



EXTRACTION OF DNA FROM SOIL USING NANOPARTICLES BY MAGNETIC BIO-SEPARATION

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Abstract : DNA extraction from soil is an emerging technology to understand the microbial diversity of an environment or an effective tool to study environmental microbiology. Soil DNA extraction using standard methods results in low quality and poor yield, making it unsuitable for polymerase chain reaction (PCR) analysis. Two strategies have been developed to improve DNA recovery regarding yield, purity and unbiased representation of microbial diversity. The first approach consists of directly extracting nucleic acids from soil through in situ cell lysis followed by DNA purification. The alternative approach is based on the separation of bacteria from the soil particles followed by cell lysis and then DNA purification. The protocol relies on the use of superparamagnetic silica magnetite nanoparticles for the isolation and purification of DNA from soil samples. DNA suitable for use in molecular biology applications was obtained from several soil samples. This article presents the methodologies relies on the isolation and purification of DNA from soil samples by using nanoparticles, to develop a rapid, simple and inexpensive protocol. The main contribution of this work lies on the DNA isolation from soil samples based on purity, yield, PCR suitability and restriction digestion. This study suggests that the methodology or protocol described in this report is cost-effective, less time consuming, rapid, high-yielding and good-quality DNA from soil. The DNA extracted using the tested method successfully permitted the PCR amplification of a fragment of the bacterial 16S rDNA gene. The extracted DNA could also be restriction endonuclease digested. The protocol reported here is simple and permits rapid isolation of PCR-ready soil DNA. The method requires only small quantities of soil samples, and is scalable and suitable for automation.

Keywords: DNA, magnetic bio-separation, metagenome, PCR amplification, rapid techniques, soil, superparamagnetic silica magnetite nanoparticles, restriction endonuclease digestion, SoilMaster DNA extraction kit.

INTRODUCTION

Extraction of bacterial nucleic acids from natural environments where they exist has become useful method to detect bacteria that cannot be cultured under natural conditions to determine the fates of selected bacteria(Zhou et al., 1996). DNA-based methodologies have become widespread protocol for soil microbiological analyses. DNA extraction from the soil is a key step for these approaches; but it is a challenge for researchers as it is still both expensive and time consuming(Chiodi et al., 2020). The isolation of environmental metagenomes that is the genetic content of any group of microorganisms has been recognized as the powerful approach to novel genetic resources in the natural environments(Sebastianelli et al., 2008). Therefore, the efficient extraction of nucleic acids, especially DNA from soil samples provide a method to access the entire soil metagenome. The two important requirements for metagenomic DNA extraction are efficient cell lysis and purification of DNA from an environmental sample(Devi et al., 2015). Extraction of DNA from natural environments has become an essential method for constructing metagenomic DNA to reveal the genotypic diversity(Sagar et al., 2013). More than 99% of the microorganisms present in natural environments are not cultivable, therefore not accessible for basic research processes (F. Fatima et al., 2014). Efficient methods of extraction of DNA is an emerging subject in research, as purified DNA is extremely important in medical and biotechnological fields. As Currently, DNA extraction methods from soil rely on the use of phenol or chloroform, but the process is multi-step, time consuming, toxic and utilizes alcohol precipitation, organic solvent extraction. This method can be inconvenient or impossible in case of either small amounts of DNA or large amount of samples(Majid et al., 2018). In the case of bio-separation and purification, magnetic carrier technology has become popular or effective method for the separation of biomolecules like DNA, RNA and proteins and this method is previously reported for the fabrication of superparamagnetic silica magnetite nanoparticles and their use in the bacterial nucleic acids extraction(Sen et al., 2018). As an important part of nanomaterials, magnetic nanoparticles widely used in various processes because of their high

surface area, good dispersion, low cost, easy separation and signal detection. Based on these characteristics of nanoparticles, it's application in nucleic acid extraction and detection was reviewed(Nanobiotechnol et al., 2020).

Objectives of this study is to evaluate and improve DNA isolation and purification methods for speed and simplicity, DNA yields, DNA fragment size and applicability to variety of soils(Zhou et al., 1996). The new procedure carried out yields a superior quantity and at least equal quality of DNA, compared with other procedures that is amenable to manipulation by molecular methods. The aim of this study was to develop an automated high-throughput methodology for DNA isolation from soil.

To validate the methodology, yield, purity and amplifiability of the isolated DNA is tested. DNA (Ansari et al., 2018)extraction from soil has three requirements:

- (i) Extraction of high molecular weight DNA,
- (ii)Extraction of DNA free from inhibitors,
- (iii)Representative lysis of microorganisms(F. Fatima et al., 2011).

The phenol/chloroform method is toxic, time-consuming and multi-step and utilizes organic solvent extraction, alcohol precipitation, as well as centrifugation. The method has limitation while dealing with small amounts of DNA. The use of magnetic carrier technology has become widespread technique for the separation of biomolecules(Zhou et al., 1996). There are certain criteria for optimum DNA extraction protocol, which includes, safety, DNA yield, DNA quality or safety, cost of running experiments, user friendliness and time(Hearn & Arblaster, 2010).

The demand for high quality of therapeutic proteins but at the same time low cost associated therapies brought the need for exploring different types of separation techniques, in order to improve downstream processing known to be responsible for a significant percentage of the total manufacturing costs. MNPs appear as a viable choice for biotechnological industries, especially for the bio-separation processes since this option can overcome many problems, specifically cost reduction and process integration(Daniela & Santana, 2011). The extraction of DNA from soil, followed by the application of Polymerase Chain Reaction (PCR) to amplify a gene common to all organisms can provide information about microbial community structure, microbial diversity, evolution and taxonomy. Thus different methods were developed and compared for DNA extraction from the soil and evaluated on the basis of PCR-based 16S ribosomal DNA analysis(F. Fatima et al., 2011).

Currently, nanoparticles have gained a huge attention for analytical biochemistry, medicine, and biotechnology applications, due to their unique size and physical properties(Katz, 2020). In the context of bio-separation and purification, a probable solution is to replace micro-particle with nanoparticles as they have high surface to volume ratio. Advancement of integrated systems to isolate DNA are developed which employ solid-phase supports among which magnetic nanoparticles have received great attention due to their easy manipulation and cost-effectiveness. Magnetic nanoparticles has become an increasingly popular technique for the separation of biomolecules namely DNA, RNA, and proteins(Ansari et al., 2018). In the present study, the protocol described for the isolation of DNA from soil samples is a rapid, inexpensive and scalable method based on use of silica magnetite nanoparticles, where the DNA obtained is suitable for PCR method and endonuclease restriction digestion.

1.SOIL SAMPLE COLLECTION

Soil samples were collected from ground soil at depths between 0 and 5 cm using an aluminium spoon. Samples were sieved using a 2mm mesh for removing stones, roots and large debris from soil prior to DNA extraction and stored in sealed plastic bags at 4°C for further analysis. Inter-sample contamination due to collection of sample was avoided by cleaning the spoon with ethanol between each sampling(Sebastianelli et al., 2008).

2.DNA EXTRACTION

(i) PHENOL-CHLOROFORM METHOD:

The phenol-chloroform DNA extraction method is a widely accepted DNA extraction method. The basic principle of this extraction method is based on liquid-liquid extraction of biomolecules. This method of DNA extraction is more effective along with isoamyl alcohol. Therefore, sometimes it is also called as phenol-chloroform isoamyl alcohol (PCI)method of DNA extraction. Role of each chemical is given below:

PROCEDURE: (Shown in fig.1)

1.Phenol-chloroform(organic) extraction uses sodium dodecyl sulphate (SDS) and proteinase K for enzymatic digestion of proteins and non-nucleic acid cellular components.

2.A mixture of phenol: chloroform: isoamyl alcohol in the ratio of 25: 24: 1 is then added to it to promote the partitioning of lipids and cellular debris into the organic phase, leaving isolated DNA in the aqueous phase.

3. Following centrifugation at 10,000 rpm for 5 minutes, the aqueous phase containing the purified DNA can be transferred to a clean tube for analysis. DNA can also be recovered by ethanol precipitation or by use of a centrifugal filter unit, which allows additional purification and concentration of the extracted DNA.

4. Purified nucleic acids were dissolved in 200 µl of nuclease free water. (Zhou et al., 1996)

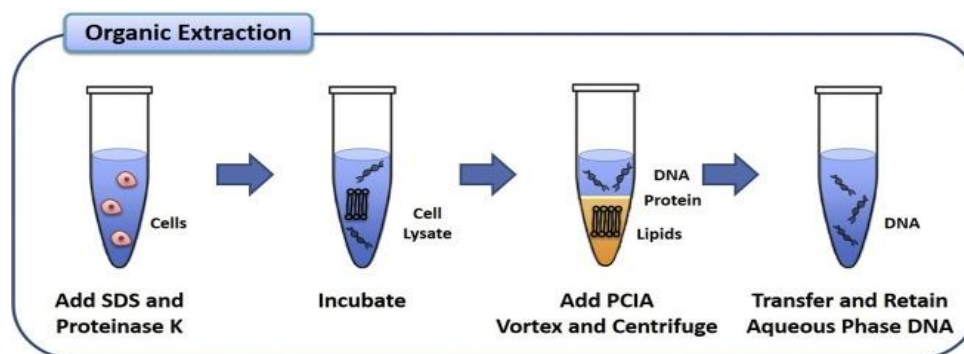


Fig.1. Schematic representation of phenol-chloroform extraction.

(ii) COMMERCIAL KIT EXTRACTION METHOD:

The SoilMaster™ DNA Extraction Kit (Table.1.) provides all the reagents necessary to recover PCR-ready DNA from soil samples. The kit utilizes a hot detergent lysis process combined with a chromatography step, which removes enzymatic inhibitors known to co-extract with DNA from soil and sediment samples.

Table.1. SoilMaster™ DNA Extraction Kit Contents

SoilMaster™ DNA Extraction Kit Contents	
• Soil DNA Extraction Buffer.....	12.5 ml
• Proteinase K (50 µg/µl)	100 µl
• Soil Lysis Buffer.....	2.5 ml
• Protein Precipitation Reagent.....	3 ml
• Inhibitor Removal Resin.....	55 ml
• Spin Columns.....	50
• DNA Precipitation Solution.....	300 µl

PROCEDURE:

1. Weigh out 100mg of soil sample into a tube.
2. Add 250 µl of Soil DNA Extraction Buffer and 2 µl of Proteinase K and mix gently.
3. Add 50 µl of Soil Lysis Buffer into it and mix briefly.
4. Incubate the tube at 65°C for 10 minutes.

5. Centrifuge at 1000 rpm for 2 minutes.
6. Transfer the supernatant to a new tube and discard the pellet.
7. Add 600 µl Protein Precipitation Reagent, mix thoroughly by inverting the tube.
8. Incubate the tube on ice for 8 minutes. Centrifuge the tube for 8 minutes at maximum speed.
9. Carefully transfer the supernatant into the prepared Spin Column.
10. Centrifuge at 2000 rpm for 2 minutes. Discard the column.
12. Add 6 µl of DNA Precipitation Solution and mix gently. Incubate the tube at room temperature for 5 minutes.
13. Centrifuge the tube at maximum speed for 5 minutes. Carefully discard the supernatant.
14. Wash the pellet with 500 µl of Pellet Wash Solution. Centrifuge at maximum speed for 3 minutes. Carefully discard the supernatant.
15. Repeat the wash and centrifuge.
16. Re suspend the pellet in 300 µl of Tris-EDTA (TE) buffer.
17. Extracted nucleic acids were resuspended in 200 µl nuclease free water. (Dna et al., 1993)

(iii) MAGNETIC BIO-SEPARATION:

The rapidly growing field of biotechnology has a critical need for simple, fast and high-throughput processes for the extraction of biomolecules. Isolation and purification of various biomolecules like nucleic acids, proteins, antibodies and antigens in highly purified form is a challenging task. Several bio-separation techniques have been proposed as advanced alternatives to the basic separation methods like precipitation, centrifugation, ligand fishing and chromatography. Magnetic bio-separation is simple, flexible and powerful in working, having important attention in biomedical applications. This is due to important properties of magnetic nanoparticles such as low toxicity, biocompatibility and large surface to volume ratios. All the separation steps of magnetic separation can take place in a single test tube. The interaction between magnetic nanoparticles and targeted molecule with a magnetic force enables separation of targeted molecules, and this is the major advantage of magnetic bio-separation (H. Fatima & Kim, 2017).

STEPS INVOLVED IN MAGNETIC BIOSEPARATION: (Shown in fig.2)

I) SYNTHESIS OF MAGNETIC NANOPARTICLES (MNPs):

First step for magnetic bio-separation is the synthesis of MNPs that involve specific synthesis steps to get specified MNPs based on the target molecules.

II) MODIFICATION OF MNPs:

After synthesis of MNPs, these are modified to inhibit MNPs reactions and agglomeration in the aqueous phase. Surface modifications of MNPs enhance its multifunctional properties like bio-compatibility, colloidal stability and bio-targeting.

III) ADSORPTION STEP:

Modified MNPs are mixed well with the target molecules present in the sample solution and incubated it for few minutes. The modified MNPs will bind with target molecules.

IV) SEPARATION STEP:

Switching on the magnetic field retains the modified MNPs along with target molecules, while undesired molecules are separated.

V) SEVERAL WASHING STEPS:

Washing buffer is allowed to enter into the column. Mixture is passed through several on-off magnetic field cycles. During the off-period, modified MNPs with target molecules again suspended, while during the on-period, they are recollected from the washing buffer.

VI) ELUTION STEPS:

After washing, the target molecules are recovered by the addition of elution buffer.

VII) RECYCLING STEP:

After each separation, the MNPs are then incubated with a specified solution to introduce fresh binding sites for the next separation that is washing of magnetic core-shell $\text{Fe}_3\text{O}_4@ \text{SiO}_2@ \text{polystyrene-alt-maleic anhydride}$ spheres enriched with Ni-NTA using EDTA solution release Ni^{2+} . The particles are then incubated with Ni $(\text{CH}_3\text{COO})_2$ solution to introduce fresh Ni-NTA sites for the separation(H. Fatima & Kim, 2017).

Table 2. Comparison of the different synthesis methods of magnetic nanoparticles

METHODS	SOLVENT	MORPHOLOGY
Co-precipitation	Water	Spherical
Hydrothermal synthesis	Water-ethanol	Spherical
Microemulsion	Organic	Spherical or cubic
Laser pyrolysis	Organic	Spherical less large
Spray pyrolysis	Organic	Spherical but aggregated
Thermal decomposition	Organic compounds	Spherical
Sonochemical	Water	Spherical and rod shaped
Arc discharge	Organic	Spherical or less large

PROCEDURE:

1.Sieved soil was suspended in 1ml lysis buffer [100 mmol l⁻¹ Tris-HCl pH 8.0,100 mmol l⁻¹ EDTA Ph 8.0, 1 mol l⁻¹ NaCl, 2% w/v sodium dodecyl sulphate (SDS), 100 µg ml⁻¹ RNase A] in a 15 ml Falcon tube and incubated at 60°C for 30 minutes with end-over-end rotation.

2.Subsequently, the sample was centrifuged at 10,000 rpm for 5 minutes and the supernatant was transferred to a clean Eppendorf tube containing 1 mg of silica-magnetite nanoparticles to which was added the same volume of binding

buffer [20% w/v polyethylene glycol (molecular weight:8000) in 4 mol l⁻¹ NaCl] and the mixture incubated for 5 minutes at room temperature with end-over-end rotation.

3.The silica-magnetite nanoparticles were subsequently immobilized using a magnetic stand and the supernatant is discarded.

4.The nanoparticles washed three times by adding 200 µl of 70% v/v aqueous ethanol and incubating for 2 minutes at room temperature with end-over-end rotation.

5.The nanoparticles were magnetically immobilized, the aqueous ethanol discarded and the nanoparticles left to air dry.

6.The DNA was eluted from the particles by adding 100 µl nuclease free water and incubating the suspension for 5 minutes at room temperature with end-over-end rotation (maximum DNA recovery was observed to be obtained when the water pH 7.0 and 8.5).

7.The nanoparticles were then immobilized and the DNA-containing supernatant transferred to a sterile Eppendorf tube. The elution process was repeated twice and the two eluates combined. The resulting DNA was stored at -20°C until required.(Sebastianelli et al., 2008)

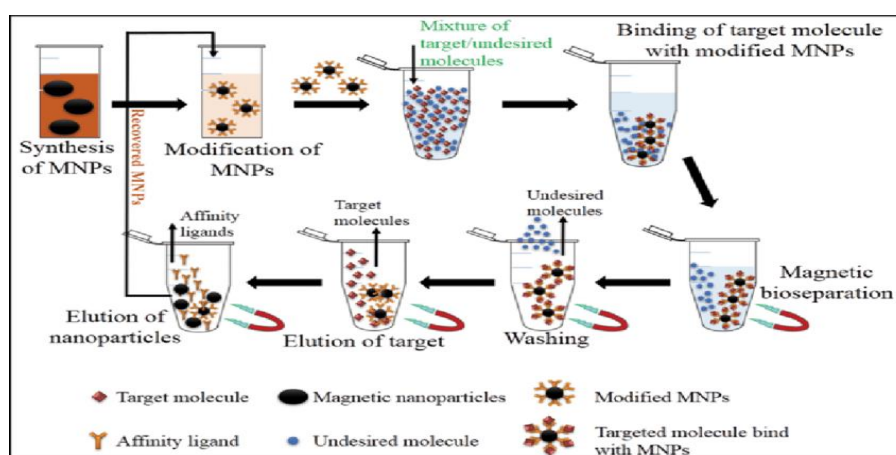


Fig.2. Schematic diagram of bio-separation by magnetic nanoparticles.

CRITERIA FOR OPTIMUM DNA EXTRACTION PROTOCOL:

- Safety: The protocol must be appropriate for use by children from the age of eleven upward.
- DNA yield: The main objective of the DNA workshop is to produce an easily visible quantity of DNA. Unlike many clinical techniques, the precise quantification of DNA yield is not relevant in this context as the DNA will not be processed further.
- DNA quality/safety: The appearance of precipitated DNA is stereotypically characterized as ‘white fluffy strands’ and it would be useful for students if the product conformed to this stereotype.
- Cost: The cost of running DNA extraction experiments can be relatively high, prohibiting the regular use of the practical in educational settings. The primary purpose of this research is to minimize the cost per participant, allowing wide use of the protocol.
- User friendliness: The protocol should be easy to follow and quick to carry out as this allows more time to explain the science behind each step. The protocol should be easily carried out without any previous knowledge of practical laboratory techniques
- Time: This is the biggest constraint since the workshop must run in 1 hr, including time to explain background to the practical as well as the practical itself. The protocol is only feasible if it can be conducted in approximately 30 minutes as this allows the required time for teaching and logistics.

3.PCR AMPLIFICATION

The polymerase chain reaction (PCR) is a method of cloning DNA without the use of microorganisms. PCR allows the rapid selection, isolation and amplification of DNA regions of interest from small amounts of tissue and can be used to help prepare DNA for sequencing.

The PCR reaction takes place in three basic steps:

- i) Template denaturation
- ii) Primer annealing
- iii) New strand extension.

The reaction mixture contains template DNA, two primers each complementary to opposite strands, buffer, the four nucleotides in equal proportions and a thermo-stable polymerase enzyme (Taq polymerase).(Chris Simon, Adrian Franke, 1991)

PROCEDURE:

PCR amplification of the 16S rDNA was performed for all the methods (one sample per triplicate) using the universal forward primer and the reverse primer. The reaction mix consisted of 5µl purified DNA as template, 0.2 mmol l⁻¹ each primer (341f / 907r amplifying a 566-bp fragment of the bacterial 16S rDNA gene), 150 µg bovine serum albumin, 1× Optimized DyNAzme EXT buffer, 0.8 mmol l⁻¹ dNTPs mix, 2 mmol l⁻¹ MgCl₂ and 2 U DyNAzyme™ EXT DNA Polymerase (NEB). The final mixture was adjusted to 50 µl by addition of sterile, purified water.

The amplification steps include following steps; (fig. 3)

- 1.Initial denaturation at 95°C for 5 minutes, 50 cycles of denaturation at 95°C for 1 minutes.
- 2.Annealing at 55°C for 30 seconds.
- 3.Extension at 72°C for 1 minutes with the final extension of 72°C for 10 minutes.

Amplification products were confirmed by loading 5 µl of samples along with 1Kb DNA ladder on 1% agarose gel.(Devi et al., 2015)

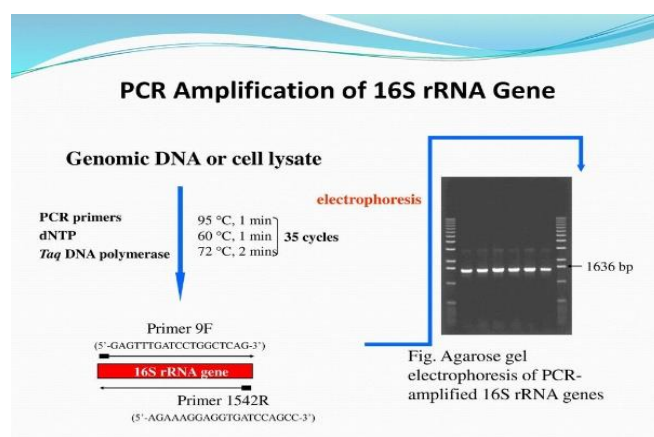


Fig. 3. PCR amplification of 16S rDNA

4. RESTRICTION ENDONUCLEASE DIGESTION

Restriction endonucleases recognize short DNA sequence and cleave double-stranded DNA at specific sites within or adjacent to the recognition sequences. Restriction endonuclease cleavage of DNA into discrete fragments is one of the most basic procedures in molecular biology. Cleavage is accomplished simply by incubating the enzyme with the DNA in appropriate reaction conditions. The amounts of enzyme and DNA, the buffer and ionic concentrations, and the temperature and duration of the reaction will vary depending upon the specific application.

PROCEDURE:

1. Pipette out 1 µg purified DNA, 2 µl 1× restriction digestion buffer and H₂O into a clean microcentrifuge tube.
2. Add restriction endonuclease (2 U EcoRI) and incubate the reaction mixture 4 hour at 37°C.
3. The reaction was terminated by heating at 65°C for 10 minutes.
4. Prepare it for agarose or acrylamide gel electrophoresis by adding 5 µl 10× loading buffer. (Bloch, 1992)

In the experiments reported here, this methodology compared the quality and quantity of nucleic acids, particularly DNA, isolated using magnetic bio-separation method from soil samples with those isolated using a standard phenol/chloroform method (Zhou et al., 1996) and a commercially available kit (SoilMaster DNA extraction kit). The efficiency of soil microbial DNA extraction depends on soil qualities, including the cell content, pH value, and humic acid content (Islam et al., 2012). In this study, three different methods for the isolation of DNA from pure cultures, soil samples and soil samples spiked with pure cultures were compared with respect to purity, yield, efficiency and selectivity of DNA extraction from different species (Kauffmann et al., 2004).

DNA extraction from soil has three requirements:

- i) Extraction of high molecular weight DNA,
- ii) Extraction of DNA free from inhibitors for subsequent molecular biological manipulations to be performed,
- iii) Representative lysis of microorganisms.

It is important to select an extraction and purification method, which yields DNA of suitable quantity and purity. The extraction methods are strongly influenced by several parameters, such as incomplete cell lysis, DNA adsorption to soil surfaces, extraction of humic acid contaminants, and DNA degradation (F. Fatima et al., 2011). Extractions from three soil samples using each method were attempted and Fig. 4a shows that using the silica magnetic nanoparticles DNA was obtained from the three samples and that most of the material was high molecular weight and no RNA was present. The phenol/chloroform method was also successful in extracting nucleic acids from all three samples but in this case both DNA and RNA were present (the later represented by the diffuse band at the bottom of the gel). Some degradation of DNA was also observed

(Fig. 4a).

The amount of DNA isolated using silica magnetic nanoparticles was greater than that from samples extracted using other methods (Table 3). Very little nucleic acid/DNA was extracted using commercial kit. This methodology tested the ability of the extracted nucleic acids to act as a substrate in the PCR amplification of a fragment of the bacterial 16S rDNA could be successfully amplified in all DNA extracted using the magnetic bio-separation approach but that in the case of phenol/chloroform no amplification products were observed (Fig. 4b). PCR products were visible after amplification of nucleic acid extracted using the SoilMaster DNA method but only in two of the three samples (Fig. 4b).

In another series of experiment no significant difference in the digestion patterns for DNAs obtained by the magnetic separation and phenol/chloroform methods was observed (Fig. 5c), whilst DNA samples extracted using the SoilMaster DNA extraction kit could not be used in this experiment because of their low concentrations (Table.3) (Sebastianelli et al., 2008).

Table. 3 Comparison of DNA extraction method from soil samples

	Method		
	Magnetic-bioseparation	Phenol/chloroform*	SoilMaster™ DNA extraction kit†
A ₂₆₀ /A ₂₈₀ ‡	0.98 ± 0.03	1.09 ± 0.04	1.58 ± 0.37
A ₂₆₀ /A ₂₃₀ ‡	0.5 ± 0.03	0.78 ± 0.03	0.57 ± 0.28
DNA yield (µg)§	9.37 ± 2.3	4.62 ± 0.67	0.60 ± 0.12¶
No. of samples	3	3	3
Time required	2 h	5 h	2 h
Cost per sample (£)	1.5	3.0	8.0
Method	Easy	Difficult	Easy

*Zhou et al. 1996.

The kit was obtained from Epicentre Biotechnologies.

DNA was diluted 1: 100 in deionized water for spectrophotometric analysis.

DNA yields were determined by comparison of sample band intensities with those of co-electrophoresed ready-to-use DNA standard.

Direct extraction of DNA from soil samples is also known to result in co-extraction of other soil components mainly humic acid, which are known to inhibit Taq polymerase and restriction endonuclease activities (Bloch, 1992). These contaminants can also be very difficult to remove from the extracted nucleic acids because they share similar physico-chemical properties. The above results tend to suggest that this was not the case in our experiments.

However, as it is known that humic acids absorb UV light at 230 nm (whereas DNA absorbs at 260 nm and protein at 280 nm) we analysed for the presence of both protein and humic acids in the extracted nucleic acids by determining samples A₂₆₀/A₂₃₀ (DNA/humic acids) and A₂₆₀/A₂₈₀ (DNA/protein) ratios. A high A₂₆₀/A₂₃₀ ratio (>2) would be indicative of little humic acids contamination and a high A₂₆₀/A₂₈₀ ratio (>1.7) of low protein contamination. We observed relatively little difference in either the A₂₆₀/A₂₃₀ or the A₂₆₀/A₂₈₀ ratios for samples extracted using either the magnetic, phenol/chloroform or SoilMaster (Epicentre) approaches, suggesting that all samples contained 'more or less' equal amounts of protein and other contaminants (Table.3).

Effectiveness of soil DNA extraction procedures may be influenced by various parameters such as incomplete cell lysis, DNA sorption to soil surfaces, extraction of humic contaminants, and DNA degradation. Thus, extraction of high molecular weight DNA, proper lysis of microbes, and inhibitor-free DNA are the major requirements for any protocol used for metagenomic study. Soil DNA extraction procedures should therefore be free from PCR inhibitors of their concentration must be low enough so that they do not interfere with enzymatic reactions. Usually organic matter is the major source of inhibitors that may be coextracted with the microbial DNA present with the soil. Majorly, humic acids create considerable problem like interference activity of DNA polymerase used for PCR reactions. As humic acid contains the same charge and size characteristics like DNA, it exhibits absorbance at both 230 and at 260 nm and hence interferes in quantization of DNA. This characteristic can be used to find out the level of contamination of humic acid in an isolated DNA sample (F. Fatima et al., 2014).

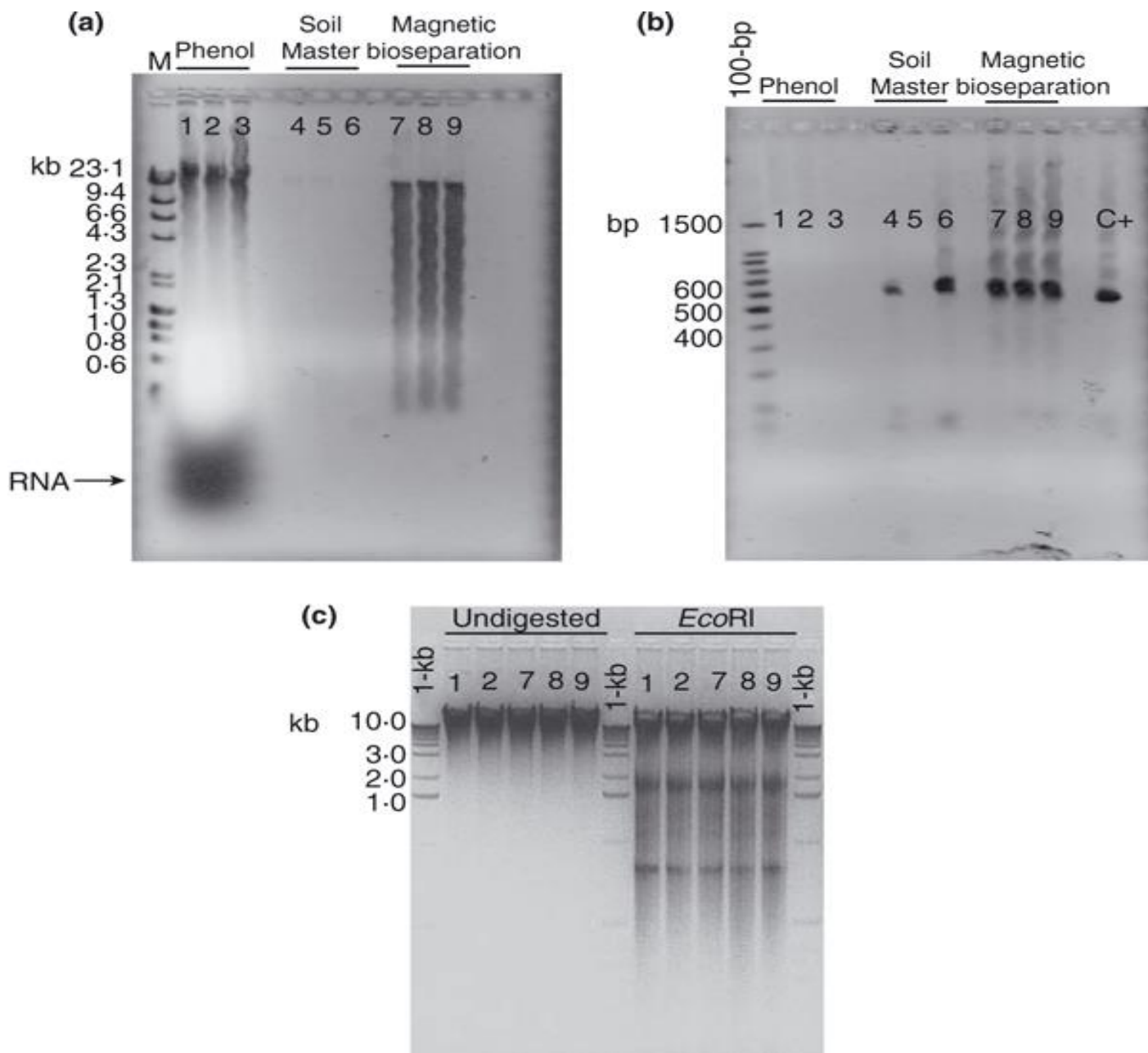
In conclusion, a new method for the isolation/purification of DNA from soil samples that relies on magnetic bio-separation has been successfully developed and its performance has been compared against other methods including a commercial kit.

The new procedure can be carried out rapidly (2 h), yields a superior quantity and at least equal quality of DNA (Fig. 4a, Table. 3) compared with the other procedures that is amenable to manipulation by molecular methods. The new method is also considered less expensive than the alternatives (Table 3).(Sebastianelli et al., 2008).

Figure. 4 Comparison of soil DNA extracted by magnetic bio-separation, phenol/chloroform and SoilMaster DNA extraction methods.

- (a) Soil DNA from the same set of samples was isolated in parallel using the phenol/chloroform (lanes 1-3), the SoilMaster DNA extraction kit (lanes 4-6) and the magnetic bio-separation (lanes 7-9) methods. Ten microlitres of each sample were electrophoresed on a 0.8% w/v agarose gel.
- (b) PCR amplification of a 566-bp bacterial 16S rDNA gene fragment from samples isolated using the phenol/chloroform (lanes 1-3), the SoilMaster DNA extraction kit (lanes 4-6) and the magnetic bio-separation (lanes 7-9) method.
- (c) One microgram of soil DNA isolated by phenol/chloroform (lanes 1-2) and magnetic bio-separation (lanes 7-9) was digested with EcoRI restriction enzyme and electrophorized on a 0.8% w/v agarose gel.





CONCLUSION

Various methods used for soil DNA extraction can cause problems in molecular analysis of populations in natural environment due to variable efficiencies of different methods. DNA extraction from soil has two basic requirements: lysis of representative microorganisms and extraction of high molecular weight, inhibitor-free DNA for subsequent molecular techniques. It is important to select an extraction method which yields DNA of suitable quality and purity with cost effectiveness. In the present study, we have compared our novel magnetic nanoparticle- based soil DNA extraction process with conventional and commercial kit-based method. The main advantage of our method is cost effective and rapid.

Extraction of DNA from soil is tough due to the interference of various organic substances especially humic acid. Various method of soil DNA extraction are already present which include various enzyme treatments such as proteinase K, lysozyme, freeze-thawing, bead beating, organic precipitation, etc. These methods are time consuming, costly, produce some chemical hindrance during amplification and amount of extracted soil DNA is very low. Here, we have compared the magnetic nanoparticle- based approach with the conventional and commercial kit- based soil DNA extraction processes on the basis of quality and quantity of DNA yield. Protein and organic component contaminations were checked at A260/280 and A260/230, respectively. Extracted DNA from various processes was also checked in agarose gel electrophoresis for DNA quality. Thus, the investigation indicates that the conventional method yields minimum quantity of DNA whereas both kit-based method and magnetic bio-separation method yield good quality DNA. Kit-based soil DNA extraction is very much costly as compared to other method.

In conclusion, we report a comparative study of existing conventional protocol and commercially available kit of soil DNA extraction with the method approaching magnetic nanoparticles. Our method yields a good quality, un-sheared, high amount DNA which is comparable to commercially available kit whereas conventional method yields poor quality DNA. Method of soil DNA extraction needs no surface functionalization of the nanoparticle, no RNAase or proteinase K treatment and does not require any organic solvent or hazardous chemicals. Magnetic nanoparticle-based soil DNA extraction does not involve any sophisticated instrument rather only a magnet can perform the extraction process under any laboratory circumstances. Soil DNA extracted by this method is potential in under-going critical molecular biology techniques such as PCR, qPCR and DGGE which are utilized as major tools to explore microbial community of particular environments. Thus, we present a simple, less time consuming, cost effective method of direct soil DNA extraction which can contribute to study various microbial diversity and exploiting them for industrial, environmental and agricultural applications. Thus, a repetitive experiment with diverse soil sample is required to extrapolate the work.

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