Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography A, 1130 (2006) 115–121
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Isolation of polymerase chain reaction-ready bacterial DNA from Lake Baikal sediments by carboxyl-functionalised magnetic polymer microspheres

Alena Španová [a], Bohuslav Rittich [a,*], Igor Štyriak [b], Iveta Štyriaková [c], Daniel Horák [d]

[a] *Masaryk University, Faculty of Science, Institute of Experimental Biology, Tvrdého 14, CZ-602 00 Brno, Czech Republic*
[b] *Slovak Academy of Sciences, Institute of Animal Physiology, Department of Microbiology, Soltesovej 4–6, Kosice SK-04001, Slovak Republic*
[c] *Slovak Academy of Sciences, Institute of Geotechnology, Department of Biotechnology, Watsonova 45, Kosice SK-04353, Slovak Republic*
[d] *Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovský Sq. 2, CZ-162 06 Prague, Czech Republic*

Available online 9 August 2006

Abstract

Carboxyl group-containing magnetic nonporous poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) (P(HEMA-*co*-EDMA)) microspheres were used for the isolation of polymerase chain reaction (PCR)-ready DNA from samples of Baikal sediments. DNA was isolated using the phenol extraction method or the Soil DNA Isolation Kit. The occurrence of false-negative results in PCR caused by the presence of extracellular inhibitors in DNA samples was solved using solid phase reversible DNA immobilisation. PCR-ready DNA was reversibly adsorbed to the microspheres in the presence of 8.0% (w/v) poly(ethylene glycol) (PEG 6000) and 2.0 M sodium chloride concentrations. The adsorbed DNA was released from the microspheres in a low ionic strength TE buffer. The quality of isolated DNA was checked by PCR amplification.

Keywords: Magnetic microspheres; P(HEMA-*co*-EDMA); Lake Baikal sediments; Microbial DNA; Magnetic separation; Polymerase chain reaction (PCR)

1. Introduction

Knowledge of microbial diversity in natural ecosystems has been limited as only a minority of occurring microorganisms can be isolated, cultured, and identified using standard techniques. Approximately, 1% of the soil bacterial population can be cultured by standard laboratory methods [1]. Direct, noncultivation-based molecular methods for detecting microorganisms in environmental samples are powerful techniques for studying complex microbial communities.

Polymerase chain reaction (PCR)-based methods have been widely used for this purpose. The identification of bacteria implies several steps: preparation of PCR-ready DNA (lysis of cells, extraction and purification of DNA, ethanol precipitation and RNase A treatment), and identification of bacterial strains by PCR and other molecular methods. The methods developed for extracting soil microbial DNA are of two types: bacterial cell separation methods and/or direct lysis of cells. The isolation of microbial cells from sample matrix is more time-consuming in comparison with the direct lysis method. Direct lysis methods do not require cell isolation and are more often used than the cell extraction methods because greater numbers of microorganisms are subjected to lysis [2]. Nonmicrobial DNA is also isolated with the direct lysis method in addition to microbial DNA. Major disadvantage of the proposed method is the fact that falsely negative results can occur in PCR identification due to the presence of extracellular PCR inhibitors, such as humic and fulvic acids in the samples [2–4]. Thus, additional purification steps are required. The problem of PCR inhibitors can be solved using various chromatographic methods [5–7] or adsorption of genomic DNA on solid phase systems [8–10]. Further progress is achieved by the application of magnetically responsive particles [11–16]. The solid-phase reversible immobilisation method (SPRI) employing carboxyl groups on the surface of magnetic particles was previously used for the isolation of short DNA fragments [12,13] and high-molecular bacterial DNA [14–16]. In our case, ferrimagnetic particles were used. If magnetic components are small enough, they respond to the magnetic field, but they

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

0021-9673/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2006.07.034

are incapable of becoming independently magnetic. As there is no magnetic remanence, the microspheres are not attracted to each other and can be easily re-suspended in homogenous mixture in the absence of external magnetic field [17]. After the incubation period, during which the target molecule binds to the affinity group on the microsphere surface, the particles can be easily removed from the suspension with a magnetic separator. Magnetic separation exposes analytes to a very little mechanical stress compared with the other methods (e.g., centrifugation). For the purpose of this study, the term 'magnetic' is used instead of ferrimagnetic, in order to emphasise the generally intense magnetic behaviour of the microspheres studied.

Lake Baikal, located in eastern Siberia in the Russian Federation, is one of the oldest (25 million years) and deepest (maximum depth 1637 m) freshwater lakes on Earth. It represents an ecosystem with unique characteristics. Significant seasonal changes in temperature take place in the top layers of water only up to the depths of 200–250 m. The average annual temperature of the subsurface water is below 4 °C [18]. The lake can harbour unique microorganisms. There have been some studies on the ecology of bacteria in water [19,20] and sediments in Lake Baikal [21,22]. The methods used for studying the soil microbial diversity were summarised in the literature [1]. Molecular techniques give a complete picture of the composition of complex communities. A useful way for the study of microbial heterogeneity is utilising rRNA sequence heterogeneity. The fluorescent in situ hybridisation (FISH) method is based on the detection of rRNA [23]. Because the rRNA content is associated with the metabolic state of the organism, FISH results are influenced by the activity of the cells. In microbial ecology the use of universal bacterial primers is preferred to amplify a rDNA and then to separate the obtained amplicons in temperature gradient gel electrophoresis [1,24]. Isolation of PCR-ready DNA from the environmental samples is therefore necessary.

The aim of this work was to test carboxyl-functionalised magnetic poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethacrylate) microspheres (P(HEMA-*co*-EDMA)) for the isolation of PCR-ready DNA from a complicated matrix containing PCR inhibitors, such as lake sediments. The quality of isolated DNA was checked by PCR amplification.

2. Materials and methods

2.1. Sediment samples and bacterial samples

Two short core sample collections from the southern basin of Lake Baikal (positions Posolsky Bank and Selenga Delta) were collected during the year 2003. The geographical situation of the study areas is given in the literature [25]. Altogether, 11 samples were taken from the uppermost 0–54 cm without destruction of the sediment-water interface. Samples of sediments were collected at 730–920 m depth. The temperature of the water was 5 °C. The strains of *Bacillus cereus* CCM145 and *Bacillus thuringiensis* CCM19^{T} were obtained from the Czech collection of microorganisms (CCM, Brno, Czech Republic). *Bacillus mycoides* cells were isolated from Baikal Lake sediments [26].

2.2. Chemicals

Agarose was purchased from Serva (Heidelberg, Germany), ethidium bromide from Sigma (St. Louis, MO, USA). Iron oxide was needle-like maghemite (γ-Fe_2O_3) from Société Française d'Electrométallurgie, Marseille, France. These particles show clearly ferrimagnetic behaviour, as reported earlier [27]. Monomers, 2-hydroxyethyl methacrylate (HEMA) and ethylene dimethacrylate (EDMA), both from Röhm (Darmstadt, Germany), were purified by distillation under reduced pressure in nitrogen atmosphere. Cellulose acetate butyrate (CAB) was a kind gift of Eastman (Kingsport, TN, USA). The primers for PCR were synthesised by Generi-Biotech (Hradec Králové, Czech Republic); LA or Taq 1.1 polymerase was from Top-Bio (Prague, Czech Republic), and DNA marker 100 bp ladder (100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1200, and 1500 bp long DNA fragments) for gel electrophoresis were from Malamité (Moravské Prusy, Czech Republic). DNA was isolated from sediment samples (see Section 2.4.2). Other chemicals and solvents were of analytical grade and were taken from commercial sources.

2.3. Equipment

The carboxyl group content on the microsphere surface was determined by titration on a 799 GPT Titrino (Metrohm, Herrisau, Switzerland). Mini Bead Beater was from BioSpecProduct (Bartlesville, OK, USA). Magnetic microspheres 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, MA, USA). Agarose gel electrophoreses were carried out using a Biometra P25 power supply (Biometra, Gottingen, Germany). The PCR products were visualised on a UV transilluminator EB-20E from UltraLum (Paramount, CA, USA), and photographed with a CD 34 Polaroid Camera (Polaroid, Waltham, MA, USA).

2.4. Methods

2.4.1. Preparation of microspheres

Magnetic nonporous P(HEMA-*co*-EDMA) (92/8, w/w) microspheres (diameter 1.5 μm,) were prepared by cellulose acetate butyrate-stabilised dispersion copolymerisation of 2-hydroxyethyl methacrylate and ethylene dimethacrylate in the presence of maghemite (γ-Fe_2O_3) nanoparticles [28]. Subsequently, the hydroxy groups of the microspheres were oxidised with 2% (w/v) aqueous solution of potassium permanganate under acidic conditions (2 M sulphuric acid) yielding 0.85 mM COOH/g [15]. Titration of carboxyl groups was carried out using 0.1 M NaOH after ion exchange with a 10% (w/v) aqueous solution of $BaCl_2$ [29]. A scheme of oxidation of P(HEMA-*co*-EDMA) microspheres is given in Fig. 1 and scanning electron micrograph of magnetic P(HEMA-*co*-EMDA) microspheres in Fig. 2.