Isolation and Diversity Analysis of Soil Metagenome

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ABSTRACT: Soil biology holds an important role for methodological studies. Among different niches, the highest level of prokaryotic diversity resides in the soil, and extraction of DNA from soil using metagenomic approaches can improve our knowledge about these communities. The analyses of soil biodiversity and utility undertake that the extracted DNA denotes the complete microbial community of that niche, but further elucidations are limited due to the amount of DNA recovered from the sample. To improve metagenomic approaches, the present study aimed at determining the effect of different concentrations of SDS and depth of soil sample from the surface on microbial diversity in different areas. Maximum amount of DNA (1.53 μg/g) was obtained by using CTAB along with 2% SDS. Soil samples taken from a depth of 16 cm resulted in maximum DNA isolation which was further enhanced due to vortexing procedure (402.46, 509.6 and 2289.8 in case of NS 16cm, waste soil 16cm and Durgapura soil 16cm respectively). The quality of DNA increased with the ratio $A_{260/280}$ varying from 1.30 to 1.61 for RNase based and 1.69 to 1.81 for column based purification.

Keywords: Absorbance; biodiversity; DNA; extraction; metagenomic; prokaryotic; quality; soil.

INTRODUCTION: The NGS technology is often used to explore the identity and abundance of culturable and non-culturable microbial species in its natural community and to decode the microbial genomes to investigate its functional repertoires. The soil microbial community is relatively diverse (Curtis, 2002 and Robe, 2003), with arguably the highest level of prokaryotic diversity of any environment (Roesch *et al.,* 2007). One gram of soil has been reported to contain up to 10 billion microorganisms and thousands of different species (Knietch, 2003). Currently, less than 1% of this diversity isconsidered to be cultivable by traditional techniques (Schloss and Handelsman, 2003). This limitation can be evaded by metagenomic approaches that have been applied to study various soil environments (Courtois *et al.*, 2003; Demaneche *et al.*, 2008; Rajendhran and Gunasekeran, 2008). Different DNA extraction methods used to study the soil biodiversity have limitations that restrict the study of diversity of the so-called metagenomicDNA (Carrig *et al.,* 2007; LaMontagne, 2002; Milton *et al.,* 2007; Morales *et. al.,* 2008). As a result, this hinders our knowledge of the total microbial diversity of soil.

Current available technologies provide relatively quick, deep and economic sequencing of metagenomic DNA samples (Kahvejian *et al.,* 2008; Shendure and Hanlee, 2008) however, metagenomic DNA sequencing depends entirely on the DNA extracted. To study soil function based on soil metagenome sequencing requires extraction of DNA from every soil microbial community but the problem arises because all the protocols facilitates the extraction of only a part of microbial population to the detriment of the rest. It has been estimated from a variety of methods that biodiversity of soil range from 104 species (Torsvik *et al.,* 2002; Roesch, 2007) to 107 species (Gans, 2005) per gram of sample. Therefore, to understand whether sequencing depth or DNA extraction diversity is driving diversity estimations depends on the biodiversity estimates on metagenomic access. Gupta *et al.,* 2017 compared the metagenomic genomic DNA extraction methods to explore the bacterial diversity in hot springs of Ladakh, India.

In this research problem, different metagenomic methods were combined to estimate soil microbial diversity to enlighten the soil bio diverse community. The diversity of DNA was significantly resolved using four different classes of DNA separation techniques. These techniques are based on (i) vertical soil sampling (ii) application of different concentrations of SDS, (iii) cell lysis stringency, and (iv) DNA purification.

MATERIALS AND METHODS:

Chemicals and Soil samples: All the reagents and chemicals were of the highest purity available and were obtained from HiMedia. Gel Extraction Kit was supplied by Taurus Scientific (USA), assay buffer, Taq Polymerase and dNTPs were from Bangalore Genei. Nine soil samples collected from different regions of Jaipur, Rajasthan namely Normal garden soil, Durgapura and Industrial waste, three each for same location but from various depths.

DNA Isolation: Two soil samples were used to standardize DNA isolation by using CTAB method given by Doyle & Doyle (1990) for plant DNA isolation, with various modifications like addition of different concentrations of SDS and enzyme (lysozyme) for lysis of the cell using same extraction buffer. The total yield of DNA was estimated by U.V. absorbance measurements. The integrity of isolated DNA was verified by visualization on agarose gel (0.8%) with DNA standards (Uncut lambda DNA).

Effect of depth on amount of DNA isolated: Effect of depth (3cm, 7cm and 16cm) on amount of DNA isolated was determined under grinding and vortexing conditions for different concentrations of SDS (1%, 2% and 3%).

Effect of DNA purification: Different methods like RNase treatment (CI method) and column based purification (using GEL Extraction Kit) were used to determine their effect on DNA concentration.

RAPD profiles: Soil samples from various depths were examined for Random Amplified Polymorphic DNA (RAPD) genetic marker with 12 decamer random primers (GCC, USA). Primers were screened taking DNA of two soil samples before performing RAPD analysis on all the genotypes.

RESULTS AND DISCUSSION:

Standardization of method for DNA isolation: For different concentrations of SDS (1%, 2% and 3%) along with CTAB, the quality of DNA was determined as the ratio $A_{260/280}$ which ranged from 1 to 1.9 before purification, which indicates high impurity in DNA solution. The yield of DNA varied from 0.53 to 1.53 µg/g of soil (Table l).

For the isolation of DNA from different soil samples, 2% SDS concentration was found optimum. This concentration was used further for DNA isolation of the entire sample with grinding/vortexing and to study the effect of depth of soil sample on microbial community (Table 2).

Effective method for Soil DNA purification: DNA was highly impure, brownish in color and after purification by using different methods like RNase treat-

ment (CI) and column based purification, the amount of DNA decreased. In order to obtain recordable absorbance, dilution factor was decreased from 300 to 100 because of less amount of DNA.

Table 1: Amount of DNA obtained by different methods of DNA isolation.

Method	Absorbance		Ratio $A_{260/280}$	DNA µg/µl	DNA µg/g
	260 nm	280 nm			
CTAB	0.008	0.008	1.0	0.04	0.53
	0.019	0.014	1.36	0.00	1.26
CTAB and 1% SDS	0.013	0.010	1.3	0.06	0.86
	0.011	0.009	1.2	0.05	0.73
CTAB and 2% SDS	0.023	0.017	1.35	0.11	1.53
	0.021	0.015	1.4	0.105	1.4
CTAB and 3% SDS	0.010	0.009	1.1	0.05	0.59
	0.013	0.010	1.2	0.065	0.86

Table 2: Effect of depth on amount of DNA obtained by Grinding/Vortexing with different concentration of SDS.

Sample ID	1% SDS		2% SDS		3% SDS	
	Grind-ing	Vortex-ing	Grind-ing	Vortex-ing	Grind-ing	Vortex-ing
NS 3cm	33.33	39.90	107.38	155.65	97.32	141.02
NS 7cm	83.24	131.00	141.00	215.00	137.51	196.26
NS 16cm	159.80	310.56	275.43	402.46	192.34	275.25
Waste soil 3cm	57.52	70.79	138.26	124.20	127.95	95.36
Waste soil 7cm	174.01	239.12	203.33	322.14	171.09	256.71
Waste soil 16cm	279.76	279.23	509.63	456.20	406.21	398.65
Durgapura soil 3cm	79.85	123.87	189.65	267.65	171.20	189.25
Durgapura soil 7cm	296.87	484.36	589.76	796.85	413.12	589.64
Durgapura soil 16cm	582.54	1015.78	1567.87	2289.82	749.23	1402.06

DNA yield (before purification) varied from 124.2 µg/µl to 2289.82 µg/µl, which is indicative of good amount of DNA, in comparison to earlier three methods (Table 1). The quality of DNA was determined as the ratio $A_{260/280}$ that ranged from 1.00 to 1.22 before purification, which is indicative of impure DNA (Table 3; Figure 1).

DNA yield after purification reduced in amount, varying from 44.97 µg/µl to 1998.36 µg/µl for RNase based purification and from 74.01 µg/µl to 1993.21 µg/µl for column based purification, but the quality increased with the ratio $A_{260/280}$ varying from 1.30 to 1.61 for RNase based and 1.69 to 1.81 for column

based purification. So column based purified DNA was subsequently used for RAPD analysis.

Table 3: Showing amount of DNA before and after purification step (as obtained by RNase/ Column based purification method of DNA isolation).

Samples	Ratio of $A_{260/280}$		
	No purification	RNase based	Column based
NS 3cm	1.12	1.45	1.69
NS 7cm	1.09	1.52	1.78
NS 16cm	1.13	1.30	1.72
Waste soil 3cm	1.22	1.56	1.76
Waste soil 7cm	1.08	1.60	1.80
Waste soil 16cm	1.06	1.54	1.79
Durgapura soil 3cm	1.00	1.44	1.81
Durgapura soil 7cm	1.03	1.45	1.69
Durgapura soil 16cm	1.04	1.61	1.73

PCR amplification of purified DNA: The primers taken were from GCC-l01 and GCC-112 series as per their availability in the laboratory. Out of twelve primers used for screening, three primers did not amplify any fragment. The reproducibility of the bands generated by these 9 primers was confirmed by replicating the amplification twice. PCR efficiency of column based and RNase based purification methods was also done using same primers in which column based purified samples gave good PCR amplification but RNase based sample gave either very faint band or no amplified product.

Table 4: RAPD profiles generated through various random decamers.

Genotype									
	D.P. 3cm	D.P. 7cm	D.P. 16cm	Waste soil 3cm	Waste soil 7cm	Waste soil 16cm	NS 3cm	NS 7cm	NS 16cm
Primer UBC 104	0	1	0	1	0	0	0	0	1
	0	0	0	0	1	0	0	0	0
	0	0	0	0	1	0	0	0	0
	0	0	1	0	0	1	1	0	1
	0	0	1	0	1	0	1	1	1
	0	0	0	0	1	0	0	0	0
	1	0	0	0	0	1	0	0	1
	1	1	0	0	0	0	1	0	0

DISCUSSION: The relationship between microbial biodiversity and their function in soil is attracting many researchers to explore them. At present, the more general concern is to conserve biodiversity and to study its part in preserving an efficient biosphere. To understand and manipulate the working of ecosystems and the ability of an ecosystem to withstand serious disturbances, one will be dependent on the diversity of the system. This spurt in interest to study soil microbial diversity stems from convincing demonstration that the uncultured microbial world far outsized the cultured world and that this unseen world could be studied.

Standardization of method for DNA isolation: In order to apply DNA based techniques to uncultivable microbes, DNA isolation methods from soil needs to be standardized. DNA extraction procedures involve cell extraction or direct lysis, depending on whether or not the microbial cells are isolated from their matrix. There are a number of methods used for lysis of cells in soil samples but in this study CTAB and CTAB with SDS in different concentrations was used. Nine soil samples collected from different regions of Jaipur, Rajasthan namely Normal garden soil, Durgapura and Industrial waste, three each for same location but from various depths (3 cm, 7 cm and 16 cm). DNA extracted using CTAB along with 2% SDS was better as compared to other methods. Similarly, Satyanarayana and coworkers in 2017 optimized high yielding protocol for DNA extraction from forty different forest soil samples and showed that in the protocol involving treatment with 20% SDS, 32.8 µg DNA/g soil was obtained.

Effect of lysis technique on the amount of DNA: Grinding treatment for 2-3 min produced highly sheared DNA however; if it was substituted with 2-3 min vortexing then it resulted in a good amount of DNA with least shearing. Nevertheless, 2% SDS with little vortexing method was found better in comparison to other methods in terms of DNA yield. Miller *et al.,*1999 used a lysis mixture containing chloroform, SDS, NaCl, and phosphate-Tris buffer (pH 8) for optimization of both the amount of DNA extracted and the molecular size of the DNA (maximum size, 16 to 20 kb) which was found to be the best physical lysis technique.

Purification of isolated DNA: The isolated DNA was purified using Nucleopore gel extraction kit. A higher yield of purified DNA was observed. DNA was also purified by using RNAse treatment followed by chloroform/ isoamyl alcohol purification and was also found to produce amplifiable DNA but with lesser purity and faint amplicons. However, extraction kit provided good quantity of DNA and the quality ratio

260/280 also increased. Similarly, Miller *et al.,* 1999 in their studies evaluated four different DNA purification methods (silica-based DNA binding, agarose gel electrophoresis, ammonium acetate precipitation, and Sephadex G-200 gel filtration) for DNA isolation and to remove PCR inhibitors from crude extracts. They found that Sephadex G-200 spin column purification was the best method for the removal of PCR-inhibiting substances and at the same time minimizing DNA losses during purification with 80 ± 7% recovery from agricultural soil and 95 ± 6% recovery from forest soil.

PCR amplification of purified DNA: In this study, out of twelve primers used for screening, three primers did not amplify any fragment. For the other nine primers, column based purified samples gave good PCR amplification but RNase based sample gave either very faint band or no amplified product. In a similar study, Tilwari and coworkers in 2013 investigated microbial diversity of industrially contaminated and uncontaminated agricultural field soil using RAPD analysis using four ten-mer primers namely RBa- 3, 4, 5 and 6 and observed that out of 56 DNA fragments generated with good reproducibility, 55 of
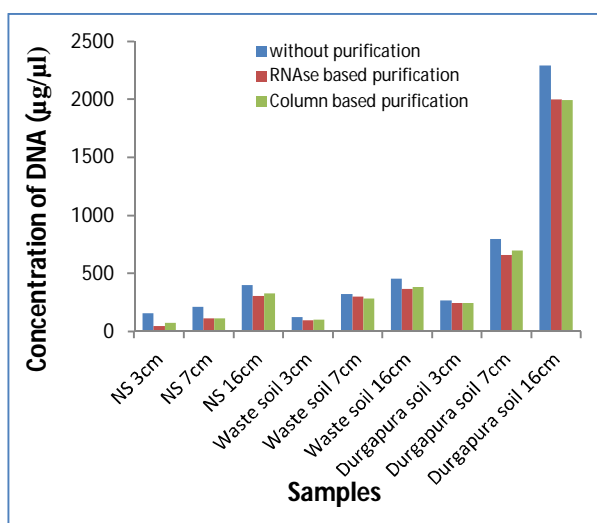
them were polymorphic (99%) and one was monomorphic (1%).

Figure 1: Amount of DNA before and after and purification.

CONCLUSION: A range of microorganisms and genes with potential biotechnological applications may be present in soil environments. Therefore, from the present investigation, it can be concluded that there is a direct effect of anthropogenic activities and depth of sample from soil surface on the microbial community of that area. Vortexing along with 2% SDS method resulted in maximum DNA isolation and column based method is the most efficient method for purification.

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