactobacillus gasseri* K7 was confirmed in all faeces samples using gassericin K7 A-specific primers LFA 185 and LFA 268 [2] (Fig. 1).

3.2. Application of Real-Time PCR for the Study of Influence of PCR Inhibitors on PCR Course

The presence of co-extracted PCR inhibitors can negatively influence the amplification reaction. The simplest approach that helps to avoid the problem of inhibition of amplification is adequate dilution of the samples containing interfering compounds. Such an approach was successfully used for the verification of the suitability of newly designed magnetic particles for their application in PCR [28,29]. Other authors [30] used real-time PCR for examination of the samples contaminated by PCR inhibitors, where comparison of the amplification efficiencies of unknown DNA samples with purified DNA standards showed the level of inhibition.

Table 1. Concentration and purity of DNA isolated from mouse faeces by methods tested

Method	Crude cell lysate (ml)	Isolated faecal DNA ($ng/\mu l$)*	Isolated faecal DNA (μg/ml crude cell lysate)	DNA purity (A_{260nm}/A_{280nm})
I	1.0	70.0	14.0	1.66
II	0.2	5.0	5.0	1.73
III	1.2	40.0	6.7	1.79

*the volume of isolated DNA was 200 μl,
I - phenol extraction, II - P(HEMA-co-GMA) microspheres, III - QIAamp DNA Stool Mini Kit.

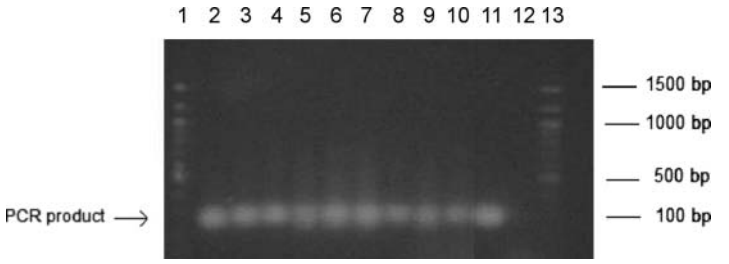

Figure 1. Agarose gel electrophoresis of PCR products (84 bp) specific to gassericin A gene of *Lactobacillus gasseri* K7 strain. Altogether, 25 ng of DNAs isolated from mouse faeces by tested methods were used as templates. Conditions: 1.8% agarose gel, TBE buffer (45 mM boric acid, 45 mM Tris-base, 1 mM EDTA (pH 8.0)). Lanes: (1) and (13) DNA 100 bp ladder, (2)–(4) method III (QIAamp DNA Stool Mini Kit), (5)–(7) method I (phenol extraction), (8)–(10) method II (P(HEMA-*co*-GMA) microspheres), (11) positive control (DNA from *Lactobacillus gasseri* K7, 10 ng/μl), (12) negative control.

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In this study, real-time PCR with LbLMa1-rev and R-16-1 primers [27] was used for the evaluation of amplification efficiency of PCR course for DNA isolated by methods I, II or III. Amplification efficiency can fluctuate due to the presence of co-extracted amplification inhibitors. A simple method to process real-time PCR data is based on Ct values. This method is very sensitive to changes in the concentration of inhibitors presented in the sample. As inhibitor concentration increases, the amplification efficiency decrease and thus the reaction curve slopes decrease [30]. Therefore, calculation of the amplification efficiency allows the detection of non-optimal assay conditions. The results of linear regression analysis are presented in Table 2.

The values of the reaction efficiency (r.e.) were calculated according to Eq. 1. When the PCR is 100% efficient, the slope of the curve (M) is -3.33. An reaction efficiency of PCR between 80 and 110% ($-3.9 >$ slope > -3.0) is acceptable. From the results given in Table 2 it follows that the reaction efficiency was lower than 100% when DNA isolated using P(HEMA-*co*-EDMA) microspheres was amplified (method II). A melting-curve analysis of PCR products consistently showed only one observable peak (melting temperature $T_m = 85^\circ$C), confirming the specificity of PCR.

Table 2. Influence of the DNA extraction methods on the efficiency of amplification in real-time PCR with SYBR Green (linear regression analysis)

Method	c DNA (ng/μl)	M	r.e.(%)	R^2	c.v. (%)
I	0.02–21.0	-3.2	106.2	0.9994	3.5–8.3
II	0.04–50.0	-3.4	95.0	0.9867	2.6–8.4
III	0.09–90.0	-3.1	111.7	0.9998	3.1–4.4

R^2 correlation coefficient,
M slope of regression curve,
r.e. reaction efficiency,
c.v. coefficient of variance,
I - phenol extraction, II - P(HEMA-co-GMA) microspheres, III - QIAamp DNA Stool Mini Kit.

The robustness of the method was estimated from the correlation coefficient of the linear regression (R^2). If R^2 is ≤ 0.985, the assay does not give reliable results for quantification. In all the reactions we succeeded to reach acceptable R^2 values. From the above-mentioned results it follows that the amplification could be partially influenced by the presence of some inhibiting compounds in DNA samples isolated by method II. This may be apparently caused by a less perfect covering of magnetic nuclei with polymer during the process of particle synthesis, as was shown earlier [24], or by the presence of some intracellular or extracellular PCR inhibitors in the samples tested. The coefficients of variance (c.v.) were in the same range (see Table 2) and corresponded with the data published [30]. It is apparent that real-time PCR enabled a more precise analysis of the presence of PCR inhibitors in the DNA samples analysed.

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4. Conclusions

The effectiveness of three different DNA isolation methods was examined for extraction of DNA from mouse faeces. All of these methods were successful. It was shown that both the quality and the quantity of total DNA isolated using magnetic P(HEMA-*co*-GMA) microspheres were sufficient for the amplification of target DNA using PCR and real-time PCR. The presence of DNA of probiotic strain *Lactobacillus gasseri* K7 was detected in all samples. On the contrary to the phenol extraction and the QIAamp DNA Stool Mini Kit, some decrease of the reaction efficiency in real-time PCR was observed after amplification of DNA isolated using carboxyl-coated P(HEMA-*co*-GMA) microspheres.

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References

[1] Cukrowska, B., Motyl, I., Kozáková, H., Schwarzer, M., Górecki, R. K., Klewicka, E., Slizewska, K., & Libudzisz, Z. (2009). *Folia Microbiol.*, *54*, 533.
[2] Majhenič, A. Č., Matijašić, B. B., & Rogelj, I. (2003). *J. Dairy Res.*, *70*, 199.
[3] Matijašić, B. B., & Rogelj, I. (2000). *Food Technol. Biotechnol.*, *38*, 113.
[4] Matijašić, B. B., Koman Rajsp, M., Perko, B., & Rogelj, I. (2007). *Int. Dairy J.*, *17*, 157.
[5] Peternel, M. Z., Majhenič, A. Č., Holo, H., Nes, I. F., Salehian, Z., Berlec, A., & Rogelj, I. (2010). *Probiotics Antimicro. Prot.*, 1.
[6] Hacin, B. (2010). Mechanisms of probiotic action of selected lactobacilli of human and animal origin. PhD thesis, University of Ljubljana, Slovenia.
[7] Wilson, G. (1997). *Appl. Environ. Microbiol.*, *63*, 3741.
[8] Sambrook, J., & Russel D. W. (2001). *Molecular Cloning* (3rd ed.), Cold Spring Harbor Laboratory Press: New York.
[9] Levison, P. R., Badger, S. E., Dennis, J., Hathi, P., Davies, M. J., Bruce, I. J., & Schimkat, D. (1998). *J. Chromatogr. A*, *816*, 107.
[10] Vogelstein, B., & Gillespie, D. (1979). *Proc. Natl. Acad. Sci. USA*, *76*, 615.
[11] Melzak, K. A., Sherwood, Ch. S., Turner, R. F. B., & Haynes, Ch. A. (1996). *J. Colloid Interface Sci.*, *181*, 635.
[12] Shi, R. B., Wang, Y. C., Hu, Y. L., Chen, L., & Wan, Q. H. (2009). *J. Chromatogr. A*, *1216*, 6382.

[13] Prodělalová, J., Rittich, B., Španová, A., Petrová, K., & Beneš, M. J. (2004). *J. Chromatogr. A*, *1056*, 43.
[14] Saiyed, Z. M., Parasramka, M., Telang, S. D., & Ramchand, C. N. (2007). *Anal. Biochem.*, *363*, 288.
[15] He, X. X., Huo, H. L., Wang, K. M., Tan, W. H., Gong, P., & Ge, J. (2007). *Talanta*, *73*, 764.
[16] Berensmeier, S. (2006). *Appl. Microbiol. Biotechnol.*, *73*, 495.
[17] Španová, A., Rittich, B., Štyriak, I., Štyriaková, I., & Horák, D. (2006). *J. Chromatogr. A*, *1130*, 115.
[18] Rittich, B., Španová, A., & Horák, D. (2009). *Food Res. Int.*, *42*, 493.
[19] Petrich, A., Mahony, J., Chong, S., Broukhanski, G., Gharabaghi, F., Johnson, G., Louie, L., Luinstra, K., Willy, B., Akhaven, E., Chui, L., Jamieson, F., Louie, M., Mazzulli, T., Tellier, R., Smieja, M., Cai, W., Chernesky, N., & Richardson, S. E. (2006). *J. Clin. Microbiol.*, *44*, 2681.
[20] Kim, S., Park, S. J., Namgoong, S., Sung, H., & Kim, M. N. (2009). *J. Virol. Methods*, *162*, 208.
[21] Rittich, B., Španová, A., Horák, D., Beneš, M. J., Klesnilová, L., Petrová, K., & Rybnikář, A. (2006). *Colloids Surf. B: Biointerfaces*, *52*, 143.
[22] Rittich, B., Španová, A., Šálek, P., Němcová, P., Trachtová, Š., & Horák, D. (2009). *J. Magn. Magn. Mater.*, *321*, 1667.
[23] Horák, D., Semenyuk, N., & Lednický, F. (2003). *J. Polym. Sci., Polym. Chem. Ed.*, *41*, 1848.
[24] Křížová, J., Španová, A., Rittich, B., & Horák, D. (2005). *J. Chromatogr. A*, *1064*, 247.
[25] Dautzenberg, H., & Philipp, B. (1974). *Faserforsch. Textiltechn.*, *25*, 469.
[26] Sinden, R. R. (1994). *DNA Structure and Function*, Academic Press: San Diego, p. 34.
[27] Dubernet, S., Desmasures, N., & Guéguen, M. (2002). *FEMS Microbiol. Letters*, *214*, 271.
[28] Španová, A., Horák, D., Soudková, E., & Rittich, B. (2004). *J. Chromatogr. B*, *800*, 27.
[29] Španová, A., Rittich, B., Beneš, M. J., & Horák, D. (2005). *J. Chromatogr. A*, *1080*, 93.
[30] Kontanis, E. J., & Floyd, F. A. (2006). *J. Forensic Sci.*, *51*, 795.

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