Role of Magnetic Nano Particles for the Extraction of DNA: A Review Paper

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ABSTRACT: ***The study of genomics post the deciphering of the human genome has led to an explosion of DNA sequencing technologies which aim to provide the result of DNA sequencing quickly using minimal steps. However, prior to DNA sequencing, the most important step is of DNA isolation. The presence of pathogens in the environment can only be detected by proper isolation of DNA samples. However, such isolation is hampered by the lack of a simple and rapid protocol for isolation of DNA from the environment. The aim of this research paper is to develop a rapid yet inexpensive technique for isolation of DNA from the samples derived from the environment. In this review paper, Iron Oxide Nanoparticles have been described for DNA isolation. Iron Oxide Nanoparticles demonstrated that the yield of DNA isolated via such nanoparticles was much higher than the Phenol/Chloroform method which is currently in use. The usage of nanoparticles in DNA isolation can thereby lead to rapid detection of pathogens in the environment.***

KEYWORDS: ***Bacteria, DNA, DNA Isolation, Environment, Magnetite Nanoparticles, Primers, Samples.***

1. INTRODUCTION

There is a large pool of microbes currently uncultivable under laboratory conditions which includes much diversity, much more that is cultivable under laboratory conditions. Many of the phylotypes display alternative metabolic pathways, different genetic codes and ribosomal compositions, and other functions which are yet to be discovered. Microbes those are present in the environment maybe phylogenetically similar or dissimilar to the ones that can be cultivated. For example in sulphur springs, DNA sequencing has revealed unclassified lineages representing novel phyla and classes. Similarly, by utilizing high throughput DNA sequencing novel microbial taxa has been discovered in the Arctic tundra soils. Many unknown functional genes may be present in the uncultivable environment. Such a genetic treasure of unknown variety maybe unlocked by DNA isolation and 16s rRNA sequencing for prokaryotes and 18s rRNA gene sequencing for eukaryotes. Table 1 and Table 2 has details of microbial enzymatic activity identified by metagenomics and bioactivities identified by metagenomics respectively[1].

Table 1: Microbial enzymes identified from bio-prospecting metagenomics from various soils[2].

Enzyme	**Origin of soil**	**Enzyme feature**
Amylase	Alluvial soil	Hydrolysis of cyclodextrin & soluble starch
Amylase	Mountainous soil	Adaptation for cold temperature
Xylanase/Cellulase	Red soil	Stable, high activity
Xylanase	Compost soil	Novel xylanase
Amidase/Esterase	Alluvial soil	Hydrolysis of florfenicol & chloramphenicol hydrolysis
Lipase/Esterase	Soil contaminated with oil	Enantioselective
Lipase	Forest soil	Thermostable lipase
ß-glucosidase	Alkaline soil	ß-glucosidase gene

The above table (Table 1) provides information about the enzymatic activities identified from soil samples collected from different sites. For identification of such activity, metagenomics was extensively used.

Table 2: Microbial bioactivities identified from identified from bio-prospecting metagenomics from various soils[3].

Source of soil	Biological role
Forest soil from Korea	Production of Indigo & Indigo
Soil from Ithaca, USA	Production of Violacein
Soil from the campus of Cornell campus	Production of long chain ester of fatty acid
Soil from desert	Production of anticancer agents
Soil from arable field	Production of alcohol
Soil from cropland	Production of Turbomycion
Soil from desert	Production of Erdacin
Soil from rice field	Production of Coproporphyrin III
Soil from garden	Production of Indigoidine

The above table (Table 2) provides information about the bioactivity of soil microbes from soil samples collected from different sites. For identification of such activity, metagenomics was extensively used. Metagenomics involves the genomic analysis of the microbial community which involves the extensive use of bioinformatics tools and software. Metagenomics yields important information not only about the identity of the microbes present in that particular sample derived from the environment but also about the phylogenetic relationship it shares with other microbes. The 70s prokaryotes consists of two subunits, 50s and 30s out of which the 16s RNA is an important component of the 30s ribosomal subunit, required for the translation of mRNA. As the 16s rRNA gene has a highly conserved nature, its sequencing provides clues to the taxonomy of a prokaryote and the phylogenetic relationship it shares with others[4]. The root of this tree is the sequence of Desulfovibrio desulfuricans ATCC 27774. Scale bars: 0.1 substitutions per nucleotide position [5]. Fig. 1 depicts an example of a phylogenetic tree construction following the sequencing of 16S rRNA gene is that from the bacterial order Myxococcales.

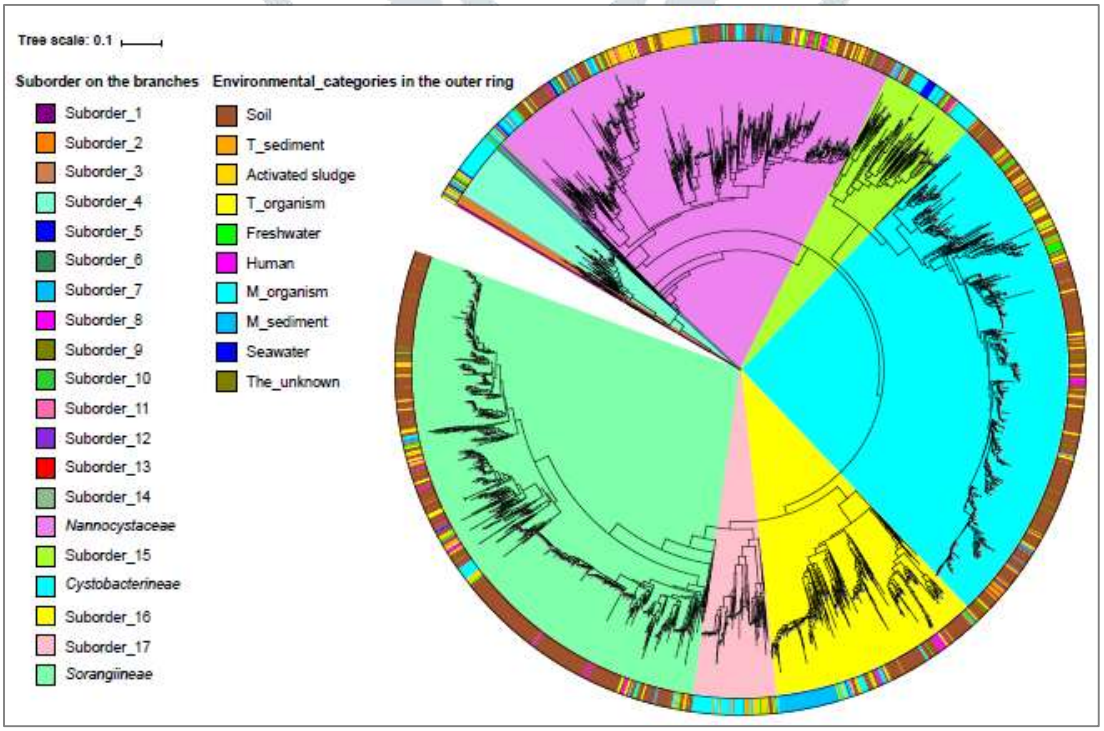

Fig. 1: An example of a phylogenetic tree construction following the sequencing of 16S rRNA gene is that from the bacterial order Myxococcales.

2. LITERATURE REVIEW

S. Basu et al. in their study discloses about limited tools for metagenomic analysis, it seemed only logical to use the popular homology tool BLAST to look for sequence similarity between sample fragments and a database. Most biologists today utilise this method without knowing how accurate BLAST is, particularly when a taxonomic class is underrepresented in the database. The goal of this research is to compare BLAST's performance in taxonomic categorization of metagenomic datasets in a supervised context, where the database contains microorganisms from the same class as the 'unknown' query fragments. We look at well-represented and under-represented taxa and phyla to see how they affect BLAST accuracy [7]. Z. J. Jay et al. in their study discloses about the identification of microorganisms in clinical and environmental samples is one of the most difficult tasks in metagenomics. While there is a large and diverse range of computational algorithms for classifying microorganisms using whole-genome shotgun sequencing data, there are few comprehensive comparisons of different methods [8].

C. T. Brown et al. in their study discusses about the ease of operation, quick setup, high sensitivity, online throughput readouts, and, most crucially, the well-understood concepts behind fluorescence spectroscopy are the primary motivators. An optical sensor using this technology typically requires sensing receptors that interact with an analyte, resulting in a change that can be connected to the identification and/or quantity of the analyte of interest. Various fluorophores are suitable to be employed as the receptor in this case, and the most recent class is fluorescent inorganic nanoparticles, which have similar fluorescence qualities to ordinary organic dyes but have unique features and nature. This work provides a systematic assessment of the evolution of these fluorescent inorganic nanoparticles in the field of optical sensing, excluding fluorescent nanoparticles based on organic fluorophores. It will go over the fundamental attributes, basic synthesis methods, engineering features, and nanoparticle characterization alternatives. This paper will present some effective instances of the use of fluorescent nanoparticles for detecting various analytes. This study provides an excellent overview of real-world uses for fluorescent inorganic nanoparticles' distinctive optical features in sensing applications[9].

3. DISCUSSION

Firstly, soil, water and food samples would be collected and DNA would be extracted from them by the conventional and the by the magnetite nanoparticle methods. Bacterial 16s rRNA primers were then used for amplifying that segment of DNA and the amplicon would be electrophoresed on a 1% Agarose Gel. Firstly as reported earlier, in an alkaline solution Fe^{2+} and Fe^{3+} ions were co-precipitated and were subsequently treated under hydrothermal conditions. After which, the resulting Fe_3O_4 nanoparticles were vacuum dried at 45 ◦ C. It was observed that even after the suspension in nanopure water, prepared MNPs were stable at room temperature (25–30◦ C) without becoming agglomerated[10]. Firstly, an aluminium spoon would be sterilized via autoclaving and then it would be used for excavating soil till a depth of 3 cm. The soil samples would then be stored in sealed plastic bags at 4°C until further use. Larger debris would be removed by sieving.

The water samples would be collected from a nearby pond and would be filtered by passing through a 0.2 mm filter unit so as to separate water from detritus. Both the filtrate and the residue would be kept in pre-sterilized 15 ml centrifuge tubes at 4°C until further use. Food samples collected from market would be deliberately exposed to air for 5 hours. Phenol and Chloroform was used for the Phenol/Chloroform method. Lysis buffer was prepared by using 100 $mmol^{-1}$, Tris–HCl pH 8.0, 100 $mmol^{-1}$, EDTA pH 8.0, 1 mol^{-1} NaCl, 2% w⁄v sodium dodecyl sulfate (SDS), 100 µg ml^{-1} RNase A. Binding buffer was prepared by adding 20% w⁄v polyethylene glycol (mol wt 8000) in 4 mol^{-1} NaCl Firstly, Phenol ⁄chloroform method of DNA isolation was used to isolate DNA from isolated from soil, water and food samples as reported earlier [9]. Similarly, DNA was isolated from the colonies that were formed on Agar plates. Isolated nucleic acids were dissolved in 250 µl of nuclease free water.

Firstly, all the samples (both the ones directly from the samples and the ones whose colony was picked) were incubated for 30 minutes in 1 ml of lysis buffer at 60°C with end-over-end rotation following which, the sample was centrifuged (10 000 g for 5 min) and the supernatant transferred to a clean 2 ml centrifuge tubes containing 1 mg of Iron Oxide Nanoparticles. Subsequently, 1 ml of the binding buffer was added to the mixture which was then incubated for 5 min at room temperature with end-over-end rotation. The Iron Oxide nanoparticles were then immobilized using a magnetic stand (Promega Ltd, Madison, WI). The supernatant was discarded and the nanoparticles were washed three times by adding 200 µl of 70% v⁄v aqueous ethanol and incubating for 2 min at room temperature with end over-end rotation. The nanoparticles were now

magnetically immobilized following which the aqueous ethanol was discarded and the nanoparticles were left to dry at air.

The DNA was then eluted from the particles by adding 100 µl of nuclease free water and incubating the suspension for 5 min at room temperature with an end-over-end rotation. The nanoparticles were then immobilized and the DNA-containing supernatant transferred to a sterile 1.5 ml centrifuge tube. The elution process was repeated twice and the two elutes combined. The resulting DNA was stored at -20°C until required. The DNA which was isolated from each sample was prepared for PCR amplification of the bacterial 16s rRNA gene. The PCR master mix contained till a final adjusted volume of 50 µl consisting of 5 µl purified DNA (approx. 10 ng DNA) as template, 1 each primer (341f/907r amplifying a 566-bp fragment of the bacterial 16S rDNA gene)at a concentration of 0.2 $mmol^{-1}$(Lane 1991), 150 µg bovine serum albumin, 1X Optimized DyNAzme EXT buffer (NEB, Herts, UK), dNTPs mix at a concentration of 0.8 $mmol^{-1}$, 2 $mmol^{-1}$ of $MgCl_2$ and 2 U of DyNAzyme™ EXT DNA Polymerase (NEB). A Progene thermal cycler (Techne, Cambridge, UK) was used for DNA Amplifications at the following parameters: 6 min at 90°C, 60 cycles of 30 s at 90°C, 30 s at 54°C, and 1 min at 72°C, followed by a final extension for 10 min at 72°C[11].

Firstly DNA isolated from soil, water (both filtrate and residue) and food samples, by the Phenol/Chloroform method. Once isolated, their 16s rRNA were amplified and were subsequently electrophoresed on a 1% Agarose gel. In another set of experiment, DNA was isolated from soil, water (both filtrate and residue) and food samples, exposed to air for up to 5 h by the Iron Oxide Nanoparticles. Once isolated, their 16s rRNA were amplified and were subsequently electrophoresed on a 1% Agarose gel. A comparison between the two figures shows that the yield of DNA and its subsequent amplification of the 16s rRNA region is greater in samples whose DNA was isolated by Iron Oxide Nanoparticles as compared to the DNA isolated by the Phenol/Chloroform method.

One of the major hindrances in isolation of DNA from soil samples is the presence of humic acid which are known to inhibit Taq polymerase and restriction endonuclease activities. Humic acid absorbs UV light at 230 nm whereas DNA absorbs at 260 nm and Protein at 280 nm. DNA samples showing a high A260/A230 and A260/A280 ratio indicates low yield of humic acid and high DNA yield respectively. As shown in Table 3, Table 4, Table 5 and Table 6, a consistent higher ratio of A260/A230 and A260/A280 were observed indicating a higher DNA content as compared to humic acid and protein in all the samples included in this experiment.

Table 3: Comparison of DNA extraction method from soil samples[2]**.**

Parameters	Magnetic Bio-separation	Phenol/Chloroform
A260/A280	1.02 ± 0.03	0.7 ± 0.04
A260/A230	0.75 ± 0.03	0.68 ± 0.03
DNA yield(µg)	9.12 ± 1.3	4.26 ± 0.07
Number of samples	5	5
Method	Easy	Difficult

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 premixed DNA standard (N. England Biolabs).

Table 4: Comparison of DNA extraction method from water-Filtrate sample[2]**.**

Parameters	Magnetic Bio-separation	Phenol/Chloroform
A260/A280	0.78 ± 0.01	0.51 ± 0.05
A260/A230	0.5 ± 0.03	0.58 ± 0.03
DNA yield(µg)	5.37 ± 0.3	1.62 ± 0.67
Number of samples	5	5
Method	Easy	Difficult

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 premixed DNA standard (N. England Biolabs).

Table 5: Comparison of DNA extraction method from water-residue sample[4]**.**

Parameters	Magnetic Bio-separation	Phenol/Chloroform
A260/A280	0.8 ± 0.01	0.6 ± 0.07
A260/A230	0.55 ± 0.03	0.35 ± 0.03
DNA yield(µg)	6.37 ± 2.3	3.62 ± 0.67
Number of samples	5	5
Method	Easy	Difficult

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 premixed DNA standard (N. England Biolabs). The major finding of this work is that irrespective of the origin and type of sample, the yield of DNA by using the Iron Oxide Nanoparticles is more than by using the conventional Phenol/Chloroform method. Consequently, the gel band intensity was higher for the 16s rRNA amplicon for the DNA isolated by using the Iron Oxide Nanoparticles is more than by using the conventional Phenol/Chloroform method.

Table 6: Comparison of DNA extraction method from food sample[6]**.**

Parameters	Magnetic Bio-separation	Phenol/Chloroform
A260/A280	0.48 ± 0.03	0.32 ± 0.04
A260/A230	0.5 ± 0.03	0.78 ± 0.03
DNA yield(µg)	9.37 ± 2.3	4.62 ± 0.67
Number of samples	5	5
Method	Easy	Difficult

4. CONCLUSION

Metagenomics is the field of genomics which involves the study of genomes isolated from the environment. Nowadays environmental studies have gained attention due to the presence of pollutants & also to know about the presence of pathogens in the environment. The field of metagenomics is thus solely dependent upon the procedure of DNA isolation which in turns is dependent upon the yield of the DNA so isolated. In this review paper, two methods of DNA isolation has been described regarding which a variety of sample types were chosen. Out of the two methods described in this paper, higher DNA yield was obtained by using the Iron Oxide Nanoparticles. Thereby, this method of DNA isolation could prove to be very useful for rapid isolation of DNA from any environment type for bio-prospecting and surveillance of pathogens.

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