Rapid isolation of genomic DNA from *E. coli* XL1 Blue strain approaching bare magnetic nanoparticles

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The aim of the present study was to develop a simple, rapid and inexpensive protocol of ultrapure bacterial genomic DNA extraction suitable for use in molecular methodology. This communication describes a protocol which uses superparamagnetic bare nanoparticles for isolation and purification of genomic DNA from overnight culture of *Escherichia coli* XL1 Blue strain. A comparison is also made with the conventional phenol–chloroform method and the commercially available DNA extraction kit. The tested method successfully yields ultrapure genomic DNA without RNA and protein contamination comparable to the extracted DNA using the commercially available kit. Additionally, the one-step magnetic method took less than 25 min to extract DNA against several hours taken by conventional protocols. Furthermore, the protocol successfully permitted the PCR amplification of a fragment of the bacterial 16S rDNA gene. The extracted DNA was also successfully digested by restriction endonuclease. The significance of this study was to establish a simple protocol for rapid isolation of PCR-ready bacterial genomic DNA.

Keywords: Bacterial genomic DNA, *Escherichia coli*, isolation and purification, magnetic nanoparticles.

THE rapidly growing field of molecular biology and biotechnology has a tremendous need for quick, simple, robust and high-throughput procedures for extraction of DNA from diverse sources. The process of genomic DNA isolation and purification has evolved considerably within the last decade. The new demands of high-throughput facilities have resulted in the development of new technologies for easier and faster DNA processing than ever before. The chemical methods for DNA extraction generally rely on the use of phenol–chloroform[1,2], but the process is toxic, time-consuming, multi-step and utilizes organic solvent extraction, alcohol precipitation as well as centrifugation[3]. In the context of bioseparation and purification, magnetic carrier technology[4] has become an increasingly popular tool for the separation of biomolecules (e.g. DNA, RNA and proteins)[5], and this group has previously reported the fabrication of superparamagnetic silica–magnetite nanoparticles and their use in the extraction of nucleic acids from bacterial cells[6–8].

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In this study, we compare the quality and quantity of isolated genomic DNA using bare magnetic nanoparticles from a cultured bacterial sample with those isolates using standard phenol–chloroform method and commercially available kit separately.

Magnetic nanoparticles were prepared by chemical co-precipitation of Fe^{2+} and Fe^{3+} ions in an alkaline solution, followed by a treatment under hydrothermal conditions[9]. Thus 2.7 g $FeSO_4 \cdot 7H_2O$ and 5.7 g $FeCl_3$ were dissolved in 10 ml nanopure water (double-distilled water filtered through 200 μm filter) separately. These two solutions were thoroughly mixed and added to double volume 10 M ammonium hydroxide with constant stirring at 25°C. Then the dark black slurry of Fe_3O_4 particles was heated at 80°C in a water bath for 30 min. The particles thus obtained exhibited a strong magnetic response. Impurity ions such as chlorides and sulphates were removed by washing the particles several times with nanopure water. Then the particles were dispersed in 20 ml nanopure water and sonicated for 10 min at 60 MHz. The yield of the precipitated magnetic nanoparticles was determined by removing known aliquots of the suspension and drying to a constant mass in an oven at 60°C. The prepared magnetic nanoparticles were stable at room temperature (25–30°C) without getting agglomerated.

Characterization of size and size distribution of the magnetic nanoparticles was made using transmission electron microscopy (FEI, Tecnai S-Twin) which reported the size of the nanoparticle to be 8 nm (Figure 1 *a*), whereas dynamic light scattering (Malvern Zetasizer) indicated monodispersity (the degree of polydispersity is 0.005) and crystallinity (Figure 1 *b*). The electron diffraction patterns also revealed the single crystal nature of these nanocrystals. The zeta potential was also measured and found to be –19.08 mV (Figure 1 *c*). The low value suggests the stability of nanoparticles because of steric repulsion and not electrostatic repulsion. X-ray diffraction on dried magnetic nanoparticle powder was performed on Siemens D500 (Siemens, Berlin) within a 2θ range of 20–80° using Cu$K\alpha$ radiation, which confirmed the presence of Fe_3O_4; the presence of Fe_2O_3 was also evident because of the oxidation of Fe_3O_4 during synthesis (Figure 1 *d*)[10]. The magnetic properties of the dried nanoparticles were obtained using vibrating sample magnometry (VSM, Lakeshore 7410) at room temperature. According to the VSM results (Figure 1 *e*), the negligible coercivity of magnetic nanoparticles showed properties of superparamagnetic materials, indicating that these nanoparticles do not retain any magnetism after removal of the magnetic field. The high magnetization and superparamagnetic properties are highly desirable for biomedical applications because larger magnetic particles form aggregates after exposure to a magnetic field.

For genomic DNA isolation, *Escherichia coli* XL1 Blue strain was inoculated in LB medium with 50 μg/ml tetracycline and incubated overnight at 37°C under shaking

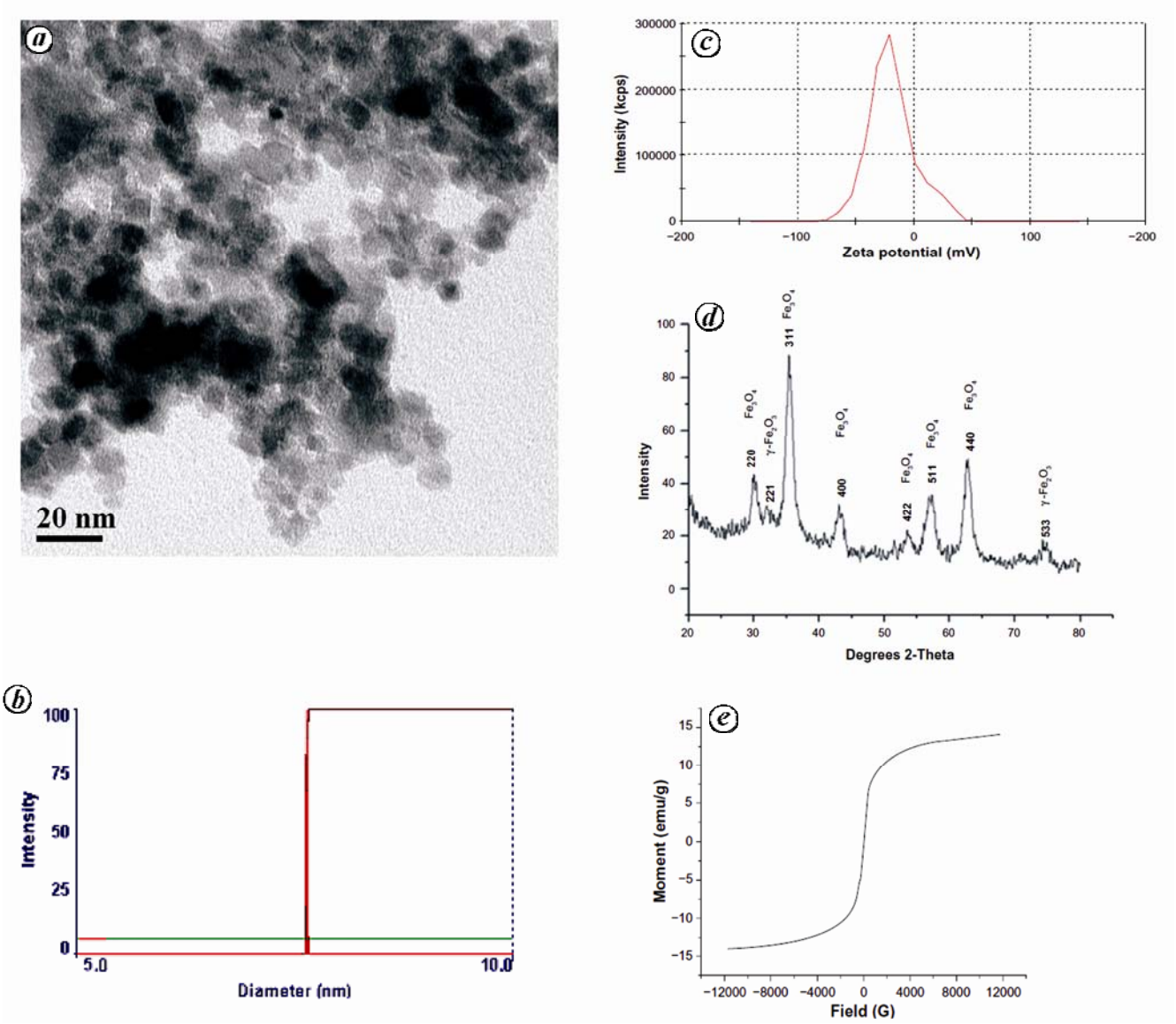

Figure 1. ***a***, Transmission electron microscopic (TEM) image of Fe_3O_4 nanocrystals taken at 120 kV in Joel TEM machine. ***b***, Dynamic light scattering data of magnetic nanocrystals showing the degree of polydispersity. ***c***, Zeta potential of magnetic nanoparticles (–19.08 mV). ***d***, X-ray diffraction pattern of iron nanoparticles. ***e***, Magnetization of nanoparticles as measured by vibrating sample magnometry at room temperature.

condition. Samples containing 10^7–10^8 cells/ml were used for the study. DNA was isolated from bacterial culture by phenol–chloroform method using a standard protocol[11] with some modifications. Here the RNAase treatment step was omitted. Genomic DNA was also isolated from 1 ml of bacterial overnight culture (10^7–10^8 cells/ml) using genomic DNA extraction kit (Chromous Biotech Pvt Ltd, Bangalore, India) according to the manufacturer's instructions. Nucleic acids obtained by both methods were finally resuspended in 200 µl nuclease-free water separately.

For bioseparation of genomic DNA using magnetic nanoparticles, 1 ml sample was taken from overnight bacterial culture. Then 30 µl of 1.5% (w/v) sodium dodecyl sulphate solution was added in 30 µl sample for cell lysis. The solutions were mixed by gentle inversion two to three times and incubated at 50°C in a water bath for 90 s. Lysis of cells was indicated by the formation of a whitish viscous suspension. After incubation, 10 µl (stock 100 mM) of freshly prepared, water-dispersed, washed and well sonicated (at least 20 min at 60 MHz) magnetic nanoparticles was added to the cell lysate, followed by addition of 75 µl binding buffer (1.25 M NaCl, 10% PEG-6000). The suspension was mixed by gentle inversion and incubated at room temperature for 3–5 min. The magnetic pellets were immobilized using a magnetic stand (Promega Ltd.), the supernatant discarded, and the nanoparticles washed with 95% ethanol followed by 70% ethanol and allowed to completely air dry at room temperature. The particles were then totally resuspended in

Table 1. Quality and quantity of bacterial genomic DNA extracted using different methods

Parameters	Method		
	Magnetic bioseparation	Phenol–chloroform	DNA extraction by commercial kit
$A_{260/280}$ #	1.8	1.5	1.83
DNA yield (μg)*	18–20	18–20	20–22
Time required	25 min (MNP preparation took 1 h)	6 h overnight	2 h
Cost/sample (in INR)	10	20	100
Presence of RNA without RNAase treatment	Nil	Present in large amounts	–

#DNA was diluted 1:100 in deionized water for spectrophotometric analysis.
*DNA yields were determined by comparison of sample band intensity with that of co-electrophoresed ready-to-use DNA standard (Bangalore Genei, Bangalore, India).

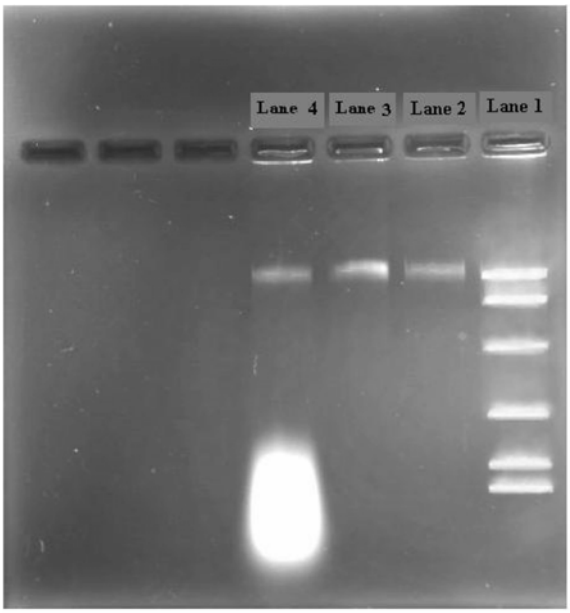

Figure 2. Agarose gel elctrophoresis of bacterial genomic DNA extracted using bare magnetic nanoparticles (lane 2), by commercial DNA extraction kit (Chromous Biotech; lane 3) and phenol–chloroform method (lane 4). Lane 1, DNA molecular weight marker (lamda phage DNA/*Hin*dIII digest).

30 μl of TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.3) and the DNA was eluted from the magnetic particles by incubation at 65°C with continuous agitation. Finally, the supernatant was transferred to a fresh tube by removing the magnetic nanoparticles (MNPs) with the help of an external magnetic field. If the DNA-containing solution appears to be cloudy (i.e. contaminated with very little MNPs), the last step may be repeated to get a clear solution of DNA. The DNA solution was electrophoresed on 0.7% agarose gel.

The quality of DNA extracted from 1 ml overnight bacterial culture using bare magnetic nanoparticles was compared with DNA extracted using the phenol–chloroform method and also using a commercially available kit electrophoretically (Figure 2). A high molecular weight DNA was obtained from the magnetic extraction process which was qualitatively comparable with the DNA isolated using the commercial kit. The phenol–chloroform method was also successful in extracting nucleic acids from the bacterial culture, but in this case both DNA and RNA were present (the latter represented by the diffuse band at the bottom of the gel). The quality of DNA isolated by these three methods was compared (Table 1). As it is known that the DNA and protein absorb in the wavelength of 260 and 280 nm respectively, the extent of protein contamination in the extracted nucleic acid was detected by the A_{260}/A_{280} ratio, spectrophotometrically. A high A_{260}/A_{280} ratio (>1.7) that indicates least protein contamination was obtained by the magnetic bioseparation and commercially available DNA isolation kit methods. The A_{260}/A_{280} ratio for phenol–chloroform method was 1.5, which indicates protein contamination. Nanoparticles as solid phase support yielded 18–20 μg genomic DNA, while it was about 20–22 μg and 18–20 μg in commercial kit extracted and phenol–chloroform extracted DNA respectively. The molecular mass of the isolated DNA was 23.1 kb, as the migrated band is comparable with the 23.1 kb band of the lambda phage/*Hin*dIII molecular weight marker (Figure 2).

The ability of the extracted nucleic acids to act as a substrate in the PCR amplification of a fragment of the bacterial 16S rDNA gene was also tested. PCR reaction was conducted using a standard method[12] for amplification of approximately 1250-bp fragment of the bacterial 16S rDNA gene. Each PCR reaction mixture (final volume of 50 μl) consisted of 5 μl purified DNA (approximately 10 ng DNA) as template, 2.5 μl of 0.2 mM of each primer 338-F (5′tcctacgggaggcagc3′) and 1392-R (5′acgggcggtgtgtrc3′; where $r = a/g$), 5 μl of *Taq* polymerase buffer, 2 μl of 10 mM dNTPs mix, and 1.5 μl of 3U DNA *Taq* polymerase. Primer amplification was performed in a thermal cycler (Applied Biosystem 2700) as follows: 5 min at 94°C, 30 cycles of 30 s at 94°C, 45 s at 56°C, and 1.30 min at 72°C, followed by a final extension for 6 min at 72°C. The PCR-amplified products were analysed directly after electrophoresis on 1% agarose gel. The results (Figure 3) clearly indicated that a 1250 bp fragment of the bacterial 16S rDNA could be successfully amplified for DNA separated by all approaches.

Restriction digestion of the isolated DNA was carried out using 1 µl purified DNA, 1× restriction digestion buffer (Bangalore Genei) and 2U *Eco*RI, and was incubated for 1 h at 37°C. The reaction was terminated by heating at 65°C for 10 min. The digestion mixture was analysed directly after electrophoresis on 1% agarose gel that showed no significant difference in the digestion patterns for DNA obtained using the magnetic separation and Commercial kit methods (Figure 4).

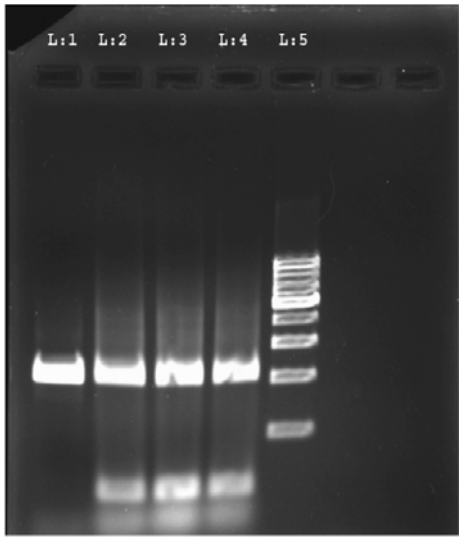

Figure 3. Agarose gel electrophoresis of 1250-bp amplicon of bacterial 16S rDNA gene fragment. Lanes 2–4, PCR product of genomic DNA isolated using the magnetic nanoparticle (lane 2), commercial kit (lane 3) and phenol–chloroform method (lane 4). Lane 5, DNA molecular weight marker (100 bp ladder). Lane 1, Standard *Escherichia coli* 16S rDNA (Bangalore Genei).

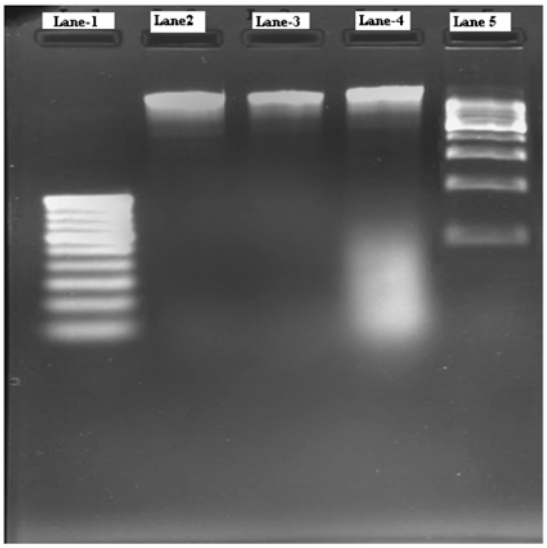

Figure 4. Restriction analysis of extracted DNA. Lanes 1 and 5, DNA molecular weight marker: 100 and 500 bp ladder respectively; lane 2, *Eco*RI digested genomic DNA isolated using magnetic nanoparticles; lane 3, Commercial kit and lane 4, phenol–chloroform method.

During recent years, techniques employing magnetic particles coated with different polymers (e.g. agarose, silica) have been used increasingly for molecular biology applications[13,14]. The purification of genomic and plasmid DNA using magnetizable support (beads or matrix) has already been attempted from different biological sources[15–20]. The applicability of these magnetic nanoparticles for elution of DNA from agarose gel was also successfully demonstrated[21]. Furthermore, carboxyl-coated magnetic particles (BioMag®) have been used as adsorbent for DNA purification under high-salt conditions[22]. All the above-mentioned extraction procedures have used coated magnetic particles, which means only the magnetic property of the particles was used to achieve quick separation. However, the use of naked (uncoated) magnetic nanoparticles (Fe_3O_4) also permits the exploitation of its property to reversibly bind DNA under specific conditions. Additionally, there are several inherent advantages regarding the use of naked particles where molecules are directly linked to the magnetic support. Due to the absence of polymer coating the prepared particle size is small (8 nm), which provides higher surface area (on a weight basis) for the binding of the biomolecules and allows the particles to have a higher magnetic susceptibility to the external magnetic field. Moreover, magnetic nanoparticles can exist as stable colloidal suspensions that will not aggregate, allowing for uniform distribution in a reaction mixture.

To check the robustness and reproducibility of the nanoparticle-based method in comparison to the phenol–chloroform and commercial kit methods, the phenol–chloroform method without RNAase treatment (Figure 2) was found to be contaminated with a large amount of RNA, whereas no low molecular weight band or smear was detected using magnetic bioseparation as well as commercial kit, indicating the absence of RNA contamination. The yield of DNA extracted using magnetic nanoparticles was comparable to that using the commercial kit method. The average $A_{260/280}$ ratio of both the isolated DNA (magnetic bioseparation and commercial kit) was 1.8, indicating that the DNA was of good quality without protein contamination[23]. This is in agreement with earlier reports which mention that in the presence of high-salt conditions or chaotropes, the adsorption of double-stranded DNA onto silica support and magnetite (Fe_3O_4) is thermodynamically favoured, whereas the adsorption of proteins and single-stranded RNA is not[24,25]. The isolated DNA was also found compatible for restriction endonuclease digestion. Additionally, successful PCR amplification indicated the absence of enzyme inhibitors. Hence, the purified DNA can be used for downstream applications.

Magnetic bioseparation has several advantages as it is simple, quick, cheap and robust, and does not require the use of organic solvents, RNAase or proteinase K. Also, the magnetic nanoparticles used for bioseparation of

genomic DNA from bacterial culture need no further functionalization/surface modification, although their extent of utilization from natural sources needs further study. The whole bioseparation procedure can be accomplished in a single tube; the separation method needs only a magnet and a heating block and can be performed in any laboratory without the requirement of sophisticated equipment. The procedure yields an ultrapure quality and at least equal quantity of DNA compared with the conventional (phenol chloroform) and commercial kit procedure that is amenable to manipulation by molecular methods. The method is also considerably less expensive than the alternatives, and yields enough DNA for 30 PCR reaction cycles in less than 25 min. Thus, this method is simple yet powerful; and it can be integrated with the existing procedures, and has the potential to contribute substantially in the automation of bacterial genomic DNA isolation. Further experimentation needs to be carried out on different microorganisms, including yeast to make this a more generalized method.

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